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ANTIBACTERIAL POTENTIAL ETHANOL EXTRACT OF KAYU RACUN LEAF (Rhinacanthus nasutus) AGAINST Staphylococcus aureus AND METHICILLIN RESISTANT Staphylococcus aureus

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Abstract

The excessive use of antibiotics in the treatment of infections or diseases caused by Staphylococcus aureus is the main cause of antibiotic resistance. Methicillin-resistant S. aureus (MRSA) strains are a serious problem because of their wide distribution in the clinical environment and the living community, so it is necessary to search for antibacterial sources from natural products and traditional medicines such as Kayu racun leaves (Rhinacanthus nasutus). Information regarding the content of the active ingredient and the antibacterial potential of the ethanol extract of Kayu racun leaves is still limited. This study aims to test the phytochemical and antibacterial activity of the ethanol extract of Kayu racun leaves against S. aureus and MRSA bacteria by invitro. The research used an experimental method and was divided into two stages, namely; Phytochemical test of the ethanol extract of Kayu racun leaves using the Thin Layer Chromatography (TLC) method and the antibacterial activity test of the ethanol extract of Kayu racun leaves against S. aureus and MRSA using the paper disc diffusion method. Based on the research results, it can be concluded that the ethanol extract of Kayu racun leaves contains alkaloids, phenols, and flavonoids. The best antibacterial activity was found at a concentration of 100 mg/ml with an inhibition zone diameter of 26.20 mm against S. aureus and 17.90 mm against MRSA.

Keywords: *phytochemistry, Kayu racun, antibacteria, S. aureus, MRSA, invitro*

INTRODUCTION

Antibiotic resistance is a global health problem (Shamsuddin et al., 2018). Excessive administration of antibiotics in controlling pathogenic microbes has triggered the emergence of resistance cases (Hussein et al., 2020).

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S. aureus is one of the species of pathogenic microbes caused by a skin infection that has good adaptability in epidermal tissue (Hua, et al., 2018; Pawar, et al., 2020). Treatment of *S. aureus* infection is generally carried out with antibiotic therapy which is still the main support of infection management. It is estimated that around 92% of Indonesians do not use antibiotics properly. So that it causes losses not only in terms of health and economy but also more worrying is the emergence of resistance or resistance to one or more antibiotics (Utami, 2011).

So far the most reported cases of resistance are Methicillin-Resistant *Staphylococcus aureus* (MRSA). MRSA is a gram-positive bacterium, a strain of *S. aureus* that has developed through horizontal gene transfer and natural selection. The spread of *S. aureus* and MRSA is generally through human contact (Kumar and Githa, 2018). MRSA strains are resistant to almost all β-lactam antibiotics, which are the most effective and widely used antimicrobial class for the treatment of infections (Oliveira dan de Lencastre, 2011; Amalia et al., 2017). These groups of antibiotics include; penicillin, tetracycline, methicillin, and vancomycin (Chakraborty et al., 2018). MRSA is also the leading cause of nosocomial infections worldwide thus increasing the length of treatment, medical costs, to the death rate (Hua et al., 2018; LanMai, 2020).

The strategy currently being developed to prevent an increase in antibiotic resistance is to combine the use of antibiotics with natural products and herbal traditional medicines (Pawar et al., 2020). Over the last decade the interest in finding natural products as antimicrobial sources has increased. Plant selection is considered the most promising because it can be selected based on ethnopharmacology and a variety of plants is quite easy to obtain (Diaz, et al., 2019). The active compounds contained in plants depend on the type of solvent used in the extraction procedure (Seleshe and Kang, 2019).

Based on local wisdom, the use of plants as medicine has been a tradition from long time ago until now. Likewise with the Simanau village community. There are 180 species and 67 families of medicinal plants used by the local community. Five species are used as drugs for skin infections, namely; *Diploterygium sp. (pakih galah), Ageratum conyzoides (silameh kambiang), Blechnum Orientale (pakih Sipasan), Greene corymbosa (Rambai Kuau), and Rhinacanthus nasutus (Kayuracun)* (Tim Ristoja Provinsi Sumatera Barat, 2012).

Research on the use of plants as medicine is gaining popularity. Several previous studies have shown that the ethanol extract of Kalanduyung leaves can inhibit S. aureus with an inhibition zone diameter of 6.875 mm, 8.5 mm, 10.175 mm, and 14.925 mm on concentration 20%, 40%, 60%, dan 80% (Trisia et al., 2018). Furthermore, Wicaksono et al., (2018) reported that the ethanol extract of *Nephelium lappaceum* leaves contained flavonoids, polyphenols, saponins, and tannins which showed antibacterial activity against MRSA at a concentration of 5% (w/v).

However, so far the information regarding the content of K*ayu racun* leaves and their potential as antibacterial agents against *S. aureus* and MRSA is still limited. So a study

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is carried out to analyze the phytochemical content of ethanol extract of *Kayu racun* leaves and test the antibacterial potential against *S. aureus* and MRSA bacteria.

METHODS

Kayu racun leaf samples were collected from *Nagari Simanau-Solok*. The research was conducted at Biota Laboratory and Microbiology Laboratory of Biology Department, Andalas University with an experimental research method. The research stages include; sample preparation, sample ethanol extraction, sample phytochemical analysis, preparation of the *S. aureus* and MRSA bacterial suspensions, and testing the antibacterial activity of the *Kayu racun* leaves ethanol extract against the two tested bacteria (Figure 1).

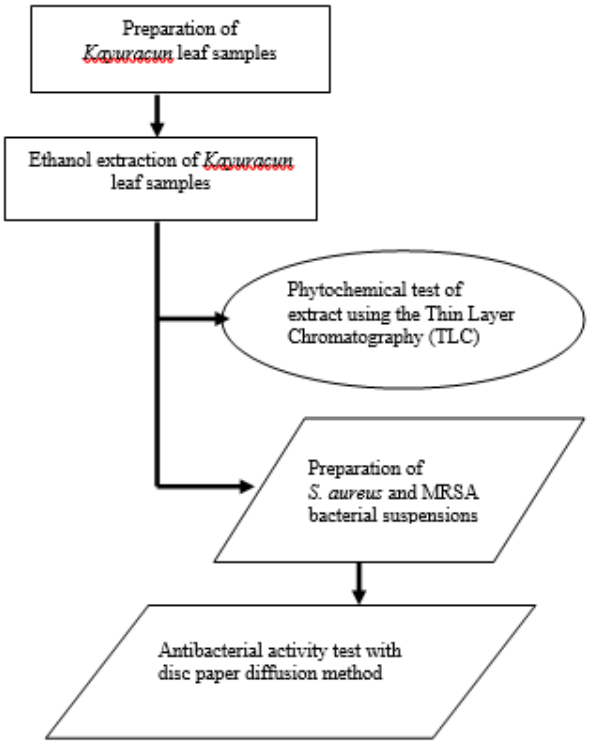

Figure 1. Research scheme

Preparation of Plant Extracts

Three kg of *Kayu racun* leaves were collected from the field. Furthermore, the leaf sample was dried, ground, and mashed in a blender. The sample is macerated in stages. The first maceration macerated *Kayu racun* leaf powder with a comparison of the sample and ethanol =1:10 (w/v). A total of 250 g of sample was macerated with 2.5 l of 70% ethanol for 18 hours. Then filtered to separate the ethanol extract of *Kayu racun* leaves from the residue, so that the ethanol extract 1 is obtained. The second maceration was carried out on the residue and ethanol by comparison 1:5 (w/v) for 24 hours. In the same way, ethanol extract 2 is obtained. The ethanol extract 1 and 2 are combined then evaporated to obtain a thick ethanol extract (crude extract).

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(Indonesia Herbal Pharmacopeia, 2012).

Phytochemical test

The phytochemical test used the Thin Layer Chromatography (TLC) method. The sample was spotted on a 60 F254 silica gel plate using a capillary tube. Furthermore, the TLC plate was sprayed with a specific spray to determine the active compound class. The spots formed on the TLC plate were observed under the light of UV254 and UV365. The specific spray reagent used is a) 1% FeCl3 to detect the presence of tannins and phenolic compounds if green, red or blue colors appear after spraying, b) Lieberman-Burchard to detect steroid compounds when blue appears and terpenoids if red appears, c) Citroborat to detect flavonoid compounds when green and yellow fluorescence appears, d) Dragendorff, to detect alkaloids when yellow fluorescence appears (Harborne, 1987; Indonesia Herbal Pharmacopeia, 2012).

Antibacterial Activity Test

Starting with the preparation of a suspension of *S. aureus* and MRSA test bacteria in a 0.9% (w/v) NaCl solution and equalizing the turbidity with a standard solution of 0.5 McFarland (10^8 CFU/ml) (Balouiri et al., 2016). Antibacterial activity test was carried out disc paper diffusion method. Each MHA medium (Mueller Hinton Agar) was poured into a sterile 10 ml petri dish, waited for it to cool and solidify. Then 100 µl of *S. aureus* and MRSA test bacteria were inoculated on each petri dish using the spread plate technique. Place the disc paper that has been dripped with the ethanol extract of *Kayu racun* leaves at the concentration tested (concentration 20, 40, 60, 80 dan 100 mg/ml), above the surface of the medium. Negative control using DMSO (dimethylsulfoxide). A positive control using the antibiotic Amoxicillin (1mg/ml). Then incubated at 37°C for 24 hours. The formed inhibition zone indicates that the ethanol extract of *Kayu racun* leaves has antibacterial activity (Purwanto, 2015).

Data Analysis

The data obtained are presented in the form of tables and figures and then described descriptively.

RESULTS AND DISCUSSION

Phytochemical Analysis of Ethanol Extract of *Kayu racun* Leaves

The use of plants as medicinal substances is related to the secondary metabolites contained in plants. The results of phytochemical analysis of the ethanol extract of *Kayu racun* leaves are shown in Table 1.

Table 1. Phytochemical test of the ethanol extract of *Kayu racun* leaves using the TLC method

Specific Spray Reagents	Result	Secondary Metabolites
Dragendorff	+	Alkaloids
FeCl3	+	Tannins and Phenol
Lieberman/ Burchad	-	Terpenoids
Citroborat	+	Flavonoids

Ket: (+) = detected, (-) = undetected

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According to Costa et al., (2012) Alkaloids, terpenoids, and phenolics, are the three major groups of secondary metabolites produced by plants and can be used as a source of medicine because they have antibacterial traits. Trisia et al. (2018) reported the phytochemical results of Kalanduyung leaves containing alkaloids, flavonoids, saponins, tannins, and steroids. The content of these compounds makes Kalanduyung leaves potential as an antibacterial agent for S. aureus. A material is categorized as antibacterial if it is inhibiting (bacteriostatic) or even kills bacteria (bactericide) (Tricia, et al., 2018; Mayer (2011).

Antibacterial Activity of *Kayu racun* Leaf Extract

Antibacterial activity test of ethanol extract of *Kayu racun* leaves was carried out to determine the response of both *S.aureus* and MRSA test bacteria. The results can be seen in Figure 2 and Figure 3.

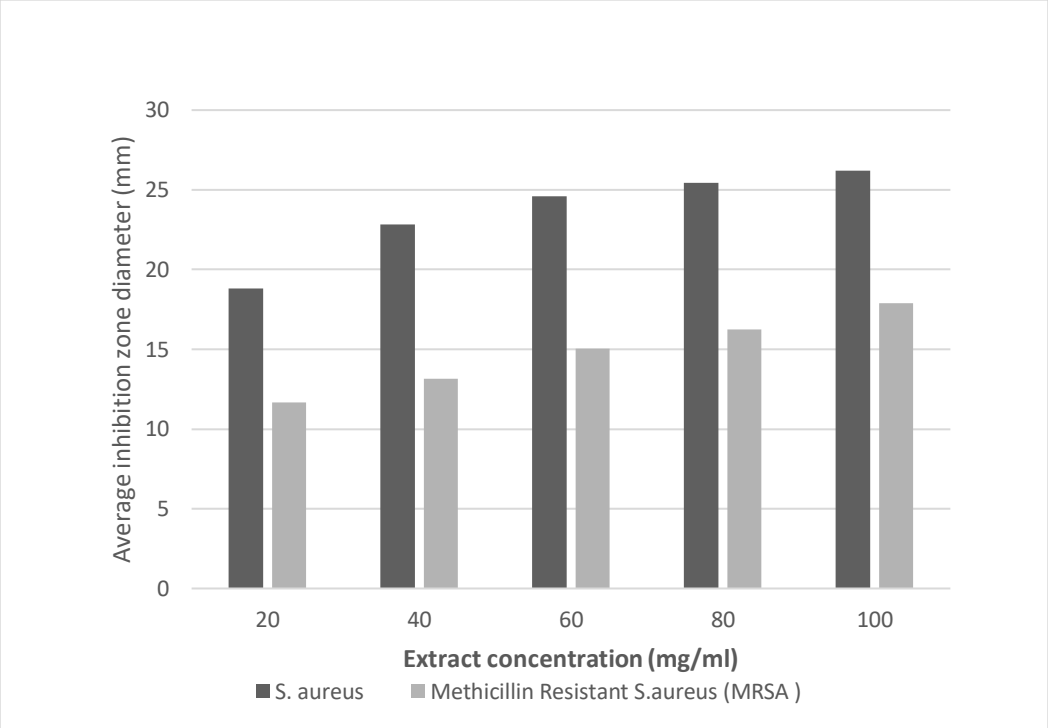

Figure 2. The mean inhibition zone of the *Kayu racun* leaf ethanol extract against *S.aureus* and MRSA.

Figure 2 shows that giving the ethanol extract of *Kayu racun* leaves at various tested concentrations can inhibit the growth of *S.aureus* and MRSA bacteria which is indicated by the formation of an inhibition zone around the disc paper. The inhibition zone is a clear area around the disc paper that shows no bacterial growth activity (Pawar, 2020).

The mean diameter of the inhibition zone of the concentration of the ethanol extract of *Kayu racun* leaves; 20, 40, 60, 80 and 100 mg/ml in *S. aureus* are 18.81; 22.84; 24.58; 25.42 dan 26.20 mm. While in MRSA

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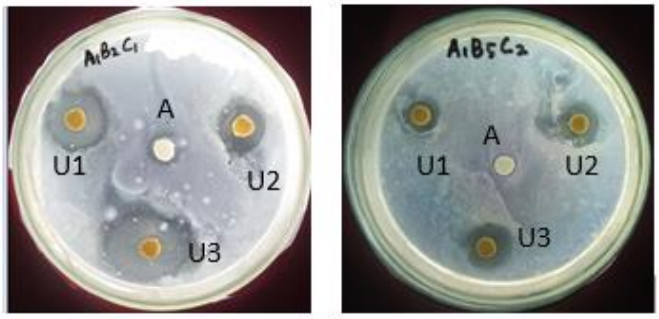
are; 11.68; 13.14; 15.06; 16.23 dan 17.90 mm. While in the negative control (K-) no inhibition zone formed. This proves that the formation of the inhibition zone is caused by the content of secondary metabolite compounds in the ethanol extract of *Kayu racun* leaves.

The diameter of the inhibition zone that was formed increased with the increase in the concentration of the *Kayu racun* leaf extract. This is because the increase in concentration is also followed by an increase in the content of active compounds contained in the extract. In addition, it is also influenced by the ability of active compounds to diffuse. Ariyanti et al., (2012) stated that the diameter size of the inhibition zone is influenced by the diffusion rate of the antibacterial agent. Diffusion of solutes from higher concentrations is faster, compared to lower concentrations. So that the growth of the test bacteria around the extract is stunted.



a b

Figure 3. The best concentration of ethanol extract of *Kayu racun* leaf (R.*nasutus*) 100 mg/ml against *S.aureus* (a) and MRSA (b).
U = repetition, A= amoxicillin 1 mg/ml

Figure 3 shows the antibacterial activity of the ethanol extract of *Kayu racun* leaves at the best concentration of 100 mg/ml with the highest average inhibition power reaching 26.20 mm against *S.aureus* which is classified as strong and 17.90 mm against MRSA which is in the moderate category.

Cavalieri et al., (2005) classified three categories based on the diameter of the inhibition zone; if the diameter ≤10 mm is categorized as resistant, between 11-19 mm is categorized as moderate, while diameter ≥20 mm is categorized as not resistant (strong).

The inhibition zone formed was greater in *S. aureus* test bacteria compared to MRSA. This is because MRSA is an infection-causing bacteria originating from an *S. aureus* strain that is resistant to several antimicrobial groups. MRSA is a strain of *S. aureus* bacteria that is resistant to all β-lactam and other antibiotics such as all Penicillin and Methicillin derivatives as well as broad-spectrum antimicrobial beta-lactamase (Okwu et al., 2019; Pawar et al., 2020).

In the positive control (K +) with Amoxicillin, a concentration of 1 mg/ml inhibition was formed 9.35 mm *against S. aureus* and 11.18 mm against MRSA. When compared to the inhibition zone of *Kayu racun* leaf ethanol extract with K (+) mathematically, it can be seen that the

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K (+) inhibition zone is equivalent to 43 times the inhibition zone of the extract at the same concentration (1 mg/ml).

Soemarie et al., (2018) reported the results of the phytochemical test of the ethanol extract of Selutui puka leaves (*Tabernaemontana macrocarpa* Jack) known to contain alkaloids, flavonoids, tannins, saponins, and the results of the test results for the antibacterial activity of the ethanol extract of selutui puka leaves against *S. aureus* shows the inhibition of 6,09 mm, 6,24 mm and 6,25 mm at concentrations of 5%, 10% and 15%, included in the moderate inhibition category.

This shows that the use of different types of medicinal plants contains different secondary metabolites with different abilities to inhibit the growth of the tested bacteria.

CONCLUSION

Based on the research that has been done, conclusions can be drawn; *Kayu racun* leaf extract contains alkaloids, phenols, and flavonoids which contribute to its antibacterial properties. The extract concentration of 100 mg/ml showed the best antibacterial activity with an inhibition zone diameter of 26.20 mm against *S. aureus* and 17.90 mm against MRSA.

The suggestion for further research is to isolate the active compound of *Kayu racun* leaves as a new antibacterial source in inhibiting *S. aureus* and MRSA.

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