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The potential of young and old *Euphorbia hirta* leaves extract as antibacterial against *Escherichia coli* and antihelminthic against *Ascaridia galli* obtained in Sentul chickens

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Abstract. *Puspitasari M, Abun, Rochana A, Widjastuti T. 2022. The potential of young and old* Euphorbia hirta *leaves extract as antibacterial against* Escherichia coli *and antihelminthic against* Ascaridia galli *obtained in Sentul chickens. Biodiversitas 23: 3243-3250.* The declining production of indigenous Sentul chickens is due to pathological microbes in the digestive tract. *Euphorbia hirta* or *patikan kebo* is a weed exhibiting active compounds that are expected to remove *Escherichia coli* and *Ascaridia galli* from the digestive tracts of chicken. This study aimed to investigate the antibacterial activity of *E. coli* and anthelmintic *A. galli* from the ethanol extract of *E. hirta*. The anthelmintic activity was analyzed in a completely randomized design with four doses (2.5, 5.0, 7.5, and 10 mg/mL) and five replicates. Mortality time series data were subjected to analysis of variance followed by the Duncan test. While a probit analysis determined the Lethal Concentration (LC_{50}) and Lethal Time (LT_{50}), experiments on antibacterial activity were performed by the microdilution method. The result showed a powerful anthelmintic activity in ethanol extracted from both young and old leaves. However, LC_{50} and LT_{50} were higher in young leaves than the old ones. While antibacterial activity against *E. coli* was apparent from ethanol extract in old and young leaves. Old leaves have a Minimum Inhibition Concentration (MIC) of 3.125% lower than young leaves (12.5%).

Keywords: Antibacterial, antihelmintic, *Euphorbia hirta*, Sentul chicken

INTRODUCTION

Sentul chicken is one of Indonesia's local chicken strains that has been cultivated for generations as a wealth of local genetic resources. Sentul chicken has good potential to be developed as a source of animal-based protein that supports national food security. Currently, Sentul chicken farming is carried out traditionally by small and medium-scale farmers. Traditional livestock farming significantly can reduce production costs and it also allows livestock to live and behave naturally which can minimize stress levels (Harahap et al. 2017; Irmaya et al. 2019). On the other hand, many factors can cause a non-significant population growth and the low productivity of Sentul chickens in traditional farming, including an unstable climate, insufficient feed and water availability, and exposure to pathogens and parasites that can occur in an uncontrolled environment.

The common pathogens that have been isolated from traditional chicken farms that caused disease are several pathogenic bacteria including *Escherichia coli* (Ayala et al. 2020). Avian pathogenic *E. coli* (APEC) is a subset of *E. coli* found on several poultry that can cause colibacillosis. Avian colibacillosis is major infectious disease in poultry. High mortality rate and decreased productivity are several impacts of this disease on all bird ages. Usually, this disease occurs simultaneously with other diseases as primary or secondary pathogenic infections (Panth 2019). APEC genetically similar to extra intestinal pathogenic *E. coli* (Ex-PEC) isolated from humans that cause extraintestinal infection, some urinary tract infection, bloodstream infection, etc. The similarity of both *E. coli* includes antimicrobial resistance patterns, virulence and antimicrobial resistance genes, and sequence type (Borges et al. 2019). The similarity between APEC and Ex-PEC suggest a possibility APEC is a potential foodborne zoonotic pathogen that can transmit from raw or processed poultry meat and egg consumed by human (Kathayat et al. 2021). In addition to the pathogenic bacteria, there are also parasites that can be found in poultry. *Ascaridia galli* is the most prevalent parasite in poultry gastrointestinal particularly in free range of traditional poultry farming systems. The infection of *A. galli* cause several clinical signs, including loss of appetite, anorexia, body weight decrease, retarded muscular and osteological development, depression and ends with increased mortality (Sharma et al. 2019). The transmission of *A. galli* is mainly by direct ingestion of eggs or larvae which are contained on the floor and ground, or mechanical vector such as earthworms which may concentrate and protect *A. galli* eggs. Intestinal hemorrhage and obstruction are the onset of *A. galli* infection. Duodenum and jejunum segment were the most severely affected. The symptoms of *A. galli* infection are low body score and fetid diarrhea (Torres et al. 2019).

In order to overcome the risk of infection by *E. coli* and *A. galli* due to the traditional Sentul Chicken farming, it is necessary to provide materials that have antihelminthic and antibacterial effects at the same time so that their handling can be efficient. Natural ingredients are the common options for farmers because they are safe for livestock and without negative effects on consumers. According to Al-snafi and Medicine (2016) herbs-based medication has gained popularity considering the level of security this method offer. The types of plants that are assumed to play a role in addressing issues in the digestive tracts include *E. hirta* or *patikan kebo*, a weed plant containing bioactive compounds of antibacterial and anthelmintic that have so far received little attention.

Euphorbia hirta is a plant with medicinal values in its chemical compounds. Studies by Al-snafi and Medicine (2017), and Perumal et al. (2012) reported that phytochemical screening of *E. hirta* reveals that the weed contains a variety of compounds that include reducing sugar, terpenoid, alkaloids, steroid, tannin, protein, fat, oil, gum, mucus, glycosides, saponin, coumarin, cardiac glycosides, anthraquinone, flavanoid and phenolic. Accordingly, this plant is the potential candidate for several medications, including for digestive tracts in chickens. Studies on *E. hirta* as the antibacterial agent have been carried out. The active compounds with antibacterial activity are flavonoids, phenol, tannins, sterols, terpenes, and saponin (Sessou et al. 2018; Parisa et al. 2019). While tannins, saponins, and flavonoids are active compounds with anthelmintic activity (Anggrahini et al. 2021). The active compounds of leaves are influenced by the leave age. Therefore, young leaves and old leaves of *E. hirta* will have different active compounds. According to Kuntoro (2010), Euphorbia hirta extract was able to significantly reduce the level of bronchial inflammation at a dose of 10 mg/day. Pujaningsih et al. (2018) research showed that old cherry leaves had greater inhibitory activity on *E. coli* and *Staphylococcus aureus* bacteria because the flavonoid content was higher in old cherry leaves than in young cherry leaves. Gupta and Gupta (2019) report that the leaf extract of *E. hirta* can inhibit the growth of *Bacillus subtilis*, *E. coli* and *S. aureus*. Investigations of the methanol extract of *E. hirta* against *E. coli* reported an amount of MIC 250 µg/mL while in ethanol extract has MIC 160 µg/mL.

The ethanol and methanol extracts of *E. hirta* showed the strongest antimicrobial activities against *Salmonella typhi* and *Pseudomonas aeruginosa* with MIC of 0,031mg/mL and 0,062mg/mL, respectively (Al-snafi and Medicine 2016). The active compounds of leaves are influenced by the leave age. Therefore, young leaves and old leaves of *E. hirta* will have different active compounds. Pujaningsih et al. (2018) research showed that old cherry leaves had greater inhibitory activity on *E. coli* and *S. aureus* bacteria because the flavonoid content was higher in old cherry leaves than in young cherry leaves. The purpose of this research is to investigate the use of ethanol extract from the old and young leaves of *E. hirta* on antibacterial activity against *E. coli* and anthelmintic against *A. galli*.

MATERIALS AND METHODS

Ethanol extract of *Euphorbia hirta* leaves

The old and young leaves of *E. hirta* were taken separately. The leaf is simple, oppositely distichous, lanceolate-oblong, and serrated; the base is asymmetric, having obliquely rounded on one side and cuneate on the other side. Leaves at the three uppermost nodes (stem has internodes) were classified as young while those below the third node were classified as old (Figure 1). *Euphorbia hirta* leaves were cleaned and air dried without exposure to direct sunlight. The dried leaves were pulverized into powder, then 200 g of the powder was macerated in 1000 mL of 96% ethanol in a dark beaker. The immersion was let sit for three days with occasional shaking. After three days, the residual was macerated two times again in 96% ethanol. The obtained substrates were combined and concentrated with a rotary evaporator. Then, the concentrate was extracted in a 50°C water bath until thick suspension was obtained Abdul and Qonitah (2019).

Phytochemical screening

The ethanol extract of *E. hirta* was analyzed to identify the active compounds using different reagents, followed by a color reaction in order to identify the presence of bioactive compounds, such as flavonoid, phenol, and tannin (Wilberforce and Olivia 2017), alkaloids, saponin and terpenoid (Roghini and Vijayalakshmi 2018).

Isolation of *Escherichia coli*

The feces of Sentul chickens were taken and put into a sterile bottle inside a cooler bag and transported to the Central Laboratory of Universitas Padjadjaran. For sample isolation and identification, the feces were dissolved in 0.1% buffer peptone water (BPW) before being grown in agar or nutrient broth media. Identification of *E. coli* was conducted by incorporating 15 mL of eosin methylene blue agar (EMBA) into a petri dish for sterilization using an autoclave at 121°C for 15 minutes. After that, the media was removed from the sterilizer and let solid at a room temperature. After dissolved, 0.1mL of the solution was put on a petri dish for incubation at 37°C for 18-24h. Following the incubation, the growing colony that showed metallic green color with a black spot in the center was considered *E. coli* colony. These colonies were collected during inoculation on a slant nutrient media for further examination (Izevbuwa and Okhuebor 2020).

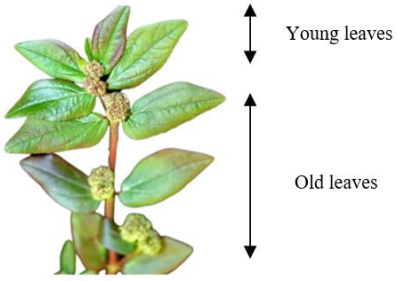


Figure 1. *Euphorbia hirta* leaves

Anthelminthic test

Worms were removed from the intestines of four Sentul chickens that were predicted to have been infected with *A. galli*. The selected chickens were those kept in a traditional cage with poor sanitation and experienced a significantly delayed growth compared to other chickens at the same age. The worms were removed from the intestines by cutting the intestines wall carefully, then the worms were washed with physiological NaCl and put into an Erlenmeyer flask filled with 0.9% of physiological NaCl. We selected worms with a relatively similar size (6-7cm). A total of 30 petri dishes were prepared and filled with treatment liquid to contain three worms each (n=90). The worms were incubated at 37°C and observed every hour. The time taken to death of individual worms was noted. Death was concluded when the worms lost their mobility (death was confirmed when the worm didn't move when transferred to water with temperature 50°C, if it moves then the worm paralysis. Paralysis was the condition when the worms did not revive even in normal saline (Soren and Yadav 2020).

The treatment used consists of the extract of old leaf (T) and young leaf (M) of *E. hirta* (T) as follows: Positive control (Piperazine), Negative control (NaCl 0.9%); T (Old leaf extract) 2.5%; T (Old leaf extract) 5.0 %; T Old leaf extract) 7.5%; T (Old leaf extract) 10%; M (Young leaf extract) 2.5%; M (young leaf extract) 5.0 %; M Young leaf extract)7.5%M (Young leaf extract) 10%

Antibacterial activities

Measuring the Minimum Zone of Inhibition (Sumitriasih et al. 2019), exactly 25mL of Nutrien agar (NA) was mixed with 25 µL of bacterial suspension (*E. coli)*, then homogenized and poured onto sterile petri dishes and let solid. Three wells (±6 mm) were made into the agar in each petri dish using a perforator. The first well was for negative control (DMSO), the second well was the positive control (0,1% Chloramphenicol) and the third well was for ethanol extract of *E. hirta*). Each well was filled with 30 µL of control extract. The ethyl acetic extract and ethanol extracts received the same treatment and incubated for 24h at 37°C, and then observed and measured for inhibitory zone using vernier calipers.

Measuring the minimum inhibitory concentration (MIC)

MIC was determined using broth dilution method in 96-well plates with the following formats: Media+Sample (Negative Control), Media+Solvent (Solvent Control), Media+Sample+Bacteria Test Sample), Media+Solvent+Bacteria (Positive Control). Then, 100µL of liquid media was put into the microplate, 100µL of the sample was placed into the first well of the microplate and then a gradual dilution was made, and 10µL of bacterial suspension was added to the microplate. Incubate at 37°C for 16-20 hours. Measure using a spectrophotometer at a wavelength of 600 nm.

After the Minimum Inhibitory Concentration (MIC) was obtained, we determined the Minimum Bactericidal Concentration (MBC) by extracting one µL of MIC media starting from the top and placed on the NA solid media in a drop plate method. Then, the NA has incubated again at 7°C for 24h. MBC occurred when the bacterial growth reached 0.1% of the total colony. MBC was indicated by zero or less than 0.1% growth of microbe in the NA medium (Prastiyanto et al. 2021).

Data of worm mortality were subjected to analysis of variance with 95% confidence interval, followed by Duncan test. While the anthelminthic test (LC_{50} and LT_{50}) were calculated using probit analysis.

RESULTS AND DISCUSSION

Phytochemical compounds

The efficacy of a plant depends on its bioactive compounds. Accordingly, it is important to perform screening to identify the compounds. The results of screening on the extract of young and old leaves of *E. hirta* are presented in Table 1.

Table 1 shows that the bioactive compounds of old and young leaves of *E. hirta* are phenolic, tannin, flavonoid, saponin, triterpenoids, steroid, and alkaloids. The difference between old and young leaves was observed from alkaloids test: young leaves were positive and old leaves were negative. This result was not significantly different from that of Ahmad et al. (2017), who reported that the phytochemical analysis *E. hirta* extract showed the presence of alkaloids, flavonoids, terpenoids, saponins, and carbohydrates. A more comprehensive analysis (Al-snafi and Medicine 2017) show that the filtered phytochemical compounds of *E. hirta* have reduced sugar, terpenoid, alkaloids, steroid, tannin, protein, fat, oil, gum, mucus, glycosides, saponin, coumarin, cardiac glycosides, anthraquinone, flavanoid and phenolic compounds.

Some bioactive compounds from these findings exhibited antibacterial or anthelminthic properties. In other words, both young and old leaves of *E. hirta* are the potential agents of antibacterial or anthelminthic properties. Furthermore, Awaad et al. (2017) reported that the extract of different species of *Euphorbia* has *different* phytochemical constituents and antimicrobial. *Euphorbia hirta* has highest antimicrobial activity compared to other species, including *Euphorbia granulate* and *Euphorbia helioscopia*. *Euphorbia hirta* has antimicrobial activity against *Klebsiella pneumonia*, *S. aureus*, and *Microsporum canis*, with the highest diameter of the inhibition zone and the lowest MIC.

Anthelmintic properties

The anthelminthic of the plants is actualized by its ability to render mortality to worms. The average lethal time (LT) of the extract of *A. galli* old or young leaves varied. The analysis of variance with 95% confidence interval showed a significant difference across LT with different doses. Table 2 illustrates the results of the Duncan test.

Table 1. Screening results of ethanol extract of *Euphorbia hirta* young leaves

Secondary metabolites	Method	Test results	
		Young leaves	Old leaves
Phenolic	$FeCl_3$ 5% Reagent	+	+
Tannin	$FeCl_3$ 1% Reagent	+	+
	Thick HCl + Mg Reagent	-	-
Flavonoid	H_2SO_4 2N Reagent	-	-
	Heated NaOH 10%	+	+
Saponin	LB Reagent	+	+
Triterpenoids	Thick H_2SO_4 +CH_3COOH	+	+
and steroid	Anhydrous Reagent	+	+
Alkaloids	Dragendorff Reagent	+	+
	Mayer Reagent	+	-

Table 2. Duncan's test for lethal time of *Ascardia galli*

Treatment (%)	Lethal time (hours)	Duncan's test
Positive control	13.44	ab
Negative control	33.11	f
T2.5	20.22	e
T5	17.67	d
T7.5	14.67	bc
T10	12.22	a
M2.5	21.11	e
M5	15.67	c
M7.5	13.44	ab
M10	13.00	ab

Note: [a-f] Different superscripts in the same row represent significant differences ($p<0.05$).

The result showed that the higher the extract of old and young leaves, the shortest the lethal time. However, we found a significant difference on the average Lethal Time between the old and young leaf extracts. On the one hand, a non-significant difference in the average lethal time was observed from the dose of 7.5mg/L and 10mg/L of old leaf extract and 7.5mg/mL of young leaf extract compared to the 7.5mg/L and 10mg/L of positive control (piperazine) on both old and young leaves. Therefore, *E. hirta* showed an excellent effect of anthelmintic compared to common medicine on the market. This finding confirmed by Kalpana et al. (2018) that the methanol extract of *E. hirta* in the water produced anthelmintic activity against earthworms (Pheretimaposthuma), which was more significant than using common medicine, i.e., Dalbendazole. Meanwhile, Kumar et al. (2015) claimed that Euphorbiaceae family exhibited a comparable anthelmintic activities with albendazole and piperazine.

Mortality in the worms is due to some compounds in the *E. hirta*. While phenol causes fatigue and death, alkaloids increase gastrointestinal activities that encourage peristaltic movement to expel the worms from the digestive tracts. According to Mubarokah et al. (2018), alkaloids as the anthelmintic agent inhibit the transportation across the cell membrane. Generally, anthelmintic activities from the alkaloids extract occur by distracting the performance of membrane cells which eventually changes the cell composition. Membrane destabilization, changes of membrane permeability, and the loss of membrane potentials have caused cells lysis that later damages the cuticle and stimulate morphological changes (damaged body surface and morphometry of the worms).

LC_{50} (lethal concentration)

LC_{50} is the concentration of extract that can kill 50% of the experiment organism. The value of LC_{50} was obtained from Probit analysis by using a linear regression curve which log10 concentration as x-axis and Probit as y-axis (Mshelia 2016).

Figure 2 shows that the linear regression equation is $y=0.4394+3.4236$, to obtain the LC_{50} value, enter a value of $y=5$ (probit of 50%) so that the LC_{50} value is 3.5 mg/mL. Figure 3 shows that the linear regression equation is $y=1.2106+0.4213$ that the LC_{50} value is 3.9 mg/mL. Accordingly, the young leaf can be better anthelmintic agent than the old leaf despite the slight difference.

The chemical compounds of leaves are different according to the plant age. Old and young leaves may have the same types of bioactive compounds, but the amount may differ. Works by Prawira-Atmaja et al. (2018), and Thi and Hwang (2014) reported a higher level of polyphenols contained in young leaf than in old leaf. Total polyphenols of dried extract of young leaf and old leaf in 80% ethanol were141.6 mgGAE/g and old leaf were 139.3 mgGAE/g, respectively.

In addition, tannin is bioactive with a high concentration in young leaf. It belongs to the polyphenol compound that consists of hydroxyl and carboxyl clusters. A higher concentrate of tannins is found in old leaves because, compared to young leaf, the old leaf is more defensive against pests, reflecting more tannins compound. Tannin is an anthelminthic agent and Esaïe et al. (2020) reported that the extracts of five plants containing tannins had caused inhibition of life development of worm larva.

LT_{50} (lethal time)

LT_{50} in this study refers to the time it takes to kill 50% of *A. galli*. LC_{50} of the old and young leaves of *E. hirta* is Illustrated in Figures 4 and 5.

The time spent to kill the worms has become shorter due to a higher dose of *E. hirta* leaf extract. The LT_{50} of young leaves and old leaves on *A. galli* would rendered approximately 50% mortality within 14.9 hours and 17 hours, respectively. The longer time in old leaf extract is due to a higher concentration of anthelmintic (tannin and polyphenol) in young leaf.

Tannin is an anthelminthic agent Esaïe et al. (2020) reported that extracts of five plants containing tannins have caused inhibition to life development of worm larva. As an anthelmintic agent, tannin will work by stimulating biochemical interactions between tannin and proline-rich proteins in the sheath nematode or cuticula that render eating disorder, metabolic disorder, and mortality to the worms. Previous findings used an electron microscope to reveal that worms exposed to thick tannin showed a direct structural damage on their cuticles. Further, the effect of tannin on the larvae of *Ascaris suum* from swine include diminished motility and sustainability. An observation using a transmission electron microscope showed that tannin damaged cuticle in the larva digestive tracts (Williams et al. 2014).

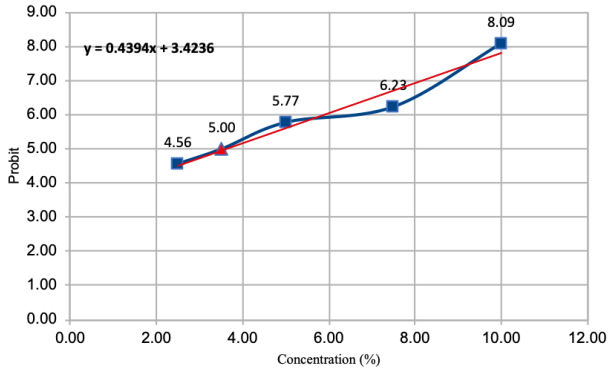


Figure 2. Linier regression of probit analysis for LC_{50} determination of young leaf extract

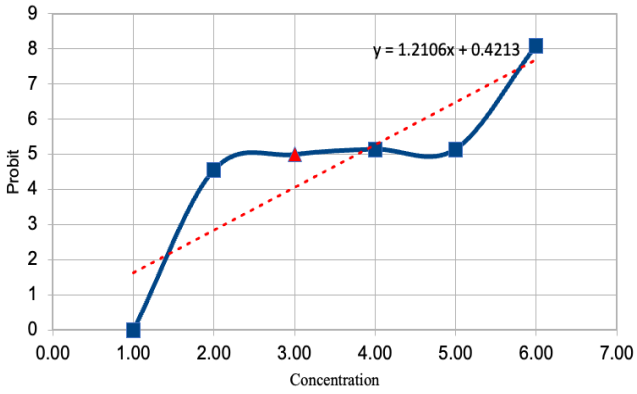


Figure 3. Linier regression of probit analysis for LC_{50} determination of old leaf extract

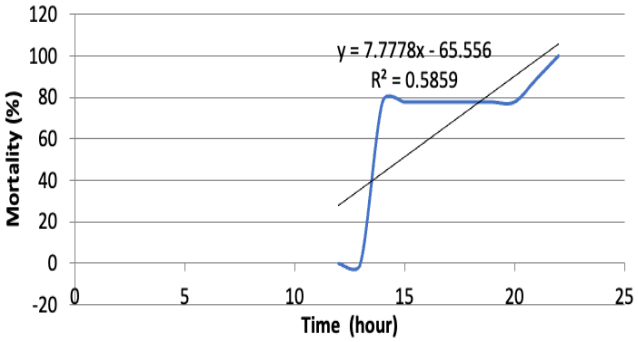


Figure 4. Regression equation of Probit analysis for LC_{50} of *Euphorbia hirta* young leaf extract

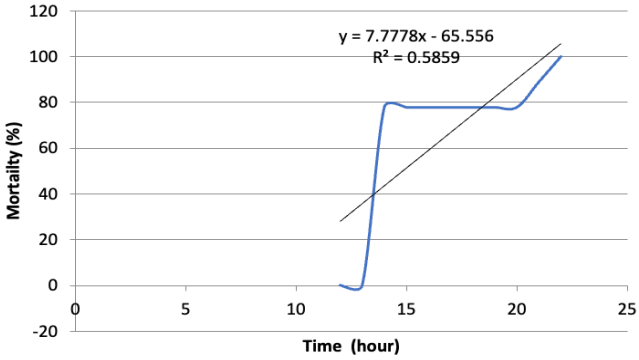


Figure 5. Regression equation of Probit analysis on LC_{50} of *Euphorbia hirta* young leaf extract

MIC of *Escherichia coli*

MIC (Minimum Inhibitory Concentration) refers to the lowest concentration to inhibit bacteria. MIC score of old and young leaves is presented in Table 4 and Table 5.

Table 4 shows the bacteriostatic activities of N-hexane extract of *E. hirta* old leaf against *E. coli* with MIC of 3.125% or 31.25 mg/mL. The reagents (DMSO 50%) exhibit bacteriostatic against *E. coli* with MIC of 12.5% or 125 mg/mL. The old leaf had a lower MIC because the bioactive compounds play more significant roles in inhibiting the activities of *E. coli*.

The N-hexane extract of young leaf shows bacteriostatic activities against *E. coli* with a minimum concentration of 6.25% or 62.50 mg/mL. Meanwhile, the reagent (DMSO 50%) shows bacteriostatic activities against *E. coli* with a minimum concentration of 12.5% or 125 µL/mL.

Tables 4 and 5 indicate that the MIC is lower in old leaf than young leaf of *E. hirta*. Bioactive compounds in *E. hirta*, such as tannin, flavonoid, terpenoid, alkaloids and polyphenol are antibacterial agents. These compounds, based on the screening result, are present but in different amount in old leaf and young leaf, resulting in different inhibitory power against bacteria. This present study showed that the minimum inhibitory power of ethanol extracts was lower in old leaf because it contained more antibacterial agents, such as flavonoid, alkaloids and flavonoid. Meanwhile, young leaf had more tannins and polyphenols. This finding was supported by Hendra et al. (2016) that the potential antibacterial agents are alkaloids and terpenoid found more abundantly in old leaf rather than young leaf. As a result, old leaf and young leaf perform different antibacterial properties.

The other antibacterial agent is flavonoid, which is more prevalent in old leaf than young leaf. Similarly, studies on tea beverage made of seagrass (*Enhalus acoroides*) (Tehubijuluw et al. 2019), avocado leaf (Felicia et al. 2017), and *Aquilaria becariana* leaf (Anwar et al. 2017) showed a higher level of flavonoid in old leaf than in young leaf.

Flavonoid in plants is formed as a response to microbial infection, Nguyen et al. (2019) reported the antibacterial activity from flavonoid compound (eriodictyol, quercitrin, and afzelin) isolated from *E. tirucalli* with the best MIC from afzelin 0.125-0.25 mg/mL.

The antibacterial activity of afzelin is associated with the phenolic structure which, according to (Thị et al. 2019) consists of hydroxyl groups in the C3 at C-ring of the flavon skeleton and without rhamnose. Other previous findings reported that the ethanol extract *E. hirta* exhibited a strong antimicrobial activity against *S. aureus* B39 with MIC of 25 mg/mL (Abdelkhalek et al. 2018).

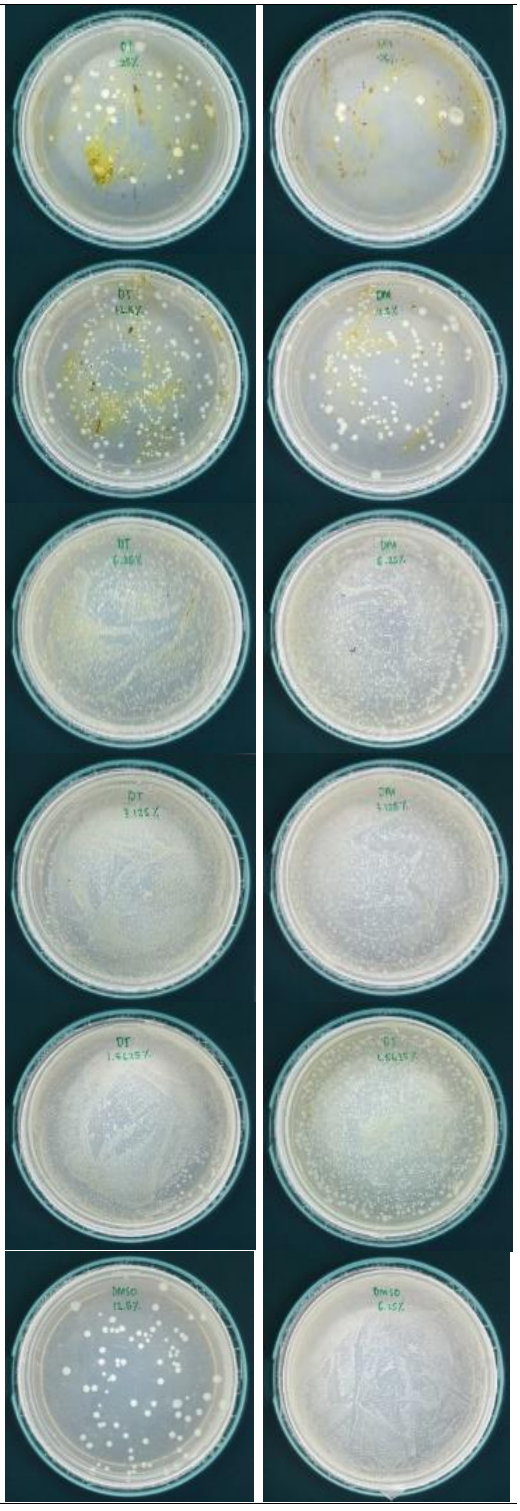
Table 4. The minimum inhibitory concentration (MIC) of old leaves *Euphorbia hirta* against *Escherichia coli*

Well	Concentration (%)											
	25	**12.5**	**6.250**	**3.125**	**1.563**	**0.781**	**0.391**	**0.195**	**0.098**	**0.049**	**0.024**	**0.012**
Media + Sample	3.85±0.01	3.84±0.05	3.91±0.17	3.75±0.13	2.89±0.08	1.95±0.12	1.09±0.18	0.59±0.11	0.34±0.23	0.216±0.21	0.134±0.22	0.093±0.09
Media + Reagent	0.05±0.12	0.05±0.15	0.05±0.16	0.05±0.05	0.05±0.24	0.05±0.15	0.05±0.12	0.05±0.21	0.05±0.18	0.048±0.15	0.048±0.32	0.047±0.22
Media + Sample + Bacteria	3.90±0.03	3.68±0.12	3.69±0.09	3.73±0.21	3.14±0.22	1.99±0.09	1.44±0.09	1.16±0.25	0.95±0.09	0.748±0.14	0.637±0.09	0.596±0.17
Media+Reagent + Bacteria	0.05±0.15	0.05±0.03	0.11±0.08	0.36±0.23	0.48±0.15	0.51±0.08	0.53±0.09	0.52±0.09	0.52±0.13	0.546±0.09	0.533±0.21	0.549±0.06
%Mortality of cells	97.77±0.14	2271.06±0.12	127.47±0.10	2141.39±0.11	-71.8±0.13	-636.09±0.21	-40.00±0.11	16.66±0.15	22.12±0.21	6.274±0.11	3.583±0.15	0.328±0.10

Table 5. The minimum inhibitory concentration (MIC) of young leaves *Euphorbia hirta* against *Escherichia coli*

Well	Concentration (%)											
	25	**12.5**	**6.25**	**3.125**	**1.563**	**0.781**	**0.391**	**0.195**	**0.098**	**0.049**	**0,024**	**0,012**
Media + Sample	3.84±0.09	3.90±0.11	3.69±0.05	3.02±0.11	2.02±0.16	1.31±0.18	0.77±0.13	0.42±0.14	0.25±0.18	0.16±.07	0,12±0.11	0,09±0.09
Media + Reagent	0.05±0.08	0.05±0.13	0.05±0.07	0.05±0.15	0.05±0.08	0.05±0.11	0.05±0.11	0.05±0.17	0.05±0.11	0.05±0.14	0,05±0.09	0,05±0.12
Media + Sample + Bacteria	3.83±0.16	3.89±0.05	3.77±0.04	3.22±0.12	2.20±0.04	1.59±0.17	0.99±0.09	0.84±0.17	0.70±0.07	0.56±0.18	0,54±0.07	0,53±0.11
Media+Reagent + Bacteria	0.05±0.08	0.05±0.07	0.14±0.10	0.31±0.17	0.43±0.05	0.48±0.11	0.50±0.05	0.48±0.08	0.49±0.08	0.50±0.12	0,51±0.05	0,53±0.15
%Mortality of cells	15.48±0.21	570.00±0.10	3.38±0.10	-32.72±0.15	-109.23±0.10	-57.900±0.16	-108.45±0.12	-2.74±0.15	2.33±0.13	-13.67±0.11	-10,40±0.12	-8,90±0.17

Table 6. MBC values of *Euphorbia hirta* against *Escherichia coli*

Conc. %	Old leaf	Young leaf
25		
12.5		
6.5		
3.125		
1.5625		
DMSO		

The inhibitory mechanism against the microorganism by antimicrobial activities is due to some factors that disturb the formation of cell wall. The cause of this mechanism is the accumulated lipophilicity components in the membrane cell that causes changes in the components of the cell wall. Bioactive components that potentially affect and disturb the integrity of cytoplasm membrane that leads to leakage in the intracellular matters and enzim inactivation. As a result, it disturbs the enzyme performance to retain the sustainability of microbial activities, so the microbes would need abundant enzymes to make the microbes energy exhausted.

The MBC test on *E. coli* showed the inhibitory power of both old and young leaf extract of *E. hirta*. This study used a range of concentrations that showed different inhibitory power where *E. coli* still grew. However, we found that the higher the dose, the less bacteria grow as illustrated in the Figure below.

MBC is the minimum concentration of an antibacterial agent that results in bacterial death that provides clear zone without microbial growth on agar media by visual observation. The results of MBC on *E. coli* showed bacterial growth at all concentrations of 1.5625% - 25% *E. hirta* with decreasing bacterial colonies with increasing concentrations of *E. hirta.* Therefore, *E. hirta* only has bacteriostatic activity.

The ethanol extract of *E. hirta l*eaf showed inhibitory power against bacteria. According to Mahmud et al. (2016), *E. hirta* extract has the potential inhibitory power against the growth of *E. coli.* The inhibited bacterial growth is due to disorder in cell conditions. Nomer et al. (2019) stated that the disturbed permeability of cell membrane due to secondary metabolite compounds will damage the function of the membrane cells, leading to cell leakage. The leakage causes the discharge of cell components which further damage the bacteria cell and render the bacteria lysis. Furthermore, the inhibited growth of bacteria were due to antimicrobial activity inhibit cell wall construction, inhibit microbial DNA replication and promote cell wall disruption and lysis (Ganesan and Xu 2017a, 2017b).

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