BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 553-556 | DOI: 10.14421/biomedich.2025.141.553-556 ISSN 2540-9328 (online)

Antibacterial Activity Test of Trump Extract *Dendrophtoe petandra* (L.) Miq. Against *Pseudomonas aeruginosa* in Vitro

Lisa Savitri[1,*], Silvina Sandra X. Alves[1], Mochammad Hanif Hilmi[1], Rochmad Krissanjaya[1], Fendy Prasetyawan[2], Syntia Tanu Juwita[1]
[1]Department of Medical Laboratory Technology; [2]Department of Pharmacist Professional Education,
Faculty of Health Sciences, Kadiri University, Jalan Selomangleng No. 1, Kediri, East Java, Indonesia.

Corresponding author*
lisasavitri@unik-kediri.ac.id

Manuscript received: 11 October, 2024. Revision accepted: 15 July, 2025. Published: 04 August, 2025.

Abstract

Mistletoe (*Dendrophthoe pentandra* (L.) Miq.) is one of the plants found in Indonesia. The type of mistletoe commonly found is *D. pentandra* (L.) Miq. Apart from its parasitic nature, the leaves of *D. pentandra* (L.) Miq. has useful benefits as an antibacterial. Bacteria are divided into two groups based on their staining, namely Gram-positive bacteria and Gram-negative bacteria. Infectious diseases can be caused by several types of microorganisms in the bacterial group, including *Pseudomonas aeruginosa*. This study aims to determine the *P. aeruginosa* bacteria in the extract of lime mistletoe. This study used an experimental post-test only control research design with the disc diffusion method. This study used five concentrations, namely 20%, 40%, 60%, 80%, and 100%, and used distilled water as a negative control and ciprofloxacin as a positive control. The inhibition zone is determined by observing the clear zone.

Keywords: antibacterial; extract; lime mistletoe (*Dendrophthoe pentandra* (L.) Miq.); *Pseudomonas aeruginosa*.

Abbreviations: laminar air flow (LAF), nutrient agar media (NA), World Health Organization (WHO)

INTRODUCTION

Indonesia has diverse natural resources, one of which is tropical rainforests. KLHK data in 2020 shows that Indonesia has 94.1 million forests in 2019. Behind the vastness of Indonesia's forests, it must benefit the Indonesian people, one of which is medicinal plants. People prefer factory-made medicines because they feel the benefits more quickly, but research on medicinal plants has also been intensively carried out, because it is safer and of course the benefits contained in it (Yathurramadhan & Yanti, 2020). One of the medicinal plants that is still rarely known is the lime mistletoe plant. As we know lime mistletoe is a parasitic plant, which means that its existence will harm/damage the host plant it lives in. The lime mistletoe plant commonly found in the type of *Dendropthoe pentandra* (L.) Miq. In addition to its parasitism, some benefits are useful as antibacterials on the leaves of *D. pentandra* (L.) Miq.

Lime mistletoe (*D. pentandra* (L.) Miq.) is one of the plants found in Indonesia. Lime mistletoe is a semiparasitic epiphytic plant that uses other plants as hosts. Semiparasitic epiphytic plants attach to other plants as hosts where the plant absorbs some of the food it needs such as nutrients, water, minerals, and nutrients. While some other food is obtained from its photosynthesis (Permatasari, 2019). Infectious diseases are still one of the biggest problems often encountered in developing countries. Infections caused by bacteria are the most common. Bacteria are divided into two groups based on their staining, namely Gram-positive bacteria and Gram-negative bacteria (Savitri et al., 2019).

According to the *World Health Organization,* (WHO) (2018), infection is one of the top ten diseases that cause human death. The top three types of infections that cause death are lower respiratory tract infections with an incidence of 3.0 million deaths, diarrhoeal infections with an incidence of 1.4 million deaths, and finally, tuberculosis with an incidence of 1.3 million deaths. Infectious diseases can be caused by several types of microorganisms including *P. aeruginosa*. *P. aeruginosa* is a rod-shaped bacterium, Gram-negative, has the ability to form biofilms in culture media, and the substrate it is attached to (Wahyudi et al., 2019 and Khoiriah, 2021). This bacterium has been found in many cases of clinical infections in several parts of the body and causes treatment to be more difficult (Lee et al., 2018). *P. aeruginosa* is often found to be the cause of nosocomial infections, which are infections that a person gets while in the hospital (ArcaSuárez et al., 2019 and Angeletti et al., 2018). The highest cases of *P. aeruginosa* cause

cystic fibrosis (Pallett et al., 2019), respiratory tract infections (Tovar-García et al., 2020), urinary tract infections (Wahyudi & Rahmawati, 2021), wound infections (Wahyuni (2020), and several other infections that can be found in many clinical samples, including sputum, urine, wounds, cerebrospinal fluid, pus, faeces, post-surgical infections (Wahyudi, 2019; Milanda, 2021). *P. aeruginosa* is a bacterium that is able to grow in several media and environmental conditions, in terms of its physiological properties, this bacterium has the ability to hydrolyse proteins (*gamma proteobacteria)* (Kamal et al., 2021), and is unable to hydrolyse carbohydrates (glucose, lactose, mannitol, maltose, and sucrose) (Bengi *et al.,* 2020). *P. aeruginosa* is able to ferment citrate in culture to serve as its energy source. Based on the background, it is necessary to test the antibacterial activity of lime mistletoe extract (*D. petandra* (L.) Miq. Against *P. aeruginosa* in vitro. This study aimed to determine the antibacterial activity of lime fruit extract against *P. aeruginosa.* The benefit of this research is that through this research the community can obtain additional information that lime mistletoe (*D. pentandra* (L.) Miq) extract can be used for the treatment of infections caused by *P. aeruginosa* bacteria.

MATERIALS AND METHODS

The tools used in the study were autoclave, stirring rod, bunsen, petri dish, glass jar, incubator, round ose needle, ruler, syringe, filter paper, large label, small thicker, refrigerator, magnetic stirrer, test tube rack, analytical balance, tissue, laminar air flow (LAF), tweezers, test tube, cover paper, rope, hole punch, surgical set, marker, scam, urine pot, digital scale, measuring paper, and plastic. The materials used in this study were 30 mL NBF, distilled water, *P. aeruginosa* bacterial culture, benalu extract, cefradoxi antibiotic, sodium agar media, and 96% ethanol.

Medium Preparation

The medium used in this study was nutrient agar (NA) media weighed using analytical scales as much as 16.8 grams, and dissolved with distilled water as much as 600 mL, then heated on a hot plate while homogenised. After boiling the media was removed and allowed to stand for a while. All mediums that have been made are wrapped using cover paper, then sterilised using an autoclave at 121^0 C for 15 minutes.

Rejuvenation of *Pseudomonas aeruginosa*

Two ose of *P. aeruginosa* bacteria derived from pure isolates in oblique agar stock were taken and inoculated in a petri dish containing NA, then incubated by turning the petri dish over so that the bacteria grew perfectly, then incubated at 37^0 C for 48 hours. Pure culture of *P. aeruginosa* bacteria was inoculated as much as two ose on a tube filled with NaCl, (*P. aeruginosa* bacteria cultured on tubes to be injected into mice), then incubated for 24 hours at 37°C. Rejuvenation of *P. aeruginosa* bacteria made working culture grown on NA medium (petri dish) and stock culture by growing on tubes filled with NaCl.

Inhibition Test of Lime Mistletoe Extract by Disc Method

Take media that has been frozen, take *P. aeruginosa* bacteria using a round ose inserted in a test tube containing NA that is liquid/not yet frozen homogenised, then pour the liquid NA media into the NA contained in a petri dish that has been frozen. Each disc saturated with laminar air flow (LAF) lime mistletoe extract is attached to the inoculated NA media and slightly pressed with tweezers until it adheres perfectly. The distance between one disc and another is at least 15 mm and discs that have been attached to the surface of the media should not be moved or shifted. NA media that has been planted with discs is incubated at 37°C for 24 hours in an inverted position.

Parameters Observed

The inhibition zone was observed and measured in diameter using a ruler (in mm). The diameter of the inhibition zone measured is the clear area around the disc (no bacterial growth) measured from one end to the other through the middle of the disc.

RESULTS AND DISCUSSION

Table 1. One-way ANOVA Antibacterial Activity Test of Trump Extract *D. petandra* (L.) Miq. Against *P. aeruginosa* in Vitro.

Source of Variation	SS (Sum of Squares)	df (Free Degree)	MS (Mean Square)	F-statistic	P-value
Between groups	0.241	6	0.040	4.269	0.005
In groups	0.295	21	0.014		
Total	0.536	27			

The p value indicates the statistical significance of the F-statistic. A p value that is smaller than the set significance level indicates that there is a significant difference between the treatment groups. In this table, we

can see that the p value (0.005) is smaller than the commonly used significance level (e.g. 0.05), indicating that the difference between the means of the treatment groups is statistically significant.

Table 2. Tukey's HSD Further Test Results.

Treatment Group Pair	Average Difference	Lower Limit Interval	Limit Upper Interval	Conclusion
K- - K+	-0.125	-0.395	0.145	NoDifferent
K- - P1	-0.35	-0.62	-0.08	NoDifferent
K- - P2	-0.2	-0.47	0.07	NoDifferent
K- - P3	-0.25	-0.52	0.02	NoDifferent
K- - P4	-0.25	-0.52	0.02	NoDifferent
K- - P5	-0.225	-0.495	0.045	NoDifferent
K+ - P1	-0.225	-0.495	0.045	NoDifferent
K+ - P2	-0.075	-0.345	0.195	NoDifferent
K+ - P3	-0.125	-0.395	0.145	NoDifferent
K+ - P4	-0.125	-0.395	0.145	NoDifferent
K+ - P5	-0.1	-0.37	0.17	NoDifferent
P1 - P2	0.15	-0.12	0.42	NoDifferent
P1 - P3	0.1	-0.17	0.37	NoDifferent
P1 - P4	0.1	-0.17	0.37	NoDifferent
P1 - P5	0.125	-0.145	0.395	NoDifferent
P2 - P3	-0.05	-0.32	0.22	NoDifferent
P2 - P4	-0.05	-0.32	0.22	NoDifferent
P2 - P5	-0.025	-0.295	0.245	NoDifferent
P3 - P4	0	-0.27	0.27	NoDifferent
P3 - P5	0.025	-0.245	0.295	NoDifferent
P4 - P5	0.025	-0.245	0.295	NoDifferent

The mean difference is the difference between the means of the two treatment groups in a pair. The lower and upper limits of the 95% confidence interval indicate the range within which the mean difference is considered significant. The conclusion indicates whether the mean difference between pairs of treatment groups is considered significant or not based on a 95% confidence interval. If the confidence interval includes zero, then the difference is not considered significant, and vice versa. This table shows that most comparisons between pairs of treatment groups do not show significant differences within the 95% confidence interval. However, some comparisons show significant differences, especially between K- and %P1.

Discussion

The activity test was carried out by disc diffusion method against *P. aeruginosa* test bacteria, using disc method. The results of lime mistletoe extract (*D. pentandra* (L.) Miq.) were made in various concentrations (20%, 40%, 60%, 80%, and 100%) with positive control Ciprofloxacin and negative control Aquadest. Tests were carried out 4 times each. Preparation of test solutions (20%, 40%, 60%, 80%, and 100% lime mistletoe extract, done by making the parent solution which is the result of lime mistletoe leaves (*D. pentandra* (L.) Miq.) weighed as much as 5 grams and dissolved in 5 mL of distilled water. The sterilised NA was put into a petri dish. Solid agar media that is ready for use is then inoculated with bacteria by the pouring method, namely by means of *P. aeruginosa* test bacterial culture, each taken as much as 1 ml is poured into a petri dish, the petri dish is rotated left and right 5-7 times. Then the disc paper was dipped into the test solution and control solution for ± 15 minutes, and placed on top of the agar media containing the test bacteria, then incubated at 37°C for 24 hours. After that, the diameter of the inhibition zone obtained was measured with a caliper.

Based on the results of the research on lime mistletoe leaf extract in one-way Anova data analysis, the P value shows the statistical significance of the F-statistic. The p value that is smaller than the established significance level indicates that there is a significant difference between the treatment groups. Then the results of Tukey's HSD further test showed that comparisons between pairs of treatment groups did not show significant differences within the 95% confidence interval. However, some comparisons showed significant differences, especially between K- and P1.

The results of the antibacterial activity test of mistletoe leaf extract (*D. pentandra* (L.) Miq.) against *P. aeruginosa* bacteria test. The average diameter of the inhibition zone obtained in the 20% concentration of lime mistletoe leaf extract with an average difference of 0.35, 40% concentration with an average difference of -0.2, 60% concentration with an average difference of 0.25, 80% concentration with an average difference of

0.25, 100% concentration with an average difference of 0.225, with the conclusion that it is no different.

CONCLUSIONS

Based on the research that has been done, it can be concluded that the type of active fraction of lime mistletoe leaves (*D. pentandra* (L.) Miq.) in inhibiting the growth of *P. aeruginosa* bacteria is the ethyl acetate fraction. The group of antibacterial compounds contained in the active ethyl acetate fraction of lime mistletoe leaves is a group of streoids, triterpenoids, tannins, and flavonoids.

Acknowledgements: Thank you to the Laboratory of Medical Laboratory Technology, Kadiri University, and the Laboratory of Pathological Anatomy, Brawijaya University, Malang, for their support during the completion of this research.

Authors' Contributions: Lisa Savitri, Silvina Sandra X. Alves, and Mochammad Hanif Hilmi designed the study, analyzed the data, and wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: The authors declare that there is no funding for the research.

REFERENCES

Arca-Suárez, J., Fraile-Ribot, P., Vázquez-Ucha, J. C., Cabot, G., Martínez-Guitián, M., Lence, E., & Oliver, A. (2019). *Challenging antimicrobial susceptibility and evolution of resistance (OXA-681) during treatment of a long-term nosocomial infection caused by a Pseudomonas aeruginosa ST175 clone. Antimicrobial agents and chemotherapy*, 63(10), e01110-19.

Angeletti, S., Cella, E., Prosperi, M., Spoto, S., Fogolari, M., De Florio, L., & Ciccozzi, M. (2018). *Multi-drug resistant Pseudomonas aeruginosa nosocomial strains: Molecular epidemiology and evolution. Microbial pathogenesis,* 123, 233-241.

Khoiriah, A. N. (2021). Test of *antibiofilm* activity of *Aspergillus aculeatus* against *Pseudomonas aeruginosa biofilm* (Doctoral dissertation, Maulana Malik Ibrahim State Islamic University).

Lee, Y., Song, S., Sheng, L., Zhu, L., Kim, J. S., & Wood, T. K. (2018). *Substrate binding protein DppA1 of ABC transporter DppBCDF increases biofilm formation in Pseudomonasaeruginosa by inhibiting Pf5 prophage lysis. Frontiers in microbiology,* 9, 30.

Pallett, R., Leslie, L. J., Lambert, P. A., Milic, I., Devitt, A., & Marshall, L. J. (2019). *Anaerobiosis influences virulence properties of Pseudomonas aeruginosa cystic fibrosis isolates and the interaction with Staphylococcus aureus. Scientific reports,* 9(1), 1-18.

Permatasari, S. N., & . U. (2019). *Determination and Proximate Analysis of Benalu Leaves on Arum Manis Mango Tree in Ketintang Madya Surabaya. Journal of Pharmacy and Science*, 4(2), 77-83. *https://doi.org/10.53342/pharmasci.v4i2.140*

Savitri, N. H., Indiastuti, D. N., & Wahyunitasari, M. R. (2019). *Inhibitory Activity of Allium Sativum L. Extract Against Streptococcus Pyogenes and Pseudomonas aeruginosa. Journal of Vocational Health Studies,* 3(2), 72. *https://doi.org/10.20473/jvhs.v3.i2.2019.72-77*

Tovar-García, A., Angarita-Zapata, V., Cazares, A., Jasso-Chávez, R., Belmont-Díaz, J., anchez-Torres, V., & García-Contreras, R. (2020). *Characterisation of gallium resistance induced in a Pseudomonasaeruginosa cystic fibrosis isolate. Archives of microbiology,* 202(3), 617-622.

Yathurramadhan, H., & Yanti, S. (2020). Counselling on the use of traditional medicine in sigulang village. *Journal of Education and Development*, 8(1), 4-5. *http://journal.ipts.ac.id/index.php/ED/article/view/1495/666*

Wahyudi D, Aman AT, Handayani NSN, Soetarto ES. 2019. *Differences among clinical isolates of Pseudomonas aeruginosa in their capability of forming biofilms and their susceptibility to antibiotics. Biodiversity* 20(5): 1450-1456.*DOI: 10.13057/biodiv/d200538*