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Chemical Composition, Antioxidant, and Antibacterial Activities of Essential Oils from *Etlingera brevilabrum* Valeton

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Abstract: Essential oils from fresh parts of *Etlingera brevilabrum* Valeton (Zingiberaceae) including rhizome, stolon, stem, and leaves were hydrodistilled, and studied for their chemical composition, antioxidant, and antibacterial activity. Characterization by GC-FID and GC-MS showed monoterpene hydrocarbons dominated the oils of the leaves and stems (57.3 and 73.4%) with oxygenated monoterpenes were dominant in the stolon and rhizomes (40.4 and 53.6%). The major compounds of the rhizome oil were perilla aldehyde (19.6%) and bornyl acetate (17.6%); the stolon oil, β-pinene (30.6%) and *p*-cymen-7-ol (25.0%); the stem oil, δ-3-carene (43.2%) and α-thujene (17.7%); and the leaf oil, α-thujene (38.1%) and *p*-cymen-7-ol (8.0%). The rhizome oil showed the highest antioxidant effects with IC_{50} = 1236.54±12.83 μg/mL in the DPPH radical scavenging activity, 22.76% inhibition in the β-carotene bleaching test at 1000 μg/mL, and IC_{50} = 878±16.54 μg/mL in the ferrous ion chelating ability. Antibacterial activity demonstrated by disc diffusion assay showed that the rhizome, stem and leaf oils inhibited *Staphylococcus aureus* and MRSA but not *Bacillus subtilis* and *Bacillus thuringiensis*. For Gram negative bacteria, the rhizome oil prevented the growth of *Proteus mirabilis*; the stem oil inhibited *Escherichia coli* and *Proteus vulgaris*; and the leaf oil was active against *E. coli*, *P. vulgaris*, and *P. mirabilis*.

Keywords: *Etlingera brevilabrum*;essential oils; GC-FID and GC-MS analyses; antioxidant; antibacteria.

1. Introduction

Zingiberaceae as a largest family of the plant kingdom is divided into four subfamilies of Hedychieae, Zingibereae, Alpineae, and Globbeae in which the *Etlingera* genus belonging to the Alpineae tribe [1]. *Etlingera* is an Indo-Pacific genus that includes more than 100 species and grows from sea level to an altitude of 2,500 m [2]. The genus is distributed from the Himalayas and southwest China through Burma, Thailand, Malaysia, and Indonesia to New Guinea and North Queensland [3].

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Various traditional and edible uses have been reported for the *Etlingera* species. Different parts of *Etlingera brevilabrum* Valeton are consumed as medicines by the tropical natives: the smoked leaves are used to treat itchy skin. Previous report on the use of essential oil include being applied to dry skin on the legs, the stem juice is used as eye drops, and the plant base is used as medicine to treat stomach aches [2]. The plant also has another medicinal use against cholera. The fruits of *E. brevilabrum* are eaten by local people. The stems, flowers, and fruits of *Etlingera elatior*, *E. rubrolutea*, and *E. littoralis* are eaten raw or cooked and also used as condiments [4, 5, 6]. Consecutive extracts from different aerial parts of *E. brevilabrum* have been shown to exhibit antibacterial activity [7]. The aims of this study was to further analyze the composition of *E. brevilabrum* focusing on the essential oils of the fresh rhizomes, stolon, stems, and leaves of using GC-FID and GC-MS, as well as to investigate their antioxidant and antibacterial oils *E. brevilabrum*.

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$), α-tocopherol, 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (ferrozine), β-carotene, polyoxyethylenesorbitanmonopalmitate (Tween 40), and butylatedhydroxytoluene (BHT) were purchased from Sigma (USA); anhydrous sodium sulfate, citric acid, ethylenediaminetetraacetic acid (EDTA), acetone, *n*-hexane, and methanol from Merck (Germany); ferrous sulfate heptahydrate from BDH (UK); and linoleic acid from Fluka (USA).

2.2. Plant material

The plant parts of the rhizomes, stolon, stems, and leaves of *E. brevilabrum* were collected in November 2010 from its natural habitat in Sabah, Malaysia. The plant species was identified by a botanist, Mr. Sani Miran, Universiti Kebangsaan Malaysia. A voucher specimen of WYA 500 for the plant was deposited at the Universiti Kebangsaan Malaysia Herbarium.

2.3. Essential oils

The fresh rhizomes, stolon, stems and leaves of *Etlingera brevilabrum* were ground (the particles size were less than one centimeter) and 300 g of each part was subjected to hydrodistillation for approximately 5 h in a Clevenger-type apparatus. The resulting essential oils were dried over anhydrous sodium sulfate and stored in tightly closed vials at -18°C before analyses.

2.4. Gas chromatography (GC) analysis

GC analyses of the essential oil constituents was carried out using a Hewlett-Packard 5890 GC equipped with a flame-ionization detector (FID) and a DB-5 (30 m × 0.25 mm i.d.; film thickness 0.1 μm) fused-silica capillary column (J.W. Scientific, USA). Helium was used as the carrier gas with a flow rate of 1.0 mL min^{-1}. The initial temperature was set at 40°C for 2 min, then heated at a rate of 3°C min^{-1} to 250°C and held isothermally for 10 min. The ion source temperature was set at 200°C and detector temperatures at 250°C. One μL of the essential oils in *n*-hexane (1:10) were injected with a split ratio of 1:20. The retention times were measured in minutes and the quantity of each component was calculated from its FID area percent.

2.5. Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil constituents were analyzed by Agilent 7890A GC coupled to an Agilent 5975C mass detector using HP-5 (30 m × 0.32 mm i.d.; 0.25 μm film thickness) (J.W. Scientific) column. The parameters of carrier gas, split ratio, and temperature program are as described in the GC analysis. Retention indices were calculated using GC data of the saturated hydrocarbon homologous series within C_8 to C_{20} and C_{21} to C_{40} carried out using the same column and conditions as mentioned in the GC analyses above. The compounds were identified by comparing the GC retention indices and mass

spectra with those found in the literature [8] and the National Institute for Standard and Technology database (2005).

2.6. Free radical-scavenging activity (RSA): DPPH assay

The RSA of the oils was measured according to the method reported previously with some modification [9, 10]. Briefly, a 1.5 mL aliquot of each oil at 200, 400, 800, 1600, and 2000 µg/mL was added to 1 mL of 0.1 mM DPPH in methanol. The mixture was agitated vigorously for 1 min, and allowed to stand in the dark for 90 min at room temperature; the absorbance value was read at 517 nm. BHT and α-tocopherol were used as reference control. All measurements were carried out in triplicate on three different days. The RSA of samples, was expressed as percentage inhibition of DPPH using the following equation: I (%) = $[(A_c\text{-}A_o)/A_c] \times 100$, where A_c is the absorbance value of the control (DPPH solution without test oil), and A_o is the absorbance value of the essential oil (DPPH solution with test oil).

2.7. The antioxidant activity:β-carotene bleaching (BCB)

The antioxidant activity of the oils was determined using β-carotene bleaching assay was measured according to [11, 12] with some modification. Briefly, 5 mL of β-carotene solution in chloroform (1 mg/mL) was added to a flask containing 50 µl of linoleic acid and 500 µl of Tween 40. The chloroform was evaporated under vacuum at 45°C for 10 min, then 125 mL of oxygenated water was added and the mixture was vigorously agitated to form an emulsion. Then 2.5 mL of the emulsion was added to 0.2 mL of the oil solution (1000 µg/mL) and the absorbance was immediately read at 470 nm. The mixture was incubated at 50°C; the absorbance was measured at 45 min intervals up to 180 min. All measurements were carried out in triplicate. BHT and α-tocopherol were used as the reference control. The antioxidant activity (AA) was evaluated in terms of β-carotene bleaching using the following formula: AA % = $[1\text{-}(A^o_0 - A^o_t)/(A^c_0 - A^c_t)] \times 100$ where A^o_0 and A^c_0 are the absorbance values of the oil and control (2.5 mL of the emulsion and 0.2 mL of methanol) at zero time, A^o_t and A^c_t are absorbance values of the oil and control after 180 min.

2.8. Determination of ferrous ion chelating (FIC) ability

The FIC ability of the oils was estimated with the method previously carried out by [4] with modification. Fifty µL of ferrous sulfate heptahydrate (2 mM) was added to a vial containing 1 mL of essential oil at 200, 400, 600, 800, and 1000 µg/mL and 2 mL of distilled water. The reaction was initiated by addition of 100 µl of ferrozine (5 mM); the reaction mixture was well agitated and incubated at room temperature for 10 min. The absorbance was measured at 562 nm. All measurements were run in triplicate. Positive controls of EDTA and citric acid were used. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following equation: % Inhibition = $[(A_c - A_o)/A_c] \times 100$, where A_c is the absorbance of the control (contains 50 µl of the ferrous sulfate, 100 µl of the ferrozine, and 1 mL of methanol), and A_o is the absorbance of the oil [13].

2.9. Antibacterial activity

Microorganisms: Test bacteria include four Gram-positive: *Bacillus subtilis* ATCC 11774, *B. thuringiensis* ATCC 10792, *Staphylococcus aureus* ATCC 25923, and Methicillin Resistant *S. aureus* (MRSA) and five Gram-negative bacteria: *Proteus vulgaris* ATCC 33420, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 10536, and *Salmonella typhimurium* ATCC 51812. Test bacteria were obtained from the culture collection of Microbiology Laboratory, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia and verified using the standard microbiology method.

Disc diffusion assay: The assay was carried out according to [14, 15] in triplicate. Sterile cotton swab was dipped in bacterial suspension (10^8 CFU/mL) and used to inoculate on the surface of Mueller-Hinton agar plates. Sterile Whatman No:1 paper discs (6 mm in diameter) were impregnated with 20 µl

(2×10 µl) essential oils (100 mg/mL) and placed on the inoculated agar. The plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the diameter of inhibition zone (in mm) produced by the oils against the microorganisms. Chloramphenicol (30 µg) was used as the positive control.

2.10. Statistical analysis

Values expressed are means of the three replicate ± standard deviation. Duncan's test was performed with values of $p < 0.05$ were considered as significantly different.

3. Results and discussion

3.1. Chemical composition of the essential oils

The essential oils from the rhizomes, stolons, stems, and leaves of *E. brevilabrum* were obtained as yellow oils. Yield of the leaf oil (0.030%) was higher than the rhizome (0.018%), stolon (0.016%), and stem (0.011%). The essential oil from the fresh rhizome in this study is lower than reported by [16] with 0.28%. The difference in yield might be attributed to the hydrodistillation time of 8 h compared to 5 h in this study.

The stem and leaf oils were marked by the presence of a high percentage of monoterpene hydrocarbons (73.4 and 57.3 %), followed by oxygenated monoterpenes (15.5 and 25.9 %) and sesquiterpene hydrocarbon (5.8 and 7.2 %) (Table 1). However, oxygenated monoterpenes dominate the rhizome and stolon oils (53.4 and 40.4%). As for the rhizome oil, sesquiterpene hydrocarbons (25.0%) and monoterpene hydrocarbons (13.2%) ranked the second and third, but this order was reversed in the stolon oil with the monoterpene hydrocarbons 36.9% and sesquiterpene hydrocarbons 15.4%. The result in this study is different from reported by Variappan et al. [16] that found oxygenated monoterpenes and sesquiterpene hydrocarbons at similar proportion in the rhizome oil of *E. brevilabrum*. Difference in chemical composition can be due to the seasonal variation [17], drying period [18] or geographical location of the plant [18, 19].

In this study, different chemical compounds have been identified from different plant parts. Gas chromatography analysis of *E. brevilabrum* essential oil resulted in the detection of 35 compounds in rhizomes, 26 compounds in stolons, 41 compounds in stem and 47 compounds in leaves (Table 1). The major compounds in the leaf oil of *E. brevilabrum* were α-thujene (38.1%), *p*-cymen-7-ol (8.0%), and β-pinene (7.8%). The stem oil was dominated by δ-3-carene (43.2%), α-thujene (17.7%), and limonene (4.1%). The stolon oil was marked by β-pinene (30.6%), *p*-cymen-7-ol (25.0%), and *exo*-fenchol (8.2%). The main components in the rhizome oil were perilla aldehyde (19.6%), bornyl acetate (17.7%), and verbenyl acetate (6.7%). The most abundant compound elemicin found by Variappan et al [16] was not found in this study. The absence of compounds in essential oil within same species for example in *E. elatior* has been reported [18, 19, 20]. Apart from the reasons mentioned above, absence of compounds might be caused by dehydration of oxygenated compounds, chemical transformations or vaporisation.

The chemical compositions of the essential oils of other *Etlingera* species have been reported previously. The rhizome oil of *E. punicea* was dominated by methyl chavicol (95.7%) [21]. Myrcene (13.5%), α-humulene (11.8%), and β-caryophyllene (10.7%) were reported as the major components of *E. elatior* leaf oil; and camphene (18.0%) and β-pinene (16.9%) in its rhizomes and roots [19]. The essential oils from *E. littoralis* were characterized by high percentages of (*E*)-methyl isoeugenol in its leaves (37.7%) and rhizomes (58.1%) [22]. Zoghbi and Andrade [19] reported that the *E. elatior* inflorescence and inflorescence axis oils were marked with dodecanol, dodecanal, and α-pinene. *Etlingera cevuga* rhizome oil contains methyl eugenol and (*E*)-methyl isoeugenol as the major compounds [23]. Yahya et al. [24] reported that 1,8-cineole (16.8%), α-phellandrene (12.7%), and β-trans-ocimene (8.9%) were the major constituents in the *E. sphaerocephala* var. *grandiflora* rhizome oil; 1,8-cineole (17.4%), α-phellandrene (9.7%), and α-pinene (9.5%) in the stem oil; and α-phellandrene (12.3%) and diprene (10.3%) in the leaf oil.

Table 1. Chemical composition of essential oils from the rhizomes, stolon, stem, and leaves of *Etlingera brevilabrum.*

Compound [a]	$I_{Ref.}$	I^{b}	Rhizome	Stolon	Stem	Leaf
α-Thujene	924	926	0.2	-	17.7	38.1
α-Pinene	932	934	0.4	-	-	1.3
α-Fenchene	945	943	6.1	-	-	0.2
Camphene	946	948	5.4	-	-	1.4
β-Pinene	974	977	-	30.6	-	7.8
Myrcene	988	990	0.8	-	-	-
δ-2-Carene	1001	998	-	5.1	-	-
δ-3-Carene	1008	1009	-	-	43.2	2.7
α-Terpinene	1014	1012	-	-	3.2	-
o-Cymene	1022	1023	0.2	1.2	2.2	-
Limonene	1024	1027	-	-	4.1	-
Eucalyptol	1026	1031	-	-	0.1	0.8
β-*trans*-Ocimene	1044	1044	-	-	1.0	0.2
γ-Terpinen	1054	1063	-	-	0.2	-
p-Mentha-3,8-diene	1068	1069	-	-	0.6	-
m-Cresol	1072	1075	-	-	0.5	-
m-Cymenene	1082	1082	-	-	1.0	1.9
Terpinolene	1086	1087	0.1	-	-	0.9
1,3,8-*p*-Menthatriene	1108	1107	-	-	-	2.7
endo-Fenchol	1114	1115	4.0	-	-	0.9
exo-Fenchol	1118	1121	-	8.2	1.5	1.9
α-Campholenal	1122	1123	-	-	-	0.9
l-Terpineol	1130	1131	-	-	-	1.5
cis-Limonene oxide	1132	1133	-	-	3.3	0.3
Camphor	1141	1141	-	-	1.4	3.7
cis-Sabinol	1144	1144	-	-	0.5	1.0
Isoborneol	1155	1153	-	-	1.0	0.3
Borneol	1165	1162	-	-	0.8	1.7
Terpinen-4-ol	1174	1172	-	-	-	0.1
trans-*p*-Mentha-1(7),8-dien-2-ol	1187	1184	-	-	-	0.3
Linalylformate	1214	1215	-	-	0.4	1.1
cis-*p*-Mentha-1(7),8-dien-2-ol	1227	1230	-	-	0.3	-
Perilla aldehyde	1269	1270	19.6	-	-	0.1
Verbenyl acetate	1280	1278	6.7	-	-	-
Bornyl acetate	1287	1287	17.6	-	3.8	-
p-Cymen-7-ol	1289	1290	0.2	25.0	0.7	8.0
Sabinyl acetate	1289	1291	-	-	-	2.2
2-Undecanone	1293	1294	0.4	0.2	-	4.7
Carvaerol	1298	1300	2.8	2.6	-	-
(2*E*,4*E*)-Decadienal	1315	1313	4.9	-	0.1	-
Myrtenyl acetate	1324	1324	0.9	0.6	-	0.5
p-Mentha-1,4-dien-7-ol	1325	1327	0.9	2.6	0.9	0.3
δ-Elemene	1335	1333		0.9	0.8	0.1
α-Cubebene	1345	1342		0.4	0.4	
α-Longipinene	1350	1352	3.8	4.2	3.3	2.6
(*Z*)-β-Damascenone	1361	1361	2.9	1.6	0.8	0.4
Carvaerol acetate	1370	1369	1.1	0.1	0.3	0.1
Isoledene	1374	1373	0.1	0.2	-	0.1
β-Elemene	1389	1390	-	5.0	0.7	-
Cyperen	1398	1399	3.4	1.4	0.2	3.4
Longifolene	1407	1408	0.7	0.3	-	0.1
β –Caryophyllene	1417	1414	1.1	0.3	0.1	0.3
cis-Thujopsene	1429	1426	0.9	-	-	0.2
α-Humulene	1452	1452	0.5	-	-	-
allo-Aromadendrene	1458	1461	4.5	-	-	-
γ-Muurolene	1478	1477	2.1	-	-	-

Compound [a]	$I_{Ref.}$	I [b]	Rhizome	Stolon	Stem	Leaf
α-Amorphene	1483	1482	0.1	-	0.2	0.1
δ-Selinene	1492	1493	1.1	-	-	-
β-Bisabolene	1505	1505	-	-	0.2	-
γ-Cadinene	1513	1510	-	-	0.1	-
(*Z*)-Nerolidol	1531	1530	1.8	1.6	-	0.9
Cedrol	1600	1602	-	-	-	0.2
epi-α-Muurolol	1640	1437	0.5	0.2	-	0.2
(*Z*)-α-Santalol	1674	1471	-	-	-	0.1
α-Bisabolone oxide	1684	1686	-	1.1	-	-
(2*Z*,6*E*)-Farnesol	1722	1725	0.6	0.2	1.2	0.3
(*Z*,*Z*)-Farnesyl acetone	1860	1861	-	2.9	-	-
Monoterpene hydrocarbons			13.2	36.9	73.4	57.3
Oxygenated monoterpenes			53.6	40.4	15.5	25.9
Sesquiterpene hydrocarbons			25.0	15.4	5.8	7.2
Oxygenated sesquiterpenes			1.2	6.0	1.3	1.6
Oxygenated non-terpenes			5.2	0.2	0.7	4.7
Total			98.2	98.9	96.7	96.7

[a]Compounds are listed in order of elution on HP-5, [b]*I* is the retention indices on HP-5. Compounds were identified based on their MS fragment pattern and retention index as compared with NIST data bases and Adams [8].

3.2. Antioxidant activity of the essential oils

Table 2 shows the antioxidant activity of the essential oils using DPPH assay, BCB and FIC ability. In the DPPH assay, all the *E. brevilabrum* oils showed higher IC_{50} values compared to control. This indicates that the essential oils from different parts of *E. brevilabrum* were poor in scavenging the DPPH free radicals. Within the different plant parts, the rhizome oil has a higher RSA compared to the stolon, leaf, and stem oils.

Antioxidant activity as demonstrated in the bleaching of β-carotene by linoleic acid system again showed low activity with rhizome oil shows a higher inhibition compared to the other oils at 1000 µg/mL (Table 2). In this study, the lipid peroxidation inhibitory activity of the essential oils were less effective as indicated by the percentages in β-carotene bleaching test. This is probably due to higher specificity of the assay for lipophilic compounds [11]. In the metal chelating ability or FIC, the essential oils showed low FIC activity at tested concentrations. All the *E. brevilabrum* oils exhibited higher metal chelating ability compared to the positive control of citric acid, but lower than EDTA as seen in Figure 1.

The rhizome oil which was rich in oxygenated monoterpenes showed the highest antioxidant activity in each of the three assays while the stem oil which was dominated by monoterpene hydrocarbons showed the lowest antioxidant activity. It is generally believed that in Zingiberaceae, antioxidants and other secondary metabolites are transported to the rhizomes where they are accumulated [8]. This implies to why rhizomes showed higher antioxidant activity than other plant parts. Furthermore, monoterpene hydrocarbons found in abundance in the stem and leaves oil in this study might relate to the ineffectiveness in antioxidant activity similar to the observation by [11] and others.

Table 2. DPPH radical scavenging activity (RSA), β-carotene bleaching (BCB), and ferrous ion chelating ability (FIC) of essential oils of the rhizomes, stolon, stems, and leaves of *E. brevilabrum*.

Oil/Standard	RSA, IC_{50} (µg/mL)	BCB (%)	FIC,IC_{50} (µg/mL)
Rhizome	1236.54±12.83^{c*}	22.76±0.48^{c}	878.39±16.54^{a}
Stolon	1522.01±27.22^{d}	7.33±1.15^{e}	960.56±11.65^{b}
Stem	1799.04±47.39^{e}	8.06±1.46^{e}	1354.09±5.94^{d}
Leaf	1525.79±38.99^{d}	9.83±1.55^{d}	1098.73±12.32^{c}
BHT	14.36±1.25^{a}	71.99±4.44^{b}	-
α-Tocopherol	11.29±1.54^{b}	87.90±2.53^{a}	-

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

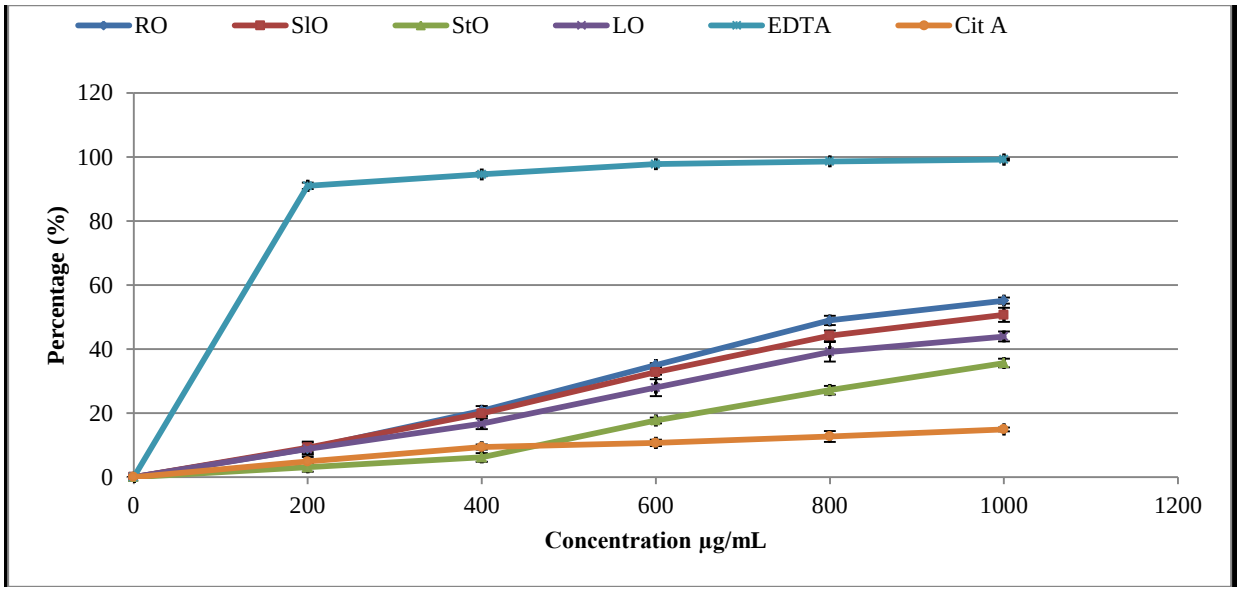

Figure 1. Ferrous ion chelating ability of essential oils of the rhizomes (RO), stolons (SlO), stems (StO), and leaves (LO) of *Etlingera brevilabrum*.

The major compounds of the rhizome oil were perilla aldehyde and bornyl acetate. Borneol and bornyl acetate may be considered as main contributors of antioxidant activity [25, 26]. Bornyl acetate in the rhizome oil might contribute to its antioxidant activity in this study. It has been reported that α-pinene and β-pinene proved to have remarkable antioxidant activity [27, 28]. However, in this study high content of β-pinene in stolon oil does not contribute to antioxidant activity. The combination of high δ-3-carene and α-thujene in the stem oil or α-thujene and *p*-cymen-7-ol in the leaf oil also does not provide antioxidant activity in both oils.

3.3. Antibacterial activity of the essential oils

The result of antibacterial investigation for the rhizome, stem, and leaf oils of *E. brevilabrum* is summarized in Table 3. Antibacterial activity of the stolon oil was not determined due to limited amount of the oil. The oils showed moderate inhibition against the tested organisms. All the oils were found to be effective against Gram-positive bacteria of MRSA and *S. aureus*, and non-active against *B. subtilis* and *B. thuringiensis*. The rhizome oil showed bigger inhibition against *S. aureus* compared to MRSA and *P. mirabilis*. The leaf oil showed inhibitory activity against *S. aureus*, MRSA, *P. vulgaris* and *P. mirabilis*.The stem oil was moderately active towards *S. aureus*, MRSA, *E. coli* and *P. vulgaris*. None of the oils inhibited *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

Our study on the *E. brevilabrum*, stolon extracts have shown that possessed more antibacterial activities compared to the stems and leaves [7]. The amount of compounds extractable from the stolon in the methanol extract where higher compared to other parts but the essential oils from this part is limited. Essential oils with higher oxygenated monoterpene amount exhibit potent antibacterial property [29]. This is proven almost true in this study as shown by the rhizome oil. The antimicrobial property of borneol as well as bornyl acetate was described previously [25, 30, 31]. The compounds available in the oil and interaction between the compounds could contribute to this antimicrobial properties. Antibacterial activity have been associated with the presence of α-pinene, β-pinene [32], borneol [33], α-terpineol, camphor, and eucalyptol (1,8-cineole) [32, 34, 35, 36]. Rhizome oil from *E. punicea* prevented the growth of *S. aureus* and *E. coli*, but not *P. aeruginosa* [2].

Table 3. Antibacterial activity of essential oils from the rhizomes, stems, and leaves of *Etlingera brevilabrum.*

Gram-positive	Rhizome oil	Stem oil	Leaf oil	Chloramphenicol[a]
MRSA	12.6±0.4[b]	10.0±0.3	8.1±0.4	22.2±0.3
S. aureus	17.7±0.5	11.2±0.6	12.6±0.5	29.8±0.5
B. subtilis	6[c]	6	6	NT[d]
B. thuringiensis	6	6	6	NT
Gram-negative				
E. coli	6	13.5±0.3	6	19.8±0.7
P. vulgaris	6	9.5±0.7	10.3±0.6	12.9±0.4
S. typhimurium	6	6	6	NT
P. mirabilis	13.2±0.6	6	16.4±0.7	16.8±0.5
P. aeruginosa	6	6	6	

[a]: Chloramphenicol (30 µg); [b]: Values are presented as means ± SD (n = 3); c: size of discs (6 mm), non-active.d: NT= not tested

4. Conclusions

The study of the chemical composition of different parts of *E. brevilabrum* illustrated a similarity in the terpenoids profile of the rhizome, stolon stem and leaf. Monoterpenes predominate in the stem and leaf oil, whereas in the rhizome and stolon oil oxygenated monoterpenes dominated. Among the oils, the rhizome oil showed higher antioxidant activities for radical scavenging and inhibition of β-carotene bleaching, and ferrous ion chelating although lower than the positive controls of BHT and α-Tocopherol. The essential oils were all effective against MRSA and *S. aureus* but variable effectiveness against Gram negative bacteria including *P. mirabilis*, *P. vulgaris*, and *E. coli.* This study successfully reports the compounds available in the essential oil from different parts of *E. brevilabrum* and the antibacterial activity.

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