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Characteristics of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) and their inhibitory response by ethanol extract of *Abrus precatorius*

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Abstract. *Mutmainnah BQ, Baktir A, Ni'matuzahroh. 2020. Characteristics of Methicillin-Resistant Staphylococcus aureus (MRSA) and Methicillin Sensitive Staphylococcus aureus (MSSA) and their inhibitory response by ethanol extract of* Abrus precatorius. *Biodiversitas 21: 4076-4085.* Three isolates of *Staphylococcus* bacteria with the code MRSA 22372, MSSA 22187 and MSSA 22366 originated from the urine of the patient at Dr. Regional General Hospital Soetomo, Clinical Microbiology Installation Surabaya, Indonesia. Differences in bacterial strains affect their sensitivity to antimicrobial agents. The active ingredient of the ethanol extract of the leaves of *Abrus precatorius* L. has the potential to inhibit bacterial growth. This study aims to further characterize the bacteria MRSA 22372, MSSA 22187 and MSSA 22366 based on morphological, biochemical and molecular characters and to compare the growth inhibitory response of these three bacteria due to the treatment of variations in the ethanol extract of *A. precatorius* of 25 mgL^{-1} - 800 mgL^{-1} leaves. The results showed there were differences in the diameter of bacterial colonies, the ability to ferment glucose and sucrose, and the production of urease and catalase. The molecular characteristics of the three bacteria have no similarity in the order of nucleotide bases or phylogenetic proximity to each other. Ethanol extract of *A. precatorius* leaves at a concentration of 800 mgL^{-1} inhibited the growth of MSSA 22187 with an inhibition zone of 41 mm and decreased the MSSA 22366 growth by 67.6%. MIC value of ethanol extract of *A. precatorius* leaves in all three bacteria was 25 mgL^{-1} with growth inhibition up to 29.4%, 35.3%, and 29.4% respectively.

Keywords: MRSA, MSSA, ethanol extract, *Abrus precatorius*

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogenic bacterium. It is found on the surface of the skin and mucosal surfaces in several human organs (Brooks and Jefferson 2012). Sakr et al. (2018) state that *S. aureus* bacteria colonize healthy individuals by 30-50% and persistently persist in those individual bodies by 10-20%. Infection that occurs in hospitals by 39-60% is a urinary tract infection (UTI) caused by the use of a catheter (Rowe and Manisha 2013). (Najar et al. 2009) states that 80% of urinary tract infections (UTIs) occur due to instrumentation by catheterization. Urinary tract infections can affect patients of all ages, with a prevalence of 5-10% in old age. (Flores-Mireles et al. 2015). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the causes of disease in humans ranging from skin infections to serious invasive infections such as pneumonia, regenerative soft-tissue infections, heart valves, and septicemia (Tong et al. 2015). MRSA infections are caused by a rise in antimicrobial resistance in the *S. aureus* river because of poor infection control and widespread use of antibiotics (Neyra et al. 2014). MRSA infection prevalence is increasing, and these infections cause more death than 40% of bacterial infections (Melzer and Welch 2013).

An increase in cases of UTIs related to the catheter was also followed by an increase in the use of antibacterial to overcome the infection. UTI treatment using appropriate and rational antibacterial can reduce the cost of treatment, prevent further complications of urinary tract infections, and prevent resistance to various antibacteria (Flores-Mireles et al. 2015). *S. aureus* was found to be resistant to penicillin class drugs and their derivatives such as methicillin (Mohammad et al. 2017). In Asia, the incidence of Methicilin Resistant *Staphylococcus aureus* (MRSA) infections reached 70% (Chen and Huang 2014). The use of antibiotics in a long time can increase the number of mutations or recombination of gene structures that occur in bacterial cells, thus forming a new generation of resistant bacteria (Peterson and Kaur 2018). Bacteria MRSA 22372, MSSA 22187, and MSSA 22366 are bacteria isolated from the patients urine at the Clinical Microbiology Installation, Dr. Soetomo Regional General Hospital, Surabaya, Indonesia. Different strains of *S. aureus* can produce varying results of activity, thus causing different inhibition of antibacterial abilities.

The preparation of natural medicines as a national cultural heritage of the Indonesian people is felt the more involved in the pattern of community life in terms of life and the economy. The public is increasingly accustomed to using natural preparations and increasingly believes in their

benefits for health. Sheikh et al. (2012) state that the use of plant extracts that have antimicrobial activity is very helpful in healing. One of the plants that have the ability as an antibacterial is *Abrus precatorius* L. *A. precatorius* is used as a phlegm thinner (mucolytic); indicative medicine for the prevention and cure of thrush, sore throat and inflammation of the tonsils; and antibacterial (Chaudhari et al. 2012; Garaniya and Bapodra 2014). *A. precatorius* contains flavonoids, terpenoids, tannins, alkaloids, and saponins which have the potential as natural antibacterial agents for the treatment of strep throat (Gnanavel and Saral 2013).

The compounds contained in *A. precatorius* plants are not only entirely polar compounds, but there are also non-polar or semi-polar and lipophilic compounds. Ethanol, ethyl acetate and n-hexane solvents are organic solvents that are widely used in the extraction process, which can dissolve flavonoid compounds, saponins, flavonoid aglycones, steroids and others (Hossain et al. 2013).

Ribka (2015) reports that ethanol extract of *A. precatorius* L. leaves had antibacterial activity in *S. aureus* of 0.093 mm at a concentration of 0.6%. Ethyl acetate fraction of ethanol extract of *A. precatorius* inhibits the growth of *S. aureus* ATCC (Ernawati 1998). Based on Mutmainnah and Ni'matuzahroh's (2017) research on the ethyl acetate extract of *A. precatorius* which inhibits the growth of MRSA 22372, it is expected that the ethanol extract of *A. precatorius* also has the ability to inhibit the growth of MRSA 22372, MSSA 22187, and MSSA 22366.

This study aims to identify the bacteria MRSA 22372, MSSA 22187, and MSSA 22366, and to compare inhibitory response by ethanol extract of *A. precatorius* at various concentrations to the three bacteria. By knowing the characteristics of the bacteria, it can be ascertained the type of strain of the genus *Staphylococcus* tested. In addition, the total flavonoid compounds contained in the ethanol extract of *A. precatorius* leaves are expected to inhibit the bacteria MRSA 22372, MSSA 22187, and MSSA 22366. Thus, the ethanol extract of *A. precatorius* leaves containing flavonoid compounds can be used as a lead compound for the development of alternative antimicrobials in controlling *S. aureus* infections.

MATERIALS AND METHODS

Materials

Staphylococcus aureus strain MRSA 22372, MSSA 22187, and MSSA 22366 were obtained from urine from three patients. Three bacterial strains are a collection of bacteria from the Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. These bacteria are isolated from the urine of patients who are resistant and sensitive to antibiotics. *A. precatorius* leaf plants were obtained from Sumenep, East Java, Indonesia.

Methods

Morphological characteristics of bacteria

Macroscopic characteristics of bacterial colonies include shape, elevation, edge, diameter, and color (Thairu 2014). While, microscopic characters of bacterial cells are carried out by Gram staining (Thairu 2014).

Biochemical characteristics of bacteria

Biochemical tests with Microbact^{TM} Identification kit were used to determine the physiological characteristics of Gram bacteria, so that genera and types of bacteria were known. Biochemical tests consisted of carbohydrate fermentation (glucose, lactose, mannitol, sucrose, xylose, rhamnose, arabinose, raffinose, and ortonitrophenyl-ß-d-galactopyranoside), oxidase (oxidase strips), motility (Sulfide Indole Motily), nitrate reduction, catalase, urease, indole, Voges Proskauer (VP), citric, sulfuric acid (H_2S), lysine, hydrolysis of gelatin, ornithine, malonic, Tryptophan Deaminase (TDA), inositol, sorbitol, adonitol, salicin, and arginine. The format was in the form of a simple test strip or micro-plate and the results were clearly seen as different color reactions that could be interpreted using Microbact. Each kit consisted of 12 (12A, 12B) miniature biochemical tests. The identification of organisms was based on changes in pH and substrate use. Identification of Gram-positive bacteria used Bergey's Manual of Determinative Bacteriology Ninth Edition (Holt et al. 2000).

16S rRNA gene PCR

The bacteria MRSA 22372, MSSA 22187, and MSSA 22366 were grown on Trypticase Soy Broth (TSB) (Merck, Germany). Two bacteria colonies of each were taken and transferred on TSB medium 5 mL and incubated at 37 °C for 24 h. About 125 µL the bacterial suspension was flattened on the FTA Card (Whatman International). The sample was dried at room temperature for 60 minutes and stored until it was ready for use. FTA discs (6 mm diameter) from dried bacterial samples impregnated on FTA cards were punched out using a Harris MicroPunch (Fitzco Inc., MN, USA), and the paper discs transferred to individual 1.5 mL microtubes. The Harris MicroPunch was cleaned during each punching by rinsing the tip with 70% industrial methylated alcohol to minimize cross-contamination of bacterial samples. Each disc was rinsed twice with 200 µL of FTA purification reagent (Whatman) and finally rinsed once with 200 µL of TE buffer (10 mM TRIS, 1 mM EDTA, pH 8.0). The TE buffer was removed and the tubes were centrifuged briefly at 16.000× g, and the remaining buffer was removed by pipetting. The FTA discs were dried at 55 °C for 15 min on a heating block, and the dry discs were transferred to individual 0.2 ml PCR amplification tubes. Amplification of 16S rDNA was carried out separately using two sets of primers to amplify two different fragment sizes (Tabel 1). Each PCR amplification was performed in a reaction volume of 50 µL consisting of a single 6 mm FTA disc immobilized with bacterial DNA, 25 µL PCR ready mix (Toyobo, Japan), 22 µL of nuclease-free water and 1 µL of each of the forward and reversed primers (10 pmol μL^{-1} each) (synthesized by MWG Biotech). A water negative control was also used for each PCR reaction. Amplification conditions for PCR were 5 min at 96 °C to denature the DNA, followed by 35 cycles of denaturation at 96°C for 45 seconds, primer annealing at

58 °C for 30 seconds and strand extension at 72 °C for 2 min on a Rotorgene thermal cycler.

The results of DNA isolation were measured for absorbance values at wavelengths of 260 and 280 nm. Calculation of DNA purity was calculated by the following formula (Lucena-Aguilar et al. 2016).

$$\text{The purity of DNA} = \frac{A_{260}}{A_{280}}$$

DNA concentration calculation was done by measuring the absorbance value of DNA isolation at a wavelength of 260 nm. DNA concentration was calculated by the following formula.

$$\text{DNA double strand concentration } \left(\frac{\mu g}{mL}\right) = A_{260} \text{ x dilution factor x 50 } \mu\text{g/mL}$$

After PCR, 2 µL of each of the PCR products were separated by gel electrophoresis using 0.8% (w/v), 10 cm horizontal agarose gels at 65 V for 45 min in 0.5×TBE running buffer (50 mmol L^{-1} Tris, 45 mmol L^{-1} boric acid, 0.5 mmol L^{-1} EDTA, pH 8.4). A 100 bp DNA molecular marker (Promega) was included for band size determination of PCR products. The gels were stained with ethidium bromide, visualized under UV transilluminator and photographed using a Syngene gel documentation system.

Analysis of bacterial phylogenetic trees

Phylogenetic trees were constructed using the 'neighbor-joining method' (Li 2015). In order to evaluate the robustness of the inferred trees, a bootstrap analysis consisting of 100 resamplings of the data was performed using Clustal W and a consensus tree was generated using neighbor-joining and the program MEGA 6.06 (Tamura et al. 2013).

Extraction of Abrus precatorius *leaves*

The conventional extraction of 30 g simplicia *A. precatorius* leaves was carried out by mixing 3000 mL of distilled ethanol in a round bottom flask and refluxed for about 5 hours. The liquid extract is obtained, separated from the solid residue by vacuum filtration, and concentrated using a rotary evaporator.

Inhibition of bacteria by using disc diffusion method

The crude ethanol extracts of *A. precatorius* were tested for antimicrobial activity using the disc diffusion method (Kirby-Bauer method) (Bauer et al. 1966). Sterile commercial blank discs (Oxoid), 6.0 mm diameter were impregnated with different dilutions of the extracts ranging from 800 mgL^{-1}/disc to 25 mgL^{-1}/disc. Extract-impregnated discs (50 µL) were placed on agar plates and incubated at 37°C for 24 hours. Aquadest (50 µL) was used as a negative control, while erythromycin discs (50 µL) were used as a positive control. Some antibiotics had been also tested such as gentamycin, penicillin G, oxacillin, cotrimoxazole, tetracycline, erythromycin, quinupristin-dalfopristin, ciprofloxacin, levofloxacin, fosfomycin, nalidixic acid, nitrofurantoin, meropenem, linezolid, daptomycin, ampicillin-sulbactam, ampicillin, chloramphenicol, and methicillin disc (50 µL) to sensitivity of antibiotic administration in MRSA bacteria 22372, MSSA 22187 and MSSA 22366. Antibacterial activities were then determined by measuring the clear zone of inhibition to the nearest millimeter (mm) ± S.E.M. The test was carried out in 3 (three) replications. Data were analyzed using SPSS 21.0 software (IBM Corp. 2012). Data were analyzed by a one-way ANOVA, followed by the Tukey HSD post-hoc test.

Inhibition of bacteria by using dilution method

The antibacterial activity of the ethanol extract of *A. precatorius* was determined by the agar dilution method described by Balouiri et al. 2016. Different concentrations of the extract ranging between 25 mgL^{-1} and 800 mgL^{-1} were prepared in molten Trypticase Soy Agar (TSA) maintained in a water bath at 50°C and used for the agar dilution assay. One hundred microlitres (100 µL) of the standardized bacterial cultures were aseptically dispensed and spread evenly on the agar plates. Other blank plates containing only TSA served as negative controls. Plates were incubated aerobically at 37°C for 24h. Each test was done in triplicate, and any test agar plate lacking visible growth was considered the minimum inhibitory concentration of the extract. Data were analyzed using SPSS 21.0 software (IBM Corp. 2012), by a one-way ANOVA, and followed by the Tukey HSD post-hoc test. Calculation of the number of living bacterial cells (CFU/mL) using the following formula (Hazan et al. 2012).

$$\text{Number of living bacterial cells (CFU/mL)} = \text{number of colonies } x \frac{1}{10^{-6}} \text{ x } 10$$

Tabel 1. Primer sequences used in this study

Primer	Sequence	Reference
8F	5'-AGAGTTTGATCCTGGCTCAG-3'	Edwards et al. (1989)
1522R	5'- AAGGAGGTGATCCAACCGCA-3'	Suzuki and Giovannoni (1996)

Determination of MIC's of the effective plant extract

Minimum inhibitory concentration (MIC) defined as the lowest concentration which resulted in maintenance or reduction of inoculums' viability was determined by serial tube dilution technique for the bacterial isolates. Different concentrations (25-800) mgL^{-1} of the crude extract and 50 mgL^{-1} of erythromycin were differently prepared by serial dilutions in the Trypticase Soy Broth (TSB) medium. Each tube was then inoculated with 100 µL of each of the adjusted bacterial strains. Two blank TSB tubes, with and without bacterial inoculation, were used as the growth and sterility controls. The bacteria-containing tubes were incubated aerobically at 37 ℃ for 24h. After the incubation period, the tubes were observed for the MICs by checking the concentration of the first tube in the series that showed no visible trace of growth. The first tube in the series with no visible growth after the incubation period was taken as the MIC.

RESULTS AND DISCUSSION

Morphological and biochemical characters

Bacterial isolates of MRSA 22372, MSSA 22187, and MSSA 22366 have almost the same morphological characters but only different in colony diameter (Table 1 and Figure 1). The bacterial colony MRSA 22372 had colony sizes of 5 to 7 mm. The diameter of bacterial colonies of MSSA 22187 and MSSA 22366 are 3-4 mm and 4-6 mm, respectively.

Gram staining of three bacteria MRSA 22372, MSSA 22187, and MSSA 22366 on TSA media showed purple-colored and circularly shaped bacteria clustered like grapes. Morphology of bacterial cells in the form of Gram-positive, coccus-shaped arranged in groups of irregular (like grapes), four-four (tetrad), a chain of three-four cells, in pairs or one at a time. After 31 biochemical characters test, isolates MRSA 22372, MSSA 22187, and MSSA 22366 only different on glucose mannitol and sucrose fermentation, urease, and catalase enzyme productions (Table 2). MRSA 22372 had the ability to ferment glucose, mannitol, and sucrose. In MSSA 22187, it only fermented sucrose. Meanwhile, the MSSA 22366 bacteria did not experience carbohydrate fermentation. In the three bacteria MRSA 22372, MSSA 22187 and MSSA 22366 had the urease enzyme. MSSA 22187 bacteria produced more urease enzymes than the two bacteria tested. Urease breaks down nitrogen and bind carbon in compositions such as amides and make ammonia final products. Ammonia will form an alkaline environment that can cause the pH of the media to become alkaline so that a change from yellow to purple (Cappuccino and Sherman 2011).

Table 2. Identification of MRSA 22372, MSSA 22187 and MSSA 22366 bacteria using the Microbact™ Identification 12A kit; and Bergey's Manual of Determinative Bacteriology, Ninth Edition (Holt et al. 2000)

Type of test	Specimen code		
	MRSA 2372	**MSSA 22187**	**MSSA 2366**
Shape	coccus	coccus	coccus
Gram	+	+	+
Oxidase	-	-	-
Motility	-	-	-
Nitrate	+	+	+
Lysine	-	-	-
Ornitine	-	-	-
H_2S	-	-	-
Glucose	+	-	-
Mannitol	+	-	-
Xylose	-	-	-
ONPG	+	+	+
Indole	-	-	-
Urease	+	++	+
VP	-	-	-
Citric	-	-	-
TDA	-	-	-
Gelatine	-	-	-
Malonate	-	-	-
Inositol	-	-	-
Sorbitol	-	-	-
Rhamnose	-	-	-
Sucrose	+	+	-
Lactose	-	-	-
Arabinose	-	-	-
Adonitol	-	-	-
Rafinose	-	-	-
Salicin	-	-	-
Arganine	-	-	-
Catalase	+	-	++
Spore	-	-	-

Table 1. Morphological characters of bacterial colonies of MRSA 22372, MSSA 22187 and MSSA 22366

Characters	Specimen code		
	MRSA 22372	**MSSA 22187**	**MSSA 22366**
Colony shape	Round	Round	Round
Pigmentation of the colony	Yellow and white	Yellow and white	Yellow and white
Colony diameter	5 mm - 7 mm	3 mm - 4 mm	4 mm - 6 mm
Cell shape	Coccus	Coccus	Coccus
Elevation	Convex	Convex	Convex
Edge	Smooth	Smooth	Smooth
Gram staining	Positive	Positive	Positive
Cell arrangement	Clustered	Clustered and *diplococcus*	Clustered and *diplococcus*

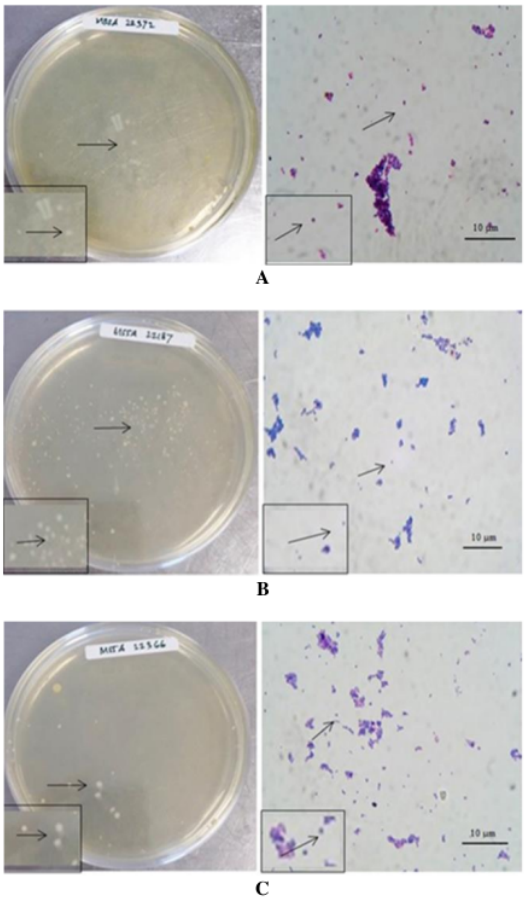

Figure 1. Morphological characters of colony and cells of MRSA 22372, MSSA 22187, and MSSA 22366. A. MRSA 22372, B. MSSA 22187, C. MSSA 22366 coccoid clusters as the best hit of *Staphylococcus*. Insert shows an enlarged image

Catalase test results on three bacteria that grew on TSA media showed that MRSA 22372 and MSSA 22366 had a positive reaction, whereas on MSSA 22187 had a negative reaction. Toelle and Lenda (2014) stated that positive catalase is shown by the presence of gas bubbles (O_2) produced by the genus *Staphylococcus*. *Staphylococcus sp*. uses catalase to protect from hydrogen peroxide (H_2O_2) by converting it to water and oxygen (Locke 2013). Hydrogen peroxide is toxic to cells because it activates enzymes in cells. Hydrogen peroxide is formed during aerobic metabolism, so microorganisms that grow in an aerobic environment must decompose the material (Ślesak et al., 2016).

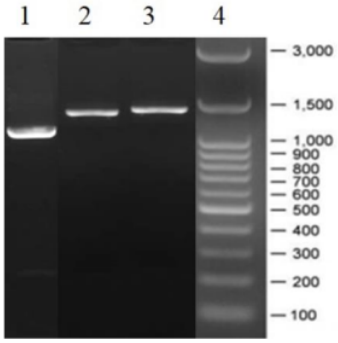

Figure 3. Electrophoresis of the amplification of 16S rRNA gene encoding for MRSA 22372, MSSSA 22187, and MSSA 22366 bacteria with primers 8F and 1522R at 72 °C. The rows of 1, 2, and 3, respectively, are the DNA bands of the 16S rRNA MRSA 2272, MSSA 22187, and MSSA 22366 gene encoding at 1426 bp, 1407 bp, and 1427 bp. Lane 4 is a marker of 100 bp DNA ladder

Phylogenetic tree

The results of the 16S rRNA gene encoding process of MRSA 22372, MSSA 22187, and MSSA 22366 can be amplified respectively of 1426 bp, 1407 bp, and 1427 bp at 72 °C annealing temperature (Figure 3).

The linear PCR method recovered almost full-length 16s rRNA gene sequences (1407-1427 nucleotides) for the three strains. The phylogenetic tree (Figure 4) demonstrated that three of the bacteria were not found to be closely related to each other. The MSSA 22187, MRSA 22372, and MSSA 22366 were not in one branch, one genus and one species (Table 4). This indicated that MSSA 22187, MRSA 22372, and MSSA 22366 had no similarity between the nucleotide base sequence and phylogenetic proximity to each other.

The bacteria MRSA 22372, MSSA 22187, and MSSA 22366 had the same root (ancestor) but undergo different changes from one another when they evolve. These three bacteria were not new bacterial species because the homology values of the three isolates are 99-100%. Větrovský and Baldrian (2013) stated that new bacterial species can be said to be in one genus-group with bacteria that are already in the Genbank data if they have homology sequences of 16S rRNA genes with values between 97-99%. If the homology value of the 16S rRNA gene sequence is less than 97%, then the bacteria cannot be called a new bacterium nor is it classified as a different genus of bacteria.

Inhibitory performance

Extraction of A. precatorius

Abrus precatorius leaf was extracted by maceration method using ethanol as solvent. During the maceration process, a diffusion process occurred. This process takes place until there is a balance between the solution that is inside and outside the plant cell. After successful completion, the diffusion process no longer runs (Khaw et al. 2017). The result of extraction with 96% ethanol solvent was obtained 39.86% of yields.

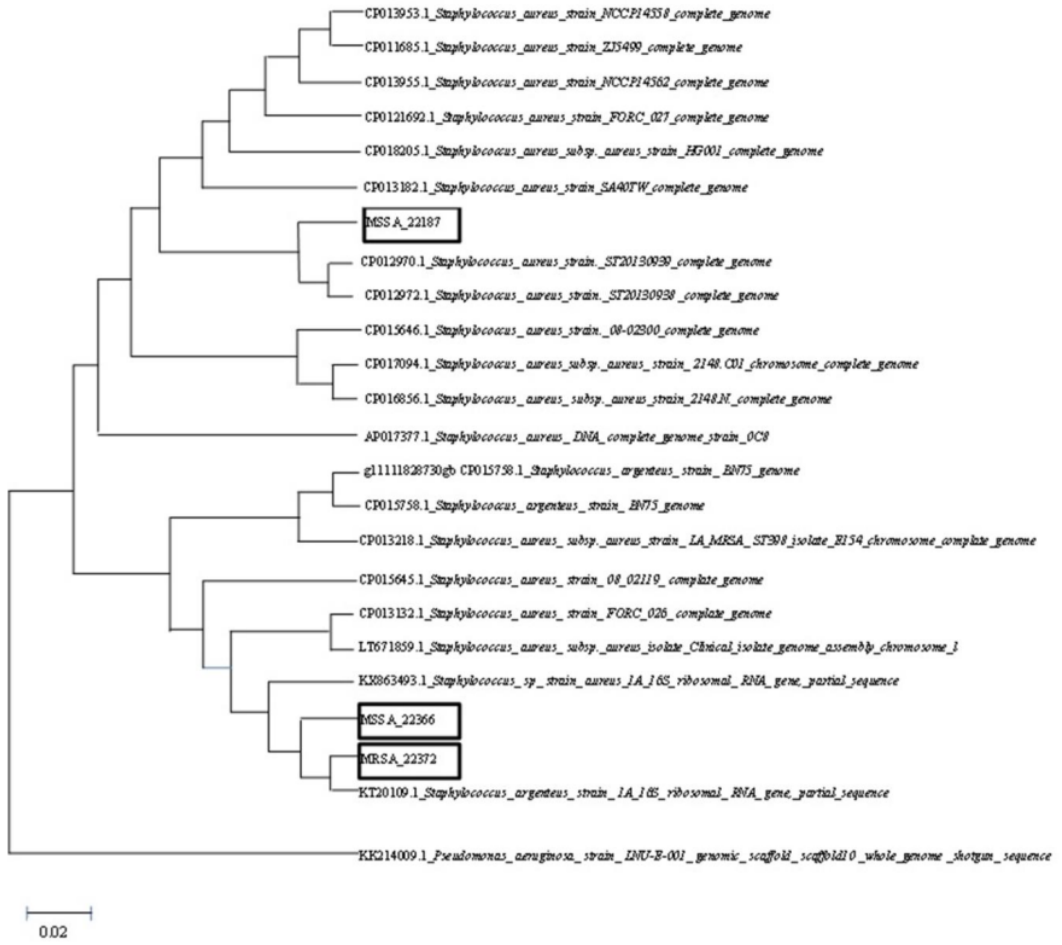

Figure 4. Relationship between the three bacteria of MRSA 22372, MSSA 22187, and MSSA 22366 by making phylogenetic trees and the position of these bacteria in several bacteria in GenBank. *Pseudomonas aeruginosa* strain LNU-E-001 genomic scaffold10, whole genome shotgun sequence was used as the out-group. The scale bar indicates 0.002 substitutions per nucleotide position

Inhibition of ethanol extract Abrus precatorius *leaves*

Antibacterial results by the disk-diffusion method (Kirby Bauer) showed that ethanol extract of *A. precatorius* leaves containing flavonoid compounds gave different results on the inhibition of test bacterial growth. This was proven by the presence of different inhibition zones in the bacteria tested (Table 5). The zone of inhibition of bacterial growth decreases in proportion to the decrease in the concentration of ethanol extract of *A. precatorius* leaves containing flavonoid compounds. This was due to the reducing content of bioactive compounds in ethanol extract of *A. precatorius* which was diluted. Increasing the amount of solvent used can reduce the number of active compounds in the extract, so the smaller the extract's ability to inhibit bacterial growth. Bacterial inhibition zone formed had varying sizes. Bacterial inhibition zone with a concentration of 800 mgL^{-1} was obtained at MSSA 22187 at 41 mm and a concentration of 50 mgL^{-1} was found at MSSA 22366 at 9 mm. Aquadest as negative control did not have antibacterial activity. This means that the antibacterial ability of the *A. precatorius* ethanol extract containing flavonoid compounds is not affected by water as the solvent for the active compound. The inhibition of the growth of the three test bacterial strains by *A. precatorius* ethanol extract was greater than the positive control. This showed that the ethanol extract of *A. precatorius* was potential in inhibiting the test bacteria because the diameter of the bacterial inhibition zone formed in the treatment was greater than that of erythromycin.

Tukey HSD analysis showed that ethanol extract of *A. precatorius* leaves on MRSA 22372 bacteria at concentrations between 800 mgL^{-1} and other concentrations had a significant difference in the formation of test bacteria inhibitory growth zones. The concentration of 100 mgL^{-1} and erythromycin showed no significant difference. It can be concluded that the concentration of ethanol extract of *A. precatorius*.L at 100 mgL^{-1} and erythromycin showed the same inhibitory effect on the growth of MRSA 22372. Likewise, between 25 mgL^{-1} concentration and distilled water did not show significant differences, which indicated the same inhibitory effect on MRSA bacterial growth 22372. The same inhibitory response was also found in testing of two other bacterial isolates, namely in MSSA 22187 and MSSA 22366 bacteria.

The sensitivity of active compounds in inhibiting bacterial growth was also evaluated based on the Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI 2012). By using erythromycin positive control, the minimum inhibitory zone that must be achieved by the active compound in ethanol extract of *A. precatorius* L can be said to be sensitive, to more than, or equal to 21 mm. In this study, the mean inhibition zone of *A. precatorius* leaves ethanol extract at a concentration of 200 mgL^{-1} was 21 mm. This proves that the active compound in this ethanol extract is sensitive in inhibiting the growth of the three test bacteria when compared with the erythromycin as criteria standard.

The sensitivity of the three bacteria MRSA 22372, MSSA 22187, and MSSA 22366 to various types of antibiotics has also been carried out (Table 3). It was found that bacterial isolates had resistance and were sensitive to the antibacterial tested. The three test bacteria that cause UTIs associated with catheters were resistant to penicillin G, tetracycline, nalidixic acid, and meropenem. This was due to the possibility that this antibacterial is a first-line antibacterial for treating UTI-related catheter cases. Resistance to antibiotics arises because of the presence of antibacterial exposure that is not optimal so that bacteria become resistant. Antibacterial resistance can occur due to various things, including changes in targets, antibacterial inactivation, decreased permeability of bacterial cell walls, blockade of antibacterial entry points, and changes in bacterial metabolic pathways (Köves et al. 2017).

The results of the antibacterial test by the dilution method evaluated by observing the number of living cells at the end of the treatment can be seen in Figure 5. The number of live bacterial cells decreased proportionally with an increase in the concentration of ethanolic extract of *A. precatorius* leaves added. Percent decrease in the number of living bacteria after administration of *A. precatorius* ethanol extract with a concentration of 800 mgL-1 in MSSA 22366 was 67.6%. Whereas, the treatment with a concentration of 25 mgL^{-1} in MSSA 22366 and MRSA 22372 was 29.4%.

Variations in the concentration of ethanol extract of *A. precatorius* L leaves affect the growth of MRSA 22372, MSSA 22187, and MSSA 22366. Tukey test results show that in all three test bacteria, variations in the ethanol extract concentration of *A. precatorius* leaves, L give a significant difference to the number of bacterial colonies. MIC values of *A. precatorius* leaf ethanol extract on the three test bacteria were obtained at a concentration of 25 mgL^{-1} with percent inhibition of bacterial growth reaching 29.4%, 35.3%, and 29.4% respectively.

This research showed that the ethanol extract of *A. precatorius* leaves containing flavonoid compounds can inhibit the growth of MRSA 22372, MSSA 22187, and MSSA 22366 and had potential as an antimicrobial alternative to Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA). Ethanol extract of *A. precatorius* containing flavonoids (Gupta and Amit 2016) can inhibit nucleic acid synthesis, cell membrane function, and energy metabolism (Hendra et al. 2011). Flavonoids that inhibit the synthesis of nucleic acids are rings A and B that play a role in the process of interconnection or hydrogen bonding by accumulating nucleic acid bases that inhibit the formation of DNA and RNA. Hydroxyl groups located in positions 2', 4' or 2', 6' are hydroxylated on ring B and 5, 7 hydroxylated on ring A plays an important role in the antibacterial activity of flavonoids. Flavonoids will cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes (Tagousop et al. 2018). Flavonoids inhibit the function of cell membranes by forming complex compounds with extracellular and dissolved proteins that can damage the bacterial cell membrane and are followed by the release of intracellular compounds (Mierziak 2014), and interferes with the permeability of cell membranes and inhibits the binding of enzymes such as ATPase and phospholipase (Epand et al. 2016). Flavonoids inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Flavonoids inhibit cytochrome C reductase so that metabolic formation is inhibited. Bacteria need energy for macromolecular biosynthesis (Kempes 2017).

Table 5. Bacterial inhibition zones of ethanol extract of *A. precatorius* leaves with various treatment concentrations using the disc -diffusion method

Treatment concentration and control (mgL^{-1})	mm ± S.E.M		
	MRSA 22372	MSSA 22187	MSSA 22366
800	31 ± 0.58^{a}	41 ± 0.58^{a}	30 ± 0.58^{a}
400	27 ± 1.00^{b}	24 ± 0.58^{b}	26 ± 0.58^{b}
200	21 ± 0.58^{c}	22 ± 0.58^{c}	23 ± 1.00^{c}
100	17 ± 1.00^{d}	20 ± 0.58^{d}	19 ± 0.58^{d}
50	10 ± 1.00^{e}	11 ± 0.58^{f}	9 ± 0.58^{f}
25	0^{f}	0^{g}	0^{g}
K (-)	0^{f}	0^{g}	0^{g}
K (+)	16 ± 0.58^{d}	15 ± 0.58^{e}	17 ± 0.58^{e}

Note: the diameter of the disc assay: 6 mm and thick 1 mm, k (-): aquadest and k (+): erythromycin (15 μg). $^{a, b, c, d, e, f}$ different letters indicate a significant difference at $p<0.05$

Table 3. Sensitivity of antibiotic administration in MRSA 22372, MSSA 22187 and MSSA 22366 bacteria

Specimen code	Type of antibiotic																		
	GM	P	OX	CTZ	TE	E	SYN	CIP	LVX	FOS	NA	FD	MEM	LNZ	DAP	SAM	AM	C	MET
MRSA 22372	R	R	R	R	R	S	R	R	R	S	R	R	R	S	S	-	-	I	R
MSSA 22187	R	R	R	R	R	R	S	R	R	S	R	S	R	-	S	R	R	-	S
MSSA 22366	S	R	S	S	R	R	S	-	-	S	R	S	R	S	S	S	R	-	S

Note: MRSA: Methicillin-Resistant *Staphylococcus aureus*, MSSA: Methicillin Sensitive *Staphylococcus aureus*, R: resistant, S: sensitive, I: intermediate, GM: gentamycin, P: penicillin G, OX: oxacillin, CTZ: cotrimoxazole, TE: tetracyclin, E: erythromycin, SYN: quinupristin-dalfopristin, CIP: ciprofloxacin, LVX: levofloxacin, FOS: fosfomycin, NA: nalidixic acid, FD: nitrofurantoin, MEM: meropenem, LNZ: linezolid, DAP: daptomycin, SAM: ampicillin-sulbactam, AM: ampicillin, C: chloramphenicol, and MET: methicillin

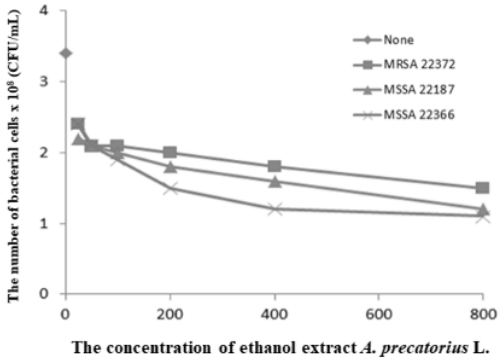

Figure 5. Inhibition of MRSA 22372, MSSA 22187 and MSSA 22366 bacteria at various concentrations of ethanol extract *A. precatorius* leaves

There was a decrease in the number of colonies in MRSA 22372, MSSA 22187, and MSSA 22366 after treatments of ethanol extract of *A. precatorius* leaves due to the presence of total phenolic and flavonoid compounds. The ethanol extract of *A. precatorius* leaves has higher inhibition of *S. aureus* compared to previous studies. (Ribka 2015) reported that ethanol extract of *A. precatorius* leaves had antibacterial activity on *S. aureus* of 0.093 mm at a concentration of 6000 mgL^{-1}. Ethyl acetate extract of *A. precatorius* can inhibit the growth of MRSA 22372 by 21 mm at a concentration of 800 mgL^{-1} (Mutmainnah and Ni'matuzahroh 2017). Ethanol extract of *A. precatorius* can inhibit the growth of *S. aureus* by 21 mm at a concentration of 1.000.000 mgL^{-1} (Mutmainnah and Ni'matuzahroh 2017). Ernawati (1998) also reported that the ethyl acetate fraction of *A. precatorius* leaf ethanol extract inhibited the growth of *S. aureus* ATCC. The mechanism of action of flavonoids as antimicrobials can be divided into 3 (three), namely inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism (Hendra et al. 2011).

All three bacteria have the ability to ferment glucose and sucrose under anaerobic conditions as an energy source for growth. They can hydrolyze urea, produce ammonia, and carbon dioxide, also produce hydrogen peroxide which can cause cell death, during aerobic respiration. The three bacteria showed a comparison that the close relationship was based on genetic distance (0.02) and similarity (83%). *Pseudomonas aeruginosa* strain LNU-E-001 genome scaffold10, all genomic rifle sequences have the farthest kinship which is an outgroup in phylogeny with genetic distance values (0.267) and similarity values (77%). The three bacteria gave different inhibitory responses after being exposed to ethanol extract of *A. precatorius* ethanol leaves containing flavonoids. Inhibition method used is a standard method of diffusion and dilution test, so it is ensured to produce accurate data. The ethanol extract of *A. precatorius* leaves used in this test is still in the form of crude extracts. Concentrations of 25 mgL^{-1} to 800 mgL^{-1} make a difference to the inhibition and growth of test bacteria.

This study provides information about the certainty of the strains of the bacteria MRSA 22372, MSSA 22187, and MSSA 22366 which were isolated from the urine of patients at the Regional General Hospital Dr. Soetomo, Clinical Microbiology Installation, Surabaya - Indonesia through morphological, biochemical and genetic characteristics using 16sRNA. The different strains in the three bacteria also gave a different sensitivity to the antimicrobial material from the ethanol extract of *A. precatorius* leaves.

Utilization of *A. precatorius* leaves as an antibacterial raw material is very prospective for use in the community. The existence of abundant *A. precatorius* L local plants in Indonesia will be able to guarantee the sustainability of the availability of raw materials for the production process.

In this study, it was concluded that the results of morphological, biochemical and genetic characterization of three bacterial isolates from the urine of patients led to *Staphylococcus* sp. the 16S gene sequence of the RNA ribosome gene, *Staphylococcus aureus* strain SA40TW genome complete, and *Staphylococcus argenteus* strain 1A_1 16S ribosomal RNA. Ethanol extract of *A. precatorius* has promising antibacterial activity by inhibiting the growth of MRSA 22372, MSSA 22187, and MSSA 22366.

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