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Research Article

Chemical Composition, Antioxidant, and Antimicrobial Activity of the Essential Oil of Leaves of *Etlingera velutina*

Behnam Mahdavi [1,2*], Wan A. Yaacob[3]

[1] *Department of Chemistry, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran*
[2] *Research Group of Phytopharmacology Systems, Hakim Sabzevari University, Sabzevar, Iran*
[3] *School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Malaysia*

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* **Corresponding Author:**

Behnam Mahdavi, Department of Chemistry, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran
Email: b.mahdavi@hsu.ac.ir

ABSTRACT

Objectives: The antimicrobial and antioxidant activities of essential oils are important because they offer a natural and effective agent. This study aims to investigate the chemical composition, antioxidant, and antimicrobial activity of the essential oil of leaves of *Etlingera velutina.*

Methods: The essential oils from the leaves of Etlingera velutina were obtained using a Clevenger-type apparatus, and the chemical compositions of the oils were identified by GC-FID and GC-MS methods. The antioxidant activity of the oil was analyzed using three common assays. The antimicrobial activity of the oil was evaluated by disc diffusion assay.

Results: The oil consists of sesquiterpene hydrocarbons with the highest percentage (63.12%). Aromadendrene (58.30%), α-pinene (10.82%), and caryophyllenyl alcohol (10.21%) were identified as the main components of the essential oil. The leaf oil exhibited antioxidant activity in all the tests: DPPH radical scavenging activity (RSA), β-carotene bleaching (BCB), and ferrous ion chelating ability (FIC). The oil presented activity against *B. subtilis, E. aerogenes, P. vulgaris,* and *Candida parapsilosis.*

Conclusion: The data suggest that the essential oil of the leaves of *Etlingera velutina* revealed moderate activity against selected microorganisms in antimicrobial and antioxidant assay. It may be considered as a natural agent for bioactivty.

Keywords: Etlingera velutina; Antioxidant Activity; Antimicrobial Activity; Essential oil

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Introduction

Essential oils are complex mixtures derived from aromatic plants and are well recognized for both their antioxidant and antimicrobial properties, which are primarily attributed to an abundance of bioactive constituents such as phenolic compounds, terpenes, and various secondary metabolites (1, 2). The presence of these compounds enables essential oils to play a pivotal role in food preservation, healthcare, and pharmaceutical applications, owing to their multifunctional properties (3). Essential oils (EOs) are well known for their broad-spectrum antimicrobial activity against bacteria, fungi, and viruses (4–6). Their effects have been established against both Gram-positive and Gram-negative bacteria, though Gram-positive organisms are generally more susceptible (7, 8). The antimicrobial effects of essential oils are primarily attributed to their ability to disrupt microbial cell membranes, leading to increased permeability, leakage of intracellular contents, and ultimately cell death (1).

Etlingera belongs to the subfamily Alpinioideae within the Zingiberaceae family, encompassing over 100 species (9). *Etlingera velutina* typically grows in shaded forest gaps and abandoned gardens, favoring moist environments such as lower slopes, valleys, or near streams at altitudes ranging from 30 to 1100 meters. The flowers of *E. velutina* are notable for having a distinctive white edge on the lateral lobes of its labellum. Locally, this species is known by names including 'Tepus kenyalang', 'Tolidus sarou', 'Tubu sia', and 'Tahau'. The plant is utilized as a flavoring agent, with young stem juice traditionally used to treat snakebites, while the shoots are edible and leaves serve as rice wrappers (10). Since there is limited data on the chemical composition and bioactivity of *E. velutina* essential oil, this study is directed toward identifying the chemical constituents in its leaf essential oil and evaluating its antioxidant and antimicrobial properties.

Methods

Essential oil extraction

The fresh leaves of *Etlingera velutina* were ground, and 300 g of each part was subjected to hydrodistillation for approximately 4 h in a Clevenger-type apparatus. The resulting essential oils were dried over anhydrous sodium sulfate and kept in tightly closed vials at -18°C before analyses.

Gas chromatography (GC) analysis

GC analysis of the essential oil constituents was carried out by 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 increased at a rate of 3°C min^{-1} to 250°C and held isothermally for 10 min. The injector temperature was set at 200°C and detector temperature at 250°C. One µL of the essential oil in n-hexane was 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. 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 analysis above.

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil constituents were analyzed by an Agilent 7890A GC coupled to an Agilent 5975C mass detector using an 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 were as described in the GC analysis. The compounds were identified by comparing the GC retention indices and mass spectra with those found in the literature. All the analysis conditions were the same as in our previous studies (11, 12).

Bioactivity evaluations

The bioactivity evaluation, including antioxidant activity and antimicrobial activity, was also conducted according to our previous studies (12–15).

Free radical-scavenging activity (RSA): DPPH assay

A 1.5 mL aliquot of each essential oil at concentrations of 200, 400, 800, 1600, and 2000 µg/mL was mixed with 1 mL of 0.1 mM DPPH solution in methanol. The mixture was vigorously shaken for 1 minute and then incubated in the dark at room temperature for 90 minutes. After incubation, the absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) and α-tocopherol were used as reference controls. All measurements were performed in triplicate over three separate days. The radical scavenging activity (RSA) of the samples was expressed as the percentage inhibition of DPPH, calculated using the following equation: RSC% = [(Ac – Ao)/Ac] × 100, where Ac is the absorbance value of the control (DPPH solution without test oil), and Ao is the absorbance value of the essential oil (DPPH solution with test oil).

The antioxidant activity:β-carotene bleaching (BCB)

A 5 mL solution of β-carotene 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 then evaporated under vacuum at 45°C for 10 minutes. Subsequently, 125 mL of oxygenated water was added, and the mixture was vigorously shaken to form an

emulsion. Next, 2.5 mL of this emulsion was mixed with 0.2 mL of the oil solution (1000 µg/mL), and the absorbance was immediately measured at 470 nm. The mixture was incubated at 50°C, with absorbance readings taken every 45 minutes for up to 180 minutes. All measurements were performed in triplicate. Butylated hydroxytoluene (BHT) and α-tocopherol served as reference controls. The antioxidant activity (AA) was assessed based on β-carotene bleaching using the following formula: AA % = $[1-(A^{o}_{0}-A^{o}_{t})/(A^{c}_{0}-A^{c}_{t})] \times 100$ 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.

Determination of ferrous ion chelating (FIC) ability

Fifty microliters (µL) of $FeSO_4$ (2 mM) was added to a vial containing 1 mL of essential oil at concentrations of 200, 400, 600, 800, and 1000 µg/mL, along with 2 mL of distilled water. The reaction was initiated by adding 100 µL of ferrozine (5 mM). The mixture was thoroughly mixed and incubated at room temperature for 10 minutes. Absorbance was then measured at 562 nm. All assays were performed in triplicate. EDTA and citric acid were used as positive controls. The percentage 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.

Antibacterial activity

Test bacteria include six Gram-positive: *Bacillus subtilis*ATCC 11774, *B. thuringiensis*ATCC 10792, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and Methicillin Resistant *S. aureus* (MRSA) and ten Gram-negative bacteria: *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 10536, *Proteus mirabilis* ATCC 12453, *Proteus vulgaris* ATCC 33420, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhimurium* ATCC 51812, and two fungi of *Candida albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019.

Disc diffusion assay

The assay was performed in triplicate. A sterile cotton swab was immersed in a bacterial suspension (10^8 CFU/mL) and used to inoculate the surface of Mueller-Hinton agar plates. Sterile Whatman No. 1 paper discs, each 6 mm in diameter, were soaked with 20 µL (2 × 10 µL) of essential oils at a concentration of 100 mg/mL and then placed onto the inoculated agar. The plates were incubated at 37°C for 24 hours. Antibacterial activity was assessed by measuring the diameter of the inhibition zones (in millimeters) formed by the oils against the bacteria. Chloramphenicol (30 µg) served as the positive control. For the disc diffusion method, two inhibition values were reported: (1) the values of the inhibition diameter zones in millimeters (mm), and (2) the values of inhibition levels in percentage (%). The inhibition level was measured by dividing the inhibition zone diameter of the sample (extracts, essential oils, or smoke liquids) by that of the antibiotic (positive control) as shown:

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

Antimicrobial activity was categorized as strong for inhibition level ≥ 70%, moderate for inhibition level 50-70%, and weak for inhibition level < 50%.

Statistical Anlysis

All statistical analyses were performed using SPSS software 21.0 (IBM, Chicago, IL, USA). All data were presented as mean ± S.D. (standard deviation). The statistical significance was determined by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A p-value less than 0.05 was considered statistically significant.

Results

Chemical composition of the essential oils

The volatile composition of the essential oil from the leaves of *Etlingera velutina* is tabulated in Table 1. The leaf oils of *E. velutina* were dominated by sesquiterpene hydrocarbons (63.12%). The predominant constituents in the leaf oil were allo-aromadendrene (58.30%), α-pinene (10.82%), and caryophyllenyl alcohol (10.21%).

Antioxidant activity of the essential oils

The antioxidant activity results of *E. velutina* essential oil are shown in Table 2. According to the results, the leaf oil showed the highest antioxidant activity for all tests. IC_{50} of 1390.66 ± 23.40 µg/mL for RSA, the percentage of 29.40 ± 1.52 for BCB, and IC_{50} of 1426.09 ± 6.61 µg/mL for FIC were the results of the leaf oil. The essential oil showed higher activity than the positive control of CitA in the FIC assay.

Antimicrobial activity of the essential oils

The antimicrobial activity of the essential oils is tabulated in Table 3. The essential oil was inactive against *B. thuringiensis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *C. albicans*. For Gram-negative bacteria *P. mirabilis* and *P. vulgaris*, the leaf oil showed activity with values of 46.4% and 60.5%, respectively. The results of the antifungal activity of the oils against *C. parapsilosis* were classified as moderate in inhibition level for the essential oil (66.0%).

Table 1. Chemical composition of the essential oils from the leaves of *Etlingera velutina*

Compound	*I**	*I***	Leaves
α-Pinene	932	933	10.82
1-Decene	986	989	0.04
trans-Linalool oxide	1084	1081	3.38
endo-Fenchol	1114	1112	7.11
exo-Fenchol	1118	1118	0.05
trans-Limonene oxide	1137	1137	0.08
β-Pinene oxide	1154	1151	0.32
n-Decanal	1201	1198	0.62
4*E*-Decen-1-ol	1259	1260	0.36
n-Decanol	1266	1266	0.24
α-Cubebene	1345	1344	0.55
allo-Aromadendrene	1458	1461	58.30
γ-Gurjunene	1477	1480	4.27
E-Nerolidol	1561	1559	0.82
Caryophyllenyl alcohol	1570	1571	10.21
Caryophyllene oxide	1582	1584	0.25
Dodecyl acetate	1607	1606	0.19
Monoterpene hydrocarbons			10.82
Oxygenated monoterpenes			10.94
Sesquiterpene hydrocarbons			63.12
Oxygenated sesquiterpenes			11.28
Non-terpene hydrocarbons			0.04
Oxygenated non-terpenes			1.41
Total			98.41

* Retention indices (*I*) from literatures(37); ** Retention indices (*I*) on DB-5 capillary column.

Table 2. DPPH radical scavenging activity (RSA), β-carotene bleaching (BCB), and ferrous ion chelating ability (FIC) of essential oil of the leaves of *Etlingera velutina*

Oil/Standard	RSA IC_{50} (µg/mL)	BCB(%)	FIC IC_{50} (µg/mL)
Leaf	1390.66±23.40^{c}	29.40±1.52^{c}	1426.09±6.61^{b}
BHT	14.36±1.25^{a}	71.99±4.44^{b}	68.18±2.56^{a}
α-Tocopherol	11.29±1.54^{b}	87.90±2.53^{a}	-
AscA	-	21.83±2.04^{d}	-
CitA	-	-	1449.21±34.05^{b}

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

Table 3. Antibacterial activity of the leaves essential oils of *Etlingera velutina* using disc-diffusion assay

Microorganisms	leaves oil	Positive Control[a]	Negative Control[b]
Gram-positive			
B. subtilis	23.5±1.2^{c}	28.9±0.8^{d}	6.0±0.0^{e}
B. thuringiensis	NA^{f}	22.3±0.3	6.0±0.0
MRSA	NA	22.2±0.3	6.0±0.0
S. aureus	NA	29.8±0.5	6.0±0.0
Gram-negative			
E. aerogenes	27.7±2.38	26.0±0.6	6.0±0.0
E. coli	NA	19.8±0.7	6.0±0.0
P. mirabilis	46.4	16.8±0.5	6.0±0.0
P. vulgaris	60.5±3.2	12.9±0.4	6.0±0.0
P. aeruginosa	NA	6.0±0.0	6.0±0.0
S. typhimurium	NA	6.0±0.0	6.0±0.0
Fungi			
C. albicans	NA	13.2±0.7	6.0±0.0
C. parapsilosis	66.0	14.1±0.4	6.0±0.0

each disc was impregnated by 20µl of essential oil at 100 mg/ml. a: Chloramphenicol 30 µg and nystatin 30 µg for fungal; b: Solvent; c: Values are inhibition levels of samples obtained according to the flowing equation: Inhibition level (%) $= \frac{\text{Inhibition zone diameter of sample}}{\text{Inhibition zone diameter of the antibiotic}} \times 100$; d: Values are Inhibition zone diameter (mm) for positive controls presented as means ± SD (n = 3), e: the size of discs (6 mm); f: Non-Active

Discussion

Previous studies on the essential oils of other *Etlingera* species reported the presence of different compounds in their essential oils as main components. β-Pinene, limonene, and 1,8-cineole were the major components in the leaf, stem, and rhizome oils of *E. brevilabrum*, respectively (11). For the fresh parts of *E. brevilabrum*, α-thujene, δ-3-carene, and perilla aldehyde were identified as the major compounds in the leaf, stem, and rhizome oils (12). α-Pinene, 1,8-cineole, β-phellandrene, and β-pinene were the main compounds of the essential oils of *E. brevilabrum* identified by GC×GC/TOFMS (16). The essential oils of *E. sayapesis* were characterized by carvone, linalool formate, and α-terpineol (15). Methyl chavicol was the main component of the rhizome oil of *E. punicea* (17). Methyl eugenol was the main component of the rhizome and leaf oils of *E. cevuga* (18, 19). The rhizome and stem oils of *E. sphaerocephala* were characterized by 1,8-cineole, and α-phellandrene was the main compound for the leaf oil (20). The leaf oil of *E. linguiforme* was dominated by the presence of 1,8-cineole; however, the oil from the rhizome of *E. linguiforme* was marked by methyl chavicol (21). The leaf oil of *E. littoralis* was characterized by the occurrence of (E)-methyl isoeugenol, while the rhizome oil also predominantly contained (E)-methyl isoeugenol. The leaf oil of *E. elatior* was marked by myrcene, and the rhizome oil was rich in camphene. The leaf oil of *E. elatior* var. Thai Queen consisted of α-pinene (22). A study on the essential oils of *E. elatior* reported that (E)-β-farnesene, β-pinene, and caryophyllene were major constituents in the leaf oil, and 1,1-dodecanediol diacetate, (E)-5-dodecene, and decanal in the stem oil (23). The essential oil from the whole plant of another Malaysian *E. elatior*, collected from Selangor, was dominated by β-pinene (24). Other studies on *Etlingera* species have described non-terpenic compounds, with (Z)-9-hexadecen-1-ol as a major compound in *E. fulgens* and dodecanoic acid as dominant in *E. venusta* (25, 26). One study on the chemical composition of the essential oils of *E. yunnanensis* from Vietnam reported germacrene D and β-pinene as the main components in the leaf, stem, and rhizome oils (27). Estragole was recognized as the major compound in the rhizome oil of *E. yunnanensis* from China in another study (28). The rhizome essential oil of *E. pavieana* was dominated by trans-anethole (13.8%) (29). For the leaf essential oil of *E. fimbriobracteata*, β- and α-pinene were the main components, while 1,8-cineole and β-pinene were dominant in the aerial stem oil, and decanal and 1,8-cineole in the basal stem oils (30).

The antioxidant activity of essential oils is fundamentally linked to their capacity to neutralize reactive oxygen species (ROS) and free radicals, stabilizing oxidative processes and mitigating cellular damage (1, 31, 32). In our previous studies, we ran the same assays to assess the antioxidant activity of E. brevilabrum and E. sayapensis oils. The results were similar to the findings of this study. It can be concluded that the essential oils of these three Etlingera species are not potent in antioxidant assays (12, 15).

The antimicrobial activity of essential oils arises from their ability to disrupt microbial structures and cellular functions (7, 8). Owing to their hydrophobic nature, essential oil molecules interact with the lipid components of microbial cell membranes, leading to increased membrane permeability, disruption of membrane proteins, leakage of cellular contents, and ultimately cell death (33–35). This broad mechanism allows essential oils to be effective against a range of microorganisms, including bacteria, fungi, and some viruses (7, 36). In our previous study, the rhizome, stem, and leaf oils of *E. brevilabrum* inhibited the growth of MRSA and *S. aureus*, but could not prevent the growth of *B. subtilis* and *B. thuringiensis*. The rhizome oil was active against *P. mirabilis*; the stem oil showed inhibitory activity against *E. coli* and *P. vulgaris*; and the leaf oil inhibited the growth of *P. vulgaris* and *P. mirabilis* (12). In contrast, the essential oils of *E. sayapensis* were more active compared to *E. velutina*. The oils inhibited the growth of 13 out of 18 selected microorganisms (15). According to another study, the rhizome oil of *E. punicea* inhibited *S. aureus*, *E. coli*, and *C. albicans*, and was inactive against *P. aeruginosa* (17).

Conclusion

In summary, this research investigated the chemical composition of essential oil extracted from the leaves of *Etlingera velutina*, along with its antioxidant and antibacterial properties. The analysis identified aromadendrene, α-pinene, and caryophyllenyl alcohol as the predominant constituents of the oil. The essential oil demonstrated moderate antioxidant effects, as evidenced by its performance in DPPH radical scavenging, β-carotene bleaching, and ferric-reducing antioxidant power assays. Additionally, it exhibited inhibitory effects against 4 out of 12 tested microbial strains, showing notable activity, particularly against *Bacillus subtilis*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Candida parapsilosis*.

Conflict of interests

The authors declare no conflict of interest.

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