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Antibacterial activity of essential oils extracted from leaf of *Euodia suaveolens* Scheff.

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Abstract
Euodia suaveolens is one of plants that has been used by ancient people in Indonesia due to its manifold activities. The essential oils of the leaf of *E. suaveolens* were extracted by steam distillation method and were analyzed utilizing GC-MS method in order to reveal the chemical constituents. The chemical components were tested to four pathogenic bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* utilizing diffusion agar method. The results showed that the main compounds extracted from the essential oils were menthofuran, p-mentha-1,8-diene, limonene, evodone, α-curcumene, globulol, longipinenepoxide, and linalool. These compounds showed antibacterial activity to inhibit the growth of four pathogenic bacteria tested, but the inhibition zones formed still lower than commercial antibiotic kanamycin.
Keywords: *E. suaveolens*, essential oil, steam distillation, antibacterial activity

Introduction
Antibiotic resistance has become a big threat to medical and food safety fields as well as nation buildings.[1] Malpractice of antibiotic usage in the third world countries such as Indonesia has weighted the problem: increase pneumonia, TB (tuberculosis), gonorrhoea, and salmonellosis infections; infectious diseases are harder to eradicate: longer stay in hospitals; health costs and death cases increases.[2,3] Indonesia which is located in the tropical area is known as megadiversity country and very popular with the usage of plants since prehistoric ancestors for daily life needs, including bacterial infectious disease cures. *Euodia suaveolens* is originally coming from West Papua and some researchers have been trying to find bioactive compounds from the plant in order to solve the crucial problem above.[4]
However, research on the antibacterial activity of the essential oils of *Euodia suaveolens* is lacking. Antibacterial potency of the compounds of the essential oils of the plant to pathogenic bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* has never been published. The ability of the bioactive compounds has never been compared to antibiotic commonly prescribed in health care practices, such as kanamycin. This present research aims to: 1) determine the bioactive compounds of the essential oils extracted from the leaf of the plant and 2) reveal the potencies of the bioactive compounds of the essential oils to pathogenic bacteria namely *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*.

Materials & Methods
Plant sample preparation
Ten kilograms of *Euodia sauveolens* fresh leaf samples (Figure 1) were rinsed with tap water, decanted, and distilled utilizing steam distillation method.[5] A number of 3.5 ml essential oils extracted was analyzed utilizing GCMS-QP2010S Shimadzu (Shimadzu Ltd., Japan)[6,7] and another 3.5 ml was utilized for antibacterial activity test.

Antibacterial activity test
Diffusion agar method was utilized in order to determine the antibacterial activity of the essential oils. Pure culture of four pathogenic bacteria was inoculated at nutrient agar plates, each plate with one culture. Every 20 µl of the essential oils was poured into a well in the nutrient agar plate and another well was filled with antibiotic kanamycin 50 µg/ml (Kimia Farma Inc., Indonesia). All of the agar plates were incubated for 24 hours at 37 °C and then the diameter of inhibition zones formed were measured.[8]

Figure 1. *Evodia suaveolens* organs (a) fruit (1), flower (2), and leaf (3). Coin diameter= 2.8 cm, (b) flower (± 0.5 cm diameter), bar= 0.5 cm, (c) young fruit (1), ripe fruit (2), bar= 0.5 cm.

Results & Discussions
The essential oils extracted from the leaf of the plant showed clear inhibition zones against four pathogenic bacteria tested (Table 1, Figure 2). *E. coli* showed the highest diameter of inhibition zones compared to the other three bacteria, while *P. aeruginosa* was the least. Diameter of inhibition zones of *S. aureus* and *S. epidermidis* did not significantly different. All of the diameter of inhibition zones formed by the essential oils extracted was still lower than antibiotic kanamycin.*E. coli* was higher in term 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 as aerobic, while *P. aeruginosa* is anaerobic bacterium.[9] Therefore, *E. coli* was more inhibited by the chemical compounds in the essential oils which were also known as volatile compounds and tend to diffuse to the surface of the agar plate.[8,9] Diameter of inhibition zones of S. aureus and S. epidermidis did not significantly different, since the two bacteria were the same genus. Bacteria with close phylogenetic relationship show higher similarities, including the ability to respond to the chemical compounds of the essential oils.[8,10]
Antibacterial activities of the essential oils extracted from the plant leaf are smaller compared to kanamycin (Table 1, Figure 2). Diameter of inhibition zones of the four pathogenic bacteria tested are significant different statistically compared to kanamycin. Kanamycin is known as wide spectrum antibiotic that is capable to inhibit both Gram negative and positive bacteria.[8,9]

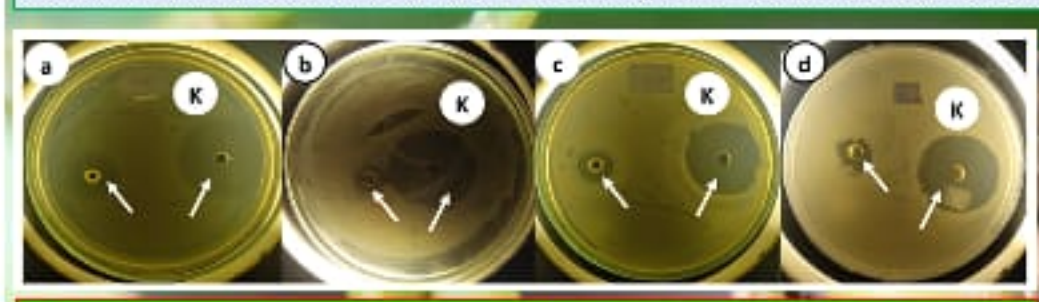

Figure 2. Inhibition zones of the essential oils against: a) *E. coli*, b) *P. aeruginosa*, c) *S. aureus*, and d) *S. epidermidis*. K is antibiotic kanamycin treatment. Arrows show the inhibition zones on the agar plate.

Table 1. Diameter (cm) of inhibition zone of the essential oils to the pathogenic bacteria tested

Sample	Pathogenic Bacteria			
	E. coli	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
Essential Oils	2.03±0.23^{b}	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}

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