ANTIBACTERIAL EFFECT OF *BOESENBERGIA PANDURATA* ESSENTIAL OILS FROM INDONESIA TOWARD *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Boesenbergiae pandurata are used as traditional medicine because it can cure stomatitis, flatulence, antibacterial and even as anticancer. Boesenbergiae pandurata are one of the medicinal plants that produce essential oils that can be used as an antibacterial. This research was an experimental study with negative control group design. The antibacterial effects of Boesenbergiae pandurata in against Escherchia coli and Staphylococcus aureus bacteria are very potent. GC-MS analysis shows that the essential oil of Boesenbergiae pandurata contains hydrophobic component mixture such as kamfen, mirsen, osimen, alpha pinen, sabinen, terpinen, fernasen, and trisiklin. In addition, it also contains hydrophilic groups such as eucalyptol, linalool, borneol, terpineol, phenyl methyl propanoate, methyl benzo propanoate, isobutyric acid, betahydroxy androsta, zerumbon, methyl hexadecanoate, methyl palmitate , hexadecanoic acid and farnesol.

Keywords: *Boesenbergia pandurata*, essential oil, *Escherchia coli* , *Staphylococcus aureus.*

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INTRODUCTION

Indonesia is a tropical country with fertile soil. There are plenty of medicinal plants in Indonesia such as ginger, galangal, finger-root and others[1]. *Boesenbergiae pandurata* essential oils contains kamfer, borneol, pinnen, seskuiterpen, zingiberon, curcumin, and zeodarin [2]. The rhizome of *Boesenbergiae pandurata* has been used as a traditional medicine to cure some of common diseases such as rheumatic disease, gastric inflammation, lender, urine laxative, malaria, mouth ulcer, diarrhea, intestinal worms, flatulence, bowel disorders, skin diseases, and tonic [2].Essential oil is a result of the plant metabolism which is volatile at room temperature. It also has a bitter taste and smells fragrance in accordance with the smell of its plant. Essential oils dissolve in organic solvents and are not soluble in water. The main components of the essential oils are camphor, trans-ocyneme, 1,8-cineol, and trans-geraniol [3].*Escherichia coli* bacteria is an intestinal bacteria included in the negative gram bacteria which found by Theodor Escherich (1885. *Escherichia coli* is a normal flora in the human intestine, commensal life in human colon and helps the formation of vitamin K which is essential for blood clotting. *Escherichia coli* can cause diarrhea if the amount in the body is excessive. *E. coli* is a member of the Enterobacteriaceae family. The cell size is 2.0-6.0 µm long and 1.1-1.5 µm wide. The initial cell shape such as coocal then forms along the filamentous size, no spores are found. These bacteria also often cause infections. The most common disease caused by *E. coli* is diarrhea[4].*E. Coli* often causes diarrhea worldwide with a variety of pathogenesis, such as EPEC (*E. Coli Enteropatogenik*), ETEC (*E. Coli Enterotoksigenik*), EHEC (E.Coli Enterohemoragik), EIEC (*E. Coli Enteroinvasif*), EAEC (*E.Coli Enteroagregatif*) [5–7]. The symptoms that often arise is a watery stool with frequency more than 4 times/day, followed by vomiting, fever and no appetite[1,8].

One of the treatment to cure diarrhea is by consuming "oralit"fluid that contains sugar and salt. But, some people drink it by adding warm tea[9,10]. Due to the abundance of finger-root on earth, it is possible for using this plant as a traditional herbal medicine. Because this plant has proved to cure stomach bloating, gastric inflammation, skin diseases, and diarrhea. In addition, it can be used as an anti inflammatory and anticancer treatment[11–15]

MATERIALS AND METHODS

Study design : This research was an experimental study with negative control group design

Plant collection : *Boesen bergia pandurata* were harvested from Karanganyar, Pacitan, Jawa Timur, Indonesia in March 2017. This research has been approved by Health Research Ethics Committee Faculty of Medicine of Universitas Muhammadiyah Surakarta with number 677/A.2/KEPK-FKUMS/IV/2017

Preparation of essential oil *Boesenbergia pandurata*: The study was divided into 5 groups. The groups can be seen in table I

Table 1

1	Negative control	:	Aquadest
2	Positive control	:	Cloramfenicol
3	Group III	:	The essential oil of *Boesenbergia pandurata* in 30% concentrations
4	Group IV	:	The essential oil of *Boesenbergia pandurata* in 50% concentrations
5	Group V	:	The essential oil of *Boesenbergia pandurata* in 75% concentrations
6	Group VI	:	The essential oil of *Boesenbergia pandurata* in 90% concentrations

Preparation of antibacterial activity test

The roots of this plant have been washed out, then dried and sterilized in an autoclave at a temperature of 121°C for 15 minutes. One ose of bacteria was taken from the culture and planted on the media. The sample was incubated for 24 hours at 37°C. Took one bacteria ose from the germ colony to each of the germ species and then each they were planted in 0.5 ml of liquid BHI medium and incubated for 5-8 hours at room temperature 37°C. Prepared 2 ml of sterile physiological Na Cl in the test tube. Then took some ose of *Staphylococcus aureus* or *Escherichia coli* bacteria from culture and inserted them into the reaction tube which contains the physiological NaCl, then compared it with the 0.5 Mc.Farland (10^8CFU / ml) suspension. The bacteria were taken with sterile lid cotton, applied to the Muller Hilton agar and flattened.

Antibacterial test

Prepared two Muller Hilton plates and then smeared *Staphylococcus aureus* that has been compared with 0.5 Mc.Farland standard on the first plate. For the second plate,smeared *Escherichia coli* that has been compared with 0.5 Mc.Farland standard. Then on the each plate, placed a disk containing essential oil finger-root (Boesenbergia pandurata) 30%, 50%, 75%, and 90%, positive control, and negative control. Arranged the distance between the wells so not too close to each other. Then, incubated on the plate at 37°C for 18-24 hours. The diameter of inhibition zone was measured by using a sliding range in millimeters (mm)

RESULTS AND DISCUSSION

The diameter of inhibition zone of antibacterial effects of essential oil (*Boesenbergia pandurata*) toward *Escherichia coli* and *Staphylococcus aureus* has been showed in table 2, and 3.

Table 2: Inhibitory growth zone of *Escherechia coli*

Escherechia coli	Diameter of inhibition zone (mm)					
	0 % (-)	30%	50%	75%	90%	Cloramphenicol (+)
1	0	10	20	25	40	25
2	0	10	15	27	40	25
3	0	15	25	30	40	25

Table 3: Inhibitory growth zone of *Staphylococcus aureus*

Staphylococcus aureus	Diameter of inhibition zone (mm)					
	0 % (-)	30%	50%	75%	90%	Amoxicilin (+)
1	0	7	13	18	25	15
2	0	10	13	20	40	15
3	0	10	14	20	24	15

Data were analyzed by paired sample T test with significance 0.05. There were significant difference of diameter of inhibition zone of *Escherechia coli* and *Staphylococcus aureus* ($P<0.05$)

DISCUSSION

Based on statistical analysis, it was showed that *Boesenbergia pandurata* essential oil with various concentrations have antibacterial effect toward *Escherechia coli* and *Staphylococcus aureus*. The inhibitory potency is significant enough compared with cloramphenicol and amoxicillin.

Figure 1: Inhibitory growth zone of *Escherechia coli* and *Staphylococcus aureus*.

The difference between inhibitory zone in *Staphylococcus aureus* (gram+) with *Escherichia coli* (gram -) is due to differences components of the cell wall of the bacterium, where *Staphylococcus aureus* (gram +) has 3 layers of cytoplasmic membrane, thick peptidoglycan layer, and loose, while *Escherichia coli* as gram (-) has a more complex and layered of cytoplasmic membranes, single peptidoglycan layer, and external membrane consisting of lipoproteins and lipopolysaccharides [4,16]. The outer membrane of *Escherichia coli*as gram (-) has an unique characteristic, the membrane is rejecting both hydrophobic and hydrophilic molecules but on the other hand it has a special channel containing a protein molecule called porin [17,18]. It facilitates passive diffusion of hydrophilic compounds with low BM such as sugars and acids amino, while the large molecule such as antibiotic molecules, include the active substance molecule essential oil extract, will have difficulty and even fail to perform penetration. It causes *Escherichia coli*as gram (-) are more resistant.[17]Research was performed by Kar *et al.,* showed that the essential oils contain seven compounds. It was camphene, 1,8-cineol, trans--ocyneme, camphor, trans-geraniol, and two unidentified peaks[19]. The main components of this essential oil are camphor, trans - - ocyneme, 1,8 - cineol, and trans - geraniol. Our research showed that the *Bosenbergia pandurata* essential oils contained hydrophobic components such as kamfen, Osimen, alpha pinnen, sabinen, terpinen, fernasen, and trisiklin. In addition, the finger-root essential oils also havehydrophilic components such as eucalyptol, linalool, borneol, terpineol, phenyl methyl propanoate, methyl benzo propanoate, isobutyric acid, betahydroxy androsta, zerumbon, methyl hexadecanoate, methyl palmitate, Hexadecanoic acid and farnesol.

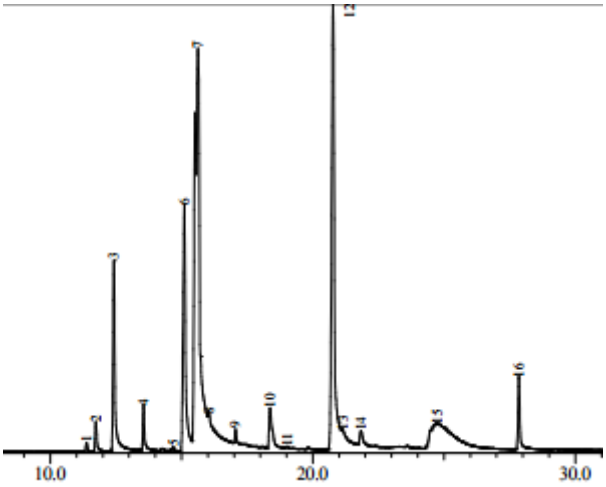

Figure 2: The results of GCMS inspection of essential oil of *Boesenbergia pandurata*

Essential oils have known to be used for treatments, because it contains antioxidants, anti-angiogenesis effect, anti-cancer effect, and inhibit growth of bacteria and molds materials. The inhibitory effect of essential oil depends on the nature and concentration of the essential oil used, as well as the type of microorganisms. The difference in inhibitory effect between these types of bacteria can be explained by the different hydrophobic properties of bacterial cell walls. Regarding the bacteria that have hydrophobic cell walls are generally more sensitive to essential oils [20].High antibacterial agent of finger-root essential oil gives the most contribution for all components in it. The hydrophobic components can be interacted with hydrophobic cell wall components, caused inflammation of the cell wall (swelling) [21,22]. Therefore, it facilitates the entry of components which initially are difficult to penetrate cell, such as components that have hydrophilic or polar groups. Components with hydrophilic groups would affect to enzymatic reactions in the cytoplasmic membranes, such as electron transport in the respiratory system, proton transport, and nutrient transport into cells that would inhibit ATP regeneration and inhibited cell growth leading to cell death [21,23–25].The phenomenon of component leakage from cell cytoplasm, can also be associated with the essential oils due to the cooperation of hydrophobic components on *Bosenbergia pandurata* essential oils and hydrophilic compound on the essential oil. The hydrophobic components can change the permeability of cell membranes, after that the hydrophilic compounds enter to cytoplasm, interact with ions, nucleic acid materials, and proteins and enzymes ofthe bacterial cells.By changing cell permeability, the material cell contents that have been disturbed can be easily escape from the cell, leading to cell leakage [18].This research was supported by previous research that shows fingerroot of *Boesenbergia pandurata* has several benefits such as a potential antioxidant due to the presence of pinostrobin, pinocembrin, essential oils, and other flavonoid compound and derivatives [2,26], *Boesenbergia pandurata* contains chalcone and potentially act as anti- inflammatory agent [11,15], *Boesenbergia pandurata* also has antibacterial activity towards *Streptococcus mutans, Lactobacillus sp,*

Candida albicans, Streptococcus sanguis and *Actinomyces viscosus* [27–29].

CONCLUSION

Based on the result of this research, it can be concluded that essential oils of *Boesenbergia pandurata* have very potential anti-bacterial effect in inhibiting growth of *Escherichia coli*and *Staphylococcus aureus* bacteria in vitro.

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