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Research Article

Antibacterial Activities of Red Mangrove (*Rhizophora stylosa* Griff.) Leaf Extract against *Klebsiella pneumoniae* ATCC 700603

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ABSTRACT

Multidrug-resistant (MDR) *Klebsiella pneumoniaeis* a critical pathogen causing severe human diseases, including pneumonia. Combating the growing threat of MDRK. pneumoniae requires innovative approaches, such as exploring plant-derived antibacterial agents. *Rhizophora stylosa* Griff., a mangrove species with traditional medicinal uses, is recognized for its bioactive compounds with potential antibacterial properties. However, research on its bioactive constituents remains limited. This study investigated the antibacterial activity of *R. stylosa* leaf extracts prepared via maceration and liquid-liquid fractionation against *K. pneumoniae* ATCC 700603. Using agar-well diffusion and cell leakage assays, the water fraction demonstrated moderate inhibition of *K. pneumoniae*, producing an average inhibition zone of 8.24 mm and a minimum inhibitory concentration (MIC) of 400 mg/ml. UV-Vis spectrophotometry revealed that the water fraction disrupted protein and nucleic acid synthesis, evidenced by leakage of cellular materials at 260 and 280 nm. Additionally, scanning electron microscopy (SEM) images of *K. pneumoniae* cells treated with the water fraction showed pore formation and structural damage. These results emphasize the antibacterial potential of the water fraction of *R. stylosa* leaves against MDRK. pneumoniae. Further investigations are necessary to isolate and identify the specific bioactive compounds responsible for these effects. Moreover, comprehensive assessments of activity and toxicity are crucial to advance *R. stylosa*-based antibacterial agents as promising alternatives for addressing the challenge of MDR bacterial infections.

1. Introduction

Klebsiella pneumoniae was initially reported in 1882 by Carl Friedlander as a bacterium isolated from the lungs of patients who had died from pneumonia (Munoz-Price *et al.* 2013; Dong *et al.* 2022). *K. pneumoniae* is a Gram-negative, encapsulated, rod-shaped, facultative anaerobic bacterium belonging to the Enterobacteriaceae family which can colonize and cause a variety of diseases in humans, notably hospital-acquired infections (Nasresfahani *et al.* 2017). It can be carried asymptomatically infections in the gastrointestinal tract, skin, nasal, and throat of healthy people. Still, it can also cause a variety of infections in hospitalized patients, most frequently pneumonia, wounds, soft tissue, and urinary tract infections, when the host immunity fails to control the pathogen growth (Holt *et al.* 2015; Nguyen *et al.* 2015). Clinically, these infections are routinely treated with β-lactams and other antibiotics that are efficacious towards Enterobacteriaceae in hospitalized or otherwise immunocompromised patients (Martin and Bachman 2018).

The emergence of *K. pneumoniae* strains that have acquired new genetic features and become either hypervirulent (HvKp) or carbapenem-resistant (CR-

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Kp) has caused alarming circumstances (Paczosa and Mecsas 2016; Chang *et al.* 2021). *K. pneumoniae* is often characterized by two basic mechanisms of antibiotic resistance: (1) The production of extended-spectrum β-lactamases (ESBLs) confers resistance to all penicillin, third-generation cephalosporins, and aztreonam, but not to cephamycins on bacteria (Shaikh *et al.* 2015), (2) The production of carbapenemases which renders bacterial resistant to nearly all available β-lactams, including carbapenems, is an even more worrisome resistance mechanism (Gulumbe and Ajibola 2020). Due to this challenging global threat, further studies on the potential antibacterial agents and the implementation of preventative measures are required to reduce the incidence and spread of *K. pneumoniae* infections, as well as the associated morbidity and mortality.

Medicinal plants are promising resources since it is known that they contain bioactive compounds with several pharmacological activities, including inhibiting the growth of Enterobacteriaceae by interacting with them through several mechanisms (Rai *et al.* 2010; Castronovo *et al.* 2021). Medicinal plants, with their various compounds, have been used to treat human ailments for centuries with minimal adverse effects, making medicinal plants a promising source for the discovery of new antibacterial agents (Panda *et al.* 2009; Sofowora *et al.* 2013; Vaou *et al.* 2021).

Red mangrove (*Rhizophora stylosa* Griff.) belonging to the family Rhizophoraceae contain numerous phytochemical compounds with significant medicinal potential, including diterpenoids, triterpenoids, sesquiterpene, daucosterol, atranorin, palmitone, polyphenols, polymeric tannins, and hydrolyzable tannins (Wu *et al.* 2009; Kalasuba *et al.* 2023). *R. stylosa* Griff. has been shown by conventional medicine to be effective in the treatment of hematuria, wound healing, rheumatism, and liver diseases (Abubakar *et al.* 2019).

Although many mangrove species have been used for centuries to treat ailments in accordance with local traditions in numerous countries, many of them have not yet been subjected to extensive scientific investigation, and their medicinal characteristics have therefore not been fully verified, especially *R. stylosa* (Gopal *et al.* 2019; Kalasuba *et al.* 2023). However, some earlier studies, for example, Seepana *et al.* (2016) had investigated the antibacterial activity of the Rhizophoraceae family, particularly the leaves of *R. apiculata* and *Bruguiera gymnorrhiza*, against Gram-negative and Gram-positive microorganisms. The results demonstrated that both species of mangroves inhibited the growth of *K. pneumoniae* with a zone of inhibition measuring 23 mm in diameter.

There is a significant lack of research on the chemical compounds of *R. stylosa*. However, in a previous study, an extract of *R. stylosa* was utilized to inhibit the growth of *Escherichia coli* effectively. The leaves of *R. stylosa* exhibited antibacterial activity against *E. coli*, with inhibition zones spanning between 11 and 19 mm (Mouafi *et al.* 2014), indicating their potential as a broad-spectrum antibacterial agent against pathogenic bacteria (Kainuma *et al.* 2015). In this study, *R. stylosa* leaves were used to evaluate the antibacterial activity against the *K. pneumoniae* bacterium. Moreover, a scanning electron microscope (SEM) micrograph might reveal structural damage caused by metabolites in the leaf fraction of *R. stylosa* toward *K. pneumoniae* cells. Therefore, the purpose of this study was to investigate the potential secondary metabolites derived from the active fraction of *R. stylosa* leaves to inhibit *K. pneumoniae* ATCC 700603 and the effects of antibacterial exposure on the morphological structure of the bacterium.

2. Materials and Methods

2.1. Materials

K. pneumoniae ATCC 700603 was obtained from the Microbiology and Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran, Indonesia. The experimental material consisted of fresh red mangrove (*R. stylosa* Griff.) leaves collected from Mangrove Forest, Karangsong Coast, Indramayu, West Java, Indonesia, in March 2022.

2.2. Mangrove Samples Extraction

The fresh leaf samples were rinsed with water to remove dirt and then dried for seven days in indirect sunlight. The desiccated samples were then crushed and powdered to increase the surface area, and then the pulverized samples were extracted at room temperature using modified maceration techniques (Joel and Bhimba 2010). Briefly, 900 g of powdered samples were soaked in 1,000 ml of 96% ethanol and stirred to produce ethanol extracts. Maceration was performed for three days, with the solvent being replaced every twenty-four hours. Afterward, the complete extracts were filtered with Whatman No. 1 filter paper. Using a rotary evaporator [BUCHI B-480, Switzerland] at 45°C, the filtrate was concentrated to obtain crude ethanol extracts. The ethanol extracts were then separated using a liquid-liquid extraction method with three different solvents:

n-hexane, ethyl acetate, and water. Prior to *in vitro* antibacterial screening, the extracts were stored at 4°C in airtight glass vessels.

2.3. Partial Purification of Bioactive Compound by Thin-layer Chromatography (TLC)

Further TLC was performed using silica gel 60 F254 [Merck, Germany] to characterize the bioactive compounds. Each extract was eluted with various selected eluent compositions according to the method described by Harbone (1984) for optimizing gradient polarities, i.e., *n*-hexane: ethylacetate (7:3), ethylacetate: methanol (9:1), and ethylacetate: methanol (1:1). The TLC results were observed after heat treatment and under 254 nm and 365 nm UV light. Afterward, individual fractions were collected and screened for antibacterial activity using the method described originally.

2.4. Antibacterial Activity Test

Antibacterial activity assay on each fraction adopted the Well Diffusion Susceptibility method. Nutrient Agar (NA) [HiMedia Laboratories LLC, Maharashtra, India] inoculated with a suspension of *K. pneumoniae* ATCC 700603 was used as the medium for this test. Then, a hole with a diameter of 6 to 8 mm is punctured aseptically with a sterile cork borer or a tip. A volume (50-100 µL) of the antimicrobial agent or fractions solution at concentrations 600 mg/ml, 700 mg/ml, 800 mg/ml, 900 mg/ml, and 1,000 mg/ml were added to the well and allowed to absorb. Antibiotic Ceftriaxone [Interbat, East Java, Indonesia] 30 µg/ml was used as the positive control, and distilled water was used as a negative control. The test samples were then incubated at 37°C for 18 to 24 hours under aerobic conditions. The existence of a clear zone surrounding the well indicates that this extract inhibits growth (Balouiri *et al.* 2016).

2.5. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The determination of MIC values of each fraction extract of *R. stylosa* leaf against *K. pneumoniae* ATCC 700603 was evaluated qualitatively by the microdilution method on a 96-well plate [Nest Biotechnology Co., Ltd, China], which was modified according to a previous method by Kang *et al.* (2019). Concentrations of 400 to 700 mg/ml of each fraction were used to determine the MIC_{50}. The fractions were put into a microtube with 3 ml of Mueller-Hinton Broth (MHB) [Merck, New Jersey, USA] media and were homogenized using a vortex. Then, 100 µL of the fraction extract was added to each well. 10 µL of a *K. pneumoniae* inoculum suspension adjusted to match the turbidity of the McFarland 0.5 scale (1×10^8 cells/ml) was added to each 96-well plate and incubated at 37°C for 24 hours. After incubation, the presence (or lack) of growth was visually determined. The MIC refers to the lowest concentration at which visible growth is completely inhibited.

The two lowest concentrations of inhibiting bacteria were used to determine the MBC. A total of 50 µL of the fraction extract with MIC value concentration was sub-cultured on NA agar plates. The MBC was found after 24 h of incubation at 37°C based on growth control. Minimum bactericidal was defined as the lowest fraction of extract concentration that limits the bacterial population's growth and viability.

2.6. Leakage Assay based on Protein and Nucleic Acid Level

The microbial biomass was obtained by centrifuging a suspension of test bacteria aged 18 to 24 hours at 3,500 rpm for 15 to 20 minutes. After discarding the filtrate, the active fraction of *R. stylosa* leaf was added until the final concentrations of 1 MIC and 2 MIC were reached. The bacterial pellet was then suspended in a 7.4-pH phosphate buffer solution to a final volume of 10 ml. After exposing the active fraction to the test bacteria for 24 hours at 37°C, the presence of protein and nucleic acid content was determined. The suspension was centrifuged for 15 minutes at 3,500 rpm. Then, the supernatant was separated and analyzed using a PerkinElmer UV-Vis Spectrophotometer Lambda 25 [PerkinElmer LLC, US] with two wavelengths, 260 and 280 nm, to determine the protein and nucleic acid content (Jamal *et al.* 2013). Pure concentrations of 1 MIC and 2 MIC were used as a blank solution. For the measurement of the exposure effect of the active fraction, the increase of protein and nucleic acid on the media was used as an indicator of membrane cell leakage in the tested bacterial cell.

2.7. Bacterial Morphological Structure Analysis with Scanning Electron Microscope (SEM)

K. pneumoniae suspension was incubated at 37°C for 24 hours at concentrations of 0 MIC, 1 MIC, and 2 MIC. Bacterial cells were then rinsed three times with phosphate-buffered saline (PBS) pH 7.0 [HiMedia

Laboratories LLC, Maharashtra, India] after the cell culture medium was removed. The cells were then fixed in 2% glutaraldehyde, rinsed, and progressively dehydrated with ethanol [Merck, New Jersey, USA]. The processed carbon tape containing bacterial cells was then transferred to a metallic stub and left to air-dry beneath a hood (Ali *et al.* 2021). The sample was coated with sputter gold and observed using SEM [JEOL JSM-IT300, USA] at Badan Riset dan Inovasi Nasional, Cisitu, Bandung, Indonesia.

3. Results

3.1. Prediction of Bioactive Compounds by Thin-Layer Chromatography Studies

Thin-layer chromatography (TLC) analysis of each fraction revealed the presence of six predominant compounds (Table 1). When an H_2SO_4 solution was sprayed on the chromatograms of both the hexane and ethyl acetate fractions, a captivating spectrum of colors was observed. Various colors of brownish-red, green, and blue were observed, indicating the possible presence of diverse bioactive compounds. These marks indicate the presence of triterpenoid compounds, derivatives of monoterpenes, lignans, and steroids. Furthermore, we observed distinct yellow regions in the chromatogram of the ethyl acetate fractions, suggesting a flavonoid compound. Additionally, when the Dragendorff reagent was sprayed on the chromatogram of the water fraction, vibrant orange-red regions appeared. This transformation strongly implies the existence of alkaloid compounds in our samples, broadening the scope of our studies.

3.2. Antibacterial Activities of *R. stylosa* Leaves Fractions

The antibacterial activity of the fractions extract of *R. stylosa* Griff. was evaluated at concentrations of 1,000 mg/ml, 900 mg/ml, 800 mg/ml, 700 mg/ml, and 600 mg/ml. The results of this assay are shown in Table 2, indicating that all fractions of *R. stylosa* leaf inhibited the growth of *K. pneumoniae* ATCC 700603. The average zone of bacterial growth inhibition ranged from 6.00±0.00 mm to 9.63±0.67 mm, with an average amount of the greatest inhibition occurring in the water fraction at 8.24±0.83 mm and the least occurring in the *n*-hexane fraction at 7.38±0.78.

3.3. MIC and MBC of *R. stylosa* Leaves Fractions against *K. pneumoniae* ATCC 700603

The results demonstrated that in each concentration series, neither turbidity nor clarity could be directly

Table 1. Detailed information on the phytochemical contents of each fraction of *R. stylosa* Griff, including their respective Thin-Layer Chromatography (TLC) Retention factor (R_f) values

Fractions	Observation test				Constituents
	R_f values	H_2SO_4	$AlCl_3$	Mayer's dragendorff's	
n-Hexane	0.96	Reddish-brown	-	-	Triterpenoid
	0.89	Green	-	-	Monoterpene derivates
	0.82	Reddish-brown	-	-	Triterpenoid
	0.49	Blue	-	-	Steroid/Terpenoid
Ethyl acetate	0.89	Green	-	-	Lignan/Diterpenes
	0.81	Green	-	-	Monoterpene derivates
	0.60	-	Yellow	-	Flavonoid
Water	0.10	-	Yellow	-	Flavonoid
	0.54	-	-	Orange-red	Alkaloid

Table 2. Zone of inhibition of *R. stylosa* extract fractions against *K. pneumoniae* ATCC 700603 using the agar well diffusion method

Samples	Inhibition zone diameter (mm) ± SD				
	600 mg/ml	700 mg/ml	800 mg/ml	900 mg/ml	1,000 mg/ml
n-Hexane fraction[a]	7.89^{bc}±0.39	7.58^{bc}±1.32	7.73^{bc}±0.55	7.71^{bc}±0.39	6.00^{b}±0.00
Ethylacetate fraction[b]	7.47^{bc}±0.84	7.79^{bc}±0.82	7.89^{bc}±0.49	7.86^{bc}±0.73	8.40^{bc}±0.95
Water fraction[b]	7.41^{bc}±0.39	7.84^{bc}±0.80	8.08^{bc}±0.19	8.24^{bc}±0.87	9.63^{c}±0.67
Ceftriaxone 30 µg/mld (+)			29.98^{d}±5.55		
Distilled water (-)			0.00^{a}±0.00		

Numbers that are followed by the same letter do not differ significantly (α.05); Data are the mean values of quadruplicate and expressed as mean ± standard deviation (SD)

observed in each well. This is because the color intensity of the *R. stylosa* leaf extract fractions is excessively concentrated. The MIC and MBC values were determined by total plate count (TPC) with concentrations ranging from 400-700 mg/ml that suppressed the growth of *K. pneumoniae* compared to the number of colonies on the inoculum control. As shown in Figure 1, the MIC concentration in the hexane fraction is 600 mg/ml, while the MBC concentration is 700 mg/ml. The MIC concentration for the ethyl acetate fraction is 400 mg/ml, and the MBC concentration is 600 mg/ml. In contrast, the MIC concentration and MBC concentration for the water fraction are 400 mg/ml and 500 mg/ml, respectively.

3.4. Leakage of Proteins and Nucleic Acids

The treatment of *K. pneumoniae* with the active fraction of *R. stylosa* leaf extract, namely the water fraction, resulted in a significant discharge of protein and nucleic acid from the cell into the medium (phosphate buffer). Measurement of media absorption at 260 nm (for nucleic acid) for tested bacteria with

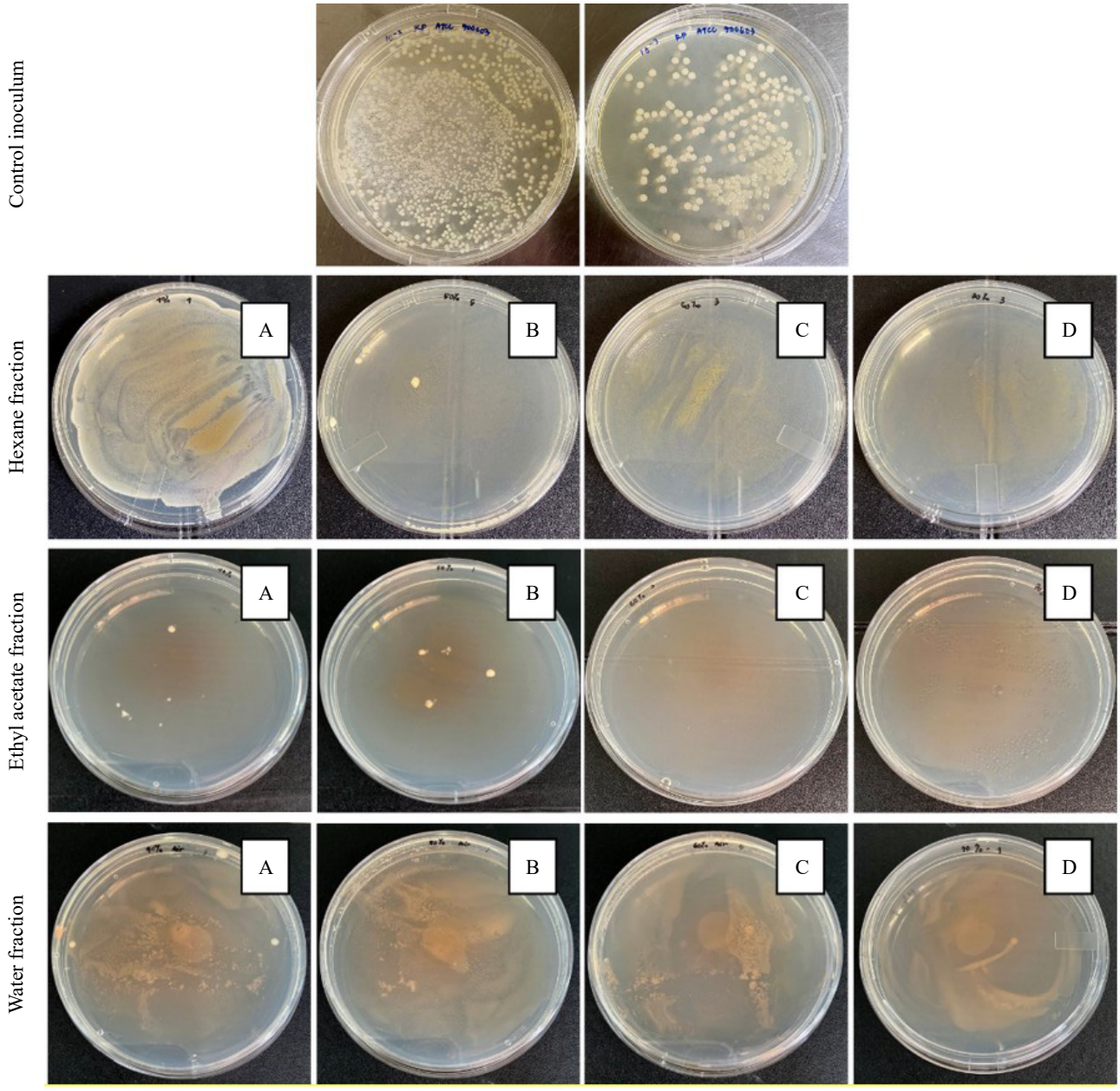

Figure 1. The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays on the *n*-hexane, ethyl acetate, and water fractions of *R. stylosa* leaves against *K. pneumoniae* ATCC 700603 were diverse: (A) 400 mg/ml, (B) 500 mg/ml, (C) 600 mg/ml, and (D) 700 mg/ml

water fraction at concentration 1 MIC revealed an increase in absorbance from 0.381 (control) to 3.514, which increased to 4.441 at concentration 2 MIC. The same thing occurred with protein content measured at wavelength 280 nm, which increased from 1.311 (control) to 3.350 and then 4.307 at a concentration of 2 MIC, as shown in Figure 2.

3.5. Changes in the Cell Morphological Structure of *K. pneumoniae* ATCC 700603

Electron micrographs of *K. pneumoniae* ATCC 700603 cells treated with the water fraction of *R. stylosa* leaves at the 1 MIC (400 mg/ml) and 2 MIC (800 mg/ml) levels (Figure 3). Untreated cells (Figure 3A) exhibited the typical appearance of short, smooth rods. Nonetheless, compared to the control cells, the treated cells exhibited substantial alterations and damage. The bacterial cells show surface irregularities, in which the rod-shaped cells have rough or wrinkly surfaces and fissures, and some of them are cavitated (Figure 3B). Damaged or leaking portions of *K. pneumoniae* cells were more visible when treated at a higher concentration, 2 MIC (Figure 3C). A closer look at Figure 3C reveals that the *K. pneumoniae* cells have completely disintegrated, with cells that are twisted, shrunken, deformed, and even fractured. The cell surface was observed to have more protrusions, leading to the formation of distinct holes, and there was a lysis of cellular debris surrounding the cells (Figure 3D).

4. Discussion

The emergence of multidrug-resistant strains and dose-limiting toxic effects has impeded antibacterial treatment (Breijyeh *et al.* 2020; Saha and Sarkar 2021). Moreover, immune-compromised and hospitalized patients are more susceptible to severe *Klebsiella* infections, which can exacerbate the patient's condition due to its ability to disseminate rapidly (Qolbi and Yuliani 2018; Arcari *et al.* 2021). Numerous researchers have scoured natural products for compounds with antibacterial properties. The utilization of mangrove plant-based ingredients as an alternative treatment for *K. pneumoniae*-related diseases has been reported previously (Rossiana *et al.* 2017).

This current study demonstrates that the fractions extract of *R. stylosa* leaf possesses promising antibacterial activity, which is likely the reason for its extensive use in traditional medicine. This is in agreement with the previous study done by Ontengco *et al.* (2003), which reported that the average growth inhibition produced by the *n*-hexane, ethyl acetate, and water fraction of *R. stylosa* leaves with mild to moderate category inhibition. In addition, compared to the *n*-hexane fraction, the ethyl acetate and water fraction has significant antibacterial activity against *K. pneumoniae*. This may be due to the presence of antibacterial compounds such as triterpenoids, monoterpene derivatives, steroids, lignans, flavonoids, and alkaloids in the *R. stylosa* fractions. Moderate inhibition might describe that *K. pneumoniae* ATCC 700603 was

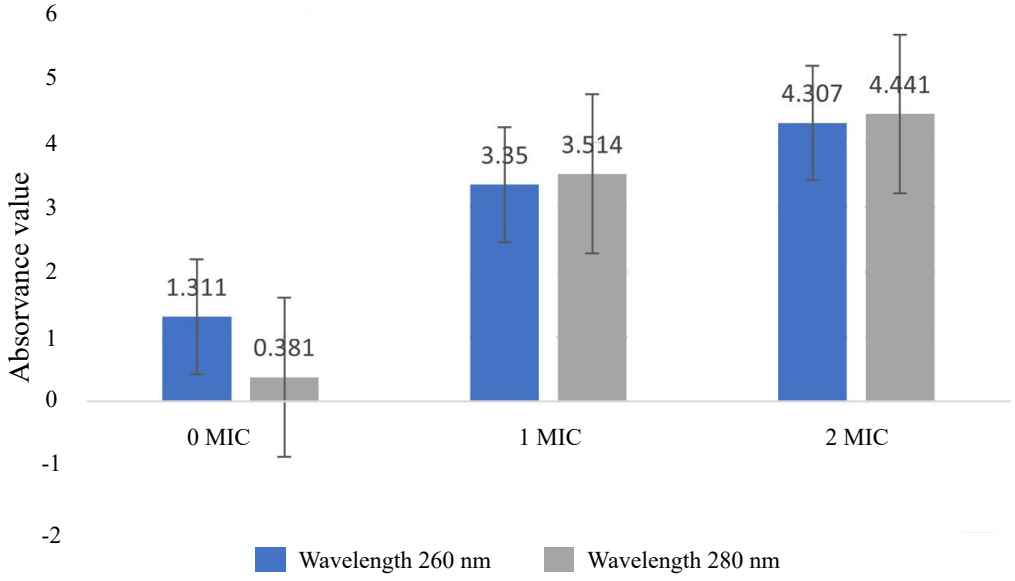

Figure 2. Appearance of 260 and 280 nm absorbing protein and nucleic acid material in the filtrate *K. pneumoniae* ATCC 700603 control suspension and after treatment with the serial concentrations of water fraction of *R. stylosa* Griff.

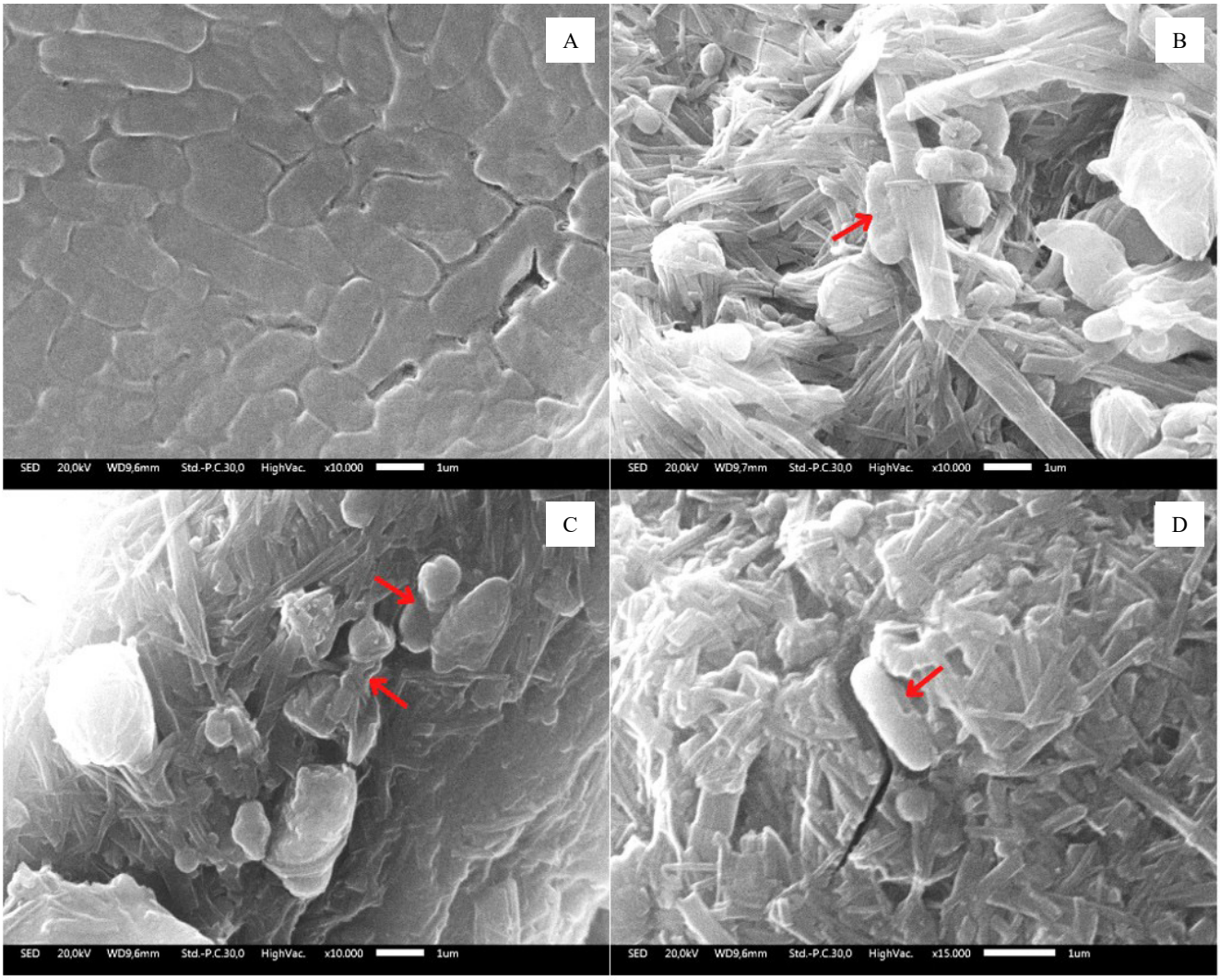

Figure 3. Electron micrographs of *K. pneumoniae* ATCC 70063 exposed to *R. stylosa* Griff's water fraction. The visual observations are as follows: (A) Untreated cells retained their integrity with a smooth surface, (B) following exposure to 1 MIC, cells displayed a crinkled appearance indicative of leakage, (C, D) At a higher concentration (2 MIC), cell rupture occurred, leading to the release of their cytoplasmic contents

moderately tolerant to the antibacterial agents of the fractions, and this strain could become resistant to the antibacterial agents.

Further tests were conducted to decipher the MIC value from *R. stylosa* fraction extract. Our study revealed that the MIC value for *K. pneumoniae* in the *n*-hexane fraction was approximately 600 mg/ml, while it was 400 mg/ml in the ethyl acetate and water fractions. Based on the confirmed test using the TPC method, fractions of extract of *R. stylosa* leaf showed bactericidal activity on *K. pneumoniae* cells at 2 MIC values. As Baquero and Levin (2021) observed, higher drug concentrations killed more microbial cells.

Several factors, including chemical composition, likely influenced the antibacterial mechanisms of *R. stylosa* water fraction, which contained secondary metabolites with antibacterial properties, such as flavonoids and alkaloids. The disruption of the cytoplasmic membrane by flavonoids will affect the loss of vital metabolites and the inactivation of the bacterial enzyme system. This damage allows nucleotides and amino acids to escape and prevents the entry of active ingredients into bacteria cells, resulting in their mortality (Sulistyani and Utami 2012; Rossiana *et al.* 2017). The antibacterial effects of alkaloid compounds disrupt the peptidoglycan component of the embryonic bacterial cell wall lining and inhibit its synthesis, thereby inhibiting cell development. This condition results in the physical and osmotic lysis of bacterial cells, leading to their death (Permatasari *et al.* 2013).

The inhibition mechanisms of *R. stylosa* fractions extracted at concentrations of 1 MIC and 2 MIC are inhibiting protein and nucleic acid synthesis and pore formation. The results demonstrated that the higher MIC value of the water fraction of *R. stylosa* leaves was proportional to the increase in the absorbance of nucleic acids at 260 nm and proteins at 280 nm. Increased protein and nucleic acid levels in bacterial cells indicate that the water fraction inhibits protein and nucleic acid synthesis (Amajida *et al.* 2019). In addition, SEM micrographs illustrated the mechanisms of the water fraction of *R. stylosa* leaf that caused pore formations in *K. pneumoniae* ATCC 700603 cells. With a higher MIC value, it was observed that the cell was injured more severely, as evidenced by the formation of shape cavities on its surface. Ibrahim *et al.* (2013) hypothesized that the formation of holes was caused by the disruption of the cell membrane and change in cell permeability, which ultimately led to the discharge of cell material.

In conclusion, this study demonstrates the antibacterial potential of the water fraction of *R. stylosa* leaves as an alternative treatment for *K. pneumoniae*-related diseases. The water fraction of *R. stylosa* leaves extract has bacteriostatic activity at low concentrations (400 mg/ml) and bactericidal activity at higher concentrations (500 mg/ml). This results in the release of cell material, which is predominantly attributable to the disruption of the cytoplasmic membrane, as well as observable changes in cell morphology. In addition, further phytochemical studies are required to isolate the pure constituents and assess their antibacterial activity against a wide range of infectious disease-causing bacteria.

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References

Abubakar, S., Kadir, M.A., Wibowo, E.S., Akbar, N., 2019. Benefits of mangrove for pharmacitic inventory in Mamuya Village, East Galela District, East Halmahera Regency (ethnopharmacological review). *J. Enggano*. 4, 12-25. https://doi.org/10.31186/jenggano.4.1.12-25

Ali, R., El-Boubbou, K., Boudjelal, M., 2021. An easy, fast, and inexpensive method of preparing a biological specimen for scanning electron microscopy (SEM). *MethodsX*. 8, 101521. https://doi.org/10.1016/j.mex.2021.101521

Amajida, H., Purwoko, T., Susilowati, A., 2019. Antibacterial activity of ethanolic and *n*-hexane extracts of *Ruellia tuberosa* leaves against *Escherichia coli* and *Bacillus subtilis* bacteria. *Biofarmasi J. Nat. Prod. Biochem.* 17, 69-80. https://doi.org/ 10.13057/biofar/f170203

Arcari, G., Raponi, G., Sacco, F., Bibbolino, G., Di Lella, F.M., Alessandri, F., Coletti, M., Trancassini, M., Deales, A., Pugliese, F., Antonelli, G., Carattoli, A., 2021. *Klebsiella pneumoniae* infections in COVID-19 patients: a 2-month retrospective analysis in an Italian Hospital. *Int. J. Antimicrob. Agents*. 57, 106245. https://doi.org/10.1016/j.ijantimicag.2020.106245

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity: a review. *J. Pharm. Analysis*. 6, 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Baquero, F., Levin, B.R., 2021. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat. Rev. Microbiol.* 19, 123–132. https://doi.org/10.1038/s41579-020-00443-1

Breijyeh, Z., Jubeh, B., Karaman, R. 2020. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve It. *Molecules*. 25, 1340. https://doi.org/10.3390/molecules25061340

Castronovo, L.M., Vassallo, A., Mengoni, A., Miceli, E., Bogani, P., Firenzuoli, F., Fani, R., Maggini, V., 2021. Medicinal plants and their bacterial microbiota: a review on antimicrobial compounds production for plant and human health. *Pathogens*. 10, 106. https://doi.org/10.3390/pathogens10020106

Chang, D., Sharma, L., Cruz, C.S.D., Zhang, D., 2021. Clinical epidemiology, risk factors, and control strategies of *Klebsiella pneumoniae* infection. *Front. Microbiol.* 12, 750662, https://doi.org/10.3389/fmicb.2021.750662

Dong, N., Yang, X., Chan, E.W.C., Zhang, R., Chen, S., 2022. *Klebsiella* species: taxonomy, hypervirulence and multidrug resistance. *EBioMedicine*. 79, 103998. https://doi.org/10.1016/j.ebiom.2022.103998

Gopal, N., Ekegbu, J., Kaur, C.P., Paulraj, P., P., R., Bhavya, K.S. 2019. Evaluation of antibacterial properties of leaves and barks of *Rhizophora stylosa* against gram-positive and gram-negative organisms. *J. Pure Appl. Microbiol.* 13, 957-965. https://doi.org/10.22207/JPAM.13.2.32

Gulumbe, B.H., Ajibola, O., 2020. Carbapenem resistant enterobacteriaceae in africa. *Borneo J. Pharm.* 3, 71-78. https://doi.org/10.33084/bjop.v3i2.1356

Harbone, J.B., 1984. *Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis*, second ed. Chapman and Hall Ltd, New York.

Holt, K.E., Wertheim H., Zadoks, R.N., Baker, S., Whitehouse, C.A., Dance, D., Jenney, A., Connor, T.R., Hsu, L.Y., Severin, J., Brisse, S., Cao, H., Wilksch, J., Gorrie, C., Schultz, M.B., Edwards, D.J., Nguyen, K.V., Nguyen, T.V., Dao, T.T., Mensink, M., Minh, V.L., Nhu, N.T., Schultsz, C., Kuntaman, K., Newton, P.N., Moore, C.E., Strugnell, R.A., Thomson, N.R., 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc. Natl. Acad. Sci. U S A.* 112, E3574 -8. https://doi.org/10.1073/pnas.1501049112

Ibrahim, D., Kassim, J., Sheh-Hong, L., Rusli, W., 2013. Efficacy of pyroligneous acid from *Rhizophora apiculata* on pathogenic *Candida albicans. J. Appl. Pharm. Sci.* 3, 7-13. https://doi.org/10.7324/JAPS.2013.3702

Jamal, Y., Irawati, P., Fathoni, A., Agusta, A., 2013. Chemical constituents and antibacterial effect of essential oil of javaneese pepper leaves (*Piper retrofractum* Vahl.). *Media Penelitian dan Pengembangan Kesehatan*. 23, 65-72. https://doi.org/10.22435/mpk.v23i2.3128.65-72

Joel, E.L., Bhimba, V., 2010. Isolation and characterization of secondary metabolites from the mangrove plant *Rhizophora mucronata. Asian Pac. J. Trop. Med.* 3, 602-604. https://doi.org/10.1016/S1995-7645(10)60146-0

Kainuma, M., Kezuka, M., Inoue, T., Chan, E.W., Tangah, J., 2015. Botany, uses, chemistry, and bioactivities of mangrove plants I: *Rhizophora stylosa. ISME/GLOMIS Electronic J.* 13, 12-17.

Kalasuba, K., Miranti., M., Rahayuningsih, S.R., Safriansyah, W., Syamsuri, R.R.P., Farabi, K., Oktavia, D., Alhasnawi, A.N., Doni., F., 2023. Red mangrove (*Rhizophora stylosa* Griff.)-A review of its botany, phytochemistry, pharmacological activities, and prospects. *Plants*. 12, 2196. https://doi.org/10.3390/plants12112196

Kang, J., Jin, W., Wang, J., Sun, Y., Wu, X., Liu, L., 2019. Antibacterial and anti-biofilm activities of peppermint essential oil against *Staphylococcus aureus. LWT*. 101, 639–645. https://doi.org/10.1016/j.lwt.2018.11.093

Martin, R.M., Bachman, M.A., 2018. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. *Front. Cell. Infect. Microbiol.* 8, 4. https://doi.org/10.3389/fcimb.2018.00004

Mouafi, F.E., Abdel-Aziz, S.M., Bashir, A.A., Fyiad, A.A., 2014. Phytochemical analysis and antimicrobial activity of mangrove leaves (*Avicenna marina* and *Rhizophora stylosa*) against some pathogens. *World Appl. Sci. J.* 29, 547-554. https://doi.org/ 10.5829/idosi.wasj.2014.29.04.13901

Munoz-Price, L.S., Poirel, L., Bonomo, R.A., Schwaber, M.J., Daikos, G.L., Cormican, M., Cornaglia, G., Garau, J., Gniadkowski, M., Hayden, M.K., Kumarasamy, K., 2013. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect. Dis.* 13, 785-796. https://doi.org/10.1016/S1473-3099(13)70190-7

Nasresfahani M., Ahadi A.M., Ayat H., Nayeri H., 2017. Evaluation of E492 microcin gene presence in *Klebsiella pneumoniae* collected from patients registered to educational hospitals of Isfahan. *Zahedan J. Res. Med. Sci.* 19, e5038. https://doi.org/10.17795/zjrms-5038

Nguyen, D.T., Lessor, L.E., Cahill, J.L., Rasche, E.S., Kuty Everett, G.F., 2015. Complete genome sequence of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* siphophage sushi. *Genome Announc.* 3, e00994-15. https://doi.org/10.1128/genomeA.00994-15

Ontengco, D., Baltazar, L., Santiago, R., Matias, R., Isacc, C., Tuazon, A.O., 2003. Methicillin-resistant *Staphylococcus aureus* isolates from *Filipino patients* (1993-2003). *Philippine J. Microbiol. Infect. Dis.* 17, 4-8.

Paczosa, M.K., Mecsas, J. 2016. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiol. Mol. Biol. Rev.* 80, 629-661. https://doi.org/10.1128/MMBR.00078-15

Panda, S.K., Thatoi, H., Dutta, S.K., 2009. Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo* L. from Similipal biosphere reserve, Orissa. *J. Med. Plant Res.* 3, 294-300.

Permatasari, G.A., Besung, I.N., Mahatmi, H., 2013. The inhibitory power of soursop leaf juice on *Escherichia coli* bacteria growth. *Indones. Medicus Veterinus*. 2, 162-169.

Qolbi, N., Yuliani, R., 2018. Antibacterial activity screening of ten leaves extracts against *Klebsiella pneumoniae. Pharmacon: J. Farmasi Indones.* 15, 8-19.

Rai, P.K., Mehta, S., Gupta, R., Watal, G., 2010. A novel antimicrobial agent, *Trichosanthes dioica. Int. J. Pharma Bio Sciences.* 1, 1-9.

Rossiana, N., Miranti, M., Kosmita, O., 2017. Antibacterial activity test of endophytic fungus from mangrove plant (*Rhizophora apiculata* L.) and (*Bruguiera gymnorrizha* (L.) Lamk.) against *Klebsiella pneumoniae* ATCC 700603. *KnE Life Sciences.* 2, 146-157. https://doi.org/10.18502/kls.v2i6.1031

Saha, M., Sarkar, A., 2021. Review on multiple facets of drug resistance: a rising challenge in the 21st century. *J. Xenobiot*. 11, 197-214. https://doi.org/10.3390/jox11040013

Seepana, R., Perumal, K., Kada, N.M., Chatragadda, R., Raju, M., Annamalai, V., 2016. Evaluation of antimicrobial properties from the mangrove *Rhizophora apiculata* and *Bruguiera gymnorrhiza* of Burmanallah coast, South Andaman, India. *J. Coastal Life Med.* 4, 475-478. https://doi.org/ 10.12980/jclm.4.2016J6-52

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.M.D., Kamal, M.A., 2015 Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology, and treatment. *Saudi J. Biological Sci.* 22, 90-101. https://doi.org/10.1016/j.sjbs.2014.08.002

Sofowora, A., Ogunbodede, E., Onayade, A., 2013. The role and place of medicinal plants in the strategies for disease prevention. *African J. Traditional, Complementary, and Alternative Med.* 10, 210–229. https://doi.org/10.4314/ajtcam.v10i5.2

Sulistyani, N., Utami, R.N., 2012. Antibacterial activity of binahong rhizome ethanol extract (*Anredera cordifolia* (Tenore) Steen) against *Salmonella thypi. In: Proceeding of International Conference on Drug Development of Natural Resources*. Yogyakarta: Ahmad Dahlan University. pp. 39-49.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., Bezirtzoglou, E., 2021. Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives. *Microorganisms*. 9, 2041. https://doi.org/10.3390/microorganisms9102041

Wu, C.H., Bernard, S.M., Andersen, G.L., Chen, W., 2009. Developing microbe-plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation, and carbon sequestration. *Microbial Biotech.* 2, 428-440. https://doi.org/10.1111/j.1751-7915.2009.00109.x