Therapeutic potential of multi-targeting phytochemicals derived from *Apium graveolens* ethanol extract in West Java, Indonesia against multidrug-resistant *Pseudomonas aeruginosa*

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Abstract. Sufa HI, Kurniati I, Dermaovan A, Abror YK, Indra AIN, Purkon DB. 2024. Therapeutic potential of multi-targeting phytochemicals derived from *Apium graveolens* ethanol extract in West Java, Indonesia against multidrug-resistant *Pseudomonas aeruginosa*. Biodiversitas 25: 2183-2190. This study aims to evaluate the antibacterial efficacy of *Apium graveolens* (AG) extract from West Java, Indonesia against MDR-*P. aeruginosa* species responsible for nosocomial infection. Several assessments of antibacterial activity in vitro were conducted, including the enumeration of bacterial colonies, the measurement of the inhibition zone by agar well diffusion, determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, and the observation of cell damage using Scanning Electron Microscopy (SEM). The results have demonstrated that AG extract from West Java, Indonesia, possesses significant potential as an antimicrobial agent against MDR-*P. aeruginosa*. The *Apium graveolens* extract, at concentrations of 50, 100, and 200 mg/mL, reduced MDR-*P. aeruginosa* colonies by 59.3%, 65.7%, and 82%, respectively. The inhibition zone sizes ranged from 7 to 12 mm for 50 mg/mL, 8 to 16 mm for 100 mg/mL, and 16 to 17 mm for 200 mg/mL. The MIC values of 100 mg/mL for PA1 and PA3, 200 mg/mL for PA2, with an MBC value of 200 mg/mL for each test. Scanning electron microscope observation indicated that treatment with 100 mg/mL of AG extract caused significant damage to bacterial cells. In conclusion, *A. graveolens* extracts show promise as potential antibacterial agents against nosocomial infections caused by MDR-*P. aeruginosa*. Further research, including in vivo studies of the antibacterial mechanism of action, is warranted.

Keywords: Antibacterial activity, *Apium graveolens*, MDR-*P. aeruginosa*, nosocomial infection

INTRODUCTION

Nosocomial infections, often referred to as Hospital-Acquired Infections (HAIs), present a substantial global healthcare concern that demands immediate and extensive focus. HAIs encompass a collection of infections that a patient does not possess before their hospital admission. These infections do not even manifest during the incubation phase; they manifest shortly after a patient's admission to the hospital, typically within 48 to 72 hours (Hoxha et al. 2019; Hazard et al. 2021). The consequences of nosocomial infections encompass a wide range of effects, extending beyond the patient's own risk of exposure, as healthcare providers, family members, and visitors may also potentially experience these infections within healthcare settings. The prevalence of HAIs between 2000 and June 2021 stands at 0.14 percent universally, with an annual increase of 0.06 percent. Notably, the highest HAI rates are observed in the African Region (AFR), while the lowest levels are found in the American (AMR) and Western Pacific (WPR) regions. Additionally, it’s worth highlighting that within the AFR, the central African subregion experiences a significantly higher prevalence, surpassing other global regions by 0.27 (with a 95% confidence interval of 0.22-0.34) (Raoufi et al. 2023). In combined hospitals, there were 250 cases of HAIs per 100,000 patient days. The most common types were urinary tract, bloodstream, and lower respiratory infections. HAI rates went up in summer (for pneumonia, respiratory, surgical, and gastrointestinal infections) and in winter (norovirus gastrointestinal infection) (Stewart et al. 2021).

Predominant bacteria causing Healthcare-Associated Infections (HAIs) include *Klebsiella pneumonia*, *Enterobacter* spp., *Staphylococcus aureus*, and *Escherichia coli*. *Pseudomonas aeruginosa*, a Gram-negative bacterium, is a major contributor to various HAIs like bloodstream, gastrointestinal, lower respiratory tract, and urinary tract infections, as well as pneumonia and surgical site infections (Stewart et al. 2021). Known for adaptability and virulence in healthcare settings, *P. aeruginosa* forms biofilms, adhering to medical surfaces, and possesses resistance mechanisms, including efflux pumps and beta-lactamase enzymes, making treatment challenging (Liao et al. 2022).

A study of 17 articles spanning 2013 to 2018 highlighted significant disparities in data availability on *P. aeruginosa* resistance in Southeast Asia. Vietnam contributed the most substantial dataset, while East Timor, Cambodia, and Laos lacked pertinent information. The investigation also revealed notable variations in *P. aeruginosa* resistance profiles within the region. Thailand emerged with the most extensive information on antibiotic resistance, showing the highest
recorded resistance rates for various antibiotics. Colistin was the only antibiotic with the lowest documented resistance rate (Yayan et al. 2015; Ciptaningtyas et al. 2019). The CDC's (2021) report noted that 785 out of 8,858 P. aeruginosa isolates were resistant, with many countries reporting high resistance, making treatment challenging and increasing the risk of complications and death. This underscores the urgent need to address the public health concern of MDR P. aeruginosa, emphasizing the development and use of antibacterial agents from natural sources as a vital strategy against bacterial resistance.

Researchers have been looking into natural sources for antibacterial agents to face MDR pathogenic bacteria. Lytic bacteriophage (Sufa et al. 2018), lactic acid bacteria (Adeniyi et al. 2015), and fungi (Demain and Martens 2017) have properties that can fight bacteria. Additionally, several studies show that plants can be valuable sources of antibacterial agents (Al-Ghanayem et al. 2017; Prastiyanto 2021). These natural sources provide a wide range of options for developing new ways against bacteria. Celery (Apium graveolens), a vegetable that belongs to Apiaceae family, has been reported to inhibit bacterial growth such as Staphylococcus aureus (Prakoso et al. 2020), S. aureus ATCC 25923, Bacillus subtilis ATCC 11774, Candida albicans ATCC 10231 E. coli and P. aeruginosa (Foudah et al. 2021), but no study has reported the antibacterial properties of A. graveolens from West Java against MDR P. aeruginosa. This study aims to fill the research gap by determining the phytochemical potential of A. graveolens from West Java against multidrug-resistant P. aeruginosa through in vitro evaluation.

MATERIALS AND METHODS

Plant collection, verification and extraction

The entire parts of celery plants (A. graveolens) were collected in April 2023 from a resident’s herbal garden in the Ciwidey area, Bandung, West Java Province, Indonesia. Before extracting this plant, species verification was conducted in the FMIPA Universitas Padjajaran laboratory based on the morphological plant determination method. Fresh A. graveolens was washed with running water to remove impurities. The washed A. graveolens was drained and weighed in its wet state. Next, it was dried in a place not exposed to direct sunlight. The partially dried samples were cut into small pieces. The plant pieces were then dried again until the moisture content was less than 10%. The dried plants were ground into powder using a blender.

The powder was subjected to maceration using 70% ethanol as the solvent, and the ratio was 1:10 (w/vol). The maceration process was carried out in a dark room at room temperature, with stirring and solvent replacement every 24 hours for five days. The supernatant was filtered with Whatman No. 1 paper, and the filtrates were evaporated using a rotary evaporator at 50°C to obtain a crude extract (Figure 1).

Reidentification of MDR P. aeruginosa

The test bacteria used consisted of three cultures: MDR-P. aeruginosa called PA1 and PA2, obtained from the Microbiology Laboratory of the Department of Medical Laboratory Technology, Bandung Health Polytechnic and MDR-P. aeruginosa ATCC® 27853, called PA3. All isolates were verified using Pseudomonas Agar Base media, Gram staining, a long biochemical test tray (12 tests), and bacterial sensitivity tests using the Clinical Laboratory Standard Institute M100S for performance standards for antimicrobial susceptibility testing (CLSI 2020).

Antibacterial efficacy of A. graveolens extract against MDR-P. aeruginosa

Total plate count

The analysis of the effectiveness of A. graveolens extract on the colony count of MDR-P. aeruginosa was conducted using the total plate count method. A volume of 1 mL of extract at a concentration of 200 mg/mL and 1 mL of the overnight test bacterial suspension (MDR-P. aeruginosa), equivalent to the turbidity of a 0.5 McFarland standard solution, were poured into sterile petri dishes. Immediately, 20 mL of sterile Plate Count Agar media at 40°C, was poured into each dish and homogenized.

Figure 1. Part of plants, powder, crude extract of Apium graveolens. A. Part of plant; B. Powder; C. Crude extract
The media were incubated for 24 hours at 37°C. The same procedure was carried out for extract concentrations of 100 and 50 mg/mL. After 24 hours of incubation, observe and count the number of colonies in the negative control dish (without bacterial addition, but only the extract), the positive control dish (test bacteria without extract addition), and the test dishes. The greater reduction in bacterial colonies in the test dish compared to the number of colonies in the positive control dish indicates the effectiveness of *A. graveolens* extract in inhibiting MDR *P. aeruginosa*.

**Agar well diffusion**

The analysis of antibacterial activity using the agar well diffusion method was performed by evenly spreading the test bacteria (which had been incubated overnight and standardized to the turbidity of a 0.5 McFarland solution) onto the surface of Mueller Hinton Agar (Oxoid) and allowing it to diffuse for 1 minute. Subsequently, *A. graveolens* extract at concentrations of 200, 100, and 50 mg/mL, each in a volume of 100 μL was introduced into wells of 6 mm diameter prepared in the MHA. This study employed Gentamicin 10 μg as the positive control and sterile distilled water as the negative control. The media were incubated at 37°C for 24 hours. The next day, the diameter of the inhibition zones formed around the wells containing the positive and negative controls, as well as the test wells, was measured. The widest zone of inhibition (mm) indicates the best antibacterial activity.

**Determination of Minimum Inhibitory Concentration (MIC)**

The MIC value for each extract was determined using the microdilution method on a microwell plate with Mueller Hinton Broth (MHB) media (CLSI 2020), following the procedure outlined by Prastiyan (2021). In each well, 100 μL of MHB was added, and 100 μL of *A. graveolens* extract was introduced into the first well, followed by serial dilutions up to the 12th well. After completing the dilutions, 10 μL of MDR *P. aeruginosa* suspension, which equated to 0.5 McFarland standards (approximately corresponding to a bacterial population of 1.5 × 10⁷ cfu/mL) was added to each well, excluding the negative control. The microwell plate was then incubated for 24 hours at 37°C. Following incubation, the MIC value was determined by identifying the lowest concentration of *A. graveolens* extract that inhibited the growth of MDR *P. aeruginosa*. This inhibition was indicated by a change in turbidity on the microwell plate, which was then compared to the control. The extract's best MIC was demonstrated by the lowest MIC value.

**Determination of Minimum Bactericidal Concentration (MBC)**

The determination of Minimum Bactericidal Concentration (MBC) followed the Minimum Inhibitory Concentration (MIC) assay. The MIC well mixture was subcultured onto Mueller Hinton Agar (MHA) and incubated at 37°C for 24 hours. MBC values were determined by examining bacterial growth on the MHA plates. MBC is defined as the minimum concentration of *A. graveolens* extract at which MDR *P. aeruginosa* shows no growth (Yin et al. 2018). The superior antibacterial activity was indicated by the lowest MBC value.

**Scanning electron microscope analysis**

Antibacterial can cause alterations in the shape of bacteria, size variations, or undergo swelling or shrinking. Some antibiotics work by damaging the bacterial cell wall. The use of SEM allows for direct observation of such damage, such as the formation of holes or the rupture of the cell wall. The SEM analysis was done using SEM Jeol JSM IT200 in the Integrated Laboratory of Bioproducts (ILab) LIPI-BRIN, Indonesia.

**Phytochemical screening of the extract**

Phytochemical screening involves qualitatively analyzing the chemical composition to identify the specific compound groups present in a plant. The investigation is centered on secondary metabolites that have health benefits, including Tannins, Saponins, Flavonoids, Terpenoids, and Alkaloids (Eve et al. 2020).

**RESULTS AND DISCUSSION**

**Yield of *A. graveolens* extract**

The extract of *A. graveolens* was obtained using the maceration method for five days with a 70% ethanol solvent. The weight of the powder used was 500 g, and 5000 mL of ethanol was used. The total extract obtained after the evaporation process using a rotary evaporator at a temperature of 50°C and a hairdryer was 31.7 g. The extraction yield obtained was 6.3%.

**Bacterial isolates characteristics**

The morphology and physiology of the three tested bacteria were very similar to the characteristics of *P. aeruginosa* (Table 1). The results of the bacterial sensitivity test to antibiotics are presented in Table 2. The sensitivity test results for the three bacteria show that PA1, PA2, and PA3 were MDR strains because they were resistant to at least three classes of antibiotics, namely carbapenem (imipenem), aminoglycosides (amikacin and gentamicin), and second-generation cephalosporin (ceftriaxone).

**The antibacterial activities of extract against MDR *P. aeruginosa***

**Total plate count**

Based on the colony growth results of the TPC method, *A. graveolens* extract with concentrations of 50, 100, and 200 mg/mL was able to reduce the colony count of PA1, PA2, and PA3 with an average decrease of 69% (Figure 2).

**Agar well diffusion**

The antibacterial effectiveness of *A. graveolens* extract was assessed using the agar well diffusion assay against MDR *P. aeruginosa* (PA1, PA2, PA3). The antibacterial impact of the extract was evaluated at concentrations of 50, 100, and 200 mg/mL by measuring the diameter of the inhibition zones (mm) against three MDR *P. aeruginosa* strains (Table 3).
The outcomes of the study indicate that *A. graveolens* extract from West Java, Indonesia, exhibited antibacterial activities against MDR-*P. aeruginosa*, as demonstrated by the presence of inhibition zones. The inhibition zone of *A. graveolens* extract against PA1 ranged from 12 to 17 mm, while its inhibition zone against PA2 ranged from 7 to 16 mm. *A. graveolens* extract demonstrated inhibition zones against PA3 ranging from 10 to 17 mm. The results showed that among the three tested concentrations, *A. graveolens* extract from West Java exhibited the highest efficacy at 200 mg/mL against PA1, PA2, and PA3.

**Table 1.** The biochemical test and characteristic of the isolates determined as *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Kind of test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth on Pseudomonas agar base</strong></td>
<td>PA1</td>
</tr>
<tr>
<td>Gram staining</td>
<td>Basil-negative Gram</td>
</tr>
<tr>
<td>Fermentation of glucose</td>
<td>Negative</td>
</tr>
<tr>
<td>Fermentation of lactose</td>
<td>Negative</td>
</tr>
<tr>
<td>Fermentation of maltose</td>
<td>Negative</td>
</tr>
<tr>
<td>Fermentation of mannitol</td>
<td>Negative</td>
</tr>
<tr>
<td>Fermentation of saccharose</td>
<td>Negative</td>
</tr>
<tr>
<td>Indole</td>
<td>Negative</td>
</tr>
<tr>
<td>Methyl red</td>
<td>Negative</td>
</tr>
<tr>
<td>Voges Proskauer</td>
<td>Negative</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>Positive</td>
</tr>
<tr>
<td>Triple sugar iron agar</td>
<td>M/M H2S- G-</td>
</tr>
<tr>
<td>Urease production</td>
<td>Negative</td>
</tr>
<tr>
<td>Motility test (semi-solid)</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Table 2.** The disk test and characteristic of the isolates determined as *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disk content (μg/mL)</th>
<th>Interpretation of inhibition zone diameter (mm)*</th>
<th>Mean of inhibition zone diameter (mm) to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>10</td>
<td>( \geq 19 ) 16-18 ( \leq 15 ) 21</td>
<td>19 21</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>( \geq 17 ) 15-16 ( \leq 14 ) 21</td>
<td>21 24</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>( \geq 17 ) 13-14 ( \leq 12 ) 19</td>
<td>21 21</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30</td>
<td>( \geq 23 ) 18-22 ( \leq 17 ) 23</td>
<td>23 24</td>
</tr>
<tr>
<td>Cefadine</td>
<td>30</td>
<td>( \geq 15 ) 13-14 ( \leq 12 ) 0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>30</td>
<td>( \geq 15 ) 13-14 ( \leq 12 ) 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>( \geq 27 ) 21-27 ( \leq 20 ) 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>25</td>
<td>( \geq 15 ) 13-14 ( \leq 12 ) 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15</td>
<td>( \geq 15 ) 13-14 ( \leq 12 ) 7</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10</td>
<td>( \geq 21 ) 15-20 ( \leq 14 ) 0 0</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

Note: *CLSI (2020). S: sensitive; R: resistant, I: intermediate.

**Figure 2.** The effectiveness of *Apium graveolens* extract in reducing MDR-*Pseudomonas aeruginosa* colony growth.
Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC value of *A. graveolens* extract against MDR-*P. aeruginosa* (PA1, PA2, PA3), suspension from MIC test wells with negative results was carried out using a streaking technique on Mueller Hinton Agar media. The smallest concentration without colony growth was established as the MBC value. The results of the MBC test of *A. graveolens* extract against MDR-*P. aeruginosa* (PA1, PA2, PA3) namely 200 mg/mL, collectively. The result can be seen in Figure 4.

Scanning electron microscope analysis

The Scanning Electron Microscope (SEM) is a tool that looks at the surface of materials with very fine detail, about 2 nm. It works by scanning the material's surface with an electron beam, and the emitted electrons create an image. The differences in height on the material create the contrasting parts of the image. The SEM image of untreated *P. aeruginosa* by magnification 50,000 × (Yi and Kim 2023) was compared to the result image of *P. aeruginosa* (PA3) after being treated with *A. graveolens* extract (100 mg/mL), shown in Figure 5.

Phytochemical content in *A. graveolens* extract

In the qualitative phytochemical examination, the crude extract of *A. graveolens* from West Java, Indonesia tested positive for the presence of tannins, saponins, flavonoids, and terpenoids/steroids (Table 4).

Table 3. The inhibition zone of *A. graveolens* to MDR-*P. aeruginosa*

<table>
<thead>
<tr>
<th>Extract concentration (mg/mL)</th>
<th>Mean of inhibition zone diameter (mm) to PA1</th>
<th>Mean of inhibition zone diameter (mm) to PA2</th>
<th>Mean of inhibition zone diameter (mm) to PA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>17</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>100</td>
<td>16</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Negative control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3. MIC test result of *Apium graveolens* extract in various concentrations listed vertically (mg/mL) against MDR-*Pseudomonas aeruginosa* (PA1, PA2, PA3) after incubation 24 hours. Positive control was done by adding bacteria in broth media without adding extract showed turbidity (K+1 = MHB+PA1, K+2 = MHB+PA2, K+3 = MHB+PA3), and negative control was done by adding extract in broth without adding bacteria, namely: K-1, K-2, K-3 = (MHB+ *A. graveolens* extract), collectively

Figure 4. The MBC result of *Apium graveolens* extract against MDR-*Pseudomonas aeruginosa*: A. PA1; B. PA2; C. PA3 was 200 mg/mL. Extract concentration was noted as: 1, 2, 3, 4, 5, 6, 7, 8 means 400; 200; 100; 50; 25; 12,5; 6,25; 3, 125 (mg/mL), respectively
Figure 5. Scanning electron microscopy images of *Pseudomonas aeruginosa*. A. Untreated; B. Treated by *Apium graveolens* extract at 100 mg/mL against MDR-*P. aeruginosa* (PA3) after 24 hours incubation. Some arrows showed cell morphological damage occurred.

Table 4. The screening result of the phytochemical qualitative test of *Apium graveolens* from West Java, Indonesia

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Tanin</th>
<th>Saponin</th>
<th>Flavonoid</th>
<th>Terpenoid/sterol</th>
<th>Bouchardat</th>
<th>Dragendorf</th>
<th>Meyer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++++/+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion

*Pseudomonas aeruginosa* is one of the predominant MDR-bacteria that causes nosocomial infection (Reynolds and Kollef 2021). *Pseudomonas aeruginosa* belongs to *Pseudomonadaceae* family, a widespread Gram-negative opportunistic pathogen in humans and is not typically recognized as a component of the normal microbiota. In this study, the bacterial isolate has been reconfirmed and showed very identic with *P. aeruginosa* as Gram-negative, non-fermenter glucose, lactose, maltose, mannitol, and saccharose, negative indole, MR and VP test, can utilize citrate as carbon source, not produce H$_2$S and urease (Hall et al. 2016). One of three isolates used in this research was apio cyanogenic strain, which did not produce unique characteristic of this bacterium, namely bluish-greenish pigment (pyocyanin). There were about 5% of strains of *P. aeruginosa* that could not produce pyocyanin (Gonçalves and Vasconcelos 2021). Research has been conducted to evaluate how pyocyanin affects various models, such as respiratory, urological, neurological, cardiovascular, and hepatic models, both in vitro and in vivo with a predominant focus on in vitro systems. These investigations have revealed the broad spectrum of effects that pyocyanin can have on the host, including pro-inflammatory and immunomodulating properties (McDermott et al. 2013; McFarland et al. 2013).

Additionally, the existence of pyocyanin in cellular systems elevates oxidative stress, leading to cellular death.

Nosocomial infection because of *P. aeruginosa* must be controlled because it can complicate the healing process. Unfortunately, most of *P. aeruginosa* has been reported as multi-drug resistant-bacteria, including amikacin, ceftazidime, gentamicin, imipenem, and meropenem (Fabian et al. 2016), colistin, tzo bactam, cefepime (Momenah et al. 2023). In this research, the three isolates were MDR-*P. aeruginosa* because all of them have been resistant to four groups of antibiotics that are commonly applied to nosocomial infection, namely carbapenem (imipenem), aminoglycosides (amikacin and gentamicin), second-generation cephalosporin (ceftiraxone). Consequently, an alternative agent is needed to treat MDR-*P. aeruginosa*.

An economical and readily available option for bacteriostatic or bactericidal agents is the utilization of extracts from potentially beneficial natural plants, such as *Apium graveolens*, commonly known as celery. *A. graveolens* grows well in Indonesia, especially in West Java. Some researchers reported the utility of this plant as an antibacterial agent, but the place to grow it is outside of West Java. *Apium graveolens* used in this research was taken from Ciwidey, West Java, Indonesia. The soil in this area is classified as latosol, a type formed through intense weathering,
rendering it both fertile and nutrient-rich. Furthermore, the soil pH falls within the range of 5 to 6.5, accompanied by an average temperature spanning 19-26°C. The specific type and condition of the soil where these plants grow can impact the phytochemical content or bioactive compounds within the plant species (Mudau et al. 2022).

Ethanol serves as the extraction solvent in this research, offering advantages over water or other solvents: (i) Its polar nature enables the extraction of polar compounds like flavonoids, alkaloids, saponins, and phenols; (ii) Ethanol's lower destructive power minimizes potential damage to extracted compounds; (iii) Ethanol is cost-effective and more readily available compared to other organic solvents; (iv) Ethanol is safe for use, being non-corrosive and not easily flammable; (v) Its efficient evaporation expedites the rotary evaporator process. Hence, ethanol proves to be a favorable choice for this study (Widyaningrum et al. 2020).

The results of this study can conclude that A. graveolens extract from West Java has the potential as an antibacterial agent against MDR-P. aeruginosa. This aligns with the results of previous studies. Celery harbors a diverse range of phytochemical compounds, including polyphenols, saponins, flavonoids, phenolic acids, alkaloids, and terpenoids, known for their antibacterial properties (Al Aboudy 2021). In this research the crude extract also contains tannins, saponins, flavonoids, and terpenoids/steroids. A variation of the phytochemical contents of A. graveolens extract in this research was observed and shown in Table 4.

Tannin is the most abundant polyphenol in edible traditional plants for medication. Saponins are natural bioorganic compounds characterized by the presence of at least one glycosidic bond (CO-glycosidic bond) at C-3 between the aglycone and the sugar chain. Flavonoids, which belong to the class of 2-phenyl-benzyl-γ-pyrene derivatives, represent secondary metabolites predominantly present in plants. Terpenoids constitute a category of organic compounds occurring naturally and are derived from the 5-carbon compound isoprene. Alkaloids are predominantly produced from amino acids, leading to a wide array of chemical structures, primarily sourced from plants. They serve vital functions in human medicine and in organisms' natural defense mechanisms. Approximately 20% of the recognized secondary metabolites in plants consist of alkaloids. The Dragendorf reagent stands as a notable method for alkaloid detection, employing bismuth nitrate and potassium iodide dissolved in glacial acetic acid solution, known for its sensitivity in identifying alkaloids (Mujeeb et al. 2014).

The result of MIC and MBC test of A. graveolens was 100 mg/mL and 200 mg/mL for both PA1 and PA3, while 200 mg/mL MIC and MBC value for PA2. This means that the phytochemical content from plants affects to these bacteria. Some plants contain flavonoids and tannins that have the potential as antibacterial agents (Prastiyanto 2021; Kováč et al. 2023). However, the results of the MIC of A. graveolens extract varied in some research studies.

The antibacterial impact of A. graveolens on MDR-P. aeruginosa induced alterations in bacterial shape, size variations, and led to cell swelling or shrinking. The A. graveolens extract at a concentration of 100 mg/mL (MIC value) was capable of disrupting bacterial cell morphology, with some exhibiting signs of lysis. Utilizing newly developed quantitative models, an article correlates changes in cell shape to modifications in the cell's physiological state, characterized by shifts in cell growth rates, protein synthesis, and proteome composition (Cykle et al. 2022).

In conclusion, the extract of A. graveolens from West Java shows potential as an antibacterial agent against MDR-P. aeruginosa. Further in vivo investigations and examinations are required to understand its mode of action.

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