Seed extracts of seven mango cultivars (*Mangifera indica* L.) in Indonesia: Evaluation of anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) causing wound infections

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Received 25 January 2023; Received in revised form 8 May 2024; Accepted 16 June 2024

ABSTRACT

**Aims:** The aim of this study was to examine the antibacterial activity of seed extracts of seven mango cultivars (*Mangifera indica* L.) in Indonesia against MRSA isolated from wounds.

**Methodology and results:** The agar well diffusion method was used to test the antibacterial activity by measuring the diameter of the inhibition zone and a microdilution method to obtain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Seed extracts of seven mango cultivars exhibited antibacterial efficacy against four MRSA bacteria isolated from wounds. The largest inhibitory area, varying from 8 mm to 20 mm, were obtained by seed extracts of seven different mango cultivars, with MIC and MBC values of 1.56 mg/mL and 6.25 mg/mL, respectively.

**Conclusion, significance and impact of study:** Seed extracts of seven mango cultivars can serve as antibacterial agents, particularly against MRSA strains causing infections. The research findings of this study could be helpful in the healthcare sector.

**Keywords:** Antibacterial activity, mango, MRSA, seed

INTRODUCTION

The skin refers to the body’s outermost organ having direct contact with the external environment (Simões et al., 2018). Its primary function is to shield the organs and underlying tissues from numerous biological, chemical and physical contaminants (Chua et al., 2016). Wounds, burns, surgical incisions and concurrent disorders such as diabetes can impair the structure and function of the skin (Fijan et al., 2019). Wounds are defined as bodily tissue damage resulting from punctures, cuts and other impacts. Infections can sometimes be transmitted through wounds. One of the health concerns induced by the entry of pathogenic microorganisms, such as bacteria, is infection.

*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, Coagulase-negative *Staphylococcus*, *Klebsiella pneumonia*, *Acinetobacter sp.*, *Staphylococcus aureus* and can all cause infections (Adhikari et al., 2020). *Staphylococcus aureus* is the most prevalent human infection-causing bacterium (Tong et al., 2015). It is commonly found in wounds and also responsible for abscesses, characterized by the buildup of pus (Rai et al., 2017). Generally, antibiotics are used to treat wound infections; however, improper administration of antibiotics might cause bacteria to develop resistance. Methicillin-resistant *S. aureus* (MRSA) infection is a global health concern, with high morbidity, mortality rates and the potential to cause bacteremia or sepsis (Hassoun et al., 2017).

MRSA is resistant to β-lactam antibiotics, including the penicillin group (oxacillin, methicillin, nafcillin). *Staphylococcus aureus* generates β-lactamase, an

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enzyme that degrades the β-lactam ring. β-lactamase breaks apart the β-lactam ring of penicillin, nullifying its antimicrobial activity (Brooks et al., 2013). The advent of bacteria resistant to many antibiotics is a significant obstacle to treating infections (Jubeh et al., 2020). Asia has the most significant reported frequency of MRSA. It is widespread in most hospitals in Asia, with a prevalence of 28% to >70% (Chen and Huang, 2014). During 2008–2010, there were 259 clinical S. aureus isolates from patients in four tertiary care hospitals in Indonesia (in Denpasar, Malang, Padang and Semarang). Seventeen (7%) of these isolates were MRSA strains, with a frequency of between 2% and 9% (Santosaningsih et al., 2016). Eight MRSA isolates from skin and soft tissue infections were recently identified among 257 S. aureus isolates (Santosaningsih et al., 2018). In Indonesia, the frequency of MRSA is exceptionally high. Accordingly, natural ingredients with antibacterial potential are highly required to limit the need for synthetic antibiotics.

Natural antibacterial agents can be obtained from seeds (Prastiyanto et al., 2020a; Prastiyanto, 2021), fungi (Prastiyanto et al., 2017; 2020b), latex (Prastiyanto et al., 2020c), plants (Prastiyanto et al., 2021), bacterial isolates from marine organisms (Prastiyanto et al., 2022b) and fruits (Prastiyanto et al., 2020d), even plasma jet (Darmawati et al., 2019). One of the natural ingredients that can be employed as an antibacterial agent is mango seed extract. Mangoes (Mangifera indica) belong to the Sapindales order and the Anacardiaceae family and grow widely throughout the world, especially in tropical countries such as Indonesia. Every part of mango trees, such as leaves, stems, skin, seeds and pulp, can be utilized in various fields as an essential source of micronutrients, vitamins and other phytochemicals (Jahurul et al., 2015). Mangoes have been reported to be rich in bioactive compounds applicable in the health sector. The phytochemical compounds demonstrate antioxidant, anti-diabetic, antibacterial, antiviral, anti-inflammatory, anti-fungal and anticancer activities (Parvez, 2016). Several studies unveiled that mango had high antibacterial activity against Gram-positive and Gram-negative bacteria (Abdullah, 2011). A previous study reported that the seed extracts of four mango varieties (Apple, Ngowe, Kent, Shabine) obtained from the Makueni and Embu areas of Kenya exhibited antibacterial and antifungal activity against E. coli, S. aureus and C. albicans (Mutua et al., 2017). Another study showed that the Kweni cultivar from Indonesia seed kernel extracts has to be developed as an antibacterial agent against MDR Pseudomonas aeruginosa, which causes wound infection (Prastiyanto et al., 2022a). This present study attempted to fill the research gap aims to determine the antibacterial potential of the seeds of seven mango cultivars (Cengkir, Golek, Kopyor, Avocado, Kweni, Manalagi and Arumanis) native to Indonesia and tested for antibacterial activity against multi-drug resistant (MDR) strain MRSA isolated from wounds.

MATERIALS AND METHODS

Sample collection and extraction

The seeds of seven mango cultivars (Cengkir, Golek, Kopyor, Avocado, Kweni, Manalagi and Arumanis) were obtained from the market in Semarang City, Indonesia (Figure 1) (Prastiyanto et al., 2022a). The mango seeds were separated from the fruit and skin. The seeds were washed with water to remove all unnecessary materials and then dried for seven days in the sunlight. Subsequently, they were crushed and then stored in an airtight container for further use in the following process.
Mango seed extracts were acquired by maceration using 96% ethanol as solvent. Mango seeds were soaked in 96% ethanol solvent in a ratio of 1:3 for 24 h at room temperature, protected from light, and stirred. The change of solvent was carried out every day until the solution was clear, with an estimate of all the active compounds in the natural ingredients had been taken. Whatman No.1 filter paper was used to filter the supernatant. Moreover, the maceration solution was concentrated using a rotary evaporator at a temperature of <50 °C for 24 h. MRSA bacterial colonies were dissolved with turbidity equivalent to 0.5 McFarland standard. Whatman No.1 filter paper was used to filter the supernatant. Moreover, the maceration solution was concentrated using a rotary evaporator at a temperature of <50 °C for 24 h. MRSA bacterial colonies were dissolved with turbidity equivalent to 0.5 McFarland standard.

Bacteria isolation

MRSA bacteria were isolated from patients’ wounds at Tugu Rejo Hospital, Semarang, Central Java, Indonesia. All isolates were identified and obtained using Vitek®2 Compact (Table 1). MRSA bacteria were subcultured on the blood agar plate (BAP) medium containing 5% blood and incubated for 16–20 h at 35 ± 2 °C MRSA bacterial colonies were suspended and adjusted to the standard McFarland 0.5 (5 × 10^8 CFU/mL) using a McFarland Densitometer.

Screening of antibacterial activity against MRSA

Inhibition zone of seed extracts

The agar-well diffusion method evaluated the anti-MRSA activity of seven mango cultivars’ seed extracts (Prastiyanto et al., 2021). MRSA bacteria in subculture on the blood agar plate (BAP) medium were incubated at 35 ± 2 °C for 24 h. MRSA bacterial colonies were dissolved with turbidity equivalent to 0.5 McFarland standard. Furthermore, MRSA bacteria were inoculated in Muller Hilton agar (MHA). The wells, with a 1 cm diameter, were prepared using a sterilized cork borer. Wells were made in each plate and filled with the seed extracts of seven mango cultivars 100 mg/mL each, then incubated aerobically at 35 ± 2 °C for 16–20 h. Oxacillin, Ampicillin, Gentamicin, Erythromycin, Levofloxacin and Tazobactam were employed as positive controls for MRSA and dimethyl sulfoxide (DMSO).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of mango seed extracts

The MIC and MBC of seed extracts of seven mango cultivars were determined using microdilution in the Mueller Hinton Broth (MHB) medium (CLSI, 2020). MHB 100 µL was introduced into 12 wells and seed extracts of seven mango cultivars (100 µL) were added to serial dilutions. Subsequently, 10^5 µL of MRSA bacterial cell suspension was added to each well. Microplates were incubated aerobically at 35 ± 2 °C for 16 h. Oxacillin was applied as a positive control for MRSA. MIC was determined by observing the lowest concentration of seven mango cultivar seed extracts that inhibited bacterial growth and could be observed visually. Then, the wells were subcultured using a 10 µL inoculation loop on BAP medium containing 5% sheep's blood and incubated at 35 ± 2 °C for 16–20 h. The lowest extract concentration not exhibiting bacterial colony growth was defined as MBC (Prastiyanto et al., 2021).

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial activity of various seed extracts of seven mango cultivars was determined by testing in vitro using the agar-well diffusion method against four MRSA bacteria isolated from wounds and S. aureus ATCC. Antibacterial activity was determined by measuring the inhibition zone diameters in mm against MRSA.

The seed extracts of seven mango cultivars with a concentration of 100 mg/mL disclosed inhibitory zones against four MRSA strains causing infections and S. aureus ATCC. The analysis results disclosed an anti-MRSA activity of the seed extracts of seven mango cultivars, indicated by inhibitory zones. The resulting inhibition zones ranged from 8 ± 0.0 to 20 ± 0.0 mm against MRSA and 11.5 ± 0.0 to 19.8 ± 0.0 mm against S. aureus ATCC (Table 2).

MIC and MBC

The MIC of seed extracts of seven mangoes was tested against four MRSA bacteria isolated from wounds and S. aureus ATCC was evaluated in vitro by the microdilution
Figure 1: MIC test results of seed extracts of seven mango cultivars using ethanol (A) Code 2C, (B) Code 5C, (C) Code 8E, (D) Code 14E, (E) ATCC Code, (F) Negative Control and (G) Positive Control for the growth of MRSA bacteria with concentrations of (1) 50 mg/mL, (2) 25 mg/mL, (3) 12.5 mg/mL, (4) 6.25 mg/mL, (5) 3.12 mg/mL, (6) 1.56 mg/mL, (7) 0.78 mg/mL, (8) 0.39 mg/mL, (9) 0.19 mg/mL, (10) 0.09 mg/mL, (11) 0.04 mg/mL, (12) 0.02 mg/mL.

Table 2: The inhibition zone diameters with 96% ethanol on the seed extracts of seven mango cultivars against the growth of MRSA bacteria and control antibiotics.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Concentration (mg/mL)</th>
<th>Inhibition zone diameter (mm)</th>
<th>S. aureus ATCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cengkir</td>
<td>100</td>
<td>11 ± 0.6</td>
<td>19.1 ± 0.9</td>
</tr>
<tr>
<td>Kopyor</td>
<td>13.5 ± 0.5</td>
<td>18 ± 0.0</td>
<td>19.8 ± 0.0</td>
</tr>
<tr>
<td>Golek</td>
<td>13.7 ± 0.9</td>
<td>18 ± 0.5</td>
<td>18 ± 0.0</td>
</tr>
<tr>
<td>Kweni</td>
<td>14.7 ± 0.5</td>
<td>17.4 ± 0.6</td>
<td>19.7 ± 0.4</td>
</tr>
<tr>
<td>Avocado</td>
<td>16 ± 0.0</td>
<td>18.5 ± 0.5</td>
<td>17 ± 0.8</td>
</tr>
<tr>
<td>Arumanis</td>
<td>12.5 ± 0.4</td>
<td>16 ± 0.0</td>
<td>11.5 ± 0.0</td>
</tr>
<tr>
<td>Manalagi</td>
<td>10.6 ± 0.4</td>
<td>17.2 ± 0.5</td>
<td>15.6 ± 0.7</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OX 5 µg</td>
<td>8 (R)</td>
<td>5 (R)</td>
<td>35 (S)</td>
</tr>
<tr>
<td>AMP10 µg</td>
<td>16 (I)</td>
<td>41 (S)</td>
<td>38 (S)</td>
</tr>
<tr>
<td>GEN 10 µg</td>
<td>25 (S)</td>
<td>24 (S)</td>
<td>28 (S)</td>
</tr>
<tr>
<td>E15 µg</td>
<td>0 (R)</td>
<td>23 (S)</td>
<td>25 (S)</td>
</tr>
<tr>
<td>LEV 5 µg</td>
<td>25 (S)</td>
<td>25 (S)</td>
<td>27 (S)</td>
</tr>
<tr>
<td>TZP 25 µg</td>
<td>22 (S)</td>
<td>37 (S)</td>
<td>34 (S)</td>
</tr>
</tbody>
</table>

R, resistant; S, sensitive; I, intermediate.

method, as displayed in Figure 2. The seed extracts of seven mango cultivars (Cengkir, Golek, Kopyor, Avocado, Kweni, Manalagi and Arumanis) acquired MIC values ranging from 1.56 mg/mL to 12.5 mg/mL of the seven extracts; the Kweni mango seed extracts produced the lowest MIC value against MRSA at concentrations of 1.56 mg/mL and 3.12 mg/mL (Figure 2).

MBC of the seed extracts of seven mangoes was tested against four MRSA bacteria isolated from wounds and ATCC was examined in vitro by microdilution. Then, the wells were subcultured on the BAP medium containing 5% sheep’s blood. The analysis unveiled that the seed extracts of seven mangoes illustrated an antibacterial activity with MBC values against MRSA between 6.25 mg/mL and 50 mg/mL (Figure 3).

Research on antibacterial agents derived from natural ingredients is necessary, in line with the increasing cases of multi-drug resistant bacteria (MDR), especially
Figure 2: MBC test results on the seed extracts of seven mango cultivars. A. Cengkir mango, B. Kopyor mango, C. Golek mango, D. Kweni mango, E. Avocado mango, F. Arumanis mango and G. Manalagi mango against the growth of MRSA and S. aureus ATCC and concentrations of (1) 50 mg/mL, (2) 25 mg/mL, (3) 12.5 mg/mL, (4) 6.25 mg/mL, (5) 3.12 mg/mL, and (6) 1.56 mg/mL.

Infections caused by MRSA. This study is more appropriate because it demonstrates relatively sizeable inhibitory zones against MRSA, classified as resistant to certain antibiotics, compared to previous studies, reporting that the mango seed extracts using methanol with a concentration of 100 mg/mL exhibited antibacterial activity against MRSA, E. coli and Vibrio vulnificus with inhibitory zone sizes of 21.25 ± 1.28 against MRSA, 17.70 ± 0.75 against E. coli and 7.8 ± 0.29 against V. vulnificus.

These results demonstrate higher antibacterial activity against MRSA (Kaur et al., 2010).

Previous research on the seed extracts of three mango cultivars (Waterlily, Lemak and Shakran) from Malaysia using ethanol unveiled an antibacterial activity against Gram-positive and Gram-negative bacteria. Waterlily mango seed extracts exhibited antibacterial activity against Staphylococcus aureus (18 mm), Bacillus subtilis (17 mm), Pseudomonas aeruginosa (14 mm) and...
E. coli (21 mm). Furthermore, Lemak mango seed extracts disclosed an antibacterial activity against S. aureus (16 mm), B. subtilis (16 mm), P. aeruginosa (13 mm) and E. coli (14 mm). Meanwhile, Shakran mango seed extracts demonstrated antibacterial activity against S. aureus (16 mm) B. subtilis (15 mm), P. aeruginosa (9 mm) and E. coli (17 mm) (Abdullah, 2011).

The research results on the antibacterial activity of the seed extracts of seven mango cultivars originating from Indonesia align with Abdullah’s research in 2011, examining the antibacterial potential of the seed extracts of mango cultivars originating from Malaysia. The results revealed that Gram-positive bacteria tended to be more sensitive to antimicrobial compounds in natural ingredients than Gram-negative bacteria. The outer membrane that surrounds the cell wall of Gram-negative bacteria will be reduced by the hydrophobic diffusion of bioactive compounds through their lipopolysaccharides. Another factor affecting the antibacterial activity is the various components of chemical compounds in natural ingredients, following their geographical origin and harvest period (Lelario et al., 2018).

Another study reported that the mango seed extracts against MRSA obtained inhibition zones of 10–13 mm using ethanol and 8-13 mm using methanol (El-Gied et al., 2012). Hence, this current study is better, with the MRSA growth inhibition zones of 8–20 mm, as displayed in Table 2. It can be associated with the variation of concentration and solvent used, the higher the concentration of the extracts, the larger the size of the resulting inhibition zone. Another factor influencing the different sizes of the inhibition zone is the type of solvent used for extraction. It is related to the nature of the polarity of the antibacterial compounds extracted with each solvent and the ability of these substances to diffuse in the medium used in the antibacterial activity test.

The antibacterial compounds in the mango seed extracts using ethanol are more soluble than methanol (Alo et al., 2012). Another study by Venkata Raju et al. (2019) reported that the mango seed extracts using methanol obtained inhibition zones of 8.5 ± 0.3 mm against S. aureus (MTCC 737), 8.2 ± 0.3 mm against E. coli (MTCC446) and 8.6 ± 0.5 mm against B. subtilis (DSM 10). Various studies have revealed that mango seed extracts had antibacterial activity against various bacterial strains, including MDR. It implies a positive effort in studies in microbiology and the treatment of infections caused by bacteria, in which the mango seed extracts can serve as an alternative natural ingredient to replace antibiotics.

This study illustrates efforts to utilize natural ingredients as an alternative to antibiotics. This research uncovered that all seed extracts of seven mango cultivars (Cengkir, Golek, Kopyor, Avocado, Kweni, Manalagi and Arumanis) exhibited antibacterial activity against MRSA isolated from wounds. The antibacterial activity is closely related to the mechanism of action of the active compounds in natural ingredients. Following the phytochemical screening, the mango seed extracts have been reported to contain flavonoid compounds, tannins, saponins, alkaloids and phenols (Somkuwar and Kamble, 2013).

The active compounds in the seeds of seven mango cultivars have different antibacterial activities in inhibiting bacterial growth, similar to other flavonoids, which work as antibacterial agents by blocking the function of cell membranes. Flavonoids also combine with intracellular proteins to generate complex molecules that can breach bacterial cell membranes and release intracellular chemicals. In addition, flavonoids can inhibit bacterial energy metabolism, which bacteria need to synthesize molecules; thus, bacterial complex molecules cannot be formed (Farhadi et al., 2019). Tannins have antibacterial activity by binding to proteins, especially in hydrophobic and hydrogen bonds. As a result, they can inhibit bacterial metabolism (Kaczmarek, 2020). Saponins are glycoside compounds exhibiting biological activity by disrupting cell membrane components, affecting membrane permeability that can cause cell leakage (Munyan et al., 2017). Alkaloids have an antibacterial mechanism by inhibiting bacterial cell wall synthesis, influencing cell membrane permeability, inhibiting bacterial metabolism, inhibiting protein and nucleic acid synthesis (Yan et al., 2021).

CONCLUSION

The seed extracts of seven mango cultivars could be a potential alternative antibacterial agent in treating infections caused by pathogenic bacteria, especially MDR. Extracts tested against MRSA isolated from wounds demonstrated antibacterial activity. Further studies should be conducted to determine the mechanism of action of the active compounds in the extracts that work as antibacterials. It is also crucial to perform in vivo tests to discover positive or negative effects, including toxicity to animal and human cells.

ACKNOWLEDGEMENTS

We acknowledge the support of the Ministry of Education, Culture, Research and Technology for Penelitian Desentralisasi (Penelitian Produk Vokasi Unggulan Perguruan Tinggi) Number 004/061026/PL/SP2H/VK.04/2023.

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