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# Chemical composition, antioxidant, and antibacterial activity of essential oils from *Etlingera sayapensis* A.D. Poulsen & Ibrahim

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ABSTRACT

**Objective:** To report the chemical composition and bioactivity (including antioxidant and antimicrobial activity) of essential oils from the rhizomes, stems, and leaves of *Etlingera sayapensis* (*E. sayapensis*) A.D. Poulsen & Ibrahim for the first time.

**Methods:** First, the essential oils were obtained using a Clevenger-type apparatus. Then, the essential oils compositions were identified by chromatography methods including GC-FID and GC–MS. For the next step, DPPH radical scavenging activity (RSA), β-carotene bleaching (BCB), and ferrous ion chelating ability (FIC) were chosen to evaluate the essential oils antioxidant activity. Finally, disc diffusion assay and minimum inhibitory concentration method (MIC) was applied to investigate antimicrobial activity of the rhizomes and leaves oils of *E. sayapensis* against 18 microorganisms.

**Results:** All of the oils contained oxygenated monoterpenes (leaves: 74.18%, stems: 75.60%, and rhizome: 54.61%), The essential oil obtained from leaves contained high amount of carvone (21.38%), *cis*-carveol (13.49%); The rhizomes oil was rich in linalool formate (25.47%), eugenol (11.84%); and the stems oil was dominated by α-terpineol (39.86%), linalool formate (30.55%). The leaves oil represented the highest ability in all of the antioxidant activity tests. For antimicrobial activity, the rhizome oil presented more active when compared to leaves oil against *Bacillus subtilis*, *Bacillus thuringiensis*, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), *Aeromonas hydrophila*, *Escherichia coli*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Shigella sonnei*, *Serratia marcescens*, *Vibrio parahaemolyticus*, *Candida albicans*, and *Candida parapsilosis*.

**Conclusions:** The most components of the essential oils belong to oxygenated monoterpenes. Linalool formate, carvone, and α-terpineol are found as the most abundant compounds in the oils of the different parts of *E. sayapensis*. The rhizomes oil can prevent the growth of wide spectrum microorganisms; however, the oils are not highly potent in antioxidant assays.

## 1. Introduction

The subfamilies of Zingiberaceae including *Hedychieae*, *Zingibereae*, *Alpineae*, and *Globbeae*, have wide variety applications in food and medicine. The plants are a prime candidate for the evaluation of their essential oils' chemical compositions and their bioactivities [1]. *Etlingera* species of Zingiberaceae family usually find in tropical regions of the world. *Etlingera* species are terrestrial plants; they are evergreen herbs and finds habitat at altitudes of sea level to 2500 m [2]. The plants of the genus are often found in lowland forest and are of numerous usages as flavoring aromatics in Sarawak – Malaysia [3]. *Etlingera elatior* (*E. elatior*) is the most commonly known species of the genus recognized with different local names such as 'Torch Ginger', 'Torch Lily', 'Porcelain Rose', and 'Philippine Waxflower'. These species have a stunning inflorescence and it is planted in all tropical

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regions due to its uses as spice [2]. In Peninsular Malaysia, the young flower shoots of *E. elatior* can be eaten raw and also used to flavor local dishes. The leaves and fruits are used to cure earaches and heal wounds. The inflorescences of the plant are also sold as cut flowers; also the leaves are mixed with other fragrant plants in water to be used for bathing to abolish body odor of post-partum women in Australia and Costa Rica [4]. In Borneo, other members of this genus are widely utilized as a salad or used in traditional medicine. *Etlingera coccinea*, which is locally known as 'Tuhau', consumed as pickles and utilized as a traditional remedy for stomach ache, food poisoning and gastric problems [5]. These abilities of different species of *Etlingera* to cure various diseases and also their uses as food flavoring are due to presences of a wide range of volatile and non-volatile compounds in the genus species as secondary metabolites. According to previous studies different groups of compounds such as oxygenated monoterpenes (1,8-cineole, perilla aldehyde, bornyl acetate, and linalool), monoterpene hydrocarbons(β-pinene, α-pinene, α-thujene, Limonene, α- and β-phellandrene), sesquiterpene hydrocarbons (β-elemene, α-selinene, and α-humulene), oxygenated sesquiterpenes (caryophyllene oxide), and phenolic compounds (methyl eugenol, (*E*)-methyl isoeugenol, eugenol) were abundant constituent of the volatile oils of different *Etlingera* species [5–10].

*Etlingera sayapensis* (*E. sayapensis*) is recognized by the plain red flower with a short labellum and the almost black stigma. The local name for *E. sayapensis* is not known. The plant grows in the mountain forest region around 1 000 m [2]. Even though there is no report on the plant uses documented in the literature, some natives in Sabah Malaysia actually consume the plant's leaves as a condiment for food flavoring. Since there has been no report found on phytochemical composition and bioactivity (antioxidant and antimicrobial activity) of *E. sayapensis* essential oils in literature, in this study we focus on identification of chemical compositions of essential oils from fresh leaves, rhizomes, and stems of the plant and evaluate their antioxidant and antimicrobial activities. We also compare our finding with the previous studies on essential oils from other *Etlingera* species to introduce the plant as an aromatic one.

## 2. Material and methods

### 2.1. Chemicals

DPPH (1,1-Diphenyl-2-picrylhydrazyl), Tween 40 (polyoxyethylene sorbitan monopalmitate), β-carotene, BHT(butylated hydroxytoluene), ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine), and α-tocopherol were purchased from Sigma (USA); CitA (citric acid), anhydrous sodium sulfate, AscA (ascorbic acid), solvents (methanol, acetone, and *n*-hexane) from Merck (Germany); linoleic acid and saturated hydrocarbon homologous series ($C_8$–$C_{20}$ and $C_{21}$–$C_{40}$) from Fluka (USA); ferrous sulfate heptahydrate from BDH (UK).

### 2.2. Plant material

The different parts of *E. sayapensis* including rhizomes, stems, and leaves were collected in May 2011 from its natural territory beside the road of Kampung Kipandi, Sabah, Malaysia with the latitude of N5°87′13″ and longitude of E116°25′02″ situated along Penampang-Tambunan Road at an elevation of 650–700 m on the Crocker range. The plant species was recognized by Mr. Sani Miran, a botanist of the Universiti Kebangsaan Malaysia (UKM). A voucher specimen of the plant (WYA 503) was deposited at the UKM Herbarium.

### 2.3. Essential oils

Three hundred grams of each fresh part of *E. sayapensis* were independently subjected to a Clevenger-type apparatus to hydrodistillate for 4 h. After drying of the yield over anhydrous sodium sulfate, the essential oils were kept in airtight vials at −18 °C before further analyses. The leaves, rhizomes, and stems of *E. sayapensis* afforded yellow essential oils with 2.37, 1.51, and 0.9 (w/w %) yields respectively.

### 2.4. Gas chromatography analysis (GC-FID)

GC-FID analysis of the volatile compounds was performed by a Hewlett–Packard GC-5890 on the fused-silica capillary column of DB-5 (30 m × 0.25 mm i.d.; film thickness 0.1 μm; from J.W. Scientific, USA). The conditions and parameters of analysis were as follows: carrier gas, Helium (rate of flow: 1.0 mL $min^{-1}$); injector temperature, 200 °C; The initial temperature, 40 °C for 2 min, raised at 3 °C $min^{-1}$ to 250 °C and held for 10 min; detector temperature, 250 °C; split ratio, 1:20; and 1 μL from the volatile oils in *n*-hexane, with ratio of 1:10, were injected into the instrument. Retention time (in term of minute) and the portion of each constituent was obtained from the area percent of the GC-FID chromatogram. The homologous series of saturated hydrocarbon ($C_8$–$C_{20}$ and $C_{21}$–$C_{40}$) were analyzed using the same column and conditions to measure the retention indices (*I*). The retention indices data were given by the equation for relative retention indices (RRI).

### 2.5. Gas chromatography–mass spectrometry analysis (GC–MS)

To identify individual components, the essential oils were subjected to Agilent GC–MS system (Agilent GC 7890A equipped with an Agilent 5975C MS detector) a HP-5 was used as the capillary column (30 m × 0.32 mm i.d.; 0.25 μm film thickness from J.W. Scientific). Framework and conditions including carrier gas, temperature program, and split ratio were the same to GC analysis as described in the last section. Then 70 eV for the ionization voltage and 40–600 amu for the scan mass range were used for the mass detector. The identity of components was assigned by comparing the GC retention indices and mass spectra of the known compounds from the literature data [11] and the National Institute for Standard and Technology database [12].

### 2.6. Bioactivity assays

The assays, which were used to screen of bioactivity of *E. sayapensis* essential oils (including antioxidant and antimicrobial activities), were according to our previous study with some modification [13–15]. For all the measurements, the essential oils were dissolved in acetone: methanol (3:7)

### 2.6.1. Antioxidant activity

For free radical-scavenging activity (RSA), a 1.0 mL of DPPH in methanol with concentration of 0.1 mM was added to a 1.0 mL aliquot part of each essential oil solution at concentrations of 200–2000 μg/mL. The mixture was shaken vigorously and incubated at room temperature about 90 min; the optical density value was measured at 517 nm. BHT and α-tocopherol were the standards. All the measurements were achieved after three replicate on 3 days. The RSA of the essential oils was calculated by the following equation and expressed in term of percentage inhibition of DPPH:

$$\text{RSA}\ (\%) = [(A_r - A_{eo})/A_r] \times 100$$

where $A_r$ is absorbance value of the reference (DPPH solution), and $A_{eo}$ is the essential oil value (DPPH solution with essential oil).

In terms of β-carotene bleaching (BCB), a 5 mL of β-carotene in chloroform (1 mg/mL) was pipetted to a container of linoleic acid (50 μL) and Tween 40 (500 μL). The mixture was placed under vacuum at 45 °C for 10 min to remove chloroform. Next, to make an emulsion, a 125 mL of oxygenated water was immediately poured into the flask and vigorously shaken. Finally, a 2.5 mL of the prepared emulsion was added to a vial containing 0.2 mL of the volatile oil solution (1000 μg/mL) and the absorbance was rapidly read at 470 nm. The combined mixture was put in an incubator at 50 °C; the absorbance value was read at 45 min periods up to 180 min. All the measurements were performed in three replicate. BHT, α-tocopherol, and AscA were the reference standards. The following formula was used to evaluate bleaching activity of the essential oils in terms of β-carotene bleaching:

$$\text{AA}\ \% = \left[1 - \left(A_0^{eo} - A_t^{eo}\right)/\left(A_0^{r} - A_t^{r}\right)\right] \times 100$$

where $A_0^{eo}$ and $A_0^{r}$ are the values of the essential oil and reference at zero time and $A_t^{eo}$ and $A_t^{r}$ are absorbance values after 180 min.

For determination of ferrous ion chelating ability (FIC), a fifty μL of $FeSO_4 \cdot 7H_2O$ solution (2 mM) and 2 mL of deionized water were pipetted to a container of essential oil solution (1 mL) at different concentration (200–1000 μg/mL). A 100 μL of ferrozine solution (5 mM) was added to the vial to initiate the reaction. The mixture was disturbed and allowed to stand at RT for 10 min. The optical density was read at 562 nm. All analyses were carried out in three replicate. CitA was the reference control. The ability of the sample to inhibit formation of the ferrozine-$Fe^{2+}$ complex was measured using the following equation in term of percentage:

$$\%\ \text{Inhibition} = [(A_r - A_{eo})/A_r] \times 100$$

where $A_r$ is the absorbance of the reference (contains ferrous sulfate, ferrozine, and methanol), and $A_{eo}$ is the absorbance of the sample.

### 2.6.2. Antimicrobial activity

For microorganisms, 6 g-positive bacteria *Bacillus thuringiensis* (*B. thuringiensis*) ATCC 10792, *Bacillus subtilis* (*B. subtilis*) ATCC 11774, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, methicillin resistant *S. aureus* (MRSA) *Staphylococcus epidermidis* (*S. epidermidis*) ATCC 12228, and *Enterococcus faecalis* ATCC 14506; 10 g-negative bacteria include: *Aeromonas hydrophila* (*A. hydrophila*) ATCC 7966, *Escherichia coli* (*E. coli*) ATCC 10536, *Enterobacter aerogenes* (*E. aerogenes*) ATCC 13048, *Proteus mirabilis* (*P. mirabilis*) ATCC 12453, *Proteus vulgaris* (*P. vulgaris*) ATCC 33420, *Salmonella typhimurium* (*S. typhimurium*) ATCC 51812, *Shigella sonnei* (*S. sonnei*) ATCC 29930, *Serratia marcescens* (*S. marcescens*) ATCC 13880, *Vibrio parahaemolyticus* (*V. parahaemolyticus*) ATCC 17802, and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 10145; and two fungi of *Candida parapsilosis* (*C. parapsilosis*) ATCC 22019 and *Candida albicans* (*C. albicans*) ATCC 90028 were used as tested microorganisms. All microorganisms were prepared from the microbiology laboratory, School of Biosciences and Biotechnology, Faculty of Science and Technology, UKM and certified by the standard microbiology method.

*For disc diffusion assay*, a sterile cotton swab was plunged in a suspension of microorganisms ($10^8$ CFU/mL) to inoculate the surface of growth medium plates (Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi). Sterile paper discs (Whatman No: 1) with a diameter of 6 mm were impregnated with 20 μL (2 × 10 μL) essential oils solution (100 mg/mL in acetone: methanol 3:7) and located on the inoculated agar. The plates were put in an incubator (24 h at 37.5 °C for bacteria or 48 h at 25 °C for fungi). The inhibition zone diameter (in mm), which was obtained by the samples against the bacteria or fungi, was determined as antimicrobial activity. Positive controls of chloramphenicol (30 μg) against bacteria, nystatin (30 μg) against fungi, and also negative control (impregnated sterile paper disc with the solvent) were used against microorganisms. The assay was run in triplicate. For this method, the inhibition level values were addressed in percentage oils with that of the antibiotic (positive control) as following show:

$$\text{Inhibition level}\ (\%) = \frac{\text{Inhibition zone diameter of sample}}{\text{Inhibition zone diameter of the antibiotic}} \times 100$$

Antimicrobial activity was classified as strong for inhibition level ≥ 70%, moderate for inhibition level 50–70%, and weak for inhibition level < 50% [16].

The minimum inhibitory concentration (MIC) assay was run on bacteria and fungi strains that exhibited sensitivity to the essential oils in the disc diffusion method. The assay was carried out according to previous reports [17–19]. First, a 100 μL of the culture media [Mueller-Hinton broth for bacteria and Sabouraud dextrose broth (for fungi)] was dispensed in each well of a plate (96-well plate), for the next step, the first one was charged with 100 μL of the essential oils solution in DMSO. Then 100 μL from each of their serial dilutions was consecutively moved into the next wells, and at the end, each well charged with 50 μL of the bacteria or fungi inoculums, which was described at microorganisms section, and 50 μL of the culture media. The final volume of mixture in each well was 200 μL in concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 mg/mL for *E. sayapensis* essential oils and 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/mL for the standard antibiotics (chloramphenicol and nystatin). Three well in the last line of the plate were used as growth controls by filling 50 μL of inoculums and 150 μL of the culture media. Also, three wells were filled by 200 μL of the culture media as negative controls. The covered plates were incubated for 24 h at 37.5 °C or 48 h at 25 °C. The turbidity of each well was then observed and

recorded. The minimum concentration that manifested no visible growth was assessed as the MIC value. All tests were carried out in triplicate.

### 2.7. Statistical analysis

The three replicates ± standard deviations were considered to express the values. Duncan's test (values of $P < 0.05$) was performed to achieve the significant differences between the outcomes of the antioxidant activity.

## 3. Results

### 3.1. Chemical composition of E. sayapensis essential oils

The leaves, rhizomes, and stems of *E. sayapensis* gave yellow essential oils with 2.37, 1.51, and 0.9 (w/w %) yields respectively. Forty-one constituents were recognized for the leaves essential oil comprising 18 oxygenated monoterpenes (74.18%), 4 phenolic compounds (12.47%), 4 oxygenated hydrocarbons (5.20%), 10 sesquiterpene hydrocarbons (4.97%), 2 monoterpene hydrocarbons (1.04%), and 3 oxygenated sesquiterpenes (0.25%), computing for 98.11% of the total leaves essential oil composition. Similar to the leaves oil, oxygenated monoterpenes were the abundant class of compounds in the rhizome and stems oils. Among 37 components in the rhizome oil 17 belonged to oxygenated monoterpenes (54.61%), 7 sesquiterpene hydrocarbons (14.55%), 2 phenolic compounds (13.46%), 2 oxygenated hydrocarbons (8.32%), 7 oxygenated sesquiterpenes (6.49%), and two monoterpenes hydrocarbon (0.15%) which accounted for 97.58%. In the stems oil, 19 compounds consisted 99.99% of the sample. This amount comprised 12 oxygenated monoterpenes (75.60%), 4 sesquiterpene hydrocarbons (15.99%), one oxygenated hydrocarbon (6.43%), one phenolic compound (1.37%), and one monoterpene hydrocarbon (0.65%) as in Table 1. The leaves oil of *E. sayapensis* was characterized by the presence of oxygenated monoterpenes of carvone (21.38%), *cis*-carveol (13.49%), terpineol (10.07%), neryl acetate (8.71%), and a phenolic compound of chavicol (8.57%) as the major compounds. The rhizome oil was rich in linalool formate (25.47%), eugenol (11.84%), *cis*-carveol (11.53%), α-cubebene (11.49%), and octyl formate (7.99%). The stems oil was dominated in α-terpineol (39.86%), linalool formate (30.55%), and δ-elemene (11.41%). As shown, linalool formate constituted more than a quarter of the rhizome, and stems oils.

### 3.2. Antioxidant activity of E. sayapensis essential oils

The results of antioxidant activity of the essential oils were exhibited in Table 2. As mentioned above, RSA, BCB and FIC were the selected assays for this propose. For the RSA assay, all the oils exhibited a higher amount of $IC_{50}$ compared to controls. This result reveals that the *E. sayapensis* essential oils were not potent to scavenge free radical of DPPH. The oils provided values ranging from (1 319.25 ± 15.35) μg/mL for the leaves oil to (1 766.06 ± 34.81) μg/mL for the stems oil, and finally (2 291.49 ± 121.18) μg/mL for the rhizome oil. Among the oils, the leaves oil [(30.75 ± 2.23) %] showed better BCB activity than the rhizomes oil [(16.91 ± 3.37) %] and stems oil

**Table 1**

Percentage composition and retention indices of the essential oils from the rhizomes, stems, and leaves of *E. sayapensis*.

| Compounds | *I* | Rhizomes | Stems | Leaves |
|---|---|---|---|---|
| Camphene | 948 | 0.10 | – | – |
| 1,8-cineole | 1 026 | 0.13 | 0.27 | – |
| *(E)*-β-ocimene | 1 044 | 0.05 | 0.6 | 0.97 |
| γ-Terpinene | 1 050 | – | – | 0.07 |
| Linalool | 1 095 | 0.04 | – | – |
| *endo*-Fenchol | 1 111 | 0.38 | 0.22 | 1.19 |
| Octyl formate | 1 125 | 7.99 | 6.43 | – |
| 1-Terpineol | 1 131 | – | – | 10.07 |
| *trans*-Pinocarveol | 1 136 | 0.48 | 0.08 | 0.80 |
| Camphor | 1 139 | 0.47 | – | 2.14 |
| Camphene hydrate | 1 145 | – | – | 2.61 |
| Pinocarvone | 1 157 | – | – | 0.23 |
| Borneol | 1 162 | – | – | 0.23 |
| α-Terpineol | 1 191 | – | 39.86 | – |
| Linalool formate | 1 217 | 25.47 | 30.55 | – |
| *cis*-Carveol | 1 227 | 11.53 | – | 13.49 |
| Nerol | 1 229 | 4.02 | – | 2.38 |
| *exo*-Fenchyl acetate | 1 231 | 4.16 | – | – |
| Tetrahydro linalool acetate | 1 232 | 2.34 | – | – |
| Neral | 1 234 | 0.05 | 0.54 | 3.20 |
| Carvone | 1 243 | – | – | 21.38 |
| Chavicol | 1 247 | – | – | 8.57 |
| Geraniol | 1 247 | 0.09 | 0.73 | 2.83 |
| Geranial | 1 267 | 0.09 | 0.19 | 0.44 |
| Nerylformate | 1 281 | – | 1.65 | 0.11 |
| Thymol | 1 293 | 1.62 | 1.37 | 0.87 |
| Geranylformate | 1 299 | 2.31 | 1.12 | 0.42 |
| Carvacrol | 1 298 | – | – | 1.61 |
| Undecanal | 1 308 | – | – | 0.92 |
| *cis*-Patchenol | 1 318 | – | 1.26 | 0.29 |
| Myrtenyl acetate | 1 325 | 2.25 | – | – |
| δ-Elemene | 1 135 | – | 11.41 | 0.75 |
| α-Cubebene | 1 348 | 11.49 | 2.72 | 2.35 |
| Eugenol | 1 354 | 11.84 | – | 1.42 |
| (2*E*)-undecanal | 1 357 | 0.33 | – | – |
| Neryl acetate | 1 360 | 0.38 | – | 8.71 |
| Decanoic acid | 1 165 | – | – | 1.61 |
| *n*-Undecanol | 1 167 | – | – | 2.59 |
| Carvacrol acetate | 1 368 | – | – | 3.21 |
| Geranyl acetate | 1 380 | 0.42 | – | – |
| (2*E*)-Hexenylcaproate | 1 388 | – | – | 0.08 |
| Cyperene | 1 400 | 0.16 | – | – |
| β-Caryophyllene | 1 417 | – | 0.60 | 0.27 |
| Linalool butanoate | 1 425 | – | 0.19 | – |
| β-Gurjunene | 1 429 | 1.61 | – | 0.56 |
| Aromadendrene | 1 437 | – | – | 0.27 |
| α-Humulene | 1 450 | 0.68 | – | – |
| Nerylpropanoate | 1 455 | – | 0.20 | 0.11 |
| *allo*-Aromadendrene | 1 458 | – | – | 0.04 |
| δ-Selinene | 1 496 | 0.16 | – | 0.06 |
| α-Selinene | 1 501 | 0.06 | – | 0.07 |
| γ-Patchoulene | 1 505 | – | – | 0.31 |
| α-Cadinene | 1 537 | 0.39 | – | – |
| (*E*)-Nerolidol | 1 565 | 0.44 | – | 0.11 |
| Spathulenol | 1 576 | 5.41 | – | – |
| Caryophyllene oxide | 1 585 | 0.29 | – | – |
| Viridiflorol | 1 592 | 0.04 | – | 0.03 |
| Epoxide-*allo*-aromadendrene | 1 636 | 0.27 | – | |
| (2*Z*,6*Z*)-Farnesol | 1 699 | 0.43 | – | – |
| (2*E*,6*Z*)-Farnesal | 1 714 | – | – | 0.11 |
| (2*E*,6*E*)-Farnesyl acetate | 1 845 | 0.31 | – | – |
| Classes of compounds | | | | |
| Monoterpene hydrocarbons | | 0.15 | 0.60 | 1.04 |
| Oxygenated monoterpenes | | 54.61 | 75.60 | 74.18 |
| Sesquiterpene hydrocarbons | | 14.55 | 15.99 | 4.97 |
| Oxygenated sesquiterpenes | | 6.49 | – | 0.125 |
| Phenolic compounds | | 13.46 | 1.37 | 12.47 |
| Oxygenated hydrocarbons | | 8.32 | 6.43 | 5.20 |
| Total | | 97.58 | 99.99 | 98.11 |

Retention indices (*I*) on DB-5 capillary column.

**Table 2**
RSA, BCB and FIC of essential oils of the rhizomes, stems, and leaves of *E. sayapensis*.

| Oil/standard | RSA, $IC_{50}$ (μg/mL) | BCB (%) | FIC, $IC_{50}$ (μg/mL) |
|---|---|---|---|
| Rhizomes | 2291.49 ± 121.18$^{e}$ | 16.91 ± 3.37$^{e}$ | 1648.76 ± 19.35$^{d}$ |
| Stems | 1766.06 ± 34.81$^{d}$ | 13.67 ± 3.24$^{e}$ | 1850.87 ± 24.11$^{e}$ |
| Leaves | 1319.25 ± 15.35$^{c}$ | 30.75 ± 2.23$^{c}$ | 1465.27 ± 18.23$^{c}$ |
| BHT | 14.36 ± 1.25$^{a}$ | 71.99 ± 4.44$^{b}$ | 68.18 ± 2.56$^{b}$ |
| α-tocopherol | 11.29 ± 1.54$^{b}$ | 87.90 ± 2.53$^{a}$ | – |
| AscA | – | 21.83 ± 2.04$^{d}$ | – |
| CitA | – | – | 1449.21 ± 34.05$^{c}$ |
| EDTA | – | – | 67.23 ± 5.21$^{a}$ |

Values are presented as means ± SD ($n$ = 3). Means with different letters are significantly different in each column ($P < 0.05$).

[(13.67 ± 3.24) %]. All the volatile oils displayed lower activity compared to the positive controls of BHT [(71.99 ± 4.44) %] and α-tocopherol [(87.90 ± 2.53) %]. Nonetheless, the leaves essential oil presented better BCB activity compared to the positive control of AscA [(21.83 ± 2.04) %]. According to the statistical analysis, no significant disparity was detected between the BCB activity of the rhizomes and stems oils. The decreasing order of the FIC ability was: leaves oil (1465.27 ± 18.23) μg/mL], rhizomes oil [(1648.76 ± 19.35) μg/mL], and stems oil [(1850.87 ± 24.11) μg/mL]. For all the oils, the FIC ability depends on the concentration. Regarding our results on the FIC ability of *E. sayapensis* essential oils, all the oils exhibited lower ability compared to the standards of EDTA and AscA; although the leaves oil ability was comparable with that of AscA.

### 3.3. Antimicrobial activity of E. sayapensis essential oils

The antimicrobial activity assays just run on the leaves and rhizomes oils, because the amount of the stem oil was not enough for the test. Table 3 represents the antimicrobial activity results. *E. sayapensis* essential oils displayed a broad spectrum of antimicrobial activity; they prevented 13 out of 18 tested microorganisms. The oils inhibited the growth of *B. subtilis*, *B. thuringiensis*, MRSA, *S. aureus*; gram-negative of *A. hydrophila*, *E. aerogenes*, *E. coli*, *P. mirabilis*, *S. marcescens*, *S. sonnei*, *V. parahaemolyticus*; and fungi of *C. parapsilosis*. The oils displayed slight difference with antimicrobial activity in which the leaves oil was active against Gram-positive bacterium of *S. epidermidis* and inactive against fungi of *C. albicans* while the results were in opposite for the rhizomes oil.

The rhizomes oil exhibited larger inhibition level compared to the leaves one for all the strains. Against the Gram-positive bacteria, the highest inhibition zone was observed for MRSA [(93.18 ± 1.19)%] followed by *B. subtilis* [(68.24 ± 3.74)%], *B. thuringiensis* [(58.33 ± 2.51)%], *S. aureus* [(47.68 ± 2.56)%]; for the gram-negative the decreasing order was *S. marcescens* [(85.87 ± 1.76)%], *E. coli* [(82.82 ± 4.99)%], *P. mirabilis* [(79.17 ± 3.96%)], *V. parahaemolyticus* [(78.39 ± 2.09)%], *A. hydrophila* [(77.47 ± 2.70)%], *S. sonnei* [(67.14 ± 1.13)%], and *E. aerogenes* [(66.87 ± 2.56)%]; and for fungi was *C. parapsilosis* [(94.98 ± 4.65)%], and *C. albicans* [(79.36 ± 2.72)%]. The leaves oil moderately inhibited *P. mirabilis* [(61.85 ± 4.29)%] *B. subtilis* [(60.91 ± 2.67)%], MRSA [(55.94 ± 2.91)%], *S. epidermidis* [(50.42 ± 2.12)%], and strongly retarded *C. parapsilosis* [(79.36 ± 2.72)%].

The MIC values of *E. sayapensis* essential oils are tabulated in Table 3. For the rhizomes oil, the lowest MIC was detected against MRSA, *S. marcescens*, and *C. parapsilosis* with a concentration of (0.52 ± 0.23) mg/mL. For the leaves oil the lowest MIC was observed for gram-positive bacterium of *B. subtilis* strain [(0.78 ± 0.00) mg/mL]; Gram-negative bacteria of *E. coli* and *S. sonnei strains* [(1.56 ± 0.00) mg/mL]; and fungal of *C. parapsilosis* [(3.64 ± 2.39) mg/mL]. For all of the strains, the essential oils activity was less than those of the antibiotics (chloramphenicol for bacteria and nystatin for fungi).

**Table 3**
Antimicrobial activity of the rhizomes and leaves essential oils of *E. sayapensis* using disc-diffusion (DD Test) and MIC assay.

| Microorganism | Rhizomes oil | | Leaves oil | | Positive control | |
|---|---|---|---|---|---|---|
| | DD Test (%) | MIC (mg/mL) | DD Test (%) | MIC (mg/mL) | DD Test (mm) | MIC (mg/mL) |
| Gram-positive | | | | | | |
| *B. subtilis* | 68.24 ± 3. 74 | 0.78 ± 0.00 | 60.91 ± 2. 67 | 0.78 ± 0.00 | 28.9 ± 0.80 | 0.02 ± 0.01 |
| *B. thuringiensis* | 58.33 ± 2.51 | 4.16 ± 1.81 | 47.11 ± 2.22 | 5.29 ± 1.88 | 22.3 ± 0.30 | 0.04 ± 0.01 |
| *Enterococcus faecalis* | NA | NT | NA | NT | NT | NT |
| MRSA | 93.18 ± 1.19 | 0.52 ± 0.23 | 55.94 ± 2.91 | 8.33 ± 3.61 | 22.2 ± 0.30 | 0.03 ± 0.01 |
| *S. aureus* | 47.68 ± 2.56 | 1.56 ± 0.00 | 33.56 ± 1.68 | 0.78 ± 0.00 | 29.8 ± 0.50 | < 0.01 |
| *S. epidermidis* | NA | NT | 50.42 ± 2.12 | 4.16 ± 1.81 | 27.2 ± 0.60 | 0.01 ± 0.00 |
| Gram-negative | | | | | | |
| *A. hydrophila* | 77.47 ± 2.70 | 3.12 ± 0.00 | 45.61 ± 2.90 | 5.21 ± 1.81 | 28.5 ± 0.50 | 0.01 ± 0.00 |
| *E. aerogenes* | 66.87 ± 2.56 | 2.60 ± 0.90 | 42.33 ± 2.44 | 2.60 ± 0.90 | 26.0 ± 0.60 | < 0.01 |
| *E. coli* | 82.82 ± 4.99 | 3.12 ± 0.00 | 44.43 ± 3.86 | 1.56 ± 0.00 | 19.8 ± 0.70 | < 0.01 |
| *P. mirabilis* | 79.17 ± 3.96 | 1.56 ± 0.00 | 61.85 ± 4.29 | 20.83 ± 7.22 | 16.8 ± 0.50 | 0.02 ± 0.00 |
| *P. vulgaris* | NA | NT | NA | NT | NT | NT |
| *P. aeruginosa* | NA | NT | NA | NT | NT | NT |
| *S. typhimurium* | NA | NT | NA | NT | NT | NT |
| *S. marcescens* | 85.87 ± 1.76 | 0.52 ± 0.23 | 44.87 ± 3.15 | 5.21 ± 1.81 | 20.5 ± 0.4 | 0.01 ± 0.00 |
| *S. sonnei* | 67.14 ± 1.13 | 6.25 ± 0.00 | 43.35 ± 3.09 | 1.56 ± 0.00 | 24.9 ± 0.3 | < 0.01 |
| *V. parahaemolyticus* | 78.39 ± 2.09 | NT | 45.96 ± 2.51 | 6.25 ± 0.00 | 28.7 ± 0.6 | < 0.01 |
| Fungi | | | | | | |
| *C. albicans* | 79.36 ± 2.72 | 2.60 ± 0.90 | NA | 4.16 ± 1.81 | 13.2 ± 0.7 | 0.01 ± 0.00 |
| *C. parapsilosis* | 94.98 ± 4.65 | 0.52 ± 0.23 | 79.37 ± 5.11 | 3.64 ± 2.39 | 14.1 ± 0.4 | 0.02 ± 0.01 |

Positive control: chloramphenicol for bacteria and nystatin for fungi. NA: non-active; NT: not tested. Values are presented as means ± SD ($n$ = 3).

## 4. Discussion

In our previous study, we analyzed the phytochemical composition of the volatile oils from the dried rhizome, stems, and leaves of *Etlingera brevilabrum* (*E. brevilabrum*). The more abundant compounds in the rhizome oil belong to oxygenated monoterpenes (40.2%) and monoterpene hydrocarbons (28.2%). For the stems and leaves, monoterpene hydrocarbons showed the highest percentage (66.8% and 89.8%) [6]. A previous study on Malaysian *E. elatior* showed that its rhizome and stem oils were chiefly composed of oxygenated monoterpenes (47.28% and 54.32%) followed by monoterpene hydrocarbons (34.96%, 33.65%) whereas sesquiterpene hydrocarbons (45.06%) and monoterpene hydrocarbons (29.75%) were found in the leaf oil [7]. Phenylpropanoid derivatives (55.5–67.4%) dominated the leaf oil of *Etlingera cevuga* (*E. cevuga*) [20]. Monoterpene hydrocarbons influenced the oils of *Etlingera sphaerocephala* var. *grandiflora* (rhizomes 55.96%, stems 42.98%, and leaves 48.61%) [9]. *Etlingera punicea* (*E. punicea*) rhizome oil was influenced by phenolic compounds (95.73%) [10].

Previous reports on *Etlingera* species essential oils showed the difference in their chemical composition compared with the plant's essential oils of this research project. For the dried parts of *E. brevilabrum*, β-pinene and α-thujene in the leaves oil, limonene and β-pinene in the stems oil, and 1,8-cineole, β-pinene in the rhizomes oil were the main compounds [6]. However, for the fresh parts of *E. brevilabrum* α-thujene, *p*-cymen-7-ol (for the leaves oil), perilla aldehyde, and bornyl acetate (for the rhizomes oil) were the major components [13]. The 1,8-cineole, β-phellandrene, α- and β-pinene were the major components of *E. brevilabrum* volatile oils those were analyzed by GC × GC/TOFMS [21]. The rhizome oil of *E. punicea* was rich in methyl chavicol [10]. The leaf oil of *E. cevuga* from different locations was dominated by methyl eugenol [20]. The other study also presented the rhizome oil of *E. cevuga* was marked by methyl eugenol [8]. The rhizomes and stems oil of *Etlingera sphaerocephala* var. *grandiflora* was characterized by 1,8-cineole and α-phellandrene. But the leaf oil mainly consisted of α-phellandrene and diprene [9]. The leaf oil of *Etlingera linguiforme* was dominated by the presence of 1,8-cineole and β-pinene. However, methyl chavicol and methyl eugenol were detected as main constituents in the rhizome oil of *Etlingera linguiforme* [22]. The leaf and rhizome oil of Malaysian *Etlingera littoralis* were characterized by the occurrence of (*E*)-methyl isoeugenol. The leaf oil of Malaysian *E. elatior* collected from Penang Botanic Garden was marked by myrcene and α-humulene; the rhizome oil was rich in camphene and β-pinene. The leaf oil of *E. elatior* var. *Thai queen* was characterized by high percentages of α-pinene and dodecanol; while camphene and dodecanol were the main components in the rhizome oil [23]. Jafar *et al.* studied the essential oils of different parts from *E. elatior* collected from diverse locations of Malaysia (Kampong Paya, Kepala Batas, and Penang). In the leaves oil (*E*)-β-Farnesene and β-pinene and in the stems one 1,1-dodecanediol diacetate and (*E*)-5-dodecene were the major constituents. The essential oil from the whole plant of the other Malaysian *E. elatior*, which collected from Selangor was dominated by *β*-pinene and 1-dodecene [24]. Other studies on *Etlingera* species have expressed non-terpenic compounds as the main chemical constituent of the essential oils. The compounds including (*Z*)-9-Hexadecen-1-ol, cyclotetradecane, n-dodecyl acetate, and cyclodecane for *Etlingera fulgens*; *and* dodecanoic acid, cyclododecane, and (*E*)-2-tetradecene for *Etlingera venusta* [25,26]. One study on the chemical composition of *Etlingera yunnanensis* from Vietnam reported the essential oils from different parts of the plant were rich in 1,8-cineole, germacrene D, and β-pinene [27]. While estragole and β-caryophyllene were recognized as the major constituents of the rhizomes oil of *Etlingera yunnanensis* from China in another study [28]. *Etlingera pavieana* rhizomes oil was prevailed by *trans*-anethole and *p*-anisaldehyde [29]. For the leaves essential oil of *Etlingera fimbriobracteata* (*E. fimbriobracteata*), β- and α-Pinene were the main components, while 1,8-cineole, β-pinene, and decanal were found as main compounds in aerial stems and basal stems oils [30].

From the investigation on the basis of chemotaxonomy, there is an outstanding similarity in the major groups of compounds in the essential oils from different *Etlingera* species. It was concluded the most of the oils are rich in oxygenated monoterpenes and monoterpene hydrocarbons compounds. However, sesquiterpene hydrocarbons, phenylpropanoid derivatives, and phenolic compounds are recognized as the major groups of compounds in some species. On the other hand, a great discrepancy is observed in the majority of the essential oils compositions. Nonetheless, α-pinene, β-pinene, and 1,8-cineole were the common prime compounds in volatile oils from most plants of the genus.

The capacity of the essential oils, which are considerable influence with the presences of terpenoid compounds, to scavenge free radical or inhibit of lipid peroxidation reported previously. The capability of the essential oils to scavenge free radical of DPPH and bleaching of β-carotene related to the synergistic or antagonistic response from various compounds as their chemical composition [31–35].

In our previous study, we carried out the same assays to recognize the antioxidant activity of *E. brevilabrum* oils. The $IC_{50}$ of RSA for rhizomes, stems, and leaves oils were (236.54 ± 12.83), (1 799.04 ± 47.39), and (1 525.79 ± 38.99) μg/mL; the percentage of BCB were (22.76 ± 0.48), (8.06 ± 1.15), and (9.83 ± 1.55) % for rhizomes, stems, and leaves oils; and $IC_{50}$ for FIC ability were measured (879.39 ± 16.54), (1 354.09 ± 5.94), and (1 098.73 ± 12.32) μg/mL for rhizomes, stems, and leaves oils respectively [13]. The rhizomes oil *E. fimbriobracteata* exhibited the higher RSA with $IC_{50}$ of 228.27 mg/mL compared to basal stems oil ($IC_{50}$ = 251.31 mg/mL), aerial stems oil ($IC_{50}$ = 301.16 mg/mL), and leaves oil ($IC_{50}$ = 518.29 mg/mL) of the plant [30].

The ability of essential oils of *E. sayapensis* to prevent the growth of both Gram-positive and Gram-negative bacteria was supported by previous studies that found out that either Gram has little effect on the ability of the plant essential oils to inhibit the bacteria strains. The chemical composition, functional groups, the structural configuration of the components, their relative proportion, and synergistic effects of the constituents are the factors to response antimicrobial activity of a plant volatile oils of [36–38]. It can be concluded that the antimicrobial activity of *E. sayapensis* essential oils depends on their chemical composition and also on their synergistic effects. Antimicrobial activity of some individual compounds such as linalool, nerol, borneol, carvacrol, eugenol, thymol, 1,8-cineole, neral, aromadendrene, α-phellandrene, α-humulene, and caryophyllene oxide, which were abundant compounds in the tested essential oils, were previously established [39,40].

As mentioned above oxygenated monoterpene of carvone is a major compound in the leaves oil of *E. sayapensis*. This compound has been reported as a fragrance and flavor, sprouting inhibitor, an antimicrobial and antitumor agent, possessing medical relevance, repellent, a building block for some compounds, and it can also be used as a biochemical environmental indicator [41,42]. Tadtong *et al.* (2009) reported inhibition zones of 11.8, 8.7, 7.8, and mm against *C. albicans*, *S. aureus*, and *E. coli* for the rhizome oil of *E. punicea*. The oil was inactive against *P. aeruginosa* [10]. The rhizomes, stems, and leaves oils of *E. brevilabrum* inhibited the growth of MRSA [(12.6 ± 0.4), (10.0 ± 0.3), (8.1 ± 0.4) mm respectively] and *S. aureus* [(17.7 ± 0.5), (11.2 ± 0.6), (12.6 ± 0.5) mm respectively] and could not prevent the growth from *B. subtilis*, *B. thuringiensis* bacteria. The antimicrobial activity of *E. brevilabrum* oils also was evaluated against gram-negative bacteria of *E. coli*, *P. vulgaris*, *S. typhimurium*, *P. mirabilis*, *P. aeruginosa*. The rhizomes oil was susceptible to *P. mirabilis* [(13.2 ± 0.6 mm)]; the stems oil showed inhibitory activity against *E. coli* [(13.5 ± 0.3) mm] and *P. vulgaris* [(9.5 ± 0.7) mm]; and the leaves oil repel the growth of *P. vulgaris* [(10.3 ± 0.6) mm], and *P. mirabilis* [(16.4 ± 0.7) mm] [13]. *E. fimbriobracteata* essential oils prevented the growth of *E. coli*, *B. subtilis*, *Bacillus spizizenii*, *S. aureus*, *C. albicans*, and *Saccharomyces cerevisiae*. The maximum activity is reported for the rhizomes oil with MIC range of 2.4–156 μg/mL [30]. Similar to our results the essential oils from *E. fimbriobracteata* were unable to inhibit the growth of *P. aeruginosa*.

In conclusion, this study outcome indicated that oxygenated monoterpenes are abundant compounds of *E. sayapensis* essential oils, which are obtained from the rhizome, stems, and leaves of the plant. The rhizome oil contained a high amount of linalool formate and eugenol; the leaves oil was dominated by carvone and *cis*-carveol; however, the stems oil was rich in α-terpineol and linalool formate. The results revealed some differences with the previous study on the essential oils chemical composition of other *Etlingera* species. Among the oils of *E. sayapensis*, the leaves one showed the highest antioxidant activity for all selected assay including RSA, BCB and FIC. In contrast, the rhizome oil exhibited the best antimicrobial activity. The rhizomes oil prevented 13 bacteria out of 18 tested bacteria strains including gram-positive, gram-negative, and fungi. According to our findings in this research paper, *E. sayapensis* can be considered as an aromatic plant and also antimicrobial agent because of the bioactivity of some volatile oil constituents such as carvone reported previously.

## Conflict of interest statement

We declare that we have no conflict of interest.

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