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Antibacterial activity of leaves, flowers, and fruits extract of *Etlingera elatior* from Nagan Raya District, Indonesia against *Escherichia coli* and *Staphylococcus aureus*

ERNILASARI[1], KAMALLIANSYAH WALIL[1], FITMAWATI[2], DEWI INDRIYANI ROSLIM[2], ZUMAIDAR[3], SAUDAH[1,♥], RAYHANNISA[4]

[1]Faculty of Teacher Training and Education, Universitas Serambi Mekkah. Jl. Unmuha, Batoh, Banda Aceh 23245, Aceh, Indonesia. Tel./fax.: +62-651-26160, ♥email: saudah@serambimekkah.ac.id, danayulisma@gmail.com

[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau. Kampus Bina Widya, Jl. H.R. Subrantas Km. 12,5, Simpang Baru, Tampan, Pekanbaru 28293, Riau, Indonesia

[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. Jl. Tgk. Syech Abdul Rauf No. 3, Darussalam, Banda Aceh 23111, Aceh, Indonesia

[4]Mathematics and Applied Science Graduate Program, Universitas Syiah Kuala. Jl. Tgk. Syech Abdul Rauf No. 3, Darussalam, Banda Aceh 23111, Aceh, Indonesia

[5]Biology Graduate Program, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. Jl. Tgk Chik Pante Kulu No. 5, Kopelma Darussalam, Syiah Kuala, Banda Aceh 23111, Aceh, Indonesia

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Abstract. *Ernilasari, Walil K, Fitmawati, Roslim DI, Zumaidar, Saudah, Rayhannisa. 2021. Antibacterial activity of leaves, flowers, and fruits extract of* Etlingera elatior *from Nagan Raya District, Indonesia against* Escherichia coli *and* Staphylococcus aureus. *Biodiversitas 22: 4457-4464. Etlingera elatior* is a medicinal plant that has been used by people in Indonesia, especially Acehnese people. Based on its secondary metabolites, *E. elatior* can be used as antibacterial agents against Gram-positive and Gram-negative. However, to determine the antibacterial activity of *E. elatior*, the parts of *E. elatior* that have been used are leaves, flowers, and fruits. This study was aimed to determine the best concentration of ethanol extract of leaves, flowers, and fruits of *E. elatior* as an inhibitor against *Escherichia coli* and *Staphylococcus aureus*. The extract was made by the maceration method with 70% ethanol as a solvent. Antibacterial activity test was carried out by the diffusion agar method using concentrations of 0.5%, 1%, 1.5% and 2%. The results showed that the fruit extract of *E. elatior* has antibacterial activity with an effective inhibitory zone at a concentration of 2% is 8.4 mm (*E. coli*) and 2.4 mm (*S. aureus*). Meanwhile, antibacterial activity of the extract of leaves and flowers against *E. elatior* cannot determine yet. Identification of leaves, flowers and fruits extract of *E. elatior* using GC-MS (gas chromatography-mass spectroscopy) showed 56 compounds were detected.

Keywords: Antibacterial test, *Escherichia coli*, *Etlingera elatior*, *Staphylococcus aureus*, Suak Bugis

INTRODUCTION

Indonesia is one of the countries which is rich in biodiversity, including medicinal plants that have been used in healthcare, protect health and disease prevention since time immemorial (Schroeder 2011; Sofowora et al. 2013). Indonesia is the second-highest number of indigenous medicinal plants, after the Amazon rain forest (Elfahmi et al. 2014). Hence, there is a high potential for pharmaceutical and biotechnological research opportunities. Recently, using traditional medicine as an alternative treatment has increased significantly (World Health Organization 2004). Traditional medicines have no side effects, are more affordable than most conventional medicines, as well as availability and compatibility as plant-derived drugs (Fatima and Nayeem 2016; Juwita et al. 2018). *Etlingera elatior* (Jack) R. M. Sm.) is one type of plant in Indonesia that is widely used as traditional medicine by local people (Silalahi 2016).

E. elatior Jack. RM is a species of plant belongs to Zingiberaceae. *E. elatior* which has various local names, i.e., *Kecombrang* in Java, *Kincung* in North Sumatera, *Honje* in West Java, *Sikala* in Southeast Sulawesi, *Bongkot* in Bali, etc (Poulsen 2012; Sabilu et al. 2017; Oktavia et al. 2019). Meanwhile, in Aceh, these species are known as *Bak Kala* and *Bungong Kala*. *E. elatior* is a medicinal plant that has been used by people in Indonesia, especially Acehnese people. Empirically, *E. elatior* is used as traditional medicine by the Gayo tribe, Central Aceh District for the treatment of various diseases such as coughs, bloating, fatigue and fatigue syndrome, abdominal pain, as well as postpartum medicine (Saudah et al. 2021).

According to (Jackie et al. 2011; Maimulyanti et al. 2015), *E. elatior* contains secondary metabolites such as flavonoids, saponins, phenols, terpenoids, tannins, glycosides, steroids, which have bioactivities. This bioactive compound has been reported to exhibit antimicrobial, antidiabetic, anticancer, antioxidant, antiaging, as well as anti-inflammatory (Habsah et al. 2005; Lachumy et al. 2010; Zan et al. 2011; Srey et al. 2014; Ghasemzadeh et al. 2015; Nor Asiah et al. 2019). Previous studies reported that *E. elatior* leaves from aqueous extract can inhibit the

growth of *Escherichia coli* and *Staphylococcus aureus* (Ningtyas 2010). Inline to (Sukandar et al. 2011), the aqueous extract of *E. elatior* leaves has antibacterial activity against *E. coli* (10 mm/100%) and *S. aureus* (8.663 mm/20%). In addition, Sukandar (2010) also revealed that the aqueous extract of *E. elatior* flower has antibacterial activity against *E. coli* (4.8 mm/60%) and *S. aureus* (6.87 mm/20%).

Phenolic and flavonoids had been reported to have antimicrobial activity through the mechanism of action of bacterial cell-wall destruction (Manoi 2009; Mahboubi et al. 2016). The mechanism of action of saponin as an antibacterial is it can cause leakage of proteins and enzymes of the cell (Madduluri et al. 2013). Saponins including glycosides and aglycone portions, while aglycone portion is steroid and triterpenoid (Manoi 2009). Saponins are divided into three major groups as a triterpenoid, steroid or steroidal glycoalkaloid (Mert-Türk 2005). Another secondary metabolite is tannin, where tannin has ability to pass through the bacterial cell wall up to the internal membrane and inhibit the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot be formed (Dabbaghi et al. 2009).

Escherichia coli is a gram-negative bacteria or also known as *E. coli*. *E. coli* is commonly found in the lower intestine of humans and animals and causes acute diarrheal diseases in all age groups (Bettelheim 2000). To detect the presence of bacteria in surface water, *E. coli* is commonly used as indicator of fecal contamination. Meanwhile, *Staphylococcus aureus* is gram-positive aerobic organism that causes skin infections, necrosis, abscess formation, sometimes pneumonia, osteomyelitis and endocarditis (Jawetz 2005; Bush and Vazquez-Pertejo 2021). *S. aureus* is resistant to several antibiotics, including the lactamase, methicillin, nafcillin, oxacillin and vancomycin groups (Jawetz et al. 2008). Resistance is a global problem that often arises in the treatment of infectious diseases. The increase in bacterial resistance to antibiotics provides a great opportunity to obtain antibacterial compounds by utilizing bioactive compounds from the diversity of plants in Indonesia (Nuria et al. 2009).

Based on the description of *E. elatior* as medicinal plant and its potential as antibacterial agent against *E. coli* and *S. aureus*. Therefore, the aim of this study is to determine the best concentration of ethanol extracts of leaves, flowers and fruits of *E. elatior* to inhibit the growth of *E. coli* and *S. aureus* together with identification of its chemical profiles by GC-MS analysis.

MATERIALS AND METHODS

Study area

The research was conducted in Suak Bugis, Nagan Raya District, Aceh, Indonesia (Figure 1). The experiment was conducted at the Regional Health Laboratory, Jakarta and Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia, from October to December 2020.

Procedures

Sample collection

The research was used purposive sampling method. Samples of *E. elatior* were collected from seven different areas of Suak Bugis, Nagan Raya District, Aceh including leaves, flowers and fruits. The leaves, flowers and fruits were taken randomly from each stem. The sample was identified by Saida Rasnovi from Herbarium of the Department of Biology, Syiah Kuala University as *E. elatior*. The coordinate of each location is presented in Table 1.

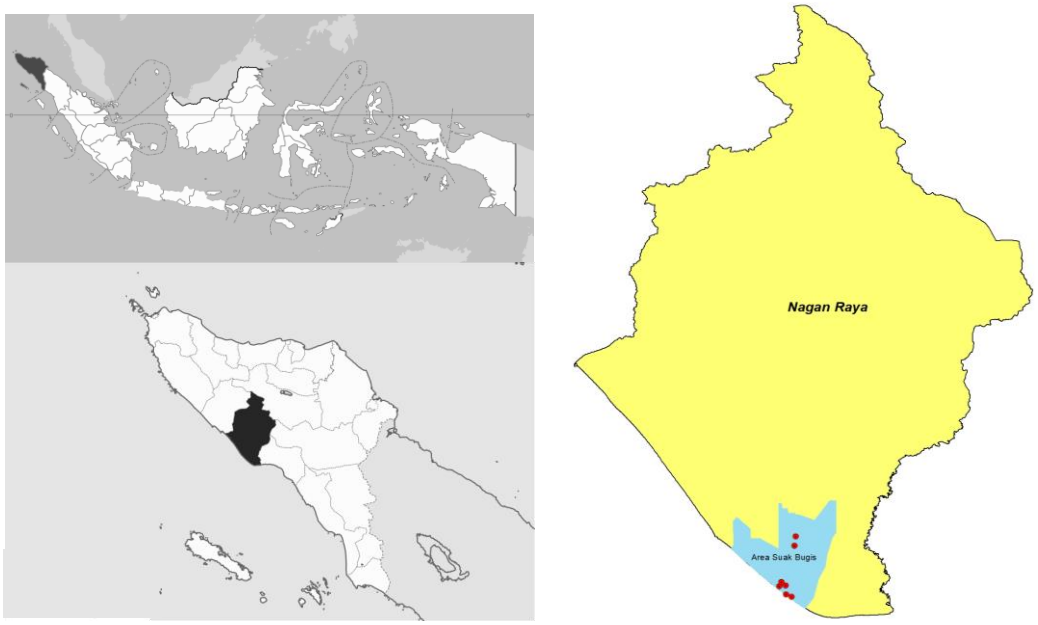

Figure 1. The collection sites of *Etlingera elatior* in Suak Bugis, Nagan Raya District, Aceh, Indonesia

Table 1. The coordinates of the *Etlingera elatior* sampling area

Species	Sample type	Sampling area	Sampling year	The coordinates	Information
E. elatior	Leaves, flowers, fruit	Suak Bugis	2020	96.49311"; 3.850495"	Point 1 (T1)
E. elatior	Leaves, flowers, fruit	Suak Bugis	2020	96.491415"; 3.836671"	Point 2 (T2)
E. elatior	Leaves, flowers, fruit	Suak Bugis	2020	96.473506"; 3.7832"	Point 3 (T3)
E. elatior	Leaves, flowers, fruit	Suak Bugis	2020	96.479313"; 3.777966"	Point 4 (T4)
E. elatior	Leaves, flowers, fruit	Suak Bugis	2020	96.470503"; 3.776872"	Point 5 (T5)
E. elatior	Leaves, flowers, fruit	Suak Bugis	2020	96.47989"; 3.764773"	Point 6 (T6)
E. elatior	Leaves, flowers, fruit	Suak Bugis	2020	96.487234"; 3.761149"	Point 7 (T7)

Sample preparation

Leaves, flowers, and fruit were harvested and washed by distilled water then cut into small pieces. Then air-dried samples in the open space. After drying process, samples were stored at 20°C and pulverized into powders.

Extraction

The extract was obtained by the maceration method with 70% ethanol as a solvent. The extraction process begins with making simplicial from leaves, flowers, and fruits that have been pulverized. The simplicial powder of about 100 grams was inserted into container, then add solvent until all ingredients were submerged and left for 24 hours. While stirring every 1 x 24 hours, after 24 hours the mixture was filtered using Whatman filter paper in a filter funnel. The filtrate was stored and the residue was soaked in a new solvent for 24 hours and repeated until the solvent was colorless. The filtrate obtained was then separated by a vacuum rotary evaporator at 50°C to separate the solvent from the thick extract filtrate of leaves, fruits, and flowers. The filtrate was stored in bottles at 20°C for further analysis.

Phytochemical screening

Phytochemical tests were performed on leaves, flowers and fruit extract of *E. elatior* to determine the content of flavonoids, saponins, tannins, alkaloids, triterpenoids, and steroids qualitatively.

Alkaloid testing

A total of 3 mL of each sample extract was added by the reagents of Mayer, Dragendorff, and Wagner. The positive result of Mayer reagent was confirmed by white precipitate, red precipitate (Dragendorff) and brown precipitate (Wagner).

Phenolic testing

A total of 3mL of extract was put into a test tube, then one drop of 1% $FeCl_3$ solution was added to 3 mL of the extract solution. A positive result of phenolic was indicated by the formation of a yellow, orange or red color.

Flavonoid testing

A total of 3 mL of sample extract was put into a test tube, then added 0.1 gram of magnesium powder, 1 mL of concentrated HCl and 1 mL of amyl alcohol. The mixture was shaken vigorously and allowed the layers to separate. The presence of flavonoids is indicated by the formation of red in the amyl alcohol layer.

Saponin testing

A total of 3 mL of sample extract was put into a test tube, then shaken vigorously and left for 10 minutes. The presence of saponin is indicated by the formation of stable foam.

Tanin testing

A total of 3 mL of sample extract was put into a test tube, then add 3 drops of 1% $FeCl_3$. The formation of blue or greenish-black color indicates the presence of tannins compounds.

Steroids and triterpenoids testing

A total of 3 mL of sample extract was dissolved in hot ethanol, heated for 5 minutes, then the solution was filtered. The filtrate result was dried by heating, then 1 mL of diethyl ether was added. A total of 3 drops of the ether fraction were transferred to a drip plate, then add 3 drops of acetic anhydride and 1 drop of concentrated sulfuric acid. Formation of reddish or violet color indicates the presence of triterpenoids, whereas the formation of green or blue color indicates the presence of steroids.

Gas Chromatography-Mass Spectroscopy (GC-MS) test

The analytical method using Gas Chromatography-Mass Spectroscopy (GC-MS) can measure the type and content of compounds in a sample both qualitatively and quantitatively. Leaves, flowers and fruits extract of *E. elatior* were analyzed using GC-MS Agilent 19091S-436 HP-5MS. 1 µL of the extract was injected into GC-MS which was operated using a glass column length of 25 m, diameter: 0.25 mm and thickness of 0.25 µm with the CP-Sil stationary phase. 5CB with an oven temperature between 70°C up to 325°C with an increased rate of 10°C/ min, a carrier gas of helium with a pressure of 16.30 kPa, a total rate of 40 mL/min and a split ratio of 1:50.

Antibacterial activity test by disc diffusion method

Antibacterial activity test was carried out by the disc diffusion method using paper discs (about 6 mm in diameter) for *E. coli* and *S. aureus* bacteria. The antibacterial testing was carried out in three repetitions. The paper discs were immersed in samples with concentrations of 0.5%, 1%, 1.5% and 2%, then placed on culture medium (Mueller Hinton Agar) that has been inoculated with a suspension of the pathogen. Incubated at 37ºC for 2x24 hours.

Observations were made by measuring the inhibition zone diameter of inhibition zone based on the paper discs (Banjara et al. 2012; Chusri et al. 2011). The negative control used in this study is DMSO 10% (v/v).

Data analysis

The presence of bioactive compound was analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) and antibacterial activity test was analyzed by quantitative method. The measurement of inhibition zone was determined by a caliper in each concentration.

RESULTS AND DISCUSSION

Identification of active compounds

Phytochemical test is a qualitative test of the content of the groups of secondary metabolites in a sample. Analysis of the chemical content of the leaves, flowers, and fruits of *E. elatior* was conducted at the Regional Health Laboratory, Jakarta by observing the presence or absence of precipitation reactions and color changes in the test tube. The analysis of phytochemical screening is presented in Table 2.

Antibacterial activity test against *E. coli*

The results of the antibacterial activity test of the ethanol extracts of *E. elatior* fruit on the growth of *E. coli* are presented in Table 3. Inhibition zones around disc paper were indicated antibacterial activity. The observation of the inhibition zone was carried out by diffusion agar method. The results of the antibacterial activity test on the three test extracts with concentrations of 0.5%, 1%, 1.5%, and 2% on *E. coli* were shown in Figure 2.

Antibacterial activity test against *S. aureus*

The results of the antibacterial activity test of the ethanol extracts of *E. elatior* fruit on the growth of *S. aureus* were presented in Table 4. The results of the antibacterial activity test on the three test extracts with concentrations of 0.5%, 1%, 1.5% and 2% on *S. aureus* can be seen in Figure 3.

GC-MS analysis

The results of the GC-MS analysis of *E. elatior* leaf, flower and fruit extracts are presented in Table 5, Table 6 and Table 7.

Discussion

The results of phytochemical analysis showed that the leaves extract of *E. elatior* contained alkaloids, phenolic, flavonoids, saponins, tannins and steroids contents. The flowers extract of *E. elatior* contains alkaloids, phenolic, flavonoids, saponins, tannins and terpenoids. While the fruit extract of *E. elatior* contains alkaloids, phenolic, flavonoids, tannins and terpenoids (Table 2). These results following with (Effendi et al. 2019), where the results of phytochemical testing showed that the leaves powder of *E. elatior* contains alkaloid, flavonoid, saponin, steroid, tannin and phenolic. *Kecombrang* flower (*E. elatior*) contains flavonoid, saponin, tannin and terpenoid (Farida and Maruzy 2016). Meanwhile, the phytochemical compounds contained in the *Kecombrang* fruit (*E. elatior*), i.e., alkaloids, polyphenols, flavonoids, saponins and essential oils (Handayani 2010). Ahmad et al. (2015) also revealed that the total phenolic content for *E. elatior* fruit was 2.29 mg GAE/g extract, while the total flavonoid content for *E. elatior* fruit was 1.78 mg QE/g extract.

The presence of alkaloids, phenolic, flavonoids, saponins, tannins and terpenoids in this study indicated that extract of leaves, flowers and fruit of *E. elatior* has the potential as an antibacterial and antimicrobial. In line with Abdallah (2011) and Sadeek and Abdallah (2019), the bioactive compounds in *E. elatior* are known as potential sources for antibacterial agents. According to Juwita et al. (2018), the high potential antimicrobial activity possessed by *E. elatior* is due to the presence of phenolic, terpenoid and flavonoid contents.

Table 3. The results of antibacterial activity tests of the ethanol extracts of *Etlingera elatior* fruit against *Escherichia coli*

Concentrations (%)	Average (mm)	Diameter zone of inhibition to nearest mm
0.5	0	Weak
1	2.5	Weak
1.5	7.8	Intermediate
2	8.4	Intermediate

Table 4. The results of antibacterial activity tests of the ethanol extracts of *Etlingera elatior* fruit against *Staphylococcus aureus*

Concentrations (%)	Average (mm)	Diameter zone of inhibition to nearest mm
0.5	0	Weak
1	0.7	Weak
1.5	1.9	Weak
2	2.4	Weak

Table 2. Qualitative phytochemical analysis *Etlingera elatior*

Phytochemical screening (extract)	Alkaloids			Phenolic	Flavonoids	Saponins	Tannins	Steroids	Terpenoids
	Mayer	Dragendorff	Wagner						
E. elatior fruits	+	+	+	+	+	-	+	-	+
E. elatior leaves	+	+	+	+	+	+	+	+	-
E. elatior flowers	+	+	+	+	+	+	+	-	+

Table 5. The results of GC-MS analysis of leaves extract of *Etlingera elatior*

Peak	Retention time	Quality	Chemical components	Content (%)
1	26.045	94	Neophytadiene	2,35
2	27.258	47	Hexadecanoic Acid, Methyl Ester	1,48
3	27.803	42	Ethyl 4-methyl octanoate	5,81
4	28.307	99	n-Hexadecanoic acid	3,73
5	28.541	89	n-Hexadecanoic acid	1,68
6	28.706	70	phytol	4,05
7	29.334	89	2-Aminoethanethiol Hydrogen Sulfate (Ester)	3,71
8	29.513	49	Myrrhine	2,11
9	29.706	702	Phenol, 2-(1-methyl-2-butenyl)-4-methoxy-	1,86
10	29.989	46	Phenol, 2-(1-methyl-2-butenyl)-4-methoxy-	3,04
11	30.934	53	Undeca-3,4-diene-2,10-dione,5,6,6-trimethyl-	7,02
12	31.134	58	(+)-(9.Beta.H)-Labda-8 (17), 13 €-Diene-5-OL	2,90
13	31.458	90	Z,E-3,13-Octadecadien-1-olacetate	2,77
14	31.733	99	Squalene	3,92
15	32.002	58	1-Benzyl-4,6-Dimethoxy-2,3-Diphenylindole	1,61
16	32.120	48	Cholesta-4,6-Dien-3-OL, 6-Fluoro-(3.Beta)-	1,93
17	32.451	46	1,3-Dithiolen, 2-(28-Norurs-12-EN-17-YL)-	1,16

Table 6. The results of GC-MS analysis of flower extract of *Etlingera elatior*

Peak	Retention time	Quality	Chemical components	Content (%)
1	6.773	38	Erythritol	7,66
2	10.875	93	2-Methoxy-4-Vinylphenol	1,14
3	15.302	95	1-Decene	4,68
4	19.232	81	Tridecyl acetate	3,09
5	27.183	96	Hexadecanoic Acid, Methyl Ester	4,76
6	27.989	38	8-Thiabicyclo [3,2,1]octan-3-ol-6-methoxy-, (3-endo, 6-exo)-	9,39
7	28.238	99	n-Hexadecanoic acid	5,15
8	28.479	74	Cis-13-Octadecenoic acid, emthyl ester	9,30
9	29.217	99	9,12-Octadecadienoic acid	5,79
10	29.520	70	1,3;2,5-Dimethlene-4-methyl-d-rhamnitol	3,31
11	29.872	92	2-Dodecen-1-yl(-)succinic anhydride	3,47
12	30.292	60	2,15-Hexadecanedione	2,28
13	30.672	97	1-Docosane	3,87
14	30.982	44	10H-Phenothiazine	3,54
15	31.444	78	14-.Beta.-H-Pregna	6,99
16	31.865	78	Methanone, (2-Aminophenyl) (2-Methoxyphenyl)-	6,49
17	32.265	56	Hexadecanoic acid, 2-hydroxy-, methyl ester	1,23
18	32.499	96	1-Docosane	4,57
19	33.016	64	Anthranilic Acid, N-Methyl-N-Phenyl-	1,65
20	33.568	47	1,3-Dioxolone, 2,2,4-trimethyl-5-tridecyl-	2,40
21	33.795	97	(9Z)-9,17-Octadecadienal	2,53
22			3 (5)-(2hydrophenyl)-5(3)-Phenylpyrazole	1,55
23	49.013	56	Tricosane	1,70

Table 7. The results of GC-MS analysis of fruit extract of *Etlingera elatior*

Peak	Retention time	Quality	Chemical components	Content (%)
1	5.538	27	1-Methyl-4-Methylenecyclohexane	1,42
2	6.428	72	6-Azabicyclo [3.2.1] octane	3,98
3	7.407	38	3-Ethoxy.Gamma.Butyrolactone	6,14
4	11.392	37	2-N-Propoxyamphetamine	1,11
5	12.785	27	1-Cyclopentene-1-1methanol, .Alpha.-Ethyl-	3,77
6	25.762	30	(Tetrahydroxy Cyclopentadienone) Trcarbonyliron (0)	2,51
7	27.045	50	(3Z)-3-Ethyl-2-Methyl-1,3-Hexadiene	7,45
8	27.189	97	Hexadecanoic Acid, Methyl Ester	5,09
9	27.762	64	Ethyl 3-Cyclohexylpropanoate	5,01
10	28.279	95	n-Hexadecanoic acid	10,01
11	28.493	99	9-Octadecanoic acid (Z)-, methylester	8,21
12	28.865	55	2-Aminoethanethiol Hydrogen Sulfate (Ester)	5,24
13	29.244	98	Oleic Acid	16,31
14	30.706	59	Cis-11-Hexadecal	4,86
15	30.934	91	Octadecenoate	2,67
16	31.451	95	14-.Beta. –H-Pregna	5,09

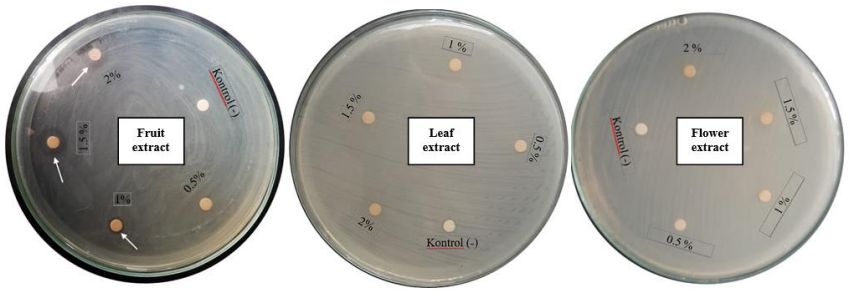

Figure 2. The diameter of the inhibition zone of *Escherichia coli* from the fruit, leaf and flower extract of *Etlingera elatior* with concentrations of 0.5%, 1%, 1.5% and 2%.

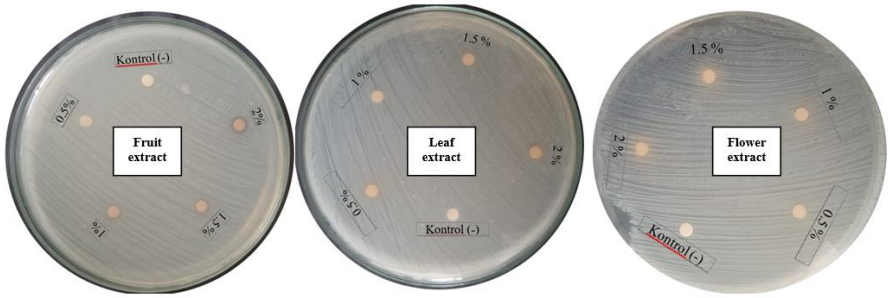

Figure 3. The diameter of the inhibition zone of *Staphylococcus aureus* from the fruit, leaf and flower extract of *Etlingera elatior* with concentrations of 0.5%, 1%, 1.5% and 2%.

In this study, *E. elatior* was obtained at various of latitudes (Table 1). Where the latitude can affect the production of secondary metabolites in plants. These results following with Demasi et al. (2018), which stated that the distance of sea (latitude) had an influence on both morphological and phytochemical traits. The contents of active substances in a plant were influenced by their environment (Liu et al. 2016). The presence or absence of certain secondary metabolites in medicinal plants are influenced by a variety of factors such as light, climate, temperature, groundwater, soil fertility and salinity (Giweli et al. 2013). The concentrations of phenolic compounds in plant were influenced by temperature, soil composition, ultraviolet radiation and rainfall (Kouki and Manetas 2002; Monteiro et al. 2006). The tannin's content can be influenced by environmental changes and development of the plant (Hatano et al. 1986; Salminen et al. 2001).

The data in Table 3 showed that the fruit extract of *E. elatior* has antibacterial properties to inhibit the growth of *E. coli* with an average zone of inhibition size of 8.4 mm/2%, 7.8 mm/1.5% and 2.5 mm/1%. The fruit extracts of *E. elatior* are seen by the diameter of inhibition formed around the paper discs (Figure 3). However, the best extracts of *E. elatior* fruit to inhibit the growth of *E. coli* was at a concentration of 2% (8.4 mm). Those values translated to categories of intermediate. A higher concentration of ethanol extract, which may will produce a larger zone of inhibition. According to Morales et al. (2003), the activity of the antimicrobial inhibition zone was grouped into four categories, i.e., weak activity (<5 mm), intermediate (5-10 mm), strong (>10-20 mm) and very strong (>20-30 mm). Previous study revealed that the *Kecombrang* fruit peel formula has antimicrobial activity to inhibit the growth of *E. coli* bacteria with ranges from 24,103-26,877 mm (Naufalin 2013).

The data in Table 4 showed that the fruit extract of *E. elatior* also has antibacterial properties to inhibit the growth of *S. aureus* with an average zone of inhibition size of 2.4 mm/2%, 1.9 mm/1.5% and 0.7 mm/1%. However, those values translated to categories of weak. In line with Pan et al. (2009), the inhibition zone of 0-3 mm includes the weak category, 3-6 mm good, and ≥6 mm strong. Simangunsong (2019) reported that the fruit extract of *E. elatior* has antibacterial activity to inhibit the growth of *S. aureus* at a concentration of 500 mg/mL with the diameter of clear zone of 22.06 mm. Meanwhile, the negative control used in this study is DMSO 10% (v/v). DMSO is a surfactant that can dissolve polar and nonpolar materials. It also showed no antibacterial activity in Figure 3 and Figure 4 (Brito et al. 2017).

The difference in diameter of inhibition zones at each concentration possibly was due to differences in the magnitude of active substances contained in the extract. In addition, the diameter of the inhibition zone was also influenced by the level of sensitivity of the bacteria. *E. coli* had higher antibacterial activity than *S. aureus*. This is related to the differences in the constituent components of the cell structure. Gram-positive bacteria contain 90% peptidoglycan and a thin layer of negatively charged teichoic acid and teichuronic acid. In Gram-negative bacteria, there is an outer layer of the cell wall that contains 5-20% peptidoglycan. This layer is the second lipid layer

called the lipopolysaccharide layer. This layer is composed of phospholipids, polysaccharides, and proteins (Madigan et al. 2000).

The result of GC-MS analysis of leaves extract of *E. elatior* in Table 5, it showed that the main components of the extract are undeca-3,4-diene-2,10-dione,5,6,6-trimethyl-with content of 7,02% and ethyl 4-methyl octanoate with content of 5,81%. The main components of the extract of *E. elatior* flower are 8-Thiabicyclo [3,2,1]octan-3-ol-6-methoxy-, (3-endo, 6-exo)- with content of 9,39% and cis-13-Octadecenoic acid, methyl ester with content of 9,30% (Table 6). In addition, the main components of the extract of *E. elatior* fruit are oleic acid with content of 16,31% and n-Hexadecanoic acid with content of 10,01%. GCMS is a method of separating organic compounds using gas chromatography (GC) and mass spectrometry (MS). The principle of separation is based on differences in the volatile ability of the compound and based on interactions with the stationary phase (capillaries).

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