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Original Article

GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF BIOACTIVE COMPOUNDS IN ESSENTIAL OILS OF LEAF OF *EUODIA SUAVEOLENS* SCHEFF

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ABSTRACT

Objective: Essential oils extracted from the leaf of *Euodia suaveolens* have been reported. However, there is no published data on comprehensive report on the chemical constituents of the essential oils and the methods utilized to extract the essential oils from the plant. This research aimed to reveal and determine the chemical constituents of essential oils from the leaf of *E. suaveolens*.

Methods: Essential oils of leaf of *E. suaveolens* were extracted by steam distillation and were analyzed utilizing Gas Chromatography-Mass Spectrometry (GC-MS) methods.

Results: The GC-MS analysis revealed the presence of twenty-five different chemical constituents from the essential oils. The main chemical constituents of essential oils extracted from *E. suaveolens*' leaves were as follows menthofuran (50.38 %), p-mentha-1,8-diene (14.34 %), limonen (10.99 %), evodone (5.55 %), α-curcumene (4.65 %), globulol (1.88 %), longipinenepoxide (1.66 %), and linalool (1.40 %). This present research found three compounds, namely p-mentha-1,8-diene, globulol, and longipinenepoxide that have never been reported by any researchers working with this plant.

Conclusion: The results showed that *E. suaveolens* contains essential oils that are potential to be explored further and utilized as medicinal products against some ailments.

Keywords: GC-MS analysis, *Euodia suaveolens*, Bioactive compounds, Essential oils

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INTRODUCTION

Indonesia has huge plant biodiversity that has been utilized by their ancestor in their daily life activities (ethnopharmaceutica). Many plant species have become the source of medicinal herbs and one of it is zodia (local name; *Euodia suaveolens* Scheff. is its scientific name, which is synonym with *Euodia hortensis* Forst.). The plant is originally growth in the West Papua and its vicinities such as Papua New Guinea, Samoa, Tonga, and Niue [1, 2].

Commonly, the plant was introduced to other places in Indonesia as ornamental and garden decorations. In some areas such as Sepik, Papua New Guinea scrapping of leaf added with water was drank to relief cold; in Solomon island crushed leaves were used to treat boils, roots and leaves were mixed with *Areca catechu* nuts and lime as malaria treatment; in Pacific islands infusion of barks or leaves were utilized to relief fever, leaves were chewed to treat toothache and remedial bathing; leaf decoction was a favorite treatment to cure stomachache, fever, and menstrual illness; decoction of barks was more potent to relief stomach ulcers, reduce menstrual and childbirth pains; leaves and in florescenses were worn as a personal decoration for social occasions; the woods were used as house construction [1].

Euodia suaveolens has been very popular as mosquito repellent in Indonesia, specifically against *Aedes aegypti*, mosquito species that became the vector of dengue fever [3, 4]. Some research topics were utilizing a combination of the plant extract with other herb extracts for instance lemon grass (*Cymbopogon citratus*) [5] or rosemary (*Rosmarinus officinalis*) [6]. *E. suaveolens* has been tested to eradicate red flour beetle (*Tribolium castaneum*) pest [7] and has also been determined its toxicity utilizing Brine Shrimp Lethality Test (BSLT) [8]. Many research activities on *E. suaveolens* were done to find bioactive compounds and produce several antimosquito formulations, namely lotion, gel, electric mat, etc [3]. Some other researchers have been trying to prove its bioactive compounds as antibacterial l [9, 10].

Research on essential oils has been increasingly done by many researchers, since the chemical constituents in the essential oils showed important aspects to human life [11–13]. Some chemical constituents of the essential oils extracted from plants have been utilized not only as fragrances, flavors, and perfumes but also as antibacterial, antifungal, antioxidant, antitumor, anticancer, anti-inflammatory, etc [14-18]. However, published data on the chemical constituents of the essential oils from the leaf of *E. suaveolens* are dearth. Therefore, this research aimed to reveal the chemical constituents of the essential oils from the leaf of *Euodia suaveolens* and to determine their potencies as pharmaceutical resources.

MATERIALS AND METHODS

Preparation of materials

Leaves of *E. suaveolens* were collected from Sleman district, Yogyakarta province, Indonesia. The plant was authenticated and identified by plant taxonomist at Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta and was deposited as herbarium at the Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta. The leaves were selected based on good quality such as green in color, no insect bites, clean (no dirt), and fresh conditions.

Experimental procedures

Fresh *E. suaveolens* leaves sample (10 kg) were collected, washed thoroughly, decanted to reduce the water, and put into distillation apparatus and were steamed for four hours to extract its essential oils. Ten kg of the leaf sample was put into a biomass holding chamber of the distillation unit. The water steam was produced by heating the water in a vessel and the steam flows into a chamber where the leaf sample was put. The steam passed through the leaf samples and created vapor, which then condensed further and flowed into a receiver vessel. The essential oils layer was separated and kept in a clean bottle [11, 19, 20].

The essential oils were then analyzed utilizing GCMS-QP2010S Shimadzu (Shimadzu Ltd., Japan) equipment with the following conditions: column type Rtx 5, column length: 30 m, film: 0.25 µm, carrier gas: Helium, ionizer: EI 70 Ev, column oven temperature:

50.0 °C, injection temperature: 300.0 °C, injection mode: split, flow control mode: pressure, pressure: 13.0 kPa, total flow: 79.3 ml/min, column flow: 0.55 ml/min, linear velocity: 26.8 cm/sec, purge flow: 3.0 ml/min, split ratio: 139.0, ion source temperature: 250.00 °C, interface temperature: 300.00 °C, solvent cut time: 3.00 min, detector gain mode: Absolute, detector gain: 1.50 kV, threshold: 0. The chemical constituents of the essential oil were identified by comparing the results of the chromatogram and retention time reference from Wiley mass spectra library (Wiley229. LIB) [21-23].

RESULTS AND DISCUSSION

GC-MS chromatogram of the main chemical constituents of essential oils extracted from *E. suaveolens*' leaves were as follows menthofuran (50.38 %), p-mentha-1,8-diene (14.34 %), limonen (10.99 %), evodone (5.55 %), α-curcumene (4.65 %), globulol (1.88 %), longipinenepoxide (1.66 %), and linalool (1.40 %). GC-MS analysis found 25 chemical constituents from the essential oils sample examined (table 1). Some of the chemical constituents identified were reported by earlier researchers to have bioactive applications such as antibacterial, antifungal, antitumor, anticancer, anti-inflammatory, etc. (table 2). These chemical constituents showed high potencies to be explored further as pharmaceutical products.

The chemical constituents revealed in table 1 were also found in the essential oils of *Euodia sauveolens* (and its synonym *Euodia hortensis*) in some researches, but with some degree of differences. Both similarities and differences were found in term of chemical species and percentage area of the chemical constituents reported (table 3). Menthofuran has the highest percentage area and similar findings were reported by some researchers [9, 10, 24]. Other chemical constituents that were revealed by some researchers were limonen, evodone, α-curcumene, and linalool [1, 9, 10, 24, 25]. But this present research found three compounds namely p-mentha-1,8-diene, globulol, and longipinenepoxide that have never been reported by any researchers working with this plant. Steam distillation was suggested to be able to extract more chemical constituents from the plant as has been reported by some researchers [11, 20]. Further research on these three compounds will be interesting to be done, specifically to determine their bioactivities in health care applications.

The differences in the percentage area of the chromatogram reported by some researchers was due to the differences in the research method as well as the analysis method applied [13]. Some researchers noticed that the differences might probably be caused by the difference of plant origins (geography), environment conditions (ecology), growth conditions (physiology) [9], and plant species collected (genetics) [1, 2].

Table 1: Chemical compounds identified from the essential oils of *E. suaveolens*

No.	Name of compounds	RT	Formula	Molecular weight
1.	1,6-Octadiene, 7-methyl-3-methylene-(CAS) 2-Methyl-6-Methylene-2,7-Octadiene	13.111	$C_{10}H_{16}$	136
2.	Cyclohexene, 1-methyl-4-(1-methylethenyl)-(CAS) 1-P-Mentha-1,8-Diene	14.598	$C_{10}H_{16}$	136
3.	Benzofuran, 4,5,6,7-tetrahydro-3,6-dimethyl-(CAS) Menthofuran	19.454	$C_{10}H_{14}O$	150
4.	1-Cyclohexene-1-methanol, 4-(1-methylethenyl)-(CAS) Perillol	20.472	$C_{10}H_{16}O$	152
5.	Evodone	24.951	$C_{10}H_{12}O_2$	164
6.	Bicyclo[3.2.1]octan-3-one	25.118	$C_8H_{12}O$	124
7.	Tricyclo[4.4.0.0(2,7)]dec-3-ene, 1,3-dimethyl-8-(1-methylethyl)-, stereoisomer (CAS) Tricyclo[4.4.0.0(2,7)]dec-3-ene, 1,3-dimethyl-8-(1-methylethyl)-, st (CAS) Copaene	25.823	$C_{15}H_{24}$	204
8.	Limonen-10-yl acetate	26.740	$C_{12}H_{18}O_2$	194
9.	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, acetate, (1. alpha.,2. beta.,5. alpha.)-(CAS) Dihydrocarveol acetate	26.958	$C_{12}H_{20}O_2$	196
10.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-(CAS) l-Caryophyllene	27.136	$C_{15}H_{24}$	204
11.	Trans-Carvyl Acetate	27.358	$C_{12}H_{18}O_2$	194
12.	beta.-Caryophyllene	28.098	$C_{15}H_{24}$	204
13.	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-(CAS). alpha.-Farnesene	28.550	$C_{15}H_{24}$	204
14.	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-(CAS) ar-Curcumene	28.668	$C_{15}H_{22}$	202
15.	3a,7-Methano-3aH-cyclopentacyclooctene, 1,4,5,6,7,8,9,9a-octahydro-1,1,7-trimethyl-, [3aR-(3a. alpha.,7. alpha.,9a. beta.)]-(CAS) Cloven	28.998	$C_{15}H_{24}$	204
16.	Longipinenepoxide	29.303	$C_{15}H_{24}O$	220
17.	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-(CAS) 1-Formyl-2,6,6-trimethyl-1-cyclohexene	31.159	$C_{10}H_{16}O$	152
18.	1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)-(CAS) 4,7,10-Cycloundecatriene, 1,1,4,8-Tetramethyl-, ALL-CIS	31.300	$C_{15}H_{24}$	204
19.	1,6-Octadien-3-ol, 3,7-dimethyl-(CAS) Linalool	31.491	$C_{10}H_{18}O$	154
20.	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-(CAS) ar-Curcumene	31.592	$C_{15}H_{22}$	202
21.	Aromadendrenepoxide-(II)	31.814	$C_{15}H_{24}O$	220
22.	7-Oxabicyclo 4.1.0 heptane, 1-methyl-4-(1-methylethenyl)-	32.173	$C_{10}H_{16}O$	152
23.	Globulol	32.552	$C_{15}H_{26}O$	222
24.	2,6-Octadien-1-ol, 3,7-dimethyl-, (E)-(CAS) Guaniol	33.907	$C_{10}H_{18}O$	154
25.	3,5-Octadiene, 4,5-diethyl-3,6-dimethyl-(CAS)	47.681	$C_{14}H_{26}$	194

Note: RT= Retention time

Table 2: Bioactivity of chemical constituents identified from essential oils of *E. suaveolens* leaf

No.	Compound name	Bioactivity	Reference
1.	Menthofuran	antioxidant	[12]
2.	p-mentha-1,8-diene	insect repellent, flavoring ingredients	[12]
3.	Limonen	antitumor, anticancer, antioxidant, anti-inflammatory, anti-stress	[16]
4.	Evodone	insect (mosquito) repellent	[9, 24]
5.	α-Curcumene	flavoring ingredients, perfumes and fragrances, insect repellent	[18]
6.	Globulol	antimicrobial, antifungal, antibacterial	[15]
7.	Linalool	cosmetics, perfumes and fragrances, biocides (disinfectants, pest control)	[14]

Table 3: Comparison of chemical constituents of essential oils extracted from *E. suaveolens* leaf

No.	Compound name	Method, plant, country	Researcher (s)
1.	menthofuran, evodone (4-ketomenthofuran), limonene	steam distillation, GLC analyzed, *E. hortensis*, Fiji	[24]
2.	prenilated acetophenon, monoterpene furano (evodone), caryophyllene, α-copaen, ar-curcumene	method not mentioned, *E. hortensis*, Papua New Guinea	[1]
3.	evodone, menthofuran, limonene, curcumene, fonenol	steam distillation, GC-MS analyzed, *E. suaveolens*, Indonesia	[10]
4.	limonene	Ethanol maceration and hydrodistillation, *E. suaveolens*, Indonesia	[25]
5.	menthofuran, evodone, linalool, citronellol, α-(2) gurjunene	hydrodistillation, GCMS analyzed, *E. hortensis*, Fiji	[9]
6.	linalool	steam distillation and TLC analyzed, *E. suaveolens*, Indonesia	[4]
7.	menthofuran, p-mentha-1,8-diene, limonen, evodone, α-curcumene, globulol, longipinenepoxide, linalool	steam distillation, GCMS analyzed, *E. suaveolens*, Indonesia	Sidharta and Atmodjo (this present research)

CONCLUSION

This present research found twenty-five chemical constituents of essential oils identified by GC-MS from the leaf of *E. suaveolens,* which is higher in numbers of compounds compares to earlier research findings. The results also showed that *E. suaveolens* contains essential oils that are potential to be explored and utilized as medication to some ailments. These findings give strong support in term of scientific bases to the practices done by the ancestors (ethnopharmaceutical) to the plant. Therefore, continuous research on the essential oils of the plant will bring more results and more chances to utilize as pharmaceutical products that will give benefits to more peoples.

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ABBREVIATIONS

cm= centimeter, Ev= electron volt, kg= kilogram, kPa= kilo Pascal, kV= kilovolt, m= meter, min= minute, ml= millilitre, sec= second, µm= micrometer

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AUTHORS CONTRIBUTIONS

Both authors have contributed equally.

CONFLICT OF INTERESTS

Authors declared no conflict of interests.

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