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Antibacterial Activity of *Marchantia paleacea* Extract against *Klebsiella pneumoniae* strains from Urinary Tract Infection Patients

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Abstract
Marchantia paleacea grows mainly in humid places and is widespread in mountainous areas in Indonesia. According to several studies, this species contains bioactive compounds that can be used as antimicrobials. This study aims to determine the effect of *Marchantia paleacea* extract on *Klebsiella pneumoniae*. The method used in this research is the Kirby-Bauer well diffusion method. The research was carried out by giving eight treatments, namely A (0.1 mg/mL), B (0.2 mg/mL), C (0.4 mg/mL), D (0.6 mg/mL), E (0.8 mg/mL), F (1 mg/mL), Aquades (negative control), and ciprofloxacin (positive control). The best average results for the clear zone diameter parameter of *Klebsiella pneumoniae* were found at concentrations of 0.4 mg/mL, 0.6 mg/ml, and 0.8 mg/ml. *Marchantia paleacea* extract can inhibit the growth of *Klebsiella pneumoniae*, but its effectiveness is low. Further research is needed on *Marchantia paleacea* extract at higher doses or using pure extract for better results.

Keywords: Antimicrobial, *Klebsiella pneumoniae*, *Marchantia paleacea*

Introduction

Urinary tract infections, a prevalent issue in the community, pose a significant global health concern, especially for women and individuals with compromised immune systems (Ibrahim *et al*., 2018) [8]. *Klebsiella pneumoniae*, a Gram-negative bacterium known for its high resistance to various antibiotics, is a major contributor to these infections, further complicating their treatment (Zhang *et al*., 2020) [25]. The escalating problem of antibiotic resistance underscores the pressing need for effective alternative therapies. However, amidst this challenge, there is a glimmer of hope in the potential of natural resources, such as the Marchantia paleacea extract, to offer practical solutions in the battle against antibiotic resistance.

Marchantia is a group of moss that has properties that are beneficial to humans. The ingredients contained in Marchantia include active secondary metabolites such as alkaloids, flavonoids, steroids, triterpenoids, coumarins, etc. One natural resource that has attracted attention is Marchantia paleacea, a liverwort species used in traditional medicine in some cultures. (Purkon *et al*., 2022; Gomes *et al*., 2022) [19, 7]. *Marchantia paleacea* has adaptogenic and anti-inflammatory properties and contains various bioactive compounds with potential therapeutic applications (Gomes *et al*., 2022) [7]. The unique chemical composition of *M. paleacea*, including terpenoids, flavonoids, and saponins, has been reported to have multiple health benefits. The use of *M. paleacea* in this context is driven by the potential of its bioactive compounds, which have not been fully explored, and the need to evaluate its ability to combat urinary tract infections caused by antibiotic-resistant pathogens.

Previous research has shown that some plant extracts have significant antimicrobial potential, inhibiting pathogenic bacteria such as *K. pneumoniae* (Arsène *et al*., 2020) [1]. Examples include studies on traditional plant extracts like *Echinacea purpurea* and *Allium sativum*, which have demonstrated antimicrobial activity against various pathogenic bacteria (Njimoh *et al*., 2015; Zazharskyi *et al*., 2019) [16, 24]. In addition, research on *M. paleacea* has also suggested its potential as a source of antimicrobial compounds (Zazharskyi *et al*., 2019; Oumar *et al*., 2021) [24, 17]. However, this information has not yet been exploited optimally, so knowledge about Marchantia paleacea, including the bioactive components in this moss plant, is still lacking.

As science and technology develop, *M. paleacea* as a natural product can be developed as an effective medicinal plant. Various studies have shown the potential of Marchantia paleacea as an antimicrobial for gram-negative and gram-positive bacteria. However, there has never been any report on the inhibitory effect of the antimicrobial *M. paleacea* on the growth of *K. pneumoniae* in sufferers of urinary tract infections.

Therefore, this research was carried out to provide information regarding the inhibition of Marchantia paleacea antimicrobial substances against the growth of *K. pneumoniae* in patients with urinary tract infections. The findings of this research could provide valuable insights into the development of novel antimicrobial agents derived from natural sources to address the challenge of antibiotic resistance.

Materi and Method

Plant and material

M. paleacea was collected from cliffs and rocks at Curug Cilember, Bogor, West Java. The plant has been identified in the Laboratory of Botany of the Faculty of Sciences, University of Indonesia. The plants were washed and dried in the laboratory under air conditioning (0˚C ± 2˚C) until analysis. *K. pneumonia* was collected in the Microbiology Laboratory of FMIPA IPB. Natrium Agar, Natrium broth, LA, and EMBA were purchased from Merck. ciprofloxacin 500 mg was used as a positive control.

Sample extraction

Ten grams of dried Simplicia were macerated with 100 mL of methanol for 48 hours with occasional stirring. The extract was then filtered with Whatman filter paper No. 1. The experiment was repeated by dissolving 100 mL of methanol into the remaining extract from the first maceration. The resulting solvent was separated from the extract with a Vacuum Rotary Evaporator (EL131) at 40ºC. The three extracts were stored at -20˚C until use.

Inoculums preparation

Bacterial strains were subcultured overnight at 37ºC with nutrient agar slants. Bacterial growth was harvested using 5 ml of sterile 9% NaCl; the absorbance was set at 580 nm, and evaporation to reach the Mc Farland standard of 10^6 CFU/ml using a spectrophotometer.

Antimicrobial activity assay

This study used the Kirby-Bauer agar well diffusion method with modifications. 100 mg of *M. paleacea* concentrated extract was dissolved in 10 ml of distilled water to reach 10,000 ppm. The solution was then carried out in graded dilutions to reach concentrations of 0.1 mg/mL (A), 0.2 mg/mL (B), 0.4 mg/mL (C), 0.6 mg/mL (D), 0.8 mg/mL (E), and 1 mg/mL (F). The antibiotic 5 µL ciprofloxacin (G) was used as a positive control, and distilled water (H) as a negative control. The sterile plates were filled with 15 ml of stock bacteria and given sodium agar media. Sodium Agar media (7.5 ml) was poured into a sterile cup, then left to solidify slightly (15 minutes), then sprinkled with 100 µL of bacterial suspension and spread evenly. Once the media is solid, make holes using a sterile cord driller with a diameter of 6 mm. The wells were given 25 µL of extract. Once completed, the cup was incubated at 35 °C for 24 hours. A zone of inhibition was measured with a caliper, recorded, and considered an indication of antibacterial activity.

Data analysis

The inhibition zone data obtained was subjected to ANOVA analysis to see differences in bacterial inhibition in all treatment groups. If the data tested gives significant results, continue with the t-test to see the differences between groups in *K. pneumonia* inhibitory power.

Results and Discussion

The inhibitory power test of *M. paleacea* extract against *K. pneumonia* measured the clear zone around the well using the average value of each treatment. The larger the diameter of the clear zone produced, the greater the effect of *M. paleacea* extract. Our results show that the average diameter of the clear zone resulting from the activity of Marchantia extract compounds is above 10 mm.

Table 1: Average inhibitory power of moss extract against *Klebsiella pneumonia*

S. No	Consentrasion (mg/ml)	Diameter zone (mm)	Note
1	0.1	13.25	R
2	0.2	12.50	R
3	0.4	24.50	S
4	0.6	21.13	S
5	0.8	21.25	S
6	1	16.25	I
7	Control +	31.25	S
8	Control -	0	R

Note: R= Resistan; I= Intermediet; S = Susceptible

The most significant barrier zone values were found at 0.4 mg/mL, 0.6 mg/mL, and 0.8 mg/mL of *M. paleacea* extracts. These were 24.5 mm, 21.125 mm, and 21.25 mm, respectively. These results show that the extraction of the Marchantia palate influences the growth of *K. pneumoniae's* bacterial colony and could act as an antibacterial agent. Because they contain little peptidoglycan, they are more susceptible to mechanical damage. (Tortora *et al*., 2013). This makes the zones that form at 0.1 mg/mL and 0.2 mg/mL of *M. paleacea* in the 13.25 mm and 12.5 mm resistance groups. *M. paleacea* produces the largest barrier zone at a 0.4 mg/ml concentration, measuring 24.5 mm.

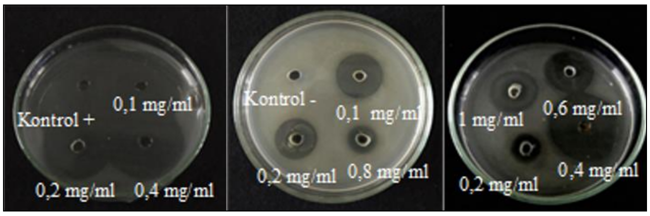

Fig 1: Diameter of the inhibition zone of *M. paleacea* extract against *K. pneumonia*

The extracts of *M. palacea* (0.4 mg/mL) can produce a barrier area with a diameter of more than 20 mm, categorized as sensitive. Faramayuda (2013) [6] asserts that the presence of a bioactive compound, such as alkaloids, flavonoids, polyphenols, henson, saponins, centroids, and monoterpenoids, contributes to the antimicrobial activity of the *M. paleacea* palate extract. The presence of phenolic compound components triggers antimicrobial activity (Fadhilla, 2012) [5]. Active compounds found in leaf extracts, such as hydroquinone phenols and tannins, are identified as substances with antimicrobial activity (Wiranto *et al*., 2021) [23]. Furthermore, flavonoid compounds contained in leaf infusions are recognized as the largest group of phenolic compounds (Maftuhah, 2015) [12]. Generally, phenolic compounds are more inhibitory (Cowan, 1999) [3]. The mechanism responsible for phenolic toxicity to microorganisms involves inhibiting enzymes by oxidized compounds. Another compound that can inhibit the growth of bacterial colonies is alcoholide. Rahmi (2016) stated that alcoholide inhibits bacterial growth by interfering with the constituent components of the peptidoglycan in bacteria cells. This interferes with the formation of the cell wall layer, leading to cell death.

According to a Junairiah study (2015) [10], *E. coli* at 5000 ppm concentrations, *S. aureus* at 40,000 ppm, and *C. albicans* at 60.000 ppm showed the highest diameter inhibition results. Generally speaking, the higher the concentration of the extract, the larger the diameter of the inhibitors produced. Each microbe has a different inhibitory response. We suspect that differences in the diffusion rate of the antibacterial compound on the medium, resulting from various types and concentrations, cause the decrease in the barrier zone at high extract concentrations.

Pratama (2015) [18] asserts in his research that the quality of the extract can influence the size of the formed barrier zone. Two factors affect the quality of extracts: Biological and chemical. Biological factors include plant species, plant origin, harvest time, storage of raw materials, age, and part of the plant used. The second factor encompasses both external and internal chemical factors, among others. External factors that influence this include the size of the material, the filters used in extraction, the content of heavy metals, pesticides in plants, and the extraction methods used. The following factors influence a substance's antimicrobial activity: The concentration of the substance, the time, the temperature, the pH, and the organism's properties (Susilo & Suciati., 2016) [21].

The study showed that the extract of *Marchantia paleacea* has antimicrobial properties against Klebsiella pneumoniae. This was demonstrated at a minimum barrier concentration (MIC) of 0.1 mg/ml, where the lowest concentration inhibits the growth of the bacterium *K. pulmonate*. Additionally, the extract has a barrier capacity of 24.5 mm at a concentration of 0.4 mg/mL, where the barrier diameter is a sensitive group. This indicates that the bacteria *K. pneumonia* remains sensitive to the extracts of the Marchantias palm.

Conclusion

The best average antibacterial activity of the *M. paleacea* liver marrow extract was at a concentration of 0.4 mg/ml, 24.5 mm. The antimicrobial compound that plays a role in inhibiting the growth of the bacteria *K. pneumoniae* is phenolic. For better results, further research on the Marchantia paleacea extract is needed, and it is hoped that this research will provide information on antimicrobial substances.

References

1. Arsène MMJ, Irina VP, Linda DAK, Ngah E, Alexander NT. Urinary tract infections: Virulence factors, resistance to antibiotics, and management of uropathogenic bacteria with medicinal plants: A review. Open Science Publishers LLP, July 5, 2020. Doi: https://doi.org/10.7324/japs.2021.110701
2. Asakawa Y. Recent Advances of Biologically Active Substances from the Marchantiophyta. SAGE Publishing. 2008; 3(1):1934578X0800300-1934578X0800300. Doi: https://doi.org/10.1177/1934578x0800300116
3. Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999; 12(4):564-582.
4. Dabur R, Gupta A, Mandal T, Singh DP, Bajpai VK, Gurav AM, *et al*. Antimicrobial Activity of Some Indian Medicinal Plants. African Traditional Herbal Medicine Supporters Initiative (ATHMSI). 2008; 4(3):313-313. Doi: https://doi.org/10.4314/ajtcam.v4i3.31225
5. Fadhilla R, *et al*. Aktivitas antibakteri ekstrak tumbuhan lumut hati (Marchantia paleacea) terhadap bakteri patogen dan perusak masakan. Jurnal Teknologi dan Industri Pangan. 2012; 23(2):126-131. Doi: 10.6066/jtip.2012.23.2.126.
6. Faramayuda F, Ratnawati J, Dewi AM, *et al*. Isolasi Senyawa Sitotoksik dari Lumut Hati (*Marchantia paleacea Bertol.*). Prosiding Seminar Nasional Tumbuhan Obat Indonesia. 2013; 44:433-446.
7. Gomes PWP, Martins LHDS, Gomes EJL, Muribeca ADJB, Pamplona S, Komesu A, *et al*. Antiviral Plants from Marajó Island, Brazilian Amazon: A Narrative Review. Multidisciplinary Digital Publishing Institute. 2022; 27(5):1542-1542. Doi: https://doi.org/10.3390/molecules27051542
8. Ibrahim NNN, Usup G, Ahmad A. Antimicrobial activity screening of marine bacteria isolated from Port Klang and Port Tanjung Pelepas. American Institute of Physics, 2018. Doi: https://doi.org/10.1063/1.5027992
9. Jetty A, Iyengar DS. Antimicrobial Activity of Millingtonia Hortensis Leaf Extract. Taylor & Francis. 2000; 38(2):157-160. Doi: https://doi.org/10.1076/1388-0209(200004)3821-1ft157
10. Junairiah, Saadiyah M, Salamun. Identifikasi Metabolit Sekunder dan Aktivitas Antimikrob Ekstrak Etil Asetat Dumortiera hirsute. Sains dan Matematika. 2015; 3(2):45-49.
11. Limsuwan S, Subhadhirasakul S, Voravuthikunchai SP. Medicinal plants with significant activity against important pathogenic bacteria. Taylor & Francis. 2009; 47(8):683-689. Doi: https://doi.org/10.1080/13880200902930415
12. Maftuhah A, Bintari HS, Mustikaningtyas D. Pengaruh Infusa Daun Beluntas (*Plucea indica L.)* Terhadap Pertumbuhan Bakteri *Stapphylococcus epidermis.* Unnes Journal Science Life. 2015; 4(1):60-65.
13. Meurer-Grimes B, McBeth DL, Hallihan BH, Delph S. Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. Taylor & Francis. 1996; 34(4):243-248. Doi: https://doi.org/10.1076/phbi.34.4.243.13220
14. Mukesh S, Vimal M, Maneesha A, Sharma M. Antimicrobial potential of the phytoextracts of some Nyctaginaceae members. Academic Journals. 2010; 9(46):7942-7947. Doi: https://doi.org/10.5897/ajb09.247
15. Mutai C, Bii C, Ioannou E, Abatis D, Roussis V. Antimicrobial activity of Acacia mellifera extracts and lupane triterpenes. Elsevier BV. 2009; 123(1):143-148. Doi: https://doi.org/10.1016/j.jep.2009.02.007
16. Njimoh DL, Assob JCN, Mokake SE, Dinga JN, Yinda CK, Sandjon B. Antimicrobial Activities of a Plethora of Medicinal Plant Extracts and Hydrolates against Human Pathogens and Their Potential to Reverse Antibiotic Resistance. Hindawi Publishing Corporation, 2015, 1-15. Doi: https://doi.org/10.1155/2015/547156
17. Oumar YS, Delphine ME, Kalamourou S, Traore M, Idrissa D, Joseph DA, *et al*. Phytochemical Study and Antibacterial Activity of Ethanolic and Aqueous Extracts of Barks of Myrianthus Holstii Engl.

(CECROPIACEAE). 2021; 9(11):1143-1150. Doi: https://doi.org/10.21474/ijar01/13852
18. Pratama Eka Y. Aktivitas Antimikroba Ekstrak Daun dan Buah Ginje (*Thevetia peruviana*) Terhadap *Staphylococcus aureus* Dan *Candida albicans* Secara *In vitro*.[Skripsi]. Universitas Muhammadiyah Surakarta: Surakarta, 2015.
19. Purkon DB, Fadhlillah FM, Maigoda TC, Iwo MI, Soemardji AA, Nadhifah A, *et al*. Phytochemical, Use in Ethnomedicine, and Therapeutic Activities of Marchantia Genus. Airlangga University. 2022; 5(3):174-174. Doi: https://doi.org/10.20473/jvhs.v5.i3.2022.174-185
20. Rahmi A, Cahyanto T, Sujarwo T, Lestari I. Uji Aktivitas Antibakteri Ekstrak Daun Beluntas (*Pluchea indica* (L.) LESS.) Terhadap *Propionibacterium acnes* Penyebab Jerawat. Research Gate. 2015; 9(1). Doi: 10.13140/RG.2.1.4234.9843.
21. Susilo S, Suciati R. Studies of Morphological and Secondary Metabolites Variaty of Mosses (Bryophyta) in Cibodas, West Java. International Journal of Advanced Research. 2016; 4(12):1397-1402. Doi: https://doi.org/10.21474/ijar01/2536
22. Tortora GJ, dkk. Microbiology An Introduction 11th Edition. Pearson: USA, 2007.
23. Wiranto B, Husnin, Susilo. Diversity of terrestrial ferns (Pteridophytes) in Ciliwung Telaga Warna Puncak Bogor tea estate in West Java. Journal of Physics: Conference Series. 2021; 755(1):1-6. Doi: https://doi.org/10.1088/1755-1315/755/1/012031
24. Zazharskyi VV, Davydenko PO, Kulishenko OM, Боровик IB, Brygadyrenko VV. Antimicrobial activity of 50 plant extracts. Oles Honchar Dnipro National University. 2019; 27(2):163-169. Doi: https://doi.org/10.15421/011922
25. Zhang S, Yang Z, Sun L, Wang Z, Sun L, JinLi X, *et al*. Clinical Observation and Prognostic Analysis of Patients With Klebsiella pneumoniae Bloodstream Infection. Frontiers Media. 2020; 10. Doi: https://doi.org/10.3389/fcimb.2020.577244