Analysis of the Potential of Kirinyu Antimicrobial Herbal Ointment (*Chromolaena odorata*) on the Growth of *Stapphylococcus aureus* to Improve Student Soft Skills in Microbiology Courses

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Abstract. The kirinyu plant (*Chromolaena odorata*) or On Seurapoh in Acehnese has long been used by people for traditional medicine, including to treat wounds. The research aims to analyze the antibacterial potential of kirinyu ointment to the growth of *Staphylococcus aureus* bacteria in vitro in improving the soft skills of Microbiology course students. The research used a completely randomized design, with a posttest only control group design, consisting of 5 treatments, namely Po: negative control, P1: positive control (Gentamicyn), P2, P3 and P4: kirinyu ointment with concentrations of 5%, 10% and 15% with *Staphylococcus aureus* as the test bacteria. The antibacterial test of kirinyu ointment used the disc diffusion method. Research data was analyzed using Anava and continued with Duncan's Multiple Range Test at the 95% level. The influence of research on students' soft skills was analyzed descriptively. The research results show that kirinyu ointment has potential as an antibacterial to *Staphylococcus aureus* bacteria. Evidenced by the formation of a clear zone around the disc, namely kirinyu ointment with concentrations of 5%, 10% and 15% respectively are 9.59 mm, 9.69 mm (medium category) and 11.45 mm (strong category) against *Staphylococcus aureus* ($P<0.05$). Furthermore, this series of research is able to improve students' soft skills in Microbiology courses at Jabal Ghafur University. Conclusion: Kirinyu ointment contains a number of active components so it can show its potential as an antibacterial against *Staphylococcus aureus*. And able to improve students' soft skills in Microbiology courses

Keywords: *Chromolaena odorata*, *Staphylococcus aureus*, antibacterial, soft skills

1 Introduction

Skin, the outermost part of the body makes it exposed to various external agents including microorganisms, apart from the nose, the mucosal lining of the respiratory tract, the vagina and the gastrointestinal tract Most bacterial communities in the hu-

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man body live in areas of the skin other than the nose, buccal mucosa, vagina and digestive tract. Exposure to various microorganisms triggers infection. This infection occurs due to an imbalance between the ability of pathogenic microorganisms and the body's defense mechanisms. Generally, the skin is exposed to *Staphylococcus aureus* bacteria.

S.aureus is classified as Gram + and is able to ferment mannitol and glucose [22]. Indicators of infection by these bacteria are the skin looks red, edema, pain and pus forms around the wound. Having open wounds on the skin or coming into contact with people with skin diseases increases the risk of infection. *S. aureus* causes serious diseases, in the form of skin infections due to open wounds and invasive infections such as pneumonia, regenerative soft tissue infections, heart valves and septicemia. [27]. The results of previous research provide information that various activities both indoors and outdoors cause exposure to *S. aureus*. The results of skin swab examinations of several people in the Glee Gapui area were positive for containing *S. aureus* bacteria [9].

S. aureus is able to evade the immune system and cause infection in the host through several mechanisms, namely dampens the humoral response, interfering with T cell help, blocking complement factors such as SCIN (*Staphylococcal Complement Inhibitor*), which prevents opsonization and lysis of pathogen cells and produces various types of toxins to kill immune cells [36].

Infectious diseases can only be treated with antibiotics. However, long-term use of antibiotics causes resistance in these microbes. This triggers increased mutation or recombination of bacterial cell genes, thereby forming resistant bacteria. One of the commonly known resistances is MRSA or *Methicillin-Resistant Staphylococcus aureus* [25]. It is known that *S. aureus* is resistant to tetracycline, Cefotaxime sodium [5].

The formation of resistant bacteria creates new problems in the health sector, especially in the treatment of infectious diseases. This is the basis for research directed at finding alternative ingredients from plants that are effective as antibacterials. One of the plants in question is kirinyu (*Chromolaena odorata*).

Kirinyu (*Chromolaena odorata*), known as On Seurapoh in the Acehnese language, is classified as a weed in the form of a woody bush and grows quickly so it is difficult to control. The abundance value of kirinyu is very high in Aceh, especially in the Glee Gapui ecosystem, Indrajaya District, Pidie Regency, so it is very easy to find without the need for cultivation. Kirinyu belongs to the Asteraceae family with the genus *Chromolaena*. It has an incomplete leaf structure with the leaf veins reaching the edge and arranged in a curved shape. The central leaf vein is the largest while the others follow the edge of the leaf [32]. The stem is round with a hairy or hairy surface. The roots are branched taproots, large and deep. It has a long cone shape and grows straight down. People have long used kirinyu leaves as medicine to treat wounds, cholesterol and stomach diseases. Its healing ability is due to the active ingredient content in it, so it has the potential to be used as a traditional medicine. Phytochemical test results show that kirinyu plants contain alkaloids, flavonoids, steroids and saponins [24], terpenoids [10], phenolics [15]. Alkaloids and flavonoids are useful as antimicrobials [12, 11, 21]. Kirinyu is also able to accelerate wound healing [1],

as a herbicide [15], insecticide [15] and is effective as a larvicide against the *Aedes aegypti* mosquito [10]. Kirinyu's herbicidal ability is caused by allelochemical (allelopathic) compounds [32], antihypercholesterolemia, and anti-inflammatory.

Treatment using kirinyu leaves is local wisdom passed down from ancestors, which is prepared by boiling, pounding and squeezing. The juice of kirinyu leaves is used to treat wounds or other skin diseases. Inspired by local wisdom and the bioactive content of the kirinyu plant, it is very necessary to do research to analyze the antibacterial potential of Kirinyu against the growth of *S. aureus* bacteria by making a topical pharmaceutical preparation, namely ointment. Apart from being easy to use and hygienic, ointment functions as a carrier for topical medications, a skin lubricant and as a skin protector. This series of research stages is devoted to improving students' soft skills in the Microbiology Course. Microbiology courses are classified as mandatory courses in the Jabal Ghafur University Biology Education Study program. Learning Microbiology courses is accompanied by practicums as an effort to improve soft skills, scientific work skills for students. So skilled in formulating problems, developing hypotheses, designing and conducting experiments, analyzing data and drawing conclusions. Fibriana and Amalis (2016)[13] explained that learning Microbiology can be maximized by using a learning approach that fosters soft skills and hard skills. Soft skills are the result of interaction and communication in life that are not obtained in special courses, but can be trained intensively in every lesson. Aspects of soft skills are communication skills, thinking skills, problem solving skills, teamwork, ethics and leadership skills[6].

Apart from soft skills, hard skills in the form of hands-on are very much needed for prospective Biology teachers. Hands-on skills include how to use tools, pipetting techniques, sterilization and aseptic techniques, weighing materials, making solutions and media, streak and pour plate methods, painting and identifying microorganisms.

2 Method

2.1 Research Location and Time

This research was carried out from May to December 2023. The preparation of the herbal ointment of kirinyu leaves was carried out in the Pharmaceutical Laboratory and the antibacterial testing was carried out in the Microbiology Laboratory of FMIPA, Syiah Kuala University, Banda Aceh.

2.2 Tools and Materials

The tools used are mortar, memmert incubator, spectrophotometer, autoclave, digital scale, laminar air flow cabinet, rotary vacuum evaporator, water bath, test tube, tube, water bath, oven, and petridisk as well as glassware.

The materials used were clinical isolate *Staphylococcus aureus* (*S. aureus*), Nutrient Agar (NA) media, Mannitol Salt Agar (MSA) media, kirinyu leaves (*Chromolaena odorata*), Nutrient Agar (NA) media, ointment base.

2.3 Population and Sample

Testing students' soft skills abilities used students from the Jabal Ghafur University Biology Education study program who had a Microbiology Course program and totaled 10 students. The sample was determined using total sampling so that the research sample was 10 students.

2.4 Types and Research Design

he type of research is a laboratory experiment with a posttest only control group design and a Completely Randomized Design (CRD), consisting of 5 treatments and 4 replications. Treatment consisted of Po: negative control, P1: positive control (Gentamicyn), P2: 5% kirinyu ointment, P3: 10% kirinyu ointment, and P4: 15% kirinyu ointment.

2.5 Preparation of Kirinyu Leaf Samples and *S. aureus* Suspension

Samples of fresh kirinyu leaves were obtained in the Jabal Ghafur University campus ecosystem, Sigli. A total of 250 g of fresh kirinyu leaves resulting from wet sorting were washed with running water and drained. Kirinyu leaves are taken three quarters from the base of the stem [8]. Next, dry it under the sun. Previously, the entire surface of the leaves was covered with a black cloth to prevent exposure to direct sunlight so as to prevent the evaporation of the volatile active ingredients. Once dry, the kirinyu leaves are made into powder.

Clinical isolates of *S. aureus* were regenerated on Nutrient Agar (NA) media and incubated for 24 hours at 37ºC. After 24 hours, the bacteria were transferred to Mannitol Salt Agar (MSA) media and incubated at 37oC for 24 hours. Preparation of S. aureus suspension using 0.9% NaCl solution until turbidity is formed which is the same as the Mc Farland No. turbidity standard. 2, equivalent to a concentration of 6.0 x 108 CFU/ml [7].

2.6 Preparation *of S. aureus* Growth Media

The media used in this research are Nurient Agar (NA) media to regenerate bacteria and Mannitol Salt Agar (MSA) media as differential selective media for *S. aureus* bacteria. NA media was made by dissolving 3 g of nutrient agar (NA) in 100 mL of distilled water. Once homogeneous, the solution was heated and stirred used a hotplate stirrer at 180°C for 30 minutes. After sterilization, the solution is poured into petri dishes. MSA media is prepared by dissolving MSA media in sterile distilled water, heating until boiling. Next, the media was sterilized in an autoclave for 15 minutes at a temperature of 121 ºC and a pressure of 1 atm.

2.7 Making Kirinyu Herbal Base and Ointment

The process of making kirinyu herbal ointment begins with maceration of simplicia powder. 50 g of kirinyu powder was dissolved in 500 ml of alcohol 70% and left for 24 hours. The kirinyu filtrate is filtered and remacerated. The filtering results are concentrated until a thick extract is formed. Next, the ointment base is made by mixing album vaseline and adeps lanae until homogeneous. Next, the stearic acid and cera alba are melted at a temperature of 60-70ºC until melted. The two solutions were mixed until homogeneous (Table 1).

Table 1. Kirinyu Leaf Extract Ointment Base Formula

No.	Composition	Formula (g)
1	Vaselin Album	86
2	Adeps Lanae	3
3	Stearic Acid	3
4	Cera Alba	8

The preparation of kirinyu herbal ointment begins with kirinyu leaf extract according to the treatment, mixed into the ointment base and stirred until homogeneous and stored in an ointment bottle. The formula for preparing kirinyu herbal ointment is available in Table 2.

Table 2. Kirinyu Leaf Ointment Formula

No.	Extract Composition	F0	F1	F2	F3
1	Kirinyu Extract Composition (g)	-	5	10	15
2	Ointment Base up to (g)	50	50	50	50

2.8 In vitro Antibacterial Potential Test

Antibacterial potential of kirinyu (*C. odorata*) herbal ointment against the growth of *S. aureus* bacteria in vitro used the disc plate method. Inoculation of *S. aureus* bacteria was inoculated aseptically into NA media via the streak method, with an inoculum density equivalent to Mc. Farland No.2 is 6.0 x 108 CFU/ml. Next, a disc plate was placed which had previously been soaked in distilled water (P0), gentamicyn (P1), kirinyu ointment according to the concentration (P2, P3 and P4). Incubation was carried out for 24 hours at 37 ºC. Observations of the diameter of the inhibition zone were carried out after 24 hours, by measure the clear area around the disc plate (Figure 1). The measurement results were grouped based on antimicrobial inhibition zone activity (Table 2).

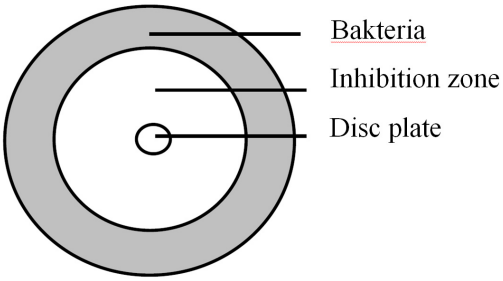

Fig. 1. Measurement of anti-microbial activity based on zone diameter observations resistor

Table 3. Classification of Growth Barrier Responses

N0	Inhibition zone diameter	Growth inhibitory response
1	> 20-30 mm	Very strong
2	> 10-20 mm	Strong
3	5-10 mm	Currently
4	< 5 mm	Weak

Source: Morales *et al*. (2003)

2.9 Soft Skill Ability Testing

Measuring students' soft skills in the Microbiology course uses a Linkert scale questionnaire instrument with a score of 4-1 for positive statements, and a score of 1-4 for negative statements, with score options: Very Good, Good, Not Good, Very Bad. The soft skill indicators measured are: Ability to communicate, Collaboration, Initiative, Not giving up easily and Ability to complete tasks [18].

2.10 Research Data Parameters and Analysis

The parameter measured was the diameter of the clear zone formed around the disc plate due to the administration of kirinyu (*C. odorata*) herbal ointment. Research data was analyzed using Analysis of Variance (ANOVA) and continued with DMRT.

Students' soft skills abilities in the Microbiology Course were analyzed descriptively.

3 Result and Discussion

3.1 Effect of Kirinyu herbal ointment on the diameter of the inhibition zone for *S. aureus* bacteria

This research uses the test bacteria *S. aureus*. *S. aureus* is a gram +bacterium, characterized by coccus shaped, + catalase test , + coagulase test, and shown by the growth of golden yellow colonies on MSA medium (Figure 2)[9]. *S. aureus* bacteria grow on Salt Agar (MSA) Media.

MSA is a differential selective media for *Staphylococcus sp* due to the NaCl salt content of 7.5% [20]. Karimela et al. (2017)[22] explained that *S. aureus* capable to ferment glucose and mannitol at MSA media. The ability of this fermentation to produce acid is shown by yellow bacteria on the media. Yellow color and produces acid and yellow color in the media [19]. Generally, bacteria cannot grow at 7.5% salinity, except for Staphylococcus sp. [20].

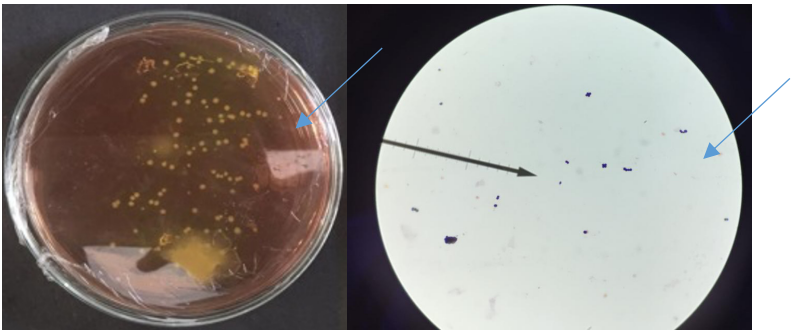

Fig. 2. *Staphylococcus sp* colonies on MSA media (a) and *Staphylococcus sp* on Gram staining (arrows) (b) [9]

The results of the study showed that kirinyu herbal ointment at various concentrations showed its potential as an antibacterial against *S. aureus*. Evidenced by the formation of a clear zone around the disc plate as a sign of inhibited growth of *S. aureus* (Figures 3 and 4). The results of measuring the diameter of the inhibition zone are shown in Table 4. The inhibition zone is formed due to the antimicrobial compound content in kirinyu leaves.

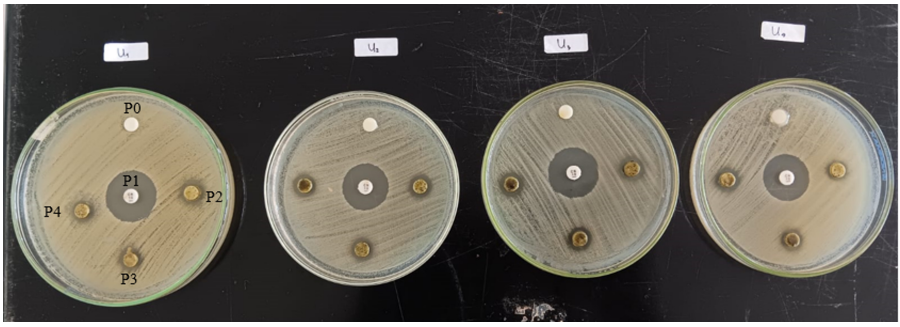

Fig. 3. Diameter of Inhibition Zones Formed in Various Treatments

Table 4. Inhibition Zone Diameter for Various Treatments

No	Treatment Group	Inhibition Zone Diameter (mm)				Average	Category
		1	2	3	4		
1	P0	0	0	0	0	0,00[a]	Weak
2	P1	21.5	20	20.7	19.5	20,43[d]	Very strong
3	P2	12	8.4	9.25	8.7	9,59[b]	Currently
4	P3	10.55	8.9	9.9	9.4	9,69[b]	Currently
5	P4	16.1	9.1	10.4	10.2	11,45[c]	Strong

Information: Po = Negative Control (test bacteria + ointment base), P1 = Positive Control (test bacteria + gentamicyn), P2 = Test bacteria + 5% kirinyu ointment, P3 = Test bacteria + 10% kirinyu ointment, P4 = Test bacteria + kirinyu ointment 15%

Measurement of the activity of antimicrobial compounds refers to Morales et al (2003) [26], which categorizes the bacterial growth inhibition response as very strong (>20-30 mm), strong (10-20 mm), currently (5-10 mm), and weak (< 5mm). Table 4 showed the highest average diameter of inhibition zone was produced by gentamicin (P1), is 20.43 mm in the very strong category and very significantly from P0, P2, P3 and P4. Administration of kirinyu ointment at various concentrations resulted in inhibitory zone diameters of 9.59 mm (P2), 9.69 mm (P3) and 11.45 mm (P4), respectively. Statistical analysis showed that an increase in the diameter of the inhibition zone occurred due to an increase in the concentration of kirinyu ointment. Treatment P4 (15% kirinyu ointment) had an inhibition zone diameter in the strong category ($P<0.05$).

The diameter of the inhibition zone formed during the kirinyu ointment treatment increased with increasing concentration. The higher the concentration of kirinyu ointment given, the wider the diameter of inhibition zone formed. The high concentra-

tion of kirinyuh ointment allows the spread of compounds to inhibit or kill S. aureus. Sudarwanto et al. (2015) [30] explained that antimicrobial activity is influenced by the type and concentration of antimicrobial ingredients. The higher the concentration, the wider the diameter of the inhibition zone formed as a result of the higher concentration of antimicrobial ingredients contained in it so that it becomes more effective. The antibacterial potential shown by administering kirinyu ointment is caused by the bioactive content in kirinyu leaves, namely flavonoids, tannins, alkaloids and steroids. Each of these components has a different actibacterial mechanism. Tannins are able to precipitate protein or combine with it. Tannins are astringent so they are used as antidiarrhea, anti-bleeding and anti-inflammatory [29]. Tannins trigger wound tissue regeneration and re-epithelialization by precipitating complex protein lipids [28]. The tannins in kirinyu have antibacterial potential by acting as an antibiofilm against *S. aureus* [34].

Flavonoids are classified as hydroxy phenols (polyphenols) with high antioxidant activity with various bioactivities, namely antioxidant [31], antibacterial, anticancer, anti-inflammatory [23].

Saponins are glycosides which are widely distributed in higher plants [2]. Saponin acts as an antibacterial [33], antifungal [3], reducing free radical activity thereby preventing biomolecular damage [17]. Alkaloids contain nitrogen atoms. Alkaloids function as antibacterial, antiviral, analgesic, hypotensive and antsidiabetic [4].

3.2 Student Soft Skill Abilities in Microbiology Courses

Data analysis of each indicator of students' soft skill abilities in studying Microbiology courses through conducting research entitled analysis of the potential of kirinyu (*C. Odorata*) herbal ointment on the growth of *S. aureus* bacteria can be seen in Table 5.

Table 5. Soft skill ability Data Analysis

No	Indicator	Percentage	Category
1	Communication skills	96.45%	Very good
2	Cooperate	92.65%	Very good
3	Initiative	84.00%	Good
4	Not easily give up	85.35%	Good
5	Completing the task	84.78%	Good

The results of data analysis of students' soft skills abilities (Table 5) through integrated soft skills research-based learning show that there has been an increase in soft skills abilities for each indicator measured. This learning is carried out by placing students in cooperative groups. Each group member is responsible for completing their research and reporting it in writing and orally to the class. Microbiology learning through a series of research is effective in improving students' soft skills for indicators of communication skills, working in teams, taking initiative, not giving up easily and being able to complete assignments [18]. Fitriana et al. [14]

explained that soft skills are really needed to determine the direction of utilizing hard skills. Good soft skills help students support academic and non-academic, and achieve balance in life.

4 Conclusion

Based on the research results, it can be concluded that Kirinyu ointment contains a number of active components so that it can show its potential as an antibacterial against Staphylococcus aureus (15% concentration shows strong antibacterial potential). A series of research activities can improve students' soft skills in the Microbiology course.

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