The Effectiveness of Kirinyuh Leaves (*Eupatorium odoratum* L.) and *Allium chinense* Extract against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: This study aims to determine secondary metabolite compounds of kirinyuh leaf extract (*Euphatorium odoratum* L) and hobo (*Allium chinense* and their antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*. The research consisted of three steps, the tirst was plant extraction and maceration using methanol as a solvent. The second, phytochemical screening to confirm secondary metabolites (alkaloids, flavonoids, terpenoids and steroids) The third, antibacterial test against *Staphylococcus aureus* and *Echerichia coli* at concentrations 0, 1, 5, 10, 15, 20 and 25 %, clyndamicin used as positive control. Data were analyzed using SPSS Version 23 software and continued statistically by Duncan Multiple Range Test. Results showed that secondary metabolites such as alkaloids, flavonoids, steroids and saponins were detected in kirinyuh. Whereas, secondary metabolites of hobo contain flavonoids, steroids and saponins. Extract concentration 25% was the most effective for kirinyuh in inhibiting *Staphylococcus aureus* and *Escheriachia coli* with the highest inhibition 15.00 to 19.03 mm, whereas, the inhibition of hobo extract effective in inhibiting *S. aureus* 12.40 mm to 15.20 mm.

1 INTRODUCTION

The use of plants as traditional medicines become increasingly widespread. One of the benefits of medicinal plants as an antimicrobe is no side effect and potential to reduce infection caused by bacteria, fungi, and parasites. The used of antibiotic to reduce diseases caused by pathogenic bacteria can cause bacterial resistance (Awoyinka et al. 2007). Therefore, the used medicinal plants as an alternative to reduce bacterial infection. Medicinal plants that can be used to treat infectious diseases are kirinyuh (*Euphatorium odoratum* L.) and hobo (*Allium chinense*).

In Thailand *kirinyuh* was used as a wound medicine, coagulant, and as an antiseptic (Irobi, 1997), in Nigeria used as a therapy for malaria (Rungnapa, 2003), while in Indonesia the plant used as a medicine for new wounds, fever, cough, and to stop the disease. bleeding (Purwati, 2003). Even so, this plant is still very rarely used by the people of Indonesia because it is considered a nuisance plant that is difficult to eradicate.

Inya-agha et al. (1987) reported that *E. odoratum* leaf extract contains tannins, phenols, saponins, and its essential oil contains α-pinene, cadinene, camphore, limonene, β-caryophyllene, cadinol isomers. Thakong (1999) reported that chloroform extract from *E. odoratum* leaves showed high activity against chloroquin-resistant *Plasmodium falciparum* (K1). The compound isolated from the chloroform extract fraction of *E. odoratum* leaves was isosacuranetin, which was inactive against *P. falciparum* at a maximum concentration of 5 μg / ml. The ethanol extract from the leaves of *E. odoratum* showed antibacterial activity against *Pseudomonas* sp., *Escherichia coli*, *B. thuringensis*, *Klebsiella* sp. and *Streptoccocus faecalis* (Irobi, 1997).

Allium chinense is the onion class commonly used for seasoning and flavoring dishes. Batak chives or onions contain nutrients that can prevent cancer and hypertension. This plant contain compounds as antioxidants, antibiotics, anti-cancer, and antibacterial agents (Rudi, 2012). The aims of the present study was to determine composition secondary metabolites crude extract of kirinyuh

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leaves and *Allium chinense* as anti-microbial against *Staphylococus aureus* and *Escherichia coli*.

2 MATERIALS AND METHOD

2.1 Sample Collection

The materials used in this study were *E. odoratum* and *Allium chinense* which were obtained from Tanah Karo area (Brastagi), North Sumatera. *Staphylococcus aureus*, and *Escherichia coli* were obtained from Biology Laboratory, Department of Biology, Medan Area University.

2.2 Extraction

The procedure used in this study consisted of three steps, the first was the extraction of kirinyuh (*Euphatorium odoratum* L) and hobo (*Allium chinense*) by maceration using methanol as solvent. The second was phytochemical screening by identifying secondary metabolites (alkaloids, flavonoids, terpenoids and steroids). The third, bioactivity test against *Staphylococcus aureus* and *Eschericia coli* using extract concentrations 0, 1, 5, 10, 15 , 20 and 25%. Extraction was started by maceration using n-hexane and methanol as solvents for 3 × 24 hours each with a solvent every 24 hours. The extract was then concentrated using rotavapor.

2.3 Phytochemical Test

Phytochemical screening for alkaloids, flavonoids, saponins, phenolics, triterpenoids and steroids was detected using reagents that specific for each compound. Screening for alkaloids using reagents such as Meyer, Bouchardat, Wagner and Drangendorff. Screening for flavonoid was conducted according to Harbone (1996). The reagents used consisted of NaOH solution, sulfuric acid (H_2SO_4), aqueous Mg-HCl solution, the reaction will form a blue violet color, with concentrated (H_2SO_4) will form a yellowish orange color, and with a dilute Mg-HCl solution with pink color. The presence of color by the addition of the reagents indicate the presence of flavonoid. Screening of triterpenoids and steroids was carried out by the Lieberman-Burchard adhesion (Harbone, 1996). The presence of triterpenoids was indicated by the presence of a red color change, while blue or purple indicates the presence of steroids. Phenolic compounds were screened using 1% $FeCl_3$ reagent (Harbone, 1996). The appearance of blue or purple blue indicates positive for phenolic. Saponin compound screening was carried out by using boiled water in a test tube and then shaking it vigorously for a while. If a permanent foam is formed for about 15 minutes with the addition of one or two drops of 2 N hydrochloric acid (HCl), it shows a positive test for saponins (Harborne, 1996).

2.4 Antimicrobial Test

To examine antimicrobial activity, extract of *Euphatorium odoratum* leaves and hobo (*Allium cinense*) were diluted in sterile distilled water with concentrations of 1, 5, 10, 15 and 20%. The bacterial culture suspension was taken from existing cultures in the laboratory. The bacterial suspension then were inoculated by spread method in petri dishes (9 cm in diameter). Five paper discs for each petri dish. Each of the disc then was dripped for each extract concentration. Petri dishes were divided 4 parts based on the concentration. All cultures were incubated at 37^{o}C for 24 hours. The presence of clear zone around the plant extract were observed. The diameter of the inhibition zone was determined using calipers according to the Kirby-bauer of Susceptibility Testing method in mm (Cappucino & Sherman, 1999).

2.5 Data Analysis

This study used a completely randomized factorial design with experimental methods. Data collected were analyzed using SPSS 23, followed by Duncan Multiple Range Test (DNMRT) for significant differences among treatments given.

3 RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Secondary metabolites of kirinyuh leaf can be seen in Table 1. The metabolites found were flavonoids, alkaloids, and saponins. Whereas, secondary metabolites of *Allium chinense* was shown in Table 2.

Table 1: Phytochemical test of methanol extract of *kirinyuh* leaves.

No	Compound identification	Reactor	Result
1	Flavonoid	$FeCl_3$ 1%	+
		Mg-HCl	-
		NaOH 10%	-
		H_2SO_4	-
2	Alkaloid	Dragendorf	+
		Bouchardat	+
		Meyer	+
		Wagner	+
3	Steroid/ Terpenoid	Salkowsky	+
		$CeSO_4$ 1% + H_2SO_4 10%	+
4	Saponin	Sample + H_2O + HCl 2N	+

Table 2: Phytochemical test of methanol extract of *Allium chinense*

No	Compound identification	Reactor	Result
1	Flavonoid	$FeCl_3$ 1%	+
		Mg-HCl	+
		NaOH 10%	-
		H_2SO_4	-
2	Alkaloid	Dragendorf	-
		Bouchardat	-
		Meyer	-
		Wagner	-
3	Steroid/ Terpenoid	Salkowsky	+
		$CeSO_4$ 1% + H_2SO_4 10%	+
4	Saponin	Sample + H_2O + HCl 2N	+

3.2 Antibacterial Test

Anti-bacterial tests were carried out to determine the ability of methanolic extract of kirinyuh and hobo in inhibiting growth of *Staphylococcus aureus* and *Escherichia coli*. The effectivity both of the extracts was indicated by the presence of a clear zone around the discs that contain plant extract (Table 3).

Table 3: Diameter of inhibition zone (mm) of methanol extract of *kirinyuh* leaves with various concentrations (%).

Treatments	*Staphylococcus aureus*		*Escherichia coli*	
(+) Control	16.97	a	19.13	a
(-) Control	0.00	f	0.00	d
1%	2.67	e	6.67	c
5%	5.00	de	6.00	c
10%	6.33	de	11.33	bc
15%	8.33	d	15.33	b
20%	13.00	c	16.67	ab
25%	15.00	ab	19.03	a

Numbers followed by the same letters not significantly different (0.5) according to Duncan New Multiple Range Test

Table 4: Diameter of inhibition zone (mm) methanol extract of *A. chinense* with various concentrations (%).

Treatment	*Staphylococcus aureus*		*Escherichia coli*	
(+) Control	16.50	a	17.50	a
(-) Control	0.00	f	0.00	g
1%	5.00	d	9.00	f
5%	7.00	c	11.00	e
10%	8.00	bc	13.00	d
15%	10.00	b	13.40	cd
20%	12.00	b	14.00	bc
25%	12.40	b	14.50	b

Numbers followed by the same letters not significantly different (0.5) according to Duncan New Multiple Range Test

Table 3 shows that the concentration of 25% is the best treatment to *S. aureus* and hobo extracts to *S. aureus* and *E. coli* with inhibition f 15.00 mm and 19.03 mm. Table 4 shows that the concentration of 25% is the best treatment of giving *S. aureus* and hobo extracts to *Staphylococcus aureus* and *Escherichia coli* with an average inhibition 12.40 mm to 14.50 mm.

Kirinyuh leaf extract (Figure 1) and hobo (Figure 2) showed positive effect in inhibiting *S. aureus* and *E. coli*. It can be seen that 25% treatment is the best treatment of kirinyuh leaf extract and hobo against *S. aureus* and *E. coli* with the average inhibition power data of 15.00 mm and 19.03 mm and 12.40 mm and 15.20 mm. Chemical compounds in kirinyuh leaf extract and raw hobo such as flavonoids, alkaloids, steroids and saponins have antibacterial activity. According to Qin and Sihotang (2020) and Simanullang et al. (2021) that secondary metabolite compounds such as flavonoids, alkaloids and saponins have potential as antimicrobial agents.

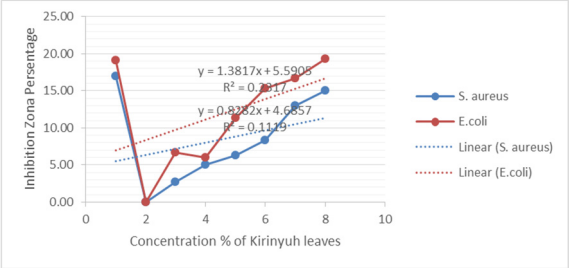

Figure 1: The percentage inhibition of bacterial growth in *kirinyuh* leaf extract.

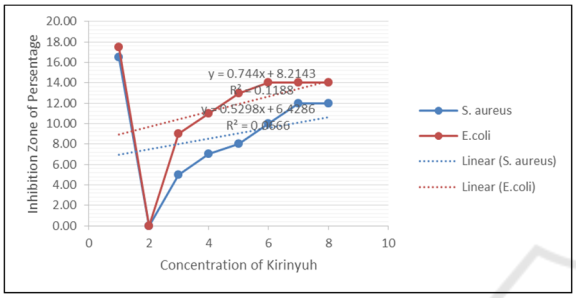

Figure 2: The inhibition zone bacterial growth on hobo extract.

According to Andriani et al., (2016) Secondary metabolite compounds such as flavonoids, alkaloids and saponins have potential as antimicrobial agents. This was confirmed by Heinrich (2009) who stated that flavonoid compounds.

Kurnonealoin compounds can cause bacterial proteins to become inactive and lose their function, while saponins can dissolve lipids in bacterial cell membranes, as a result it can reduce lipid tension, change cell permeability, abnormal cell function and eventually lysis and cause death (Ismiyati, 2014). *S. aueus* and *E. coli* are pathogenic bacterial isolates with thick cell walls because they contain a lot of peptidoglycan and are quite thick (20 -80 nm) and also contain teichoic acid and lipoteichoic acid (Heinrich et al. 2009).

This bacterial cell wall arrangement contains only one layer of plasma membrane, this is what causes its osmotic pressure to drop dramatically when given the anthracurnonealoin complex contained in aloe vera extract. So that the bacterial cell will have difficulty controlling the respiration process and ion transport from outside the cell. Based on the results of data analysis from the One-Way Anova test, the significance results were smaller than 0.05, which means that there were significant differences in the inhibitory power of various concentrations of kirinyuh leaf extract and hobo raw on the growth of *S. aureus* and *E. coli* isolates in compared to control (-) and (+), meaning that the three concentrations of kirinyuh leaf extract and hobo have antibacterial effects against *S. aureus* and *E. coli* but not as strong as the control (+).

4 CONCLUSION

Secondary metabolites of kirinyuh and hobo is a group of flavonoids, alkaloides, steroids and saponins that potential as antibacterial to inhibit *S. aureus* and *E. coli.*

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