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

Antimicrobial  
effect of Katuk  
Leaf Extract

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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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Emerald Reach Proceedings Series  
Vol. 1  
pp. 361–366  
Emerald Publishing Limited  
2516-2853  
DOI 10.1108/978-1-78756-793-1-00037

## 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  
Concentration (MBC)  
on dilution test of  
*katuk leaf extract* (*S.*  
*androgynus* (*L.*)  
*Merr.*) Against  
*Escherichia coli*  
(CFU/ml)

Repetition	Dilution	Concentration				
		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

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	$\alpha$ Score
Concentration 20%	5	99.3	0.988	0.05
Concentration 40%	5	84.8	0.188	
Concentration 60%	5	90.6	0.443	
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	$\alpha$ 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	$\alpha$ Score
Concentration 20%	127,198	14,489.28	0.00	0.05
Concentration 40%	95,222	9,637.68		
Concentration 60%	69,484	12,848.53		
Concentration 80%	48,452	8,674.80		
Concentration 100%	41,586	15,172.01		

*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).

**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%

(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 60%	Concentration 100%	53.636	0.000
	Concentration 20%	-57.714	0.000
	Concentration 40%	-25.738	0.004
Concentration 80%	Concentration 80%	21.032	0.015
	Concentration 100%	27.898	0.002
	Concentration 20%	-78.746	0.000
Concentration 100%	Concentration 40%	-46.770	0.000
	Concentration 60%	-21.032	0.015
	Concentration 100%	6.866	0.393
Concentration 20%	Concentration 20%	-85.612	0.000
	Concentration 40%	-53.636	0.000
	Concentration 60%	-27.898	0.002
Concentration 40%	Concentration 80%	-6.866	0.393

## References

- Ajizah, A. (2004). "Sensitivitas Salmonella typhiirum Terhadap Ekstrak Daun Psidium Guadjava L. Bioscientiae". *Bioscientiae*, Vol. 1, No. 1, pp. 31–38.
- Depkes, R.I. (2012). "Penelitian dan Pengembangan Kesehatan Departemen Kesehatan RI. Badan Litbangkes RI 2012". Kementerian Kesehatan Republik Indonesia, Jakarta. Available: <http://www.diskes.jabarprov.go.id/> [accessed 1 August 2016].
- Fatimah, S., Prasetyaningsih, Y. and Munandar, A. (2014). "Efektifitas Ekstrak Daun Katuk (Sauropus Androgynus L.) Dalam Menghambat Pertumbuhan Bakteri *Staphylococcus aureus* Secara In Vitro". Jogjakarta. Available: <http://jurnal.unimus.ac.id/index.php/psn12012010/article/view/1175/1228>
- Gabriela, V.Ch., Walewangko, W., Bodhi, B.J. and Kepe. (2015). "Uji Resistensi Bakteri *Escherichia coli* Yang Di Isolasi Dari Plak Gigi Menggunakan Ampisilin". *Jurnal e-Biomedik*, Vol. 3, No. 1.
- Garg, R., Felgin, R.D., Christie, A.B. and Morens, D. (2016). "Infectious Disease", *Encyclopedia Britannica*. Available: <https://www.britannica.com/science/infectious-disease> [accessed 19 August 2016].
- Handayani, T. (2013). *Apotek Hidup*. Ilmu Padi Infra Pustaka Makmur, Jakarta, p. 4.
- Hariana, A. (2015). *262 Tumbuhan Obat dan Khasiatnya*. Penebar Swadaya, Jakarta, p. 12.
- Roland, M.T. (2013). "Antibiotics Rasistantce Bacteria". Better Health Channel. Victoria State Government, Australia. Available <https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/antibiotic-resistant-bacteria> [accessed 5 September 2016].
- Rukmana, R. and Indra, M. (2003). "*Katuk Potensi dan Manfaatnya*". Kanisius, Yogyakarta, pp. 7–8.
- Schegel, H.G. (1994). *Mikrobiologi umum, Edisi 6*. Gadjah Mada Press, Yogyakarta.
- Sylvia, A. and Wilson, L.M. (2006). *Patofisiologi Konsep Klinis Proses – Proses Penyakit* (6th ed., Vol. 2). EGC, Jakarta.
- Suprayogi, A. and Meulen, U. (2004). "The Influence of Sauropusandrogynus leaves on the Production of Volatile Fatty Acids using Rumen Liquor under In-Vitro Conditions". In *Expo 2004 – Workshop, Reihe: Nachhaltige Tierproduktion, Tierernährung – Ressourcen und neue Aufgaben*, Braunschweig, p. 29.
- US National Library of Medicine National Institute of Health. (2015). "Multidrug-resistant *Escherichia coli* in Asia: Epidemiology and Management". Available: <http://www.ncbi.nlm.nih.gov/pubmed/25805210> [accessed 23 July 2016].

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