[biodiv] Editor Decision

3 messages

Smujo Editors <smujo.id@gmail.com> Thu, May 14, 2020 at 3:45 PM
Reply-To: Smujo Editors <editors@smujo.id>
To: NI'MATUZAHROH NI'MATUZAHROH <nimatuzahroh@fst.unair.ac.id>, AFAF BAKTIR <afaf-b@fst.unair.ac.id>, "BQ. MUTMAINNAH" <bmmasadepan9@gmail.com>

NI'MATUZAHROH NI'MATUZAHROH, AFAF BAKTIR, BQ. MUTMAINNAH:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Characteristics of Methicillin Resistant Staphylococcus aureus (MRSA) and Methicillin Sensitive Staphylococcus aureus (MSSA) and Their Inhibitory Response by Ethanol Extract of Abrus precatorius L.".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer D:

Herewith I would like to send the reviewed paper.
Thank you so much.

Recommendation: See Comments

Reviewer E:

For authors:

I have checked your manuscript in anti-plagiarsm web-based application, I found very low similarity with other online publication.

I have reviewed this manuscript and I put all the correcting items in the right hand of the text. I hope the authors can revised as soon as possible.

Recommendation: Revisions Required

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2 attachments

E-5919-Article Text-21779-1-4-20200427 - Reviewed #1 @ 27042020.doc
1527K

D-5919-Article Text-21779-1-4-20200427.doc
1558K

nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id> Thu, May 14, 2020 at 4:26 PM
To: Smujo Editors <editors@smujo.id>

Dear Smujo Editors

Thank you very much for the information and the opportunity given to us. We will immediately make improvements according to the advice of the reviewer and will send it back to you.

Best regards

Dr. Ni'matuzahroh
[Quoted text hidden]

Baiq Mutmainnah <bmmasadepan9@gmail.com> Sat, May 16, 2020 at 5:53 AM
To: Smujo Editors <editors@smujo.id>
Cc: NI'MATUZAHROH NI'MATUZAHROH <nimatuzahroh@fst.unair.ac.id>, AFAF BAKTIR <afaf-b@fst.unair.ac.id>

Thanks a lot.
[Quoted text hidden]

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nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id>

[biodiv] New notification from Biodiversitas Journal of Biological Diversity

3 messages

Smujo Editors <smujo.id@gmail.com> Thu, Jul 30, 2020 at 8:10 PM
Reply-To: Smujo Editors <editors@smujo.id>
To: NI'MATUZAHROH NI'MATUZAHROH <nimatuzahroh@fst.unair.ac.id>

You have a new notification from Biodiversitas Journal of Biological Diversity:

You have been added to a discussion titled "Revised paper" regarding the submission "Characteristics of Methicillin Resistant Staphylococcus aureus (MRSA) and Methicillin Sensitive Staphylococcus aureus (MSSA) and Their Inhibitory Response by Ethanol Extract of Abrus precatorius L.".

Link: https://smujo.id/biodiv/authorDashboard/submission/5919

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

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nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id> Mon, Aug 3, 2020 at 11:45 AM
To: Smujo Editors <editors@smujo.id>

Dear Editor

I hereby send the revision of our article by considering the suggestions and input of reviewers 1 and 2. We provide a color shadow in the sentences in which we have revised the article. We apologize for the delay in sending our revisions. We are ready to fix again if there are still things that are not suitable. Thank you very much for the opportunity and assistance provided to us.

Best regards

Ni'matuzahroh

[Quoted text hidden]

---

nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id> Mon, Aug 3, 2020 at 12:23 PM
To: Smujo Editors <editors@smujo.id>

Dear Editor

I hereby send the revision of our article by considering the suggestions and input of reviewers 1 and 2. We provide a color shadow in the sentences in which we have revised the article. We apologize for the delay in sending our revisions. We are ready to fix again if there are still things that are not suitable. Thank you very much for the opportunity and assistance provided to us.

Best regards

Ni'matuzahroh

[Quoted text hidden]

3 attachments

Revised article_Characteristic_MRSA_Ni'matuzahroh et al..doc
983K

Respon from Author to Reviewer D-5919-1 (1).docx
84K

Respon from Author to Reviewer E-5919-1.docx
56K

Reviewer D

No	Comment for Reviewer	Respon from Author	Note
1	✓ Better mention that characterization was performed in two ways : biochemical and molecular characters. ✓ After reading through, I just understood that ethanol is only for steroid and flavonoid extraction, but the abstract mislead me. ✓ Based on the title, it have been clear that target bacteria belonged to Staphylococcus. It should be explained in previous. for instance : Three Staphylococcus isolates had been purified with a selective medium and coded as ….. This study was a further investigation to characterized them based on biochemical and molecular characters ….,	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : Bacteria MRSA 22372, MSSA 22187 and MSSA 22366 were bacteria that were isolated from the urine of patients at the Regional General Hospital Dr. Soetomo Clinical Microbiology Installation Surabaya, Indonesia. Different strains of *S. aureus* can produce varying results of activity, thus causing different inhibition of antibacterial abilities. This study was a further investigation to characterized them based on biochemical and molecular characters.	
2	Difficult to follow, need to resentence. Were they similar or not based on biochemical and molecular characters?	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : The results showed the biochemical characteristics of the three bacteria were differences in colony diameter, glucose, urease, sucrose and catalase. The molecular characteristics	

		of the three bacteria had no similarity in the order of the nucleotide bases or phylogenetic proximity to each other.	
3	After reading it, I just noticed that several concentration of ethanol were applied. It should be mention above on the first sentence. NOTE : the abstract need to be reconstructed with a clear information of the research.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the abstract	
4	These sentences can be used in short in abstract.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the abstract	
5	Inhibition due to the residue of ethanol or due to extracted steroid/flavonoid? this sentence was ambiguous/bias	✓ The results of the improvement are in the manuscript : It is expected that ethanol extract of *A. precatorius* L. containing flavonoid compounds also has the ability to inhibit the growth of MRSA 22372, MSSA 22187 and MSSA 22366	
6	Please put a clear meaning of this statement " compare the antimicrobial activity of the ethanol extract of *A. precatorius* L. "	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : " to compare inhibitory response by ethanol extract of *A. precatorius* L."	
7	How come? Since this study examined only ethanol? Do you meant that lower/higher ethanol concentration put impact on kind of antibiotic/drug treatment? Since you did not measure the	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : " aerobic bacteria are found that lead to the genus *Staphylococcus*"	

	flavonoid compounds after ethanol extraction at different concentration. “ can choose the right antibiotic or drug to prevent or treat infections “		
8	This is very clear information for the isolates. It will be good if mention in previous.	Thank you to reviewers for the appreciation given to our information for the isolates research and the opportunity for us to improve the manuscript.	
9	Should they go the step biochemical characters?	Yes, they should. The bacteria cannot be determined based solely on morphological characteristics, it is also necessary to examine the physiological characteristics and factors that influence their growth.	
10	Please read papers that do the similar steps, and follow how they describe them Please re-correct the writing of this step and use the same grammar (past or present tense) For instance the biochemical test performed following the protocol of Microbact….Kit.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : Biochemical tests with Microbact ^{TM} Identification kit are used to determine the physiological characteristics of gram bacteria, so that genera and types of bacteria are known. Biochemical tests consisted of carbohydrate fermentation (glucose, lactose, mannitol, sucrose, xylose, ramnosa, arabinose, rafinose and Ortonitrophenyl-ß-d-galactopiranoside), oxidase (Oxidase strips), motility (Sulfide Indole Motily), nitrate reduction, catalase, urease, indole, Voges Preskauer (VP), citric, sulfuric acid (H_2S), lysine, hydrolysis of gelatin, ornitine, malonic, Triptophan Deaminase (TDA), inositol, sorbitol, adonitol, salicin and arginine. The format is in the form of a simple test strip or micro-plate and the results are clearly seen as different color reactions that can be interpreted using Microbact. Each kit consisted of 12 (12A, 12B) miniature biochemical	

		tests. The identification of organisms is based on changes in pH and substrate use. Identification of gram-positive bacteria use the book Bergey's Manual of Determinative Bacteriology Ninth Edition (Holt et al. 1994).	
11	Please read papers that do the similar steps, and follow how they describe them Please re-correct the writing of this step and use the same grammar (past or present tense)	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : **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 ^{0}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 sample 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 minimise 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 pippetting. 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 fragmentsizes (**Tabel 1**). Each PCR amplification was performed in a reaction volume of 50 µl consisting of a single 6 mm FTA disc immobilised with bacterial DNA, 25 µl PCR ready mix (Toyobo, Japan), 22 µl of nuclease free water and 1µl of each of the forward and reversed primers (10 pmol μl^{-1} each) (synthesised by MWG Biotech). A water negative control was also used in for each PCR reaction. Amplification conditions for PCR were 5 min at 96 ^{0}C to denature the DNA, followed by 35 cycles of denaturation at 96 ^{0}C for 45 seconds, primer annealing at 58 ^{0}C for 30 seconds and strand extension at 72 ^{0}C for 2 min on a Rotorgene thermal cycler.

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

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.

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

		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 DNA double strand concentration ($\frac{\mu g}{mL}$) = A_{260} x dilution factor x 50 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. Thegels were stainedwith ethidium bromide, visualised under UV transilluminator and photographed using a Syngene gel documentation system.	
13	Please read papers that do the similar steps, and follow how they describe them Please re-correct the writing of this step and use the same grammar (past or present tense)	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : Phylogenetic trees are constructed using the 'neighbor joining method'(Saitou and Nei, 1987). In order to evaluate the robustness of the inferred trees, a bootstrap analysis consisting of 100 resamplings of the data is performed using Clustal W and a consensus tree is generated using neighbor joining and the program MEGA 6.06.	

14	Please re-correct the writing of this step and use the same grammar (past or present tense)	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : The conventional extraction 30 g of simplicia plant material was mixed with 3000 mL of distilled in ethanol a round bottom flask and refluxed for about 5 h. Liquid extracts were obtained and were separated from the solid residue by vacuum filtration, were concentrated using a rotary evaporator.	
15	Please read papers that do the similar steps, and follow how they describe them Please re-correct the writing of this step and use the same grammar (past or present tense)	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : The crude extracts of *Abrus precatorius* L. sinensis 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. Aquades (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, penisilin G, oxacillin, cotrimoxazol, tetracyclin, erythromisin, quinopristin-dalfopristin, ciprofloxacin, levofloxacin, fosfomycin, nalidixic acid, nitrofurantoin, meropenem, linezoid, daptomycin, ampicillin-sulbactam, ampicillin, cholaramphenicol, 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 millimetre (mm) ± S.E.M. The test was carried out in 3 (three) replications. Data were analyzed using an one-way ANOVA, followed by the Tukey HSD post-hoc test	
16	Please read papers that do the similar steps, and follow how they describe them Please re-correct the writing of this step and use the same grammar (past or present tense)	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : **Inhibition of bacteria (Total visiable count).** To determine the antibacterial activity of the ethanol extract of *A. precatorius* L. by the agar dilution method described by Schwalbe et al. 2007, different concentrations of the extract ranging between 25 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 was aseptically dispensed and spread evenly on the agar plates. Another 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 an one-way ANOVA, followed by the Tukey HSD post-hoc test. Calculation of the number of living bacterial cells (CFU/mL) using the following formula (Waluyo 2008). Number of living bacterial cells (CFU/mL) = number of colonies $x \frac{1}{10^{-6}}$ x 10	

		Determination of minimum inhibitory concentrations (MIC's) of the effective plants 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 erythromicin 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 ˚C 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.	
17	Will it be fine if divided into 3 sub topics only ?: 1. Morfological and biochemical characters 2. Phylogenetic tree 3. Inhibitory performance	✓ Yes, it will divided into 3 sub topics according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
18	Just describe the different only : colony diameter	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	

19	Figure said nothing for the diameter colony, since there was no comparable scale	The results of the improvement are in the manuscript.	
20	This pictures said nothing. Better to took 1 clear cell arrangements : coccoid clusters as a best hit of *Staphylococcus*	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
21	Please re-write with correct grammar	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : 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, isolate MRSA 22372, MSSA 22187 and MSSA 22366 only different on glucose mannitol and sucrose fermentation, urease and catalase enzyme production (**Table 2**). MRSA 22372 had the ability to ferment glucose, mannitol and sucrose. In MSSA 22187 it only sucrose ferments. 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. Use enzyme urease that break 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).	
22	1. Please re-write with correct grammar. 2. Better explain those biochemical characters relates to the nature of *Staphylococcus*. For instance : Are they aerobic bacteria? Since they are catalase positive vacteria	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript. Catalase test results on three bacteria that grew on TSA media in this study showed that MRSA 22372 and MSSA 22366 showed a positive reaction, whereas on MSSA 22187 it showed a negative reaction. Toelle et al. (2014) stated that positive catalase is shown the presence of gas bubbles (O_2) produce by the genus *Staphylococcus*. *Stapylococcus sp* uses catalase to protect from hydrogen peroxide (H_2O_2) by converting it to water and oxygen (Locke et al. 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 (Lay 1994).	
23	Underline " antibiotic administration ?"	✓ The results of the improvement are in the manuscript : antibiotic administration such as gentamycin, penisilin G, oxacillin, cotrimoxazol, tetracyclin, erythromisin, quinopristin-dalfopristin, ciprofloxacin, levofloxacin, fosfomycin, nalidixic acid, nitrofurantoin, meropenem, linezoid, daptomycin, ampicillin-sulbactam, ampicillin, cholaramphenicol, and methicillin were also carried out on the bacteria MRSA 22372, MSSA 22187 and MSSA 22366 (**Table 3**).	

24	I am sorry I did not get this information. Was the method explaining this also? what is the purpose of this works?	✓ Yes, the method this also was explained ✓ The results of the improvement are in the manuscript.	
25	Is this for Inhibition of bacteria (Total Plate Count)	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
26	This sub topic can be involved in the phylogenetic tree	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
27	No need to present here, since this study not examined the validity of methods. This data was only raw data for obtaining excellent 16sRNA sequences.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
28	DNA band encoding gene…. (?)… No need put those information, since they had been explained on the Figure 3.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
29	Please read papers that do the similar works and follow how they describe them Please re-correct the writing and use the same grammar (past or present tense)	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript : 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 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 indicates that MSSA 22187, MRSA 22372 and MSSA 22366 had no similarity between the nucleotide base sequence and phylogenetic proximity to each other.	

30	Still unclear, the inhibition due to the ethanol concentration or due to the extracted compound after ethanol extraction	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
31	It is not necessary to explain all 3 isolates if the message were the same. from the table it was obviously significance effect of concentration for 3 isolates. Took 1 isolate and explained clear and briefly. Then put the message that the similar effects were also detected in 2 other isolates. Thus discuss why higher concentration inhibited more, with assumption based on literature study, since there is no info about the extracted bioactive	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
32	Please refer to previous comment	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	
32	Please read carefully and rewrite the conclusion	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript.	

Reviewer E

No	Comment for Reviewer	Respon from Author	Note
1	There is no background in abstract. Please add accordingly.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
2	This paragraph has little relationship with MRSA and MSSA case/situation. Be more to the point. Please change accordingly.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
3	Question: Any reason why did you choose this plant? Any previous data or story?	✓ Yes, I have reason why I choose this plant. ✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
4	Question: Did you make/hame informed consent regarding this isolated bacterium?	Yes, I made	
5	Question: How did you know if the bacteria was of MRSA and MSSA? How can you categorized those isolates were MRSA or MSSA? What was the test?	The results of the improvement are in the manuscript	
6	???	The results of the improvement are in the manuscript	
7	Question: Any citation for this formulae?	✓ Yes, there is citation for this formulae ✓ The results of the improvement are in the manuscript	

8	Please use past tense	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
9	Please use past tense.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
10	Please add citation for primer and PCR setting.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
11	Question: Any citation for this application? Who made this application?	✓ Yes, there is citation for this application ✓ The results of the improvement are in the manuscript	
12	???	✓ The results of the improvement are in the manuscript	
13	Question: Any citation for this formulae?	The results of the improvement are in the manuscript	
14	Question: What application did you use? Please add.	The results of the improvement are in the manuscript	
15	Question: Any citation for the formulae?	The results of the improvement are in the manuscript	
16	Where is the discussion part? I only see/read results. Please make discussion part, which contains (such as) strength and weakness of this research, implication of your research to the latest information, potential to product development, etc.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	

17	The photos were difficult to identify. Do you have better ones?	✓ I am sorry. I only have that photos. However, I will tried to improve it to be clearer. ✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
18	Please use past tense.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
19	Please use standar abbreviation for antibiotic names.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
20	Did you need to show this photo? I guess you automatically do this after PCR	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
21	Please construct phylogenetic tree that include outgroup to be able to understand the tree.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	Line 236-237
22	Question: If it did not meet the CLSI standard, what is the implication?	✓ The active compound extract of *A. precatorius* L. inhibiting the growth of the three test bacteria	
23	Please use past tense.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	

24	Question: What is the implication, then?	The results of the improvement are in the manuscript	
25	This is not a conclusion. This is a short summary of your findings. Please make relevan conclusion.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	
26	Please use citation manager (Mendeley/zotero, etc). Many references you used here were old enough. Could you change with newer ones? Maximum 10 year old. I highlited the old references.	✓ The author has corrected it according to the reviewer's suggestion. ✓ The results of the improvement are in the manuscript	

Characteristics of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) and Their Inhibitory Response by Ethanol Extract of *Abrus precatorius* L.

Abstract. 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* L. 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* L. 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* L. 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* L.

Running title: Characteristics of MRSA, MSSA and Inhibitory of *Abrus precatorius* L.

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 (Kasmad et al. 2010). (Samad 2014) 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. The main cause of infection is due to the presence of microorganisms that multiply in the urinary tract (Purnomo 2012). Methicillin resistant *Staphylococcus aurens* (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% and in Indonesia the prevalence in 2006 reached 23.5% (Sulistyaningsih 2010). 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 has the ability as an antibacterial is *A. precatorius* L. *A. precatorius* L. is used as a phlegm thinner (mucolytic) (Noviana 2013); indicative medicine for the prevention and cure of thrush, sore throat and inflammation of the tonsils (Indah and Darwati 2013); and antibacterial (Chaudhari et al. 2012; Garaniya and Bapodra 2014). *A. precatorius* L. 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 which contained in *A. precatorius* L. plants are not only entirely polar compounds, but there are also non-polar or semi-polar and lipophilic compounds. Ethanol, ethylacetate 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 (Siregar et al. 2012).

Ribka (2015) reportes that ethanol extract of *Abrus 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* L. 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* L. which inhibits the growth of MRSA 22372, it is expected that the ethanol extract of *A. precatorius* L. 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* L. 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* L. leaves are expected to inhibit the bacteria MRSA 22372, MSSA 22187 and MSSA 22366. Thus, the ethanol extract of *A. precatorius* L. 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. These bacteria are isolated from the urine of patients who are resistant and sensitive to antibiotics. *Abrus precatorius* L. leaf plants were obtained from Sumenep, East Java, Indonesia.

Methods

Morphological characters 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 ™ 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, ramnosa, arabinose, rafinose and Ortonitrophenyl-ß-d-galactopiranoside), oxidase (Oxidase strips), motility (Sulfide Indole Motily), nitrate reduction, catalase, urease, indole, Voges Preskauer (VP), citric, sulfuric acid (H_2S), lysine, hydrolysis of gelatin, ornitine, malonic, Triptophan Deaminase (TDA), inositol, sorbitol, adonitol, salicin and arginine. The format were 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 the book 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 ^{0}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 sample 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 minimise 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 pippetting. 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 fragmentsizes (**Tabel 1**).

Each PCR amplification was performed in a reaction volume of 50 µl consisting of a single 6 mm FTA disc immobilised with bacterial DNA, 25 µl PCR ready mix (Toyobo, Japan), 22 µl of nuclease free water and 1µl of each of the forward and reversed primers (10 pmol μl^{-1} each) (synthesised by MWG Biotech). A water negative control was also used in for each PCR reaction. Amplification conditions for PCR were 5 min at 96 ^{0}C to denature the DNA, followed by 35 cycles of denaturation at 96 ^{0}C for 45 seconds, primer annealing at 58 ^{0}C for 30 seconds and strand extension at 72 ^{0}C for 2 min on a Rotorgene thermal cycler.

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

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

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

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. Thegels were stainedwith ethidium bromide, visualised 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 *A. precatorius* L. 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 disk diffusion method

The crude ethanol extracts of *Abrus precatorius* L. 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, penisilin G, oxacillin, cotrimoxazol, tetracyclin, erythromisin, quinopristin-dalfopristin, ciprofloxacin, levofloxacin, fosfomycin, nalidixic acid, nitrofurantoin, meropenem, linezoid, daptomycin, ampicillin-sulbactam, ampicillin, cholaramphenicol, 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 millimetre (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 an 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* L. 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 was aseptically dispensed and spread evenly on the agar plates. Another 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 an 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).

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

Determination of Minimum Inhibitory Concentrations (MIC's) of the effective plants 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 erythromicin 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 ˚C 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.

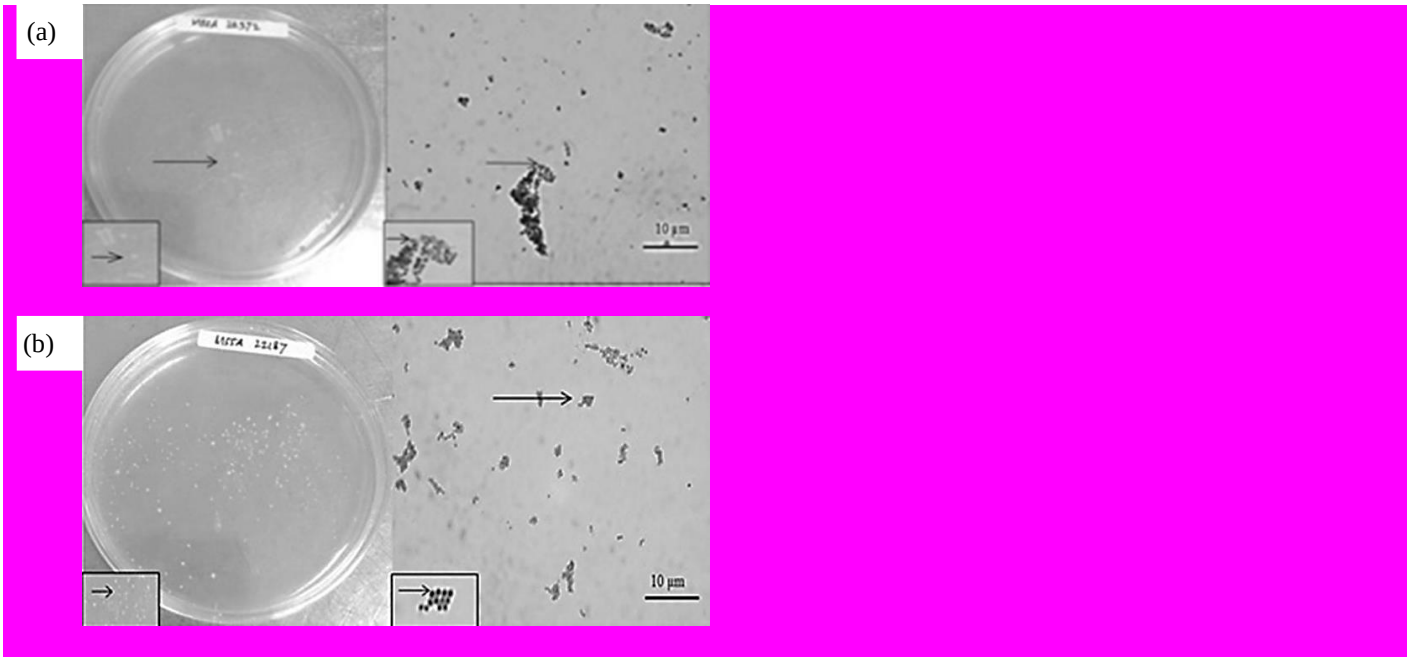
RESULTS AND DISCUSSION

Morfological 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.

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

No	Characters	Specimen code		
		MRSA 22372	MSSA 22187	MSSA 22366
1	**Colony shape**	round	round	Round
2	**Pigmentation of the colony**	yellow and white	yellow and white	yellow and white
3	**Colony diameter**	5 mm – 7 mm	3 mm - 4 mm	4 mm - 6 mm
5	**Cell shape**	coccus	coccus	coccus
6	**Elevation**	convex	convex	convex
7	**Edge**	smooth	smooth	smooth
8	**Gram staining**	positive	positive	positive
9	**Cell arrangement**	clustered	clustered and *diplococcus*	clustered and *diplococcus*



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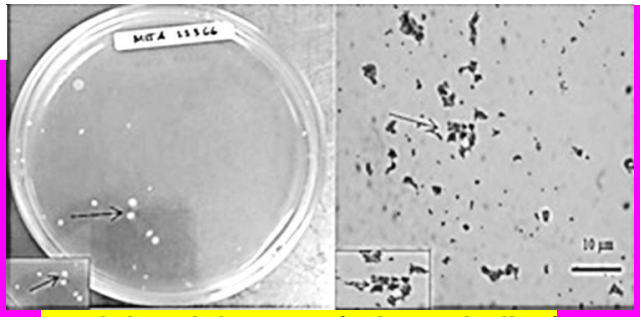

Figure 1. Morphological characters of colony and cells of MRSA 22372, MSSA 22187, and MSSA 22366. (a) MRSA 22372, (b) MSSA 22187 and MSSA 22366 coccoid clusters as a best hit of *Staphylococcus*. Insert shows an enlarged image.

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 fermentations, 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 break 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).

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).

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).

No	Type of test	Specimen code		
		MRSA 22372	MSSA 22187	MSSA 22366
1	Shape	coccus	coccus	coccus
2	Gram	+	+	+
3	Oxidase	-	-	-
4	Motility	-	-	-
5	Nitrate	+	+	+
6	Lysine	-	-	-
7	Ornitine	-	-	-
8	H_2S	-	-	-
9	Glukose	+	-	-
10	Mannitol	+	-	-
11	Xylose	-	-	-
12	ONPG	+	+	+
13	Indole	-	-	-
14	Urease	+	++	+
15	VP	-	-	-
16	Citric	-	-	-
17	TDA	-	-	-
18	Gelatine	-	-	-
19	Malonate	-	-	-
20	Inositol	-	-	-
21	Sorbitol	-	-	-
22	Rhamnose	-	-	-
23	Sucrose	+	+	-
24	Lactose	-	-	-
25	Arabinose	-	-	-
26	Adonitol	-	-	-
27	Rafinose	-	-	-
28	Salicin	-	-	-
29	Arganine	-	-	-
30	Catalase	+	-	++

31	**Spore**	-	-	-

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 ^{0}C annealing temperature (**Figure 3**).

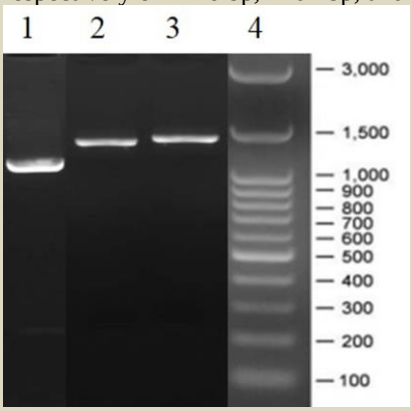

Figure 3. Electrophoresis of the amplification of 16S rRNA gene encoding for MRSA 22372, MSSSA 22187 and MSSA 22366 bacteria with primers 8F and 1522R at 72 ^{0}C. The rows of 1, 2 and 3, respectively, are the DNA bands of the 16S rRNA MRSA 2272, MSSA 22187 and MSSA 22366 gene encoding at 1426 bp, 1407 bp and 1427 bp. Lane 4 is a marker of 100 bp DNA ladder.

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 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.

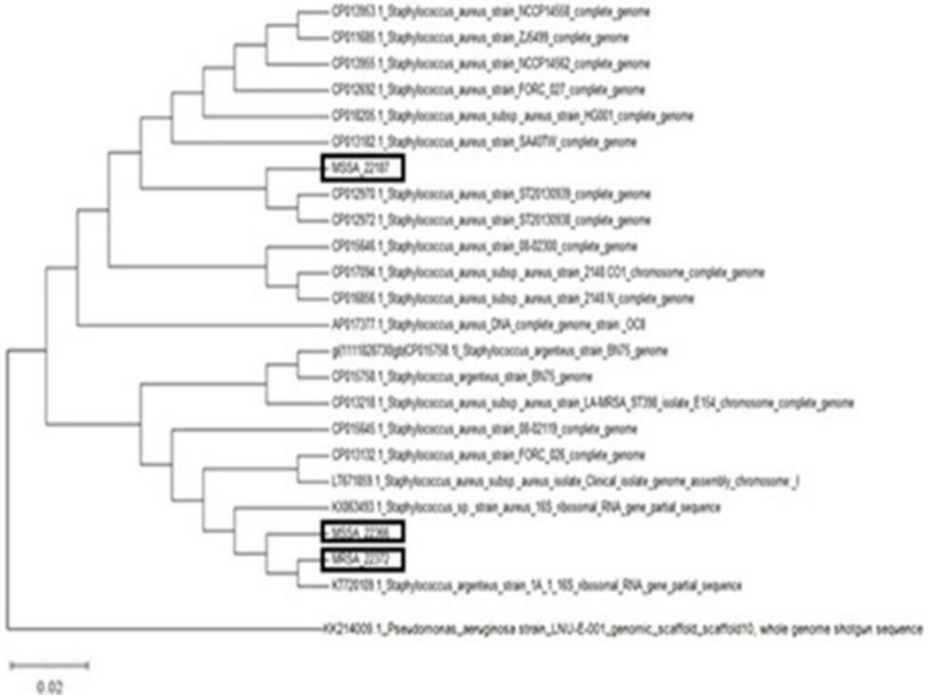

Figure 4. Relationship between the three bacteria of MRSA 22372, MSSA 22187 and MSSA 22366 by making phylogenetic trees and the position of these bacteria in several bacteria in GenBank. *Pseudomonas aeruginosa* strain LNU-E-001 genomic scaffold10, whole genome shotgun sequence was used as the out group.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

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* L.

Abrus precatorius L. 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.

Inhibition of ethanol extract *A. precatorius* L. leaves

Antibacterial results by the disk-diffusion method (Kirby Bauer) showed that ethanol extract of *A. precatorius* L. 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. pr*ecatorius L. leaves containing flavonoid compounds. This was due to the reducing content of bioactive compounds in ethanol extract of *A. precatorius* L. 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 a negative control did not have antibacterial activity. This means that the antibacterial ability of the *A. precatorius* L. 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* L. ethanol extract was greater than the positive control. This showed that the ethanol extract of *A. precatorius* L. 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.

Table 5. Bacterial inhibition zones of ethanol extract of *A. precatorius L.* 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 (+): erithromycin (15 µg). [a, b, c, d, e, f] different letters indicate a significant difference at $p<0.05$

Tukey HSD analysis showed that, ethanol extract of *A. precatorius* L. 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 erithromycin 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* L. 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 erithromycin 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)

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

No	Specimen code	Type of antibiotic																		
		GM	P	OX	CTZ	TE	E	SYN	CIP	LVX	FOS	NA	FD	MEM	LNZ	DAP	SAM	AM	C	MET
1	MRSA 22372	R	R	R	R	R	S	R	R	R	S	R	R	R	S	S	-	-	I	R
2	MSSA 22187	R	R	R	R	R	R	S	R	R	S	R	S	R	-	S	R	R	-	S
3	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 = cotrimoxazol, TE = tetracyclin, E = erythromisin, SYN = quinopristin-dalfopristin, CIP = ciprofloxacin, LVX = levofloxacin, FOS = fosfomycin, NA = nalidixic acid, FD = nitrofurantoin, MEM = meropenem, LNZ = linezoid, DAP = daptomycin, SAM = ampicillin-sulbactam, AM = ampicillin, C = chloramphenicol, and MET = methicillin.

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* L. leaves added. Percent decrease in the number of living bacteria after administration of *A. precatorius* L. 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 L.* leaf ethanol extracts 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

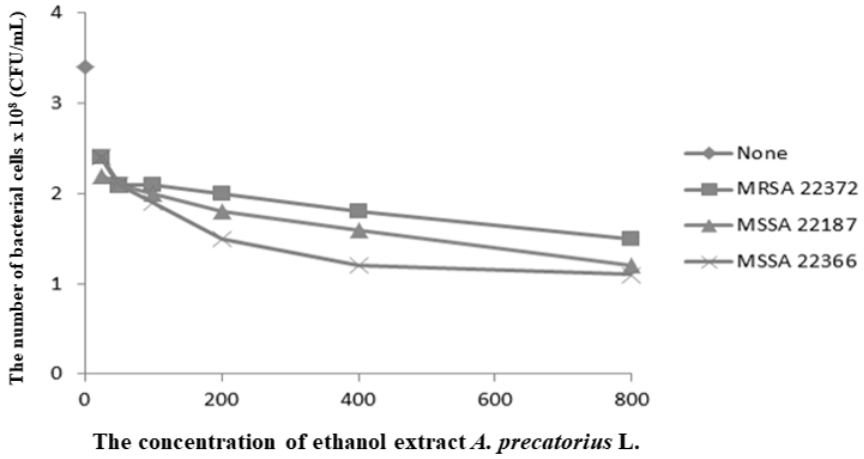

Figure 5. Inhibition of MRSA 22372, MSSA 22187 and MSSA 22366 bacteria at various concentrations of ethanol extract *A. precatorius* L. leaves.

This research showed that the ethanol extract of *A. precatorius* L. 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* L. 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).

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* L. leaves due to the presence of total phenolic and flavonoid compounds. The ethanol extract of *A. precatorius* L. leaves has higher inhibition of *S. aureus* compared to previous studies. (Ribka 2015) reported that ethanol extract of *A. precatorius* L. leaves had antibacterial activity on *S. aureus* of 0.093 mm at a concentration of 6000 mgL^{-1}. Ethyl acetate extract of *A. precatorius* L. 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* L. 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* L. 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%). P*seudomonas 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* L. 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* L. 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 L. leaves.

Utilization of *A. precatorius* L. 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 compelete, and *Staphylococcus argenteus* strain 1A_1 16S ribosomal RNA. Ethanol extract of *A. precatorius* L. has promising antibacterial activity by inhibiting the growth of MRSA 22372, MSSA 22187 and MSSA 22366.

ACKNOWLEDGEMENTS

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nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id>

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Dear Editor

Thank you for your information

Best regards

Dr. Ni'matuzahroh

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