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The Difference in the Antimicrobial effect of Katuk Leaf Extract (*Sauropus Androgynus* (*l.*) *Merr.*) Concentration against *Escherichia Coli*

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Abstract

Purpose – The aim of this study is to determine the differences in the antimicrobial activity of katuk leaf (Sauropus androgynus (L.) Merr) against *Escherichia coli*.

Design/Methodology/Approach – The method used in this study was experimental posttest using a control group design. Analysis of the effect of katuk leaf was performed in the dilution method with 20%, 40%, 60%, 80%, and 100% concentration. The data were analyzed using one-way ANOVA test ($\alpha = 0.05$) and was then tested using the least significant difference (LSD) test.

Findings – Bacterial colony counting that used total plant count found the average of *E. coli* amount at 20% of concentration (526.820 CFU/ml), 40% of concentration (449.380 CFU/ml), concentration of 60% (255.710 CFU/ml), concentration of 80% (194.110 CFU/ml), and at concentration 100% (168.600 CFU/ml). This study concluded that the katuk leaf extract at 20%, 40%, 60%, 80%, and 100% of concentration had antimicroba effect with significant influence. The 100% of concentration had the most significant effect compared with the other concentrations.

Research Limitations/Implications – Katuk leaf could be used as one of the alternative herbal choices that has a compound antimicrobial effect.

Originality/Value – This study increases the theoretical understanding of the difference of antimicrobial effectivity of katuk leaf extract (*S. Androgynus* (L.) Merr.) concentration against *E. coli*

Keywords *Escherichia coli*, antimicrobial, extract, *katuk* leaf

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1. Introduction

Infectious diseases are the leading cause of morbidity and mortality in the world. World Health Organization (WHO) in 2012 mentioned that one-third of the 25 million deaths worldwide are caused by infectious diseases (Depkes, 2012). Bacteria are one of the most common contributing microorganisms (Garg *et al.*, 2016). The high incidence of infection requires treatment in killing the bacteria, one of them by using antimicrobials, but the high use of antimicrobials is the greatest trigger for the emergence of bacterial resistance (Sylvia and Wilson, 2006). Some bacteria can cause resistance to antimicrobials, one of which is the bacterium *Escherichia coli*, causing important problems for public health (Roland, 2013). *E. coli* bacterial infections in Asia have been reported to be resistant to antimicrobial use of about 60–79% (US National Library of Medicine National Institute of Health, 2015). The results of Antimicrobial Resistance in Indonesia (AMRIN-Study) study showed that from 2,494 individuals in Indonesia, 43% of *E. coli* samples from thousands of individuals are resistant to several antimicrobials, 34% *ampicillin*, 29% *co-trimoxazole,* and 25% *chloramphenicol* (Gabriela *et al.*, 2015).

Indonesia is a biodiversity mega-country rich in medicinal plants and very potential to be developed, one of which is *katuk* leaf (Hariana, 2015). *Katuk* leaf (*Sauropus androgynus* (*L*). *Merr.*) is a plant that is utilized as a traditional medicinal ingredient due to its antimicrobial ability, improving and facilitating milk secretion, overcoming skin disorders, fever, and osteoporosis (Suprayogi *et al.*, 2004; Handayani, 2013). Results of research conducted by the National Working Group of Indonesian Medicinal Plants showed that katuk leaves contain several chemical compounds, including flavonoids and tannins that function as antibacterial (Rukmana and Indra, 2003). Previous research conducted by Fatimah *et al.* (2014) explains that leaf extract of katuk with a concentration of 60–100% can inhibit the growth of *Staphylococcus aureus* bacteria in vitro, but does not affect the concentration of 20% and 40%. Research on the effectiveness of katuk leaves at various concentrations in inhibiting *E. coli* has not obtained the data.

2. Method

This research is a laboratory experimental study using true experimental post-test only control group design. The sample of this research is *katuk* leaf (*S. androgynus* (*L.*) *Merr.*) which is obtained from Uteunkot village, Lhokseumawe, and pure culture of *E. coli* bacteria is obtained from Microbiology Laboratory of Faculty of Pharmacy, University of Sumatera Utara. The size of the sample is calculated by Federer's formula, and the result is five repetitions. The experimental materials were divided into five groups (according to concentrations of 100%, 80%, 60%, 40%, and 20%) plus two control groups (Mc Farland control and negative control of *katuk* leaf extract without suspension).

The leaves of katuk were extracted by the maceration method, until the powder was obtained about 300 g, followed by dissolution with 96% ethanol for three days, then evaporated using vacuum rotary evaporator to produce 30 ml liquid extract. The results of pure extraction were diluted with aquades to reach concentrations of 20%, 40%, 60%, 80%, and 100%. Furthermore, the dilution results are homogenized with the vortex.

Test bacteria from pure cultures that have been identified, about 0.1 ml bacterial inoculum were fed to each extract tube for each concentration, then tested the effectiveness of antimicrobial by dilution technique, which is to be assessed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Next is calculated total plate count (TPC) to get the average value of colony of *E. coli* on katuk leaf extract (*S. androgynus* (*L.*) *Merr.*) with a concentration of 20%, 40%, 60%, 80 %, and 100%.

This study used completely randomized design (RAL) with five treatments each with five repetitions. The research was analyzed using one-way ANOVA test with significance level (= 0.05).

3. Results

3.1. MIC and MBC extract of katuk leaf (s. androgynus (l.) merr.) against E. coli bacteria on dilution 10^{-2} and 10^{-3}

The result of MIC and MBC of *katuk* leaf extract (*S. androgynus* (*L.*) *Merr.*) in *E. coli* bacteria showed the lowest colony count in 100% *katuk* leaf extract (see Table 1). While not found in MBC against *E. coli* bacteria on *katuk* leaf extract (*S. androgynus* (*L.*) *Merr.*) at concentrations of 20%, 40%, 60%, 80%, and 100% both on dilution 10^{-2} and 10^{-3}.

Next TPC is calculated in Table 2 to get the average value of colony of *E. coli* on *katuk* leaf extract of various concentrations.

The result of calculation of total plate count (TPC) found that the average number of bacteria *E. coli* at a concentration of 20% had the highest amount of bacterial colony, while at 100% concentration, had the lowest amount of bacterial colony.

Table 1. The result of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Consentration (MBC) on dilution test of *katuk leaf extract (S. androgynus (L.) Merr.)* Against *Escherichia coli* (CFU/ml)

		Concentration				
Repetition	Dilution	20%	40%	60%	80%	100%
I	10^{-2}	1,180	824	524	433	622
	10^{-3}	1,062	785	478	326	419
II	10^{-2}	971	793	569	396	432
	10^{-3}	960	766	392	281	254
III	10^{-2}	1,371	896	598	378	288
	10^{-3}	872	801	422	393	263
IV	10^{-2}	1,126	822	733	446	312
	10^{-3}	888	810	417	382	303
V	10^{-2}	1,124	1,023	827	598	276
	10^{-3}	897	896	523	334	254

Table 2. The calculation of Average Total Plate Count (TPC) of *Katuk Leaf Extract* (*Sauropus androgynus (L.) Merr.*) Against *Escherichia coli*

Concentration (%)	Average Number of Colony (CFU/ml)
20	526.820
40	449.380
60	255.710
80	194.110
100	168.600

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3.2. Efficacy of antimicrobial leaf extract katuk (s. androgynus (l.) merr.) against E. coli bacteria

Normality test results show that the average percentage data of *E. coli* bacteria in TPC are normal distribution ($P > 0.05$) so that further data can be used for one-way ANOVA test (Table 3).

Furthermore, the homogeneity test is needed to determine the data variance used is the same or not. The test used is the Levene test (Table 4).

Homogeneity test results indicate that data variance in this research data is homogenous ($P > 0.05$) so that further data can be used for one-way ANOVA test.

One-way ANOVA test results in Table 5 shows a significant difference in each concentration with *P* value (0.00). The above data were then tested again using post hoc least significant difference (LSD) to determine whether or not there was a significant effect between the concentration of *katuk* leaf extract (*Sauropusandrogynus* (*L.*) *Merr.*) At concentrations of 20%, 40%, 60%, 80%, and 100%.

4. Discussion

The results of this study showed that there was KHM in all leaf *katuk* extract concentration tested against *E. coli*. While no value of minimum inhibitory level (MIC) was obtained in

Table 3. Shapiro–Wilk Normality Test on Antimicrobial Effectiveness of *Katuk* Leaf Extract (*S. androgynus* (L.) Merr.) Against *Escherichia coli* Bacteria

Group	Number of Sample	Average (%)	*P* value	α Score
Concentration 20%	5	99.3	0.988	
Concentration 40%	5	84.8	0.188	
Concentration 60%	5	90.6	0.443	0.05
Concentration 80%	5	81.7	0.110	
Concentration 100%	5	82.4	0.126	

Table 4. Levene Homogeneity Test against Percentage of Total *E. coli* Bacteria

Levene Statistic	df1	df2	*P* value	α Score
0.684	4	20	0.611	0.05

Table 5. One-Way ANOVA Test of Percentage of TPC of *Escherichia coli* Bacteria

Variable	Mean	SD	*P* value	α Score
Concentration 20%	127,198	14,489.28		
Concentration 40%	95,222	9,637.68		
Concentration 60%	69,484	12,848.53	0.00	0.05
Concentration 80%	48,452	8,674.80		
Concentration 100%	41,586	15,172.01		

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katuk leaf extract at all concentrations. TPC calculations show that extract of *katuk* leaf (*S. androgynus* (*L.*) *Merr.*) at concentrations of 20% and 40% has a weak effectiveness in inhibiting *E. coli* while at concentrations of 60%, 80%, and 100% have strong activity. Concentration of 100% is the most effective concentration in inhibiting *E. coli* compared with other concentration variations.

The higher concentration of *katuk* leaf extract (*Sauropusandrogynus* (*L.*) *Merr.*) will decrease the number of bacterial colonies that survive. This suggests that with increasing concentrations the greater the levels of active ingredients that act as antimicrobials, so that their growth in inhibiting bacteria is also greater. This is in line with research (Fatimah *et al.*, 2014) which assessed the effectiveness of *katuk* leaf extract in inhibiting the growth of *S. aureus* bacteria in vitro.

Different concentrations in variables' treatment affect the linearity of the optical density (glass and air medium) of the bacteria. Thus, the higher the concentration, the smaller the optical density, which means fewer bacteria can survive. This shows that with the increasing concentration, the greater the level of active ingredients that function as antibacterial, so that its ability in inhibiting bacterial growth is also greater (Ajizah, 2004).

The ability of an antimicrobial agent in negating the micro-organism's survival depends on the concentration of the antimicrobial agent, meaning that the amount of antimicrobial agent in a bacterial environment is critical to the life of exposed bacteria. In addition to the concentration factor, the antimicrobial type also determines the ability to inhibit bacterial growth. In this study, suspected bacterial sensitivity of *E. coli* is because of the chemical content in *katuk* leaf extract which consists of antimicrobial nature (Schlegel, 1994).

(I) Group	(J) Group	Mean Difference (IJ)	Sig.
Concentration 20%	Concentration 40%	31.976	0.001
	Concentration 60%	57.714	0.000
	Concentration 80%	78.746	0.000
	Concentration 100%	85.612	0.000
Concentration 40%	Concentration 20%	−31.976	0.001
	Concentration 60%	25.738	0.004
	Concentration 80%	46.770	0.000
	Concentration 100%	53.636	0.000
Concentration 60%	Concentration 20%	−57.714	0.000
	Concentration 40%	−25.738	0.004
	Concentration 80%	21.032	0.015
	Concentration 100%	27.898	0.002
Concentration 80%	Concentration 20%	−78.746	0.000
	Concentration 40%	−46.770	0.000
	Concentration 60%	−21.032	0.015
	Concentration 100%	6.866	0.393
Concentration 100%	Concentration 20%	−85.612	0.000
	Concentration 40%	−53.636	0.000
	Concentration 60%	−27.898	0.002
	Concentration 80%	−6.866	0.393

Table 6. Post-Hoc Test LSD on percentage of TPC of *Escherichia coli.* The result of post-hoc LSD test in Table 6 shows significant difference among concentration 20%, concentration 40%, and concentration 60%

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