International Journal of Life Science Research Archive

ISSN: 0799-6640 (Online)

Journal homepage: https://sciresjournals.com/ijlsra/

(RESEARCH ARTICLE)

Check for updates

Activity test of *Guazuma ulmifolia* Lam (Jati Belanda) methanol extract against bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae*

Rizki Zalzabillah *, Flora Ramona Sigit Prakoeswa, Sri Wahyuni and EM Sutrisna

Faculty of Medicine, University of Muhammadiyah Surakarta, Indonesia.

International Journal of Life Science Research Archive, 2023, 04(01), 216–221

Publication history: Received on 07 January 2023; revised on 03 March 2023; accepted on 06 March 2023

Article DOI: https://doi.org/10.53771/ijlsra.2023.4.1.0032

Abstract

Background: *Staphylococcus aureus* and *Klebsiella pneumoniae* bacteria are a group of bacteria that can infect the respiratory tract so that they can cause complications for humans, Jati belanda leaves *(Guazuma ulmifolia* Lam*)* is a plant that has potential as an antibacterial, because it has active substances that can stop the growth of bacterial organisms, namely flavonoids, saponins, tannins, and alkaloids are its components.

Purpose: This study aims to determine the antibacterial activity of methanol extract of Jati belanda leaves (*Guazuma ulmifolia* Lam) on the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* bacteria.

Methods: Antibacterial activity test in this study was carried out using the well method, *Staphylococcus aureus* bacteria and *Klebsiella pneumoniae* bacteria as test microbes in this study, positive control (*chloramphenicol*), 20% DMSO negative control and five concentration groups namely 100%, 50%, 25%, 12.5% and 6.25% from methanol extract of Jati belanda leaves (*Guazuma ulmifolia* Lam).

Results: From the research results it is known that the methanol extract of Jati belanda leaves (*Guazuma ulmifolia* Lam) is able to inhibit *Staphylococcus aureus* bacteria with each inhibition zone at 100% concentration of 12.31 mm, 50% concentration of 10.48 mm, 25% concentration of 7.62 mm and the positive control (*chloramphenicol*) was 33.24 mm and the *Klebsiella pneumonia* bacteria at 100% concentration was 14.27 mm, 50% concentration was 11.14 mm and the positive control (*chloramphenicol*) was 40.6 mm.

Keywords: Antibacterial; *Staphylococcus aureus*; *Klebsiella pneumonia*; Jati belanda

1. Introduction

Single-celled organisms or commonly called bacteria are organisms that often infect the human respiratory tract, in that case it is the main route for bacteria to enter the body (Sabiladiyni*et al.,*2018,.), one of the organisms or bacteria that can enter through the respiratory tract, namely gram-positive bacteria *Staphylococcus aureus* and gram negative bacteria *Klebsiella pneumoniae* is a group of bacteria that can infect the respiratory tract so that it can cause complications for humans who are infected by these bacteria. (Dewi, 2017), these bacteria can incubate in various respiratory tracts, specifically among the lower respiratory tract and these bacteria can infect the lower respiratory tract causing pneumonia, and these bacteria can cause skin infections, because the entry route of bacteria other than the respiratory tract respiration, namely in skin tissue (Ningsih, 2017),

* Corresponding author: Rizki Zalzabillah

In research using *database* multi-hospital in the United States from 2007 to 2011 there were 24,644 clinically relevant cases *Staphylococcus aureus* reported (Ondusko & Nolt, 2018). According to *World Health Organization* in 2013 deaths caused by pneumonia were in 8th place from the results of basic health research in Indonesia as many as 22,000 people died caused by pneumonia. From the results of this research, the researchers wanted to add to the existing antibacterial data in Indonesia so that the use of antibacterial for raw materials derived from plants can be used in the world of health as raw materials for standardized antibacterial production, (Trisia*et al*., 2018).

Antibacterials are interfering secondary metabolites that can inhibit or kill bacterial growth by damaging the metabolic structure so that bacterial growth is disrupted or can even kill microbes. *Guazuma ulmifolia*Lam) is a plant that can be used as an antibacterial candidate, because it has an active substance that can stop the growth of bacteria. Several studies have stated that the leaves, seeds and bark of Jati belanda (*Guazuma ulmifolia*Lam) is widely used as a medicine for worms and elephantiasis besides that it can treat stomach pain. (Trisya*et al*., 2018). In a previous study, the antibacterial activity of the ethanol extract of Jati belanda leaves (*Guazuma ulmifolia*Lam) against bacteria*E. coli* has a very significant inhibition, at a concentration of 5% inhibition of 9.67 mm, a concentration of 10% has an inhibition of up to 11 mm, and at a concentration of 20% has an inhibition of 12.67 mm, (Onduskо*et al.*, 2018)

From the results of this study it is known that the Jati belanda leaves (*Guazuma ulmifolia*Lam) has substances that are effective in inhibiting or even killing bacteria*E. coli*, the advantage in this study compared to previous research is to use methanol solvent to extract Jati belanda leaves (*Guazuma ulmifolia*Lam), as well as tested on bacteria*Staphylococcus aureus* and*Klebsiella pneumoniae*. (Trisia*et al*., 2018).

2. Material and methods

2.1 Tools and Materials

Tools include digital scales, micropipette, rotatory evaporatory vacuum, water bath, autoclave, erlenmeyer, ohse, petri dish, measuring cup, caliper, test tube, sterile cotton swab, spatula, filter paper, separating funnel, stir bar.

The material used in this study was Jati belanda (*Guazuma ulmifolia lam*), methanol, bacterial culture *Staphylococcus aureus* and *Klebsiella pneumoniae,* Standard Mc. Farland 0.5, Media Mueller hinton agar (MHA), Aqua pro injection, NaCl, DMSO 20%, Antibacterial (*Chloramphenicol*)

2.2 Determination

The determination of the Jati belanda leaf plant was carried out at the Tawangmangu Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT). The results of the determination show:

- Species : *Guazuma ulmifolia*Lam.
- Synonyms : *Bubroma guasuma* Willd.
- Family : Malvaceae*.*

2.3 Yield

The yield was carried out to find out the comparison between the simplicia of Jati belanda leaves (*Guazuma ulmifolia*Lam*)* with extract. The yield results that have been carried out are as follows:

Dry weight: 1000 gram

Viscous extract: 130 gram

Extract yield : $\frac{\text{Viscous extract}}{\text{Dry weightt}}$ x 100%

: $\frac{130}{1000}$x 100%

:13%

Based on the calculation above, it has been found that the yield of 1000 grams of Jati belanda leaves (*Guazuma ulmifolia*Lam) is 13%

2.4 Sample Size Estimation

In this study the sample was divided into 7 treatment groups. Group I, namely the positive control was Antibacterial (*Chloramphenicol*) 50 µl concentration of 10% using the disc method, group II is a negative control that is given DMSO 20% 50 µl, group III is a 100% concentration of 50 µl, group IV is a concentration of 50% of 50 µl, group V is a concentration of 25% as much as 50 µl, group VI that is 12.5% concentration as much as 50 µl and group VII that is 6.25% as much as 50 µl..

2.5 Extraction

Extraction is a method for separating compounds and metabolic active substances contained in the test sample. The extraction process was carried out by the simplicia soaking maceration method using methanol solvent for five days with a ratio of 1:3. During the maceration process, stirring is carried out every day. After the maceration or soaking process is carried out, the extract is filtered and followed by evaporation using an evaporator.

2.6 Preparation of Bacterial Suspension

The suspended bacteria are the bacteria that will be tested, namely bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae,* The bacteria came from the Microbiology Laboratory of the Faculty of Medicine, Sebelas Maret State University Surakarta, the bacteria were cultured using Mueller Hinton Agar (MHA) media for 24 hours, at room temperature (37 ^{0}C) until a bacterial colony is obtained. Furthermore, the bacterial colonies obtained from the growth results were planted in 0.5 ml of liquid BHI media, then incubated for 5 hours at room temperature (37 ^{0}C). The results of the bacterial culture were taken and put into a test tube that had been given a NaCl solution, then the turbidity level was adjusted to the 0.5% Mc Farland standard.

2.7 Antibacterial Activity Test

The antibacterial test used the spray method, the bacteria that met the Mc Farland standard of 0.5% were taken using a sterile cotton swab, rubbed slowly and evenly on Mueller Hinton Agar (MHA) media. In the media, make wells to place sample concentrations of 100%, 50%, 25%, 12.5%, 6.25%, negative control and positive control in the stew. After that the bacteria were incubated at 37 ^{0}C for 24 hours and the diameter of the inhibition zone formed at each concentration was measured using a caliper with an accuracy of 0.01mm.

3. Results and discussion

In this study, the antibacterial activity of the methanol extract of Jati belanda leaves was tested *(Guazuma ulmifolia*Lam*)* against bacterial growth *Staphylococcus aureus* and *Klebsiella pneumoniae*. Laboratory experimental research design using the method *post test only controlled group design*. In this antibacterial test using the well-diffusion method, so that the extract from Jati belanda leaves can diffuse in the agar media, besides that the well-diffusion method is quite easy to work on. In this study there were 7 treatment groups namely methanol extract of Jati belanda leaves*(Guazuma ulmifolia*Lam*)* 100%, 50%, 25%, 12.5%, 6.25%, positive control (*Chloramphenicol*) 10% and negative control *Dimethyl Sulfoxide* (DMSO) 20%. The treatment group was repeated 4 times, according to Federer's calculation formula which obtained the results (n> 3.5) so that the number of treatment groups in each bacterium was 28 groups..

Inhibition of bacterial growth *Staphylococcus aureus* in this study showed varying results in each treatment group, there were treatment groups that did not produce inhibition zones and there were those that produced inhibition zones. In the methanol extract group at concentrations of 100%, 50%, 25% and positive control (*Chloramphenicol*) 10% showed the presence of antibacterial activity, the largest inhibition zone was found in the positive control group (*Chloramphenicol*) 10% with an average inhibition zone of 33.24 mm, while the methanol extract group at a concentration of 12.5%, 6.25% and negative control *Dimethyl Sulfoxide* (DMSO) 20% did not show the results of antibacterial activity which was indicated by the absence of an inhibition zone around the wells.

Antibacterial activity test results from methanol extract of Jati belanda leaves (*Guazuma ulmifolia*Lam) against bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae,* with 4 repetitions shown in Tables 1 and 2

Table 1 Results of measuring the inhibition zone of *Staphylococcus aureus*

Treatment group	Mean ± standard
00%	12.31 ± 0.13
50%	10.48 ± 0.06
25%	7.62 ± 0.29
12,5%	0 ± 0
6,25%	0 ± 0
Chloramphenicol 10%	33.24 ± 0.05
DMSO 20%	0 ± 0

Table 2 Results of *Klebsiella pneumoniae* inhibition zone measurements

concentration	Mean ± standard
100%	14.27 ± 0.04
50%	11.14 ± 0.05
25%	0 ± 0
12,5%	0 ± 0
6,25%	0 ± 0
Chloramphenicol 10%	40.6 ± 0.05
DMSO 20%	0 ± 0

From the results of tables 1 and 2 above, the methanol extract of Jati belanda leaves is able to inhibit bacterial growth *Staphylococcus aureus* and *Klebsiella pneumonia.* Data on the diameter of inhibition obtained from the antibacterial activity test of the methanol extract of Jati belanda leaves *(Guazuma ulmifolia*Lam*)* not normally distributed and not homogeneous so that the statistical test used for the statistical test is a non-parametric statistical test. From the test results *Kruskal Wallis* on the diameter of inhibition in bacteria *Staphylococcus aureus* obtained p value = 0.000 (<0.05) and in bacteria *Klebsiella pneumoniae* p value = 0.000 (<0.05) which means that the results are significant. After doing non-parametric statistical tests*Kruskal Wallis* then proceed with the test *Mann-Whitney* to see differences between groups.

On the test *Mann Whitney* the parameter used is the value *Asymp 2-tailed*. From the test comparison group *Mann Whitney* in getting the results on the comparison between *chloramphenicol* with JB concentration of 100%,*chloramphenicol* with JB concentration of 50%,*chloramphenicol* with JB concentration 25%*, chloramphenicol* with JB concentration of 12.5%,*chloramphenicol* with JB concentration of 6.25%,*chloramphenicol* with 20% DMSO, 20% DMSO with 100% JB concentration, 20% DMSO with 50% JB concentration, 20% DMSO with 25% JB concentration, 100% JB concentration with 50% JB concentration, 100% JB concentration with 25 JB concentration %, JB concentration 100% with JB concentration 12.5%, JB concentration 100% with JB concentration 6.25%, JB concentration 50% with JB concentration 25%, JB concentration 50% with JB concentration 12.5%, JB concentration 50% with JB concentration of 6.25%, JB concentration of 25% with JB concentration of 12.5%, and JB concentration of 25% with JB concentration of 6.25%. Significantly different results were obtained, this showed that the methanol extract of Jati belanda leaves had an antibacterial effect on bacteria *Staphylococcus aureus*. While in comparison JB concentration of 12.5%, and JB concentration of 6.25% which has value p= 1 (> 0.05), meaning that the groups have insignificant differences in inhibition ability or have the same inhibition ability. The next comparison is the comparison between *chloramphenicol* with JB concentration of 100%,*chloramphenicol* with JB concentration of 50%,*chloramphenicol* with JB concentration 25%*, chloramphenicol* with JB concentration of 12.5%,*chloramphenicol* with JB concentration of 6.25%,*chloramphenicol* with 20% DMSO, 20% DMSO with 100% JB concentration, 20% DMSO with 50% JB concentration, 100% JB concentration with 50% JB concentration, 100% JB concentration with 25% JB concentration, 100% JB concentration

with JB concentration 12.5%, JB concentration of 100% with JB concentration of 6.25%, JB concentration of 50% with JB concentration of 25%, JB concentration of 50% with JB concentration of 12.5%, and JB concentration of 50% with JB concentration of 6.25 % obtained significantly different results, this shows that the methanol extract of Jati belanda leaves has an antibacterial effect on bacteria *Klebsiella pneumoniae.* Whereas in the comparison of JB concentration of 25% with JB concentration of 12.5%, and JB concentration of 25% with JB concentration of 6.25%, 12.5%, and JB concentration of 6.25% which has a value$p$= 1 (> 0.05), meaning that the groups have insignificant differences in inhibition ability or have the same inhibition ability.

Comparison of positive control with concentrations of Jati belanda leaves is between *chloramphenicol* with JB concentration of 100%, *chloramphenicol* with JB concentration of 50%,*chloramphenicol* with JB concentration 25%, *chloramphenicol* with JB concentration of 12.5%,*chloramphenicol* with JB concentration of 6.25% obtained significantly different results, this is to find out whether the potential inhibition as an antibacterial has the same strength as *chlorampenicol* or lower than *chlorampenicol*.

Comparison of negative control with concentrations of Jati belanda leaves on bacteria *Staphylococcus aureus* is between 20% DMSO with 100% JB concentration, 20% DMSO with 50% JB concentration, 20% DMSO with 25% JB concentration, 20% DMSO with 12.5% JB concentration, 20% DMSO with 6.25% JB concentration . Significantly different results were obtained in the comparison of 20% DMSO with 100% JB concentration, 20% DMSO with 50% JB concentration, 20% DMSO with 25% JB concentration, this shows the methanol extract of Jati belanda leaves has an antibacterial effect on bacteria. *Staphylococcus aureus*. Whereas in the comparison of DMSO 20% with JB concentration of 12.5%, 20% DMSO with JB concentration of 6.25% which has a value p= 1 (> 0.05), meaning that the groups have insignificant differences in inhibition ability or have the same inhibition ability.

Comparison of negative control with concentrations of Jati belanda leaves on bacteria *Klebsiella pneumoniae* are 20% DMSO with 100% JB concentration, 20% DMSO with 50% JB concentration, 20% DMSO with 25% JB concentration, 20% DMSO with 12.5% JB concentration, 20% DMSO with 6.25% JB concentration. Significantly different results were obtained in the comparison of 20% DMSO with 100% JB concentration, 20% DMSO with 50% JB concentration, this shows that the methanol extract of Jati belanda leaves has an antibacterial effect on bacteria. *Klebsiella pneumoniae*. Whereas in the comparison of 20% DMSO with 25% JB concentration, 20% DMSO with 12.5% JB concentration, 20% DMSO with 6.25% JB concentration which has a value p= 1 (> 0.05), meaning that the groups have insignificant differences in inhibition ability or have the same inhibition ability.

4. Conclusion

Antibacterial properties of Jati belanda methanol extract*(Guazuma ulmifolia lam)* varies depending on the bacteria tested where on the bacteria *Staphylococcus aureus* has an inhibition zone starting from a concentration of 25% and continues to increase up to a concentration of 100%, but the inhibition zone produced at a concentration of 100% is still lower than the positive control (*chloramphenicol*) and the treatment group on Jati belanda leaf extract on bacteria *Klebsiella pneumonia* has an inhibition zone starting from a concentration of 50% and continues to increase to a concentration of 100%, but the inhibition zone produced at a concentration of 100% is still lower than the positive control (*chloramphenicol*). Of all the extract groups, the largest inhibition zone treatment was the concentration of 100% methanol extract on bacteria *Staphylococcus aureus.*

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

References

[1] Apriliana , E. , & Hawarima , V. (2016).The content of rambutan fruit (Nephelium lappaceum L.) as an antibacterial against E. coli Causes Diarrhea The Content of Rambutan (Nephelium lappaceum L.) as Antibacterials Against E. coli Causes Diarrhea. 5(April).

[2] Dewi, (2017). Inhibition of areca seed (Areca catechu l.) infusion against *Staphylococcus aureus* bacteria.proceedings of national & international seminars, 2017: proceedings of the national education seminar, science and technology. https://jurnal.unimus.ac.id/index.php/psn12012010/article/view/3101/3006

[3] Diana. R. F, & Budiarso, F. (2015). Resistance Test of Escherichia Coli Bacteria Isolated From Dental Plaque To Mercury And Chloramphenicol Antibiotics.E-Biomedic Journal, 3(1). https://doi.org/10.35790/ebm.3.1.2015.6607

[4] Febrianasari, F. (2018). Antibacterial Activity Test of Kirinyu Leaf Extract (Chromolaena odorata) Against *Staphylococcus aureus*.Yogyakarta: Sanata Dharma University. https://repository.usd.ac.id/30997/1/141434020.pdf

[5] Fiana , F. M. , Zukhruf , N. , Kiromah , W. , & Purwanti , E. (2020).Antibacterial Activity of Ethanol Extract of Breadfruit Leaf (Artocarpus altilis) Against *Staphylococcus aureus* and Escherichia coli Antibacterial Activity of Ethanol Extract of Breadfruit Leaf (Artocarpus altilis) Against *Staphylococcus aureus* and Escher. 10–20.

[6] Hapila, A. (2020).Diversity and Characterization of Microbial Isolates from Deer Feces Using Conventional and Metagenomic Analysis. repository.unhas.ac.id. http://repository.unhas.ac.id/id/eprint/154

[7] Leksono, W. B., Pramesti, R., Santosa, G. W., & Setyati, W. A. (2018). Types of Methanol and N-Hexane Solvents on Antioxidant Activity of Gelidium sp. From Drini Beach, Gunungkidul - Yogyakarta.Tropical Marine Journal, 21(1), 9. https://doi.org/10.14710/jkt.v21i1.2236

[8] Lo, S., Bruce, W., & Jesu, C. (2019).Morphological and physiological responses of Guazuma ulmifolia Lam . to Morphological and physiological responses of Guazuma ulmifolia Lam . to different pruning dates. August 2018. https://doi.org/10.1007/s10457-017-0136-

[9] Manik1, D. F., Hertiani2, T., Anshory1, H., & . (2014). with antibacterial activity of ethanol extract and cherry leaf fractions. 1–11.

[10] Ningsih, (2017). Potency of methanol extract from leaves of Mango Bacang (Mangifera foetida L.) as an antibacterial against Enterobacter aerogenes and identification of its active compound class.research chemistry journal, flight. 2 no. 2 (2017): December, 108–117. https://ejournal.unair.ac.id/JKR/article/view/5753/4131

[11] Ondusko, & Nolt, D. (2018). *Staphylococcus aureus*. Pediatrics in Review, 39(6), 287–298. https://doi.org/10.1542/pir.2017-0224

[12] Priliani, D. I. (2012).Antibacterial and Bioautographic Activities of Ethanol Extract of Guava Leaves (Anacardium Occidentale L.) Against Pseudomonas Aeruginosa Multiresistant and eprints.ums.ac.id. http://eprints.ums.ac.id/id/eprint/18810

[13] Profile, P., & Bioactivities, H. (2021). Guava (Psidium guajava L .) Leaves : Nutritional Composition ,. 1–20.

[14] Rahmi, M., & Putri, D. H. (2020). The Antimicrobial Activity of DMSO as A Natural Extract SolventBiology Porch, 5(2), 56–58.

[15] Ratnasari, D. (2017).Inhibitory Power of Jatropha Curca L. Leaf Extract on the Growth of Pseudomonas Bacteria aeruginosa repository.unimus.ac.id. http://repository.unimus.ac.id/id/eprint/1240

[16] Sabiladiyni Agus; Djunaedi, Ali, H. A. T. (n.d.). Preliminary Test of Biotransformation Product Activity of Avicennia marina Mangrove Leaves With Fungus Isolate Against Pathogenic Bacteria Klebsiella pneumonia and Enterobacter aerogenes.977-2407769. https://ejournal3.undip.ac.id/index.php/jmr/article/view/25926/23116

[17] Trisia, A., Philyria, R., & Toemon, A. N. (2018). Antibacterial activity test of the ethanol extract of kalanduyung leaves (Guazuma ulmifolia Lam.) against the growth of *Staphylococcus aureus* using the disc diffusion methodPrevious Journal. http://journal.umpalangkaraya.ac.id/index.php/anterior/article/view/12

[18] Ulfah, V. F., Iskandar, Y., Pharmacy, F., Padjadjaran, U., & Barat, J. (2020).Farmaka Farmaka. 17, 98–104.

[19] Valentina, F. K. (2021). Scientific ability of shallot skin powder macerate (allium cepa) against the growth inhibition zone of Staphylococcus aureu bacteriarepo.poltekkes-medan.ac.id. http://repo.poltekkes.medan.ac.id/jspui/handle/123456789/4448

[20] Verdiana, M., Widarta, I. W. R., & Permana, I. D. G. M. (2018). Effect of solvent type on ultrasonic wave extraction on antioxidant activity of lemon peel extract (citrus limon (linn.) burm f.).journal of food science and technology (itepa), 7(4), 213. https://doi.org/10.24843/itepa.2018.v07.i04.p08

[21] Wael, M. U., Dewi, S. S., & Maharani, E. T. W. (2017). Inhibitory Power of Areca Seed (Areca Catechu L.) Infusion Against S. Aureus Bacteria.University of Muhammadiyah Semarang, 7–20.

[22] Wahyuni, S., Dewi, S. S., & Wilson, W. (2018). activities of ethanol extracts of lemongrass (cymbopogon citratus) towards growth *Staphylococcus aureus* and Candida albicans. Repository.Unimus.Ac.Id. http://repository.unimus.ac.id/id/eprint/3185