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Phytochemical and antibacterial analyses of essential oils extracted from the leaves of *Euodia suaveolens* Scheff

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ABSTRACT

Euodia suaveolens is one of the plants that ancient people in Indonesia used due to its manifold benefits. Earlier research on this plant was mostly done on its potency as a mosquito repellent. This present study aims to determine the phytochemical and antibacterial analyses of the essential oils (EOs) extracted from the leaves of *E. suaveolens*. The EOs of the leaves of *E. suaveolens* were extracted by steam distillation method and were analyzed phytochemically utilizing the GC-MS technique to determine the chemical constituents. The chemical components were tested on four pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* utilizing the diffusion agar method. The results showed that the main compounds extracted from the EOs were α-curcumene, evodone, globulol, limonene, linalool, longipinenepoxide, menthofuran, and p-mentha-1,8-diene. The antibacterial analysis of these compounds showed potential activities to inhibit the growth of four pathogenic bacteria tested, but the inhibition zones formed were still lower compared to commercial antibiotic kanamycin. *E. suaveolens* EOs exhibited diameter of zone of inhibition as follows 2.03±0.22, 0.50±0.49, 1.38±0.10, 1.40±0.27 cm to *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis* while kanamycin showed 3.43±0.08, 3.25±0.08, 3.38±0.12, and 3.18±0.24 cm respectively. These results recommend that the main compounds extracted from the EOs of the leaves of *E. suaveolens* be explored further to determine their potencies as new antibiotic medications.

Keywords: *E. suaveolens*, essential oils (EOs), steam distillation, antibacterial potency

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INTRODUCTION

Resistance of some bacteria to antibiotics has been increasing for the last few years and it has also forced healthcare practitioners to find new antibiotic medications. Antibiotic or antimicrobial resistance (AMR) has become a big threat to medical, food safety, and nation-building (World Health Organization., 2019). Malpractice of antibiotic usage in third-world countries such as Indonesia has weighted the problems, such as the increase in TB (tuberculosis), pneumonia, gonorrhea, and salmonellosis infections, infectious diseases are harder to eradicate; longer stay in hospitals; health costs and death cases increase (Davies & Davies, 2010; Zaman et al., 2017). The new antibiotic is hoped to suppress health costs due to the high price of antibiotics prescribed. New antibiotics can also decrease the length of stay in hospitals and increase workforce productivity (World Health Organization., 2019).

Indonesia which is located in the tropical area is known as a megadiversity country which has been utilized since their prehistoric ancestors for daily life needs, including bacterial infectious disease cures. Some of the medicinal plants in Indonesia have been known and utilized by people from other countries. *Euodia suaveolens* Scheff. (syn. *Euodia hortensis* Forster & Forster; vernacular name "zodia") is originally coming from West Papua and some researchers have been trying to find bioactive compounds from the plant to solve health problems in Indonesia (Maryuni et al., 2008; Romulo et al., 2018; Simaremare & Lestari, 2017). Furano monoterpenes (evodene) and prenylated acetophenones have been extracted from *E. suaveolens* leaves, and essential oil (EO) containing a-copaene, ar-curcumene, and caryophyllene have also been extracted from the flowers of this plant (Brophy et al., 1985; Lemmens & Bunyapraphatsara, 2003).

Research and application of the bioactive compounds of this plant as mosquito repellent has been done by many (Budiman & Rahmawati, 2015; Mirawati et al., 2018; Simaremare et al., 2017; Simaremare & Lestari, 2017; Widawati & Santi, 2013). To increase its mosquito-repellent potency, the plant extract was mixed with other plant extracts such as lemon grass (*Cymbopogon citratus*) (Mirawati et al., 2018) or rosemary (*Rosmarinus officinalis*) (Widawati & Santi, 2013). The plant extract has been utilized to eradicate warehouse insect pest *Tribolium castaneum* (Cameron et al., 2016), has been tested for its toxicity to *Artemia salina* larvae (Lestari et al., 2015), has been tested as an antiretroviral agent (Larson et al., 2014) and has also been applied as an anticancer agent (Sanora et al., 2019).

However, research on the phytochemical and antibacterial analyses of the EOs of *Euodia suaveolens* is lacking. Antibacterial potency of the compounds of the EOs of the plant against pathogenic Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, as well as Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* has never been published. The ability of the bioactive compounds has never been compared to antibiotics commonly prescribed in health care practices, such as kanamycin. Therefore, this present research aims to analyze: 1) the bioactive compounds of the EOs extracted from the leaves of the plant and 2) the potencies of the bioactive compounds of the EOs against four selected pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.

MATERIALS AND METHOD

Materials

Euodia sauveolens leaf samples were collected from a plant collector in Sleman, Yogyakarta Special Province, Indonesia. The plant leaf samples were described and identified by a plant taxonomist at the Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta. The plant samples were deposited in the herbarium at the Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta. The plant leaves were selected based on good quality sample standards such as green in color, fresh condition, no insect bites, and clean (no dirt).

The GCMS-QP2010S Shimadzu equipment (Shimadzu Ltd., Japan) was utilized in the present research with the following settings: carrier gas: Helium, column type Rtx 5, column flow: 0.55

ml/min, column length: 30 m, column oven temperature: 50.0 °C, detector gain mode: Absolute, detector gain: 1.50 kV, film: 0.25 µm, flow control mode: pressure, injection temperature: 300.0 °C, injection mode: split, ion source temperature: 250.00 °C, ionizer: EI 70 Ev, pressure: 13.0 kPa, interface temperature: 300.00 °C, linear velocity: 26.8 cm/sec, purge flow: 3.0 mL/min, split ratio: 139.0, solvent cut time: 3.00 min, total flow: 79.3 mL/min, threshold: 0.

Methods

Plant sample preparation

Ten kilograms of *E. sauveolens* fresh leaves samples were rinsed with tap water thoroughly and discharged to reduce the water. The samples were distilled utilizing the steam distillation method for four hours. The samples were then put into a biomass holding chamber of the distillation apparatus. The water steam was gained by heating the water in a vessel and the steam flowed into a holding chamber where the leaf samples were put. The water steam passed across the leaves and produced vapor, which then condensed and flowed into a clean Erlenmeyer flask. The EOs layer was separated and kept in a clean flacon bottle (Božović et al., 2017; Rassem et al., 2016).

Phytochemical analysis

Several 3.5 ml of the EOs extracted were analyzed utilizing GCMS-QP2010S Shimadzu equipment. The chemical compounds of the EOs were identified by contrasting the results of the chromatogram and retention time with the reference from the mass spectra library (Wiley229. LIB) (Ghimire et al., 2017; Handayani & Nurcahyanti, 2015). While another 3.5 mL of the EOs was utilized for the antibacterial analysis.

Antibacterial analysis

The antibacterial analysis was done utilizing the diffusion agar method to determine the potency of the EOs against four selected pathogenic bacteria. Pure cultures of four selected pathogenic bacteria were inoculated at nutrient agar plates, each plate with one culture. Every 20 µl of the EOs was poured into a well in the nutrient agar plate and another well was filled with commercial antibiotic kanamycin 50 µg/ml (Kimia Farma Inc., Indonesia). All the agar plates were incubated at 37 ^{o}C for 24 hours and then the diameters of the inhibition zones found were quantified utilizing a caliper (Simaremare & Lestari, 2017; Solórzano-Santos & Miranda-Novales, 2012).

Data Analysis

The experimentations were done in quintuplicates and data obtained were analyzed utilizing analysis of variance (ANOVA) procedure statistical method at $p<0.05$.

RESULT AND DISCUSSION

Phytochemical analysis

The chemical compounds of the EOs extracted were put in sequence based on the percentage of chromatogram area as follows linalool (1.40 %), longypynenepoxyde (1.66 %), globulol (1.88 %), α-curcumene (4.65 %), evodone (5.55 %), limonene (10.99 %), p-mentha-1,8-diene (14.34 %), and menthofuran (50.38 %) (Table 1). The total chemical compounds revealed were 25, but 18 other compounds with smaller percentage area (Figure 1).

There were similarities and differences compared to previous research findings, both the kinds of and the percentage area of each chemical compound revealed. Menthofuran was the compound with the highest percentage area extracted from the EOs as reported by (Brophy et al., 1985; Chand et al., 2016; Maryuni et al., 2008). In general, menthofuran and furans are chemical substances that are vulnerable to oxidative metabolism by cytochrome P450 enzymes (Khojasteh et al., 2010). More recent research shows that menthofuran can inhibit severe acute respiratory syndrome coronavirus (SARS-CoV-2) replication in the infected cells (Sanja et al., 2022). Menthofuran is highly demanded

in the aroma industry and estimated that the demand for menthofuran is 150–200 mt/year (Kumar et al., 2014). Other EO compounds that were found in the plant were limonene, evodone, α-curcumene, and linalool (Brophy et al., 1985; Chand et al., 2016; Maryuni et al., 2008), while longypynenepoxide, globulol, and p-mentha-1,8-diene have never been reported by any researchers who were working with *E. suaveolens*.

The difference in terms of the percentage area of every EO compound was due to the difference in distillation and analysis methods utilized (Dhifi et al., 2016; Lahlou, 2004). Some researchers noticed that the difference was influenced by the origin (geographical), environmental condition (ecological), growth condition (physiological) (Chand et al., 2016; Maharaj et al., 2016), and the species or subspecies or varieties of the plant used (Lemmens & Bunyapraphatsara, 2003).

Table 1. Characteristics of the essential oils compounds extracted from the plant

Peak#	R Time	Area	Area %	Compound Name
1	13.111	119467	0.29	β-myrcene
2	14.598	5893898	14.34	p-mentha-1,8-diene
3	19.454	20706148	50.38	menthofuran
4	20.472	243132	0.59	β-pinene
5	24.951	2281197	5.55	evodone
6	25.118	387444	0.94	1,4-heptadiene, 3-methyl
7	25.823	135389	0.33	α-copaene
8	26.740	4516886	10.99	limonene
9	26.958	283879	0.69	1-p-menthen-9-al
10	27.136	257174	0.63	β-caryophyllene
11	27.358	106995	0.26	carvyl acetate
12	28.098	309811	0.75	α-humulene
13	28.550	347119	0.84	α-farnesene
14	28.668	1912747	4.65	α-curcumene
15	28.998	145463	0.35	gurjunene
16	29.303	682229	1.66	longipinenepoxide
17	31.159	176347	0.43	β-cyclocitral
18	31.300	159377	0.39	β-terpinyl butanoate
19	31.491	576708	1.40	linalool
20	31.592	109121	0.27	tetradecane, 3-phenyl
21	31.814	292934	0.71	caryophyllene epoxide
22	32.173	165927	0.40	patchulane
23	32.552	773805	1.88	globulol
24	33.907	343305	0.84	geraniol
25	47.681	173741	0.42	3,5-dodecadiene, 2-methyl
		41100243	100.00	

Antibacterial analysis

The antibacterial analysis was done utilizing the agar well diffusion method instead of the agar disk diffusion method because the present research makes use of four pathogenic bacteria with different traits, specifically aerobic and anaerobic. In the agar well diffusion method, the antibacterial substances tested were placed into a well and diffused throughout the agar media, thus it will reach both the aerobic and anaerobic part of the media. In the agar disk diffusion method, the antibacterial substances were absorbed by the paper disk and then placed on top of the media, hence the antibiotic substances were mostly diffused horizontally (Balouiri et al., 2016).

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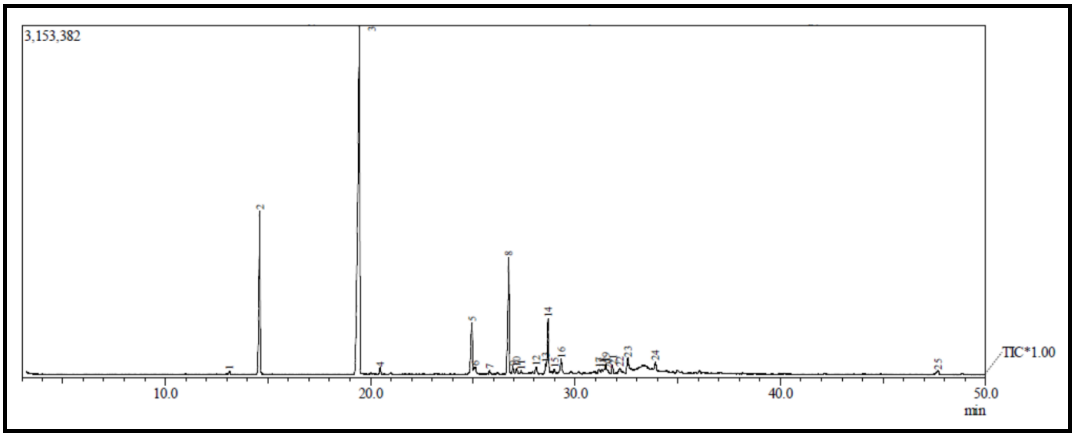

Figure 1. Chromatogram profile of the EO compounds extracted from *E. suaveolens* leaves

The EOs extracted from the leaf of the plant showed clear inhibition zones against four pathogenic bacteria tested (Table 2, Figure 2). *E. coli* showed the highest diameter of inhibition zones compared to the other three bacteria, while *P. aeruginos*a was the least. The diameter of the inhibition zones of *S. aureus* and *S. epidermidis* was not significantly different. All of the diameter of inhibition zones formed by the EOs extracted was still lower than commercial antibiotic kanamycin.

E. coli was higher in terms of diameter of inhibition zones compared to *P. aeruginosa*, though the two bacteria were grouped as Gram-negative, but differ in oxygen demand. *E. coli* is considered aerobic, while *P. aeruginosa* is an anaerobic bacterium (Andrade et al., 2014; Maharaj et al., 2016). Therefore, *E. coli* was more inhibited by the chemical compounds in the EOs which were also known as volatile compounds and tend to diffuse to the surface of the agar plate (Maharaj et al., 2016; Solórzano-Santos & Miranda-Novales, 2012). The diameter of the inhibition zones of *S. aureus* and *S. epidermidis* was not significantly different, since the two bacteria were the same genus. Bacteria with close phylogenetic relationships show higher similarities, including the ability to respond to the chemical compounds of the EOs extracted (Solórzano-Santos & Miranda-Novales, 2012). Similar findings were reported by Swamy et al., 2016 that Gram-negative bacteria were more sensitive toward the EOs of thyme (*Thymus vulgaris*).

The effect of the EOs on microorganisms may be due to the synergistic effects of the compounds which become more effective in the inhibition of a particular species of microorganisms either by causing cell lysis or death or by inhibiting the cell wall synthesis (Mahmoud et al., 2016). The plausible inhibition mechanisms showed by the EOs to the bacteria include: a) the EOs will spread on the cell wall of the bacteria, increase its permeability, and cause the loss of important compounds in the cell, b) increase the acidity inside the cell, thus ATP productions are inhibited, and c) damages of cell genetic materials which leads to the cell death (Andrade et al., 2014; Bach & Bach, 2021; Li et al., 2014; Saad et al., 2013; Turgis et al., 2009; Turina et al., 2006). One of the important characteristics of EOs is their hydrophobicities which make them easily bind to lipids in the cell membrane and mitochondria, disturb the structure, and increase membrane permeability (Chouhan et al., 2017; Lopez-Romero et al., 2015; Solórzano-Santos & Miranda-Novales, 2012).

Antibacterial activities of the EOs extracted from the plant leaf are smaller compared to kanamycin (Table 2, Figure 2). The diameter of inhibition zones of the four pathogenic bacteria tested is significantly different statistically compared to kanamycin. Kanamycin is known as a wide-spectrum antibiotic that is capable of inhibiting both Gram-negative and positive bacteria (Maharaj et al., 2016; Solórzano-Santos & Miranda-Novales, 2012). However, the potential of the chemical compounds of the EOs extracted from *E. suaveolens* as an antibacterial agent is still promising to be explored in the future.

Table 2. Diameter (cm) of inhibition zones of the EOs to the pathogenic bacteria

Sample	Pathogenic Bacteria			
	E. coli	***P. aeruginosa***	***S. aureus***	***S. epidermidis***
Essential Oils	2.03 ± 0.22^{c}	0.50 ± 0.49^{a}	1.38 ± 0.10^{b}	1.40 ± 0.27^{b}
Positive Control (kanamycin)	3.43 ± 0.08^{d}	3.25 ± 0.08^{d}	3.38 ± 0.12^{d}	3.18 ± 0.24^{d}

Note: numbers are mean ± SD from quintuplicates, numbers with the same alphabet in the row show no significant difference statistically ($p<0.05$)

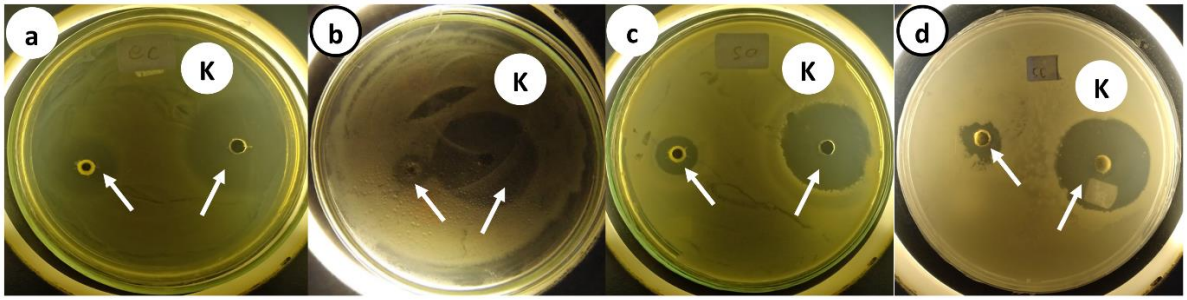

Figure 2. Inhibition zones of the EOs against a) *E. coli*, b) *P. aeruginosa*, c) *S. aureus*, and d) *S. epidermidis*. K is antibiotic kanamycin treatment. Arrows show the inhibition zones on the agar plate

CONCLUSION

The main chemical compounds revealed from the EOs extracted from the *E. suaveolens* leaves are menthofuran, p-mentha-1,8-diene, limonene, evodone, α-curcumene, globulol, longipinenepoxide, and linalool. The chemical compounds from the EOs extracted showed the ability to inhibit three pathogenic bacteria namely *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, but with low inhibition against *P. aeruginosa*.

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