Antibacterial activity of seed kernel extracts of seven mangoes (Mangifera indica) cultivars native to Indonesia against MDR-Pseudomonas aeruginosa isolated from wounds

by Ana H

Submission date: 21-Nov-2022 10:16AM (UTC+0700)
Submission ID: 1959802007
File name: 12260-Article_Text-1066112-1-10-20221109_1.pdf (855.19K)
Word count: 5588
Character count: 31956

BIODIVERSITAS
Volume 23, Number 11, November 2022
Pages: 5629-5637

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231112

Antibacterial activity of seed kernel extracts of seven mangoes (*Mangifera indica*) cultivars native to Indonesia against MDR-*Pseudomonas aeruginosa* isolated from wounds

MUHAMMAD EVY PRASTIYANTO[1,♥], SRI DARMAWATI[2,3], ANA HIDAYATI MUKAROMAH[3,4]

[1]Microbiology Laboratory, Department of Medical Laboratory Technology, Faculty of Health and Nursing, Universitas Muhammadiyah Semarang. Jl. Kedungmundu Raya No. 18, Semarang 50273, Central Java, Indonesia. Tel./Fax.: +62-24-76740296, ♥email: evy_prastiyanto@unimus.ac.id
[2]Molecular Biology Laboratory, Department of Medical Laboratory Technology, Faculty of Health and Nursing, Universitas Muhammadiyah Semarang. Jl. Kedungmundu Raya 18, Semarang 50273, Central Java, Indonesia
[3]Graduate Program of Laboratory for Clinical Science, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang. Jl. Kedungmundu Raya No. 18, Semarang 50273, Central Java, Indonesia
[4]Chemistry Laboratory, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang. Jl. Kedungmundu Raya No. 18, Semarang 50273, Central Java, Indonesia.

Manuscript received: 16 September 2022 2022. Revision accepted: 29 October 2022.

Abstract. *Prastiyanto ME, Darmawati S, Mukaromah AH. 2022. In vitro evaluation of the antibacterial activity of seed kernel extracts of seven mangoes (*Mangifera indica*) cultivars native to Indonesia against MDR-*Pseudomonas aeruginosa *isolated from wounds. Biodiversitas 23: 5629-5637. Pseudomonas aeroginusa* the most common bacterium causing wound infections, with the most common solution being antibiotics. However, excessive and inappropriate use of antibiotics will lead to the emergence of multi-drug resistant (MDR) bacterial strains. Therefore, natural ingredients are needed as alternative antibacterial agents. This study aimed to determine the antibacterial activity of seed kernel extracts from seven cultivars of mango (*Mangifera indica*) from Indonesia, i.e., Cengkir, Kopyor, Golek, Kweni, Avocado, Arumanis, and Manalagi, against MDR-*P. aeroginusa* bacteria isolated from wounds. The agar well diffusion method was carried out to determine the inhibition zone, and the microdilution method was used to determine the MIC and MBC values. The results showed that the seed kernel extracts of seven cultivars of mangoes had antibacterial activity against MDR-*P. aeruginosa*. Of the seven mango cultivars, Kweni cultivar seed kernel extracts demonstrated the lowest MIC and MBC values of ≥0.75 mg/mL and ≥12.5 mg/mL. This study concludes that Kweni cultivar seed kernel extracts have the potential to be developed as agents of anti-MDR-*P. aeruginosa* causes wound infection.

Keywords: *Mangifera indica*, MDR, *Pseudomonas aeruginosa*, seeds, wounds

INTRODUCTION

A wound is a condition in which the extracellular matrix (ECM) is damaged, resulting in the loss of the skin's protective function, with or without damage to underlying tissues (muscle, bone, and blood vessels). Wounds are caused by trauma to the skin from sharp objects or collisions. Wounds are also instigated by lacerations of membranes, most commonly the skin. Microorganisms can invade and multiply through an open wound (Nasser et al. 2018). In chronic wounds, bacteria are associated with multiple species, causing virulent tissue damage. *Pseudomonas, Staphylococcus, Enterobacter, Peptoniphilus, Stenotrophomonas, Serratia,* and *Finegoldia* are commonly found in wounds (Rahim et al. 2016). A previous study by Puca et al. (2021) reported that the wound samples examined contained Gram-negative bacteria (57%) and Gram-positive bacteria (36.6%). The Gram-positive bacteria include *Staphylococcus aureus* (79.4%), while the Gram-negative bacteria comprise *Escherichia coli* (20.7%), *Pseudomonas aeruginosa* (40.2%), *Acinetobacter baumanii/haemolyticus* (9.5%), and *Proteus mirabilis* (11.2%). *P. aeroginusa* is the most commonly found among the Gram-negative bacteria that infect wounds. *Pseudomonas aeruginosa* is an opportunistic pathogenic bacteria widely distributed in nature (Moradali et al. 2017). These bacteria can form biofilms on wounds, a major public health concern. Biofilms are communities or colonies of bacteria that grow together on the extracellular matrix (ECM), a fundamental structural component of the bacterial community that serves as a protective barrier (Ma et al. 2009). *Pseudomonas aeruginosa* colonization can result in acute skin or wound infections (Bassetti et al. 2018). The bacteria are known to infect wounds (Smolle et al. 2018). The mortality rate of patients with wounds infected with *P. aeruginosa*, particularly MDR bacteria, is higher than that of patients with uninfected wounds (Branski et al. 2009).

Antibiotics are commonly used to treat wounds. Common antibiotics used in wound care include macrolides, β lactams, tetracycline, fluoroquinolones, and aminoglycosides (Tzaneva et al. 2016). Antibiotics play a role in suppressing bacterial growth, but overuse of antibiotics causes systemic damage (Everts 2017). In addition, excessive and uncontrolled antibiotic use resulted in MDR bacterial strains. Therefore, alternative antibacterial sources from natural ingredients are needed. Natural antibacterial sources comprise marine

microorganisms (Prastiyanto et al. 2022), lactic acid bacteria (Lestari et al. 2019), mushrooms (Prastiyanto et al. 2020a), fruit (Prasnto et al. 2020c), seeds (Prastiyanto 2021), and latex (Prastiyanto et al. 2020b). Plants are the most common and potential antibacterial agents from natural sources of antibacterial agents (Prastiyanto et al. 2021), one of which is mango.

Mango (*Mangifera indica* L.) belongs to the *Anacardiaceae* family of the order *Sapindales*, an economically important tropical fruit plant, a source of traditional medicine (Kumar et al. 2021; Ghosh et al. 2022). In 2018, the global mango production was 55.4 million tonnes, with India, China, Thailand, and Indonesia leading the production (Kumar et al. 2021). There are over 1000 mango cultivars available worldwide, but only a few are traded.

Mango cultivars from various countries have been reported to have antibacterial properties. For example, a previous study showed that the Malaysian Chokanan mango seed methanol extract had antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (Kaur et al. 2010). Another study showed that ethanol and methanol extracts of Sudanese mango seed cultivars had good inhibitory activity against almost all tested strains, i.e., *Bacillus cereus, Citrobacter freundii, Escherichia coli, Listeria monocytogens, Mycobacterium senegalense, Salmonella typhi, Shigella flexnerri, Staphylococcus aureus*, *Yersinia enterocolitica* (El-Gied et al. 2012). However, there has been limited research on the antibacterial properties of Indonesian mango cultivars. This present study attempted to fill the research gap by evaluating the antibacterial activity of seven mango cultivars (Cengkir, Kopyor, Golek, Kweni, Avocado, Arumanis, and Manalagi) originated from Indonesia against MDR *P. aeruginosa* isolated from wounds.

MATERIAL AND METHODS

Collection and extraction of mango seeds

Seeds of mango cultivars of Cengkir, Kopyor, Golek, Kweni, Avocado, Arumanis, and Manalagi, were collected from local markets (Semarang, Central Java, Indonesia) (Figure 1). First, the mango seeds were washed and sun-dried for seven days. Then, the dried seeds were soaked in a 96% ethanol solution at a 1:3 ratio at room temperature for 24 hours. The ethanol solvent was changed every 24 hours until the solution became clear, indicating that the bioactive components had been extracted in ethanol. The supernatant was filtered using Whatman paper No. 1, and the filtrate was concentrated with a rotary evaporator at 40°C.

Isolation of *Pseudomonas aeroginusa* from wounds

Pseudomonas aeroginusa isolates were obtained from wound samples from patients at Tugurejo Hospital in Semarang, Indonesia. The bacteria were cultured using MacKonkey agar media and incubated at 35 ± 2°C for 24 hours. The colonies were non-lactose fermenters selected using positive catalase and oxidase testing. Vitek®MS was used for bacterial identification and susceptibility testing (Prastiyanto 2021). The following antibiotics used as positive controls were AMP (Ampicillin), TZP (Piperacillin-tazobactam), SAM (Ampicillin-sulbactam), CAZ (Ceftazidime), CFZ (Cefazolin), FEP (Cefepime), CRO (Ceftriaxone), ATM (Aztreonam), TGC (Tigecycline), MCA (Amikacin), CIP (Ciproflox), MEM (Meropenem), and GEN (Gentamicin). MDR-*P. aeruginosa* determination was based on the Clinical Laboratory Standard Institute (CLSI) of M100-S25 (CLSI 2020).

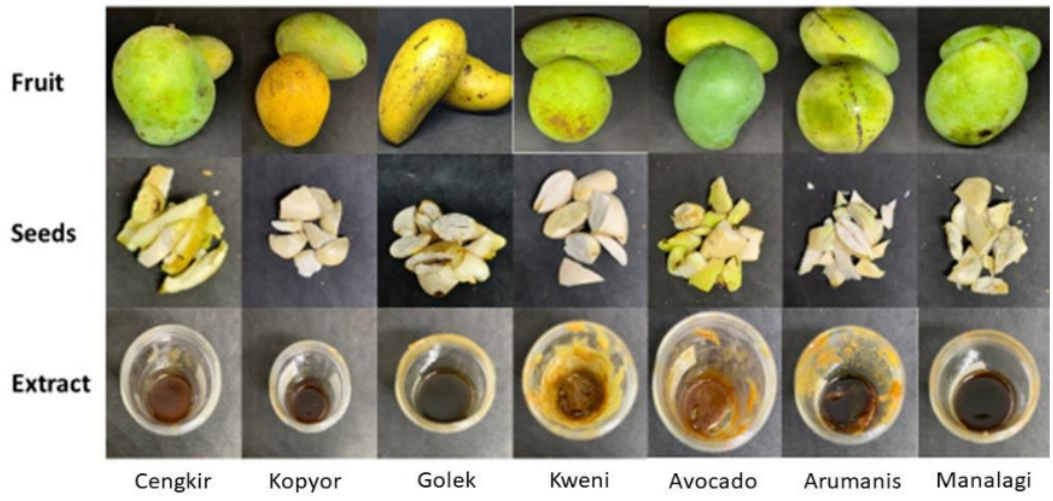

Figure 1. Fruit, seeds, and extracts of seven mango cultivars

The anti-MDR activity against *Pseudomonas aeroginusa* from mango seed extracts

Agar well diffusion

Agar well diffusion assay was used to evaluate the antibacterial activity of mango seed extracts against MDR-*P. aeruginosa* (Prastiyanto 2021). MDR-*P aeroginusa* isolates from patient wounds were cultured on a blood agar plate and then incubated for 24 hours at 35 ± 2°C. All bacterial isolates were standardized with 0.5 McFarland ($1.5x10^8$ CFU/mL). Each isolate was inoculated on Muller Hilton agar (MHA) media using a sterile cotton swab, 10 minutes incubation. The MHA media was perforated using a cork borer (0.5 cm in diameter). Four holes were made for each concentration (0.1 mg/mL, 1 mg/mL, 10 mg/mL, and 100 mg/mL). The extract was dissolved in Dimethyl sulfoxide (DMSO). Then, 100 μL of the extract was added to each well, followed by incubation at a temperature of 35 ± 2°C for 16-20 hours. The antibiotics used were Ampicillin (AMP) (10 μg), ceftriaxone (CTR) (30 μg), Tigecycline (TGC) (15 μg), Nitrofurantoin (NIT) (300 μg), Trimethoprim (TMP) (25 μg), Gentamicin (GEN) (10 μg), Meropenem (MEM) (10 μg), and Ciprofloxacin (CIP) (5 μg).

Minimum Inhibitory Concentration (MIC)

The microdilution method used Mueller Hilton Broth (MHB) media added with 0.05% 2,3,5-Triphenyltetrazolium chloride on a microwell plate to determine the MIC (Prastiyanto 2021). First, MHB (100 μL) was added to each well, and 100 μL of the extract was supplemented to the first well, then serial dilutions were made up to the 12th well. After that, 10 μL of MDR *P. aeruginosa* was added to each well, then incubated for 18-20 hours at 35 ± 2°C. The MIC value was observed by the presence of the color change on the microwell plate. The lowest MIC value of the extract was indicated as the best antibacterial activity.

Minimum Bactericidal Concentration (MBC)

Wells on MIC were subcultured on Blood Agar Plate (BAP) media and then incubated at 35 ± 2°C for 16-20 hours. The MBC value was determined by observing the growth of bacteria on BAP media. The MBC value was determined as the lowest concentration at which MDR-*P. aeruginosa* is unable to grow (Prastiyanto 2021).

Phytochemical screening of the extract

Phytochemical analysis (triterpenoids, steroids, flavonoids, alkaloids, phenolates, tannins, and saponins) of the crude extracts of mango seeds was carried out using previously described methods (Eve et al. 2020).

RESULTS AND DISCUSSION

MDR-*Pseudomonas aeruginosa* from wound

MDR-*P. aeruginosa* bacteria were isolated from the wounds and tested for their susceptibility to several antibiotics. The susceptibility test showed that the isolated bacteria were MDR *P. aeruginosa* (Figure 2) because it was resistant to at least three or more classes of antibiotics. MDR-*P. aeruginosa* strain #1 showed resistance to penicillins (Ampicillin, Piperacillin-tazobactam, and Ampicillin-sulbactam), cephalosporins (Ceftazidime, Ceftriaxone, and Cefazolin), Monobactam (Aztreonam), carbapenems (Meropenem), aminoglycosides (Amikacin), fluoroquinosides (Amikacin), (ciprofloxacin) and glycylcycline (Tigecycline). The MDR-*P. aeruginosa* strain #2 was resistant to the following classes of antibiotics: penicillins (Piperacillin-tazobactam and Ampicillin-sulbactam), cephalosporins (Ceftriaxone and Ceftazidime), Monobactam (Aztreonam), carbapenems (Meropenem), aminoglycosides (Amiconemikacin, fluoroquintacocin). MDR-*P. aeruginosa* strain #3 was resistant to the following antibiotics: penicillins (Ampicillin-sulbactam, Ampicillin, and Piperacillin-tazobactam), cephalosporins (Ceftriaxone, Ceftazidime, and Cefazolin), Monobactam (Aztreonam), carbapenems (Meropenem), aminoglycosides (Amikacinosine), fluoroquinolones (ciprofloxacin) and glycylcycline (Tigecycline). MDR-*P. aeruginosa* strain #4 was resistant to cephalosporins (Ceftriaxone, Ceftazidime, and Cefazolin), Monobactam (Aztreonam), carbapenems (Meropenem), aminoglycosides (Gentamicin, Amikacin), fluoroquinolones (ciprofloxacin) and glycylcycline (Tigecycline). *Pseudomonas aeruginosa* is a bacterium commonly found in wounds (Nagoba et al. 2013).

The results of this study revealed that four bacterial isolates from the wound samples containing *P. aeruginosa* were resistant to penicillin, cephalosporin, monobactam, and carbapenem; therefore, *P. aeruginosa* produced extended-spectrum beta-lactamase (ESBL). In addition, the four isolated strains were carbapenem-resistant *P. aeruginosa* (CRPA) strains. ESBL-*P. aeruginosa* is the main cause of wound infection (Ullah et al. 2009).

Extract yields

The yield of ethanol extract from seven cultivars of mangoes (Cengkir, Kopyor, Golek, Kweni, Avocado, Arumanis, and Manalagi) was presented in Table 1. The extract yield of the Kweni cultivar was higher than the other six cultivars, indicating that the Kweni cultivar contained phytochemicals (with relatively polar constituents) that are more soluble in ethanol. In addition, ethanol solvent produced extracts with better antibacterial potential than other solvents (Prastiyanto et al. 2020c).

Table 1. The extract yields of seed kernel of seven cultivars of mango (*Mangifera indica*)

Cultivar	Yield (%)
Cengkir	11.86
Kopyor	25.35
Golek	28.31
Kweni	33.87
Alpukat	31.33
Arumanis	23.51
Manalagi	22.22

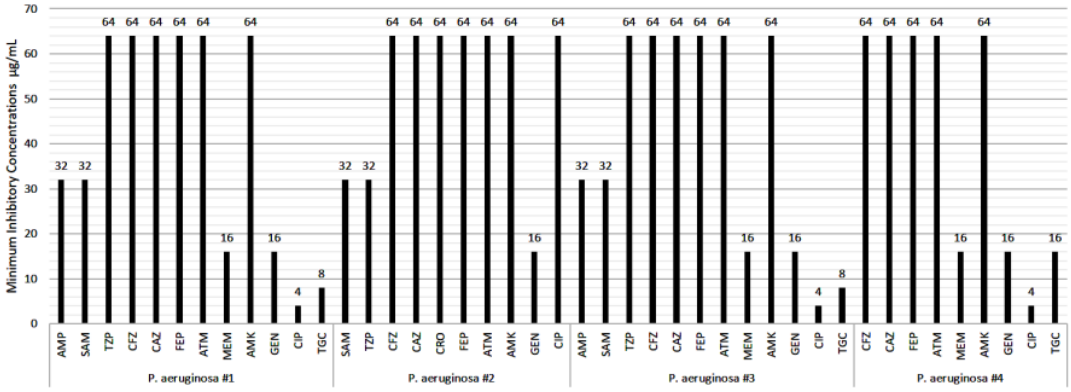

Figure 2. Susceptibility test of several antibiotics against *P. aeruginosa* isolated from wounds. AMP: Ampicillin; SAM: Ampicillin-sulbactam; TZP: Piperacillin-tazobactam; CFZ: Cefazolin; CAZ: Ceftazidime; CRO: Ceftriaxone; FEP: Cefepime; ATM: Aztreonam; MCA: Amikacin; MEM: Meropenem; CIP: ciprofloxacin, GEN: Gentamicin; TGC: Tigecycline

Antibacterial activities assay

Agar well diffusion assay

The antibacterial activity of the extract of mango seed kernels was evaluated using the agar well diffusion assay method against MDR-*P. aeruginosa* from wounds. The results showed that the extracts of seven mango cultivars had inhibitory zones (Figure 3), indicating the presence of antibacterial activity against MDR-*P. aeruginosa*. The inhibitory zones of mango seed extracts at concentrations of 0.1 mg/mL, 1 mg/mL, 10 mg/mL, and 100 mg/mL against four MDR-*P. aeruginosa* isolates from wound samples and *P. aeruginosa* ATCC 9027 were presented in Figure 4. This figure showed that seven mango seed extracts had antibacterial activity against MDR-*P. aeruginosa* wound isolates and the antibacterial activity from the extract of seven mango cultivars against MDR-*P. aeruginosa* was directly proportional to the extract concentrations. The inhibition zone of extracts at a concentration of 0.1 mg/mL ranging from 6.0 ± 0.0 mm to 11.90 ± 0.16 mm. The inhibition zone of extracts at a concentration of 1 mg/mL ranging from 11.00 ± 0.0 mm to 17.30 ± 0.19 mm, while a concentration of 10 mg/mLhad the diameter of the inhibition ranging from 18.00 ± 0.11 mm to 28.20 ± 0.16 mm. Extract concentration of 100 mg/mL had the diameter of the inhibition zone ranging from 19.75 ± 0.43 mm to 30.63 ± 0.39 mm. *Pseudomonas aeruginosa* isolates had no inhibitory zones to the following antibiotics: ceftriaxone (CTR) (30 μg), Ampicillin (AMP) (10 μg), Tigecycline (TGC) (15 μg), Nitrofurantoin (NIT) (300 μg), Trimethoprim (TMP) (25 μg), Gentamicin (GEN) (10 μg), Meropenem (MEM) (10 μg), and ciprofloxacin (CIP) (5 μg). Extracts of seven mango cultivars had wider inhibitory zones than the control antibiotics.

The results of the antibacterial activity by the agar well diffusion assay showed that seed extracts of the seven mango cultivars (Cengkir, Kopyor, Golek, Kweni, Avocado, Arumanis, and Manalagi) had the potential as antibacterial agents against 4 MDR-*P aeruginosa* bacteria isolated from wounds. The inhibitory zone of these extracts ranges from 6.00 ± 0.00 mm to 30.63 ± 0.39 mm, which is larger than previous studies. The inhibition zone diameter of mango seed kernel extract of Apple and Sabine cultivars from Kenya against *Escherichia coli* at a concentration of 100% ranges from 19.3 and 17.3 mm (Mutua et al. 2017). The study on Julie mango and John mango cultivars from Makurdi, Nigeria, against *P. aeruginosa* showed that the hexane extract of Julie mango cultivar seeds at a concentration of 100 mg/mL showed a diameter of inhibition of 6.67 ± 0.58. In comparison, extract hexane of John mango cultivars at a concentration of 100 mg/mL showed a diameter of 7.76 ± 01.00 mm against *P. aeruginosa* (Mutua et al. 2017).

Another study reported that mango seed methanol extract from Sudan at a concentration of 5 mg/mL had an inhibition zone of 6 mm against *P. aeruginosa* ATCC 27853 (El-gied et al. 2012). In addition, the MDR strain of P. aeruginosa was used in this study, while the previous study used the standard strain. The ethanol extract was more likely to be chosen as a solvent in the further study because methanol is risky due to its high toxicity.

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

The microdilution method using a microwell plate was used to determine the MIC and MBC values (Prastiyanto 2021). The pathogenic bacteria can be in direct contact with the extract in the dilution assay, so it does not depend on the diffusion properties of the material and media be tested (Estrela et al. 2001). In the serial micro dilutions, the MIC value was determined as the extract's lowest concentration, which had antibacterial properties. In this study, the MIC and MBC values of seed kernel extract from seven mango cultivars ranged from ≥0.75 to ≥25 mg/mL dan ≥12.5 to ≥50 mg/mL, respectively (Table 2). It is the first study that evaluates the antibacterial activity of seed kernel extracts of mango cultivars native to Indonesia against MDR-*P. aeruginosa* isolated from wound, as well as qualitative screening for phytochemical content.

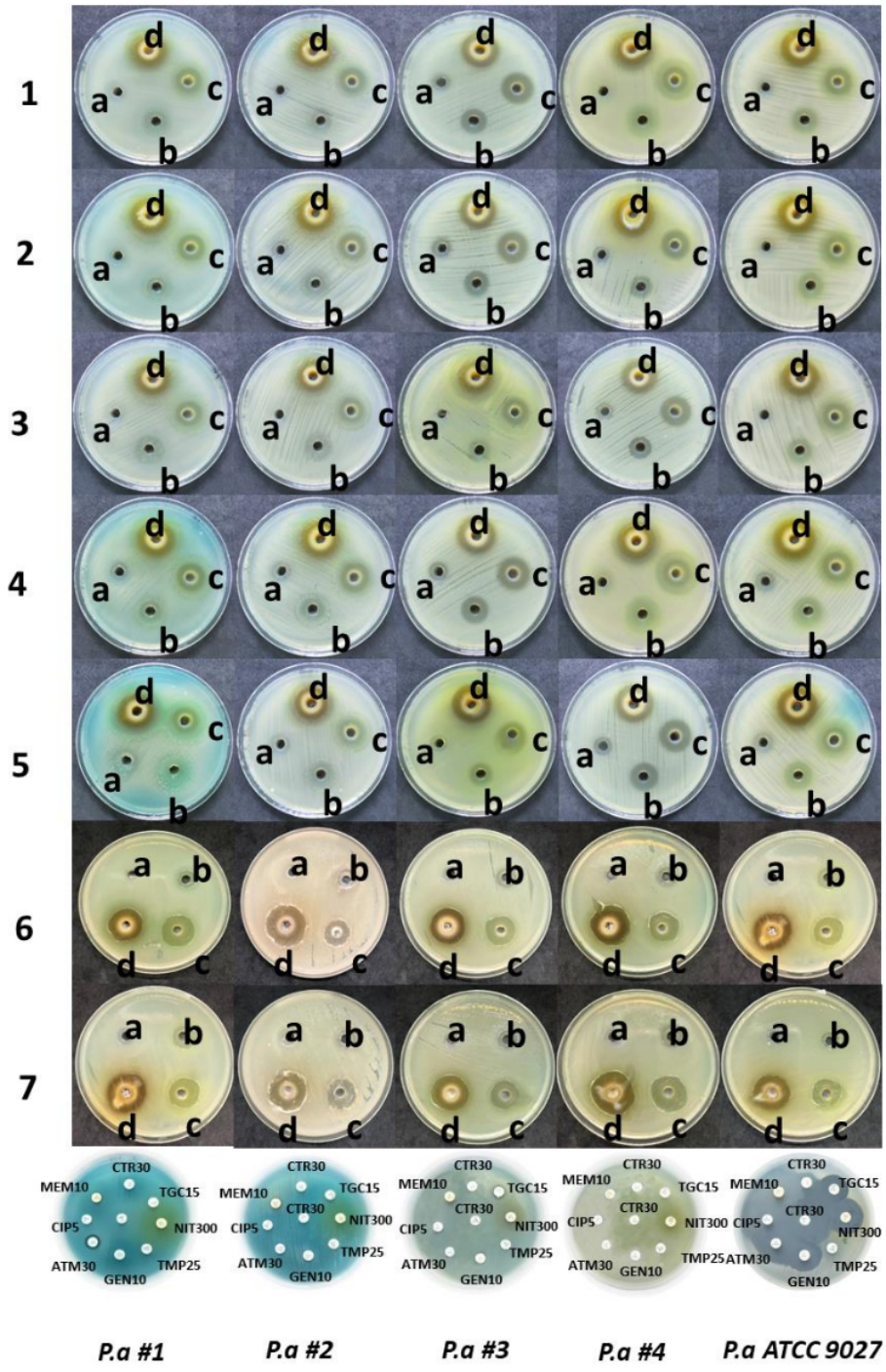

Figure 3. The inhibition zones of the extracts of seed kernels of seven mangoes (*Mangifera indica* L) cultivars; 1: Gedong, 2: Kopyor, 3: Golek, 4: Kweni, 5 Avocado, 8: Arumanis, 7: Manalagi (mg/mL) against MDR *Pseudomonas aeruginosa* (P.a); a: 0.1 mg/mL, b: 1 mg/mL, c: 10 mg/mL, d: 100 mg/mL: Ampicillin (AMP) (10 μg), ceftriaxone (CTR) (30 μg), Tigecycline (TGC) (15 μg), Nitrofurantoin (NIT) (300 μg), Trimethoprim (TMP) (25 μg), Gentamicin (GEN) (10 μg), Meropenem (MEM) (10 μg), and Ciprofloxacin (CIP) (5 μg)

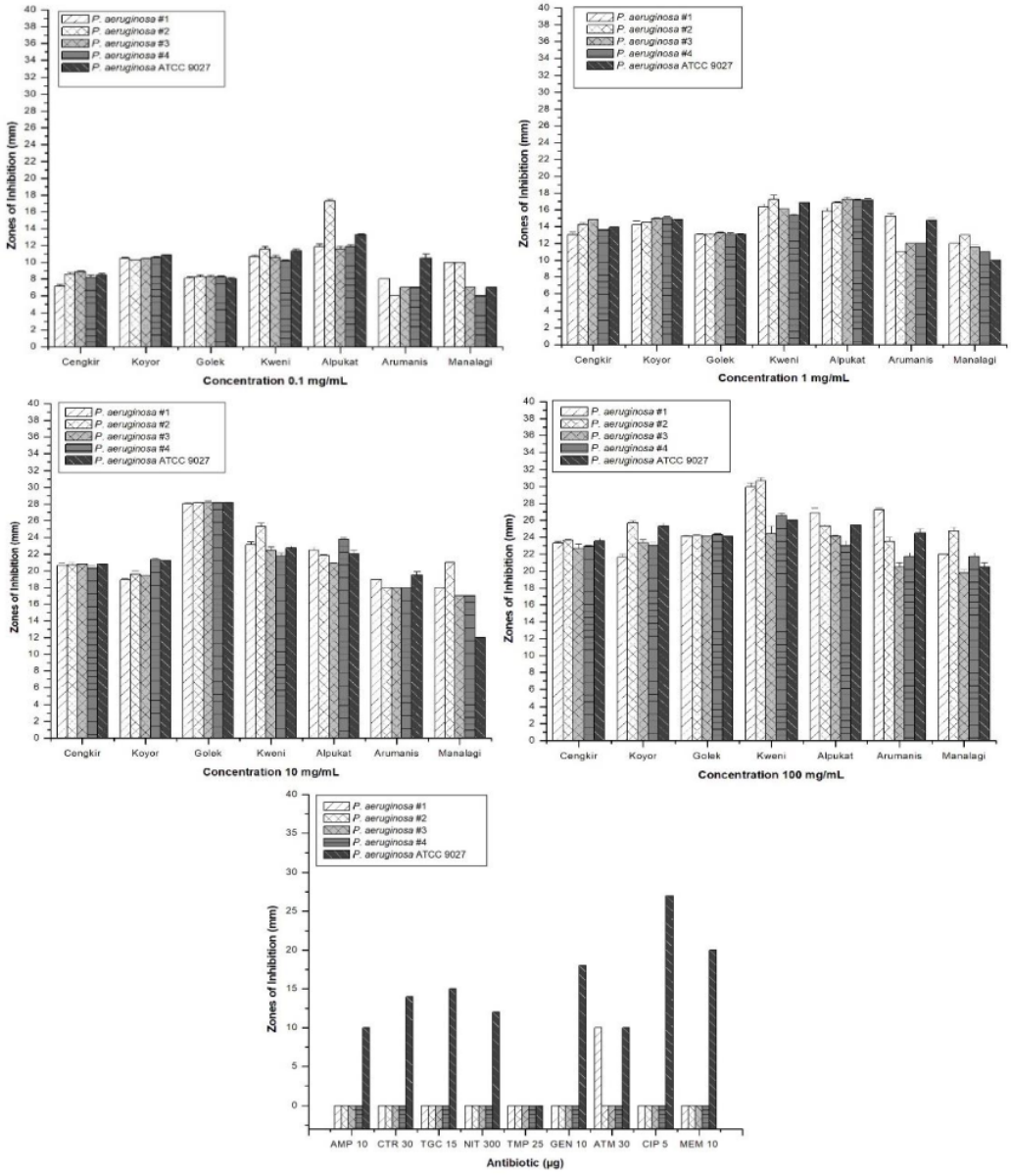

Figure 4. The diameters of the inhibition zones of seed kernels extracts of seven mangoes (*Mangifera indica*) cultivars from Indonesian

Table 2. The MIC and MBC values of seed kernel extracts of seven mangoes (*Mangifera indica*) cultivars against MDR-*P. aeruginosa*

MDR-*P. aeruginosa*	Seed kernel extracts of seven mangoes (*Mangifera indica*. L) cultivars													
	Cengkir		Kopyor		Golek		Kweni		Avocado		Arumanis		Manalagi	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	≥1.56	≥50	≥12.5	≥25	≥1.56	≥25	≥0.75	≥12.5	≥1.56	≥25	≥25	≥50	≥1.56	≥50
2	≥1.56	≥50	≥12.5	≥25	≥1.56	≥25	≥0.75	≥12.5	≥1.56	≥25	≥25	≥50	≥1.56	≥50
3	≥1.56	≥50	≥12.5	≥25	≥1.56	≥25	≥0.75	≥12.5	≥1.56	≥25	≥25	≥50	≥1.56	≥50
4	≥1.56	≥50	≥12.5	≥25	≥1.56	≥25	≥0.75	≥12.5	≥1.56	≥25	≥25	≥50	≥1.56	≥50
ATCC	≥1.56	≥50	≥12.5	≥25	≥1.56	≥25	≥0.75	≥12.5	≥1.56	≥25	≥25	≥50	≥1.56	≥50

The seed kernel extracts of the Kweni mango cultivar demonstrated better antibacterial activity than the other six mango cultivars, as indicated by lower MIC value against MDR-*P. aeruginosa* strains (Table 2). A previous study by Dzotam and Kuete (2017) reported that ethanol mango leaves extract had Antibacterial activities against *P. aeruginosa* PA01 and *P. aeruginosa* PA124 with a MIC value of 1.024 mg/mL. In this study, it was shown that the extracts of seed kernels of the Kweni mango cultivar had better antibacterial activity against MDR-*P. aeruginosa* with lower MIC and MBC values (≥0.75 mg/mL and ≥12.5 mg/mL).

Another study reported the Antibacterial potential of seed extracts of the Fahlun mango cultivar from Thailand against MRSA had an inhibitory zone of 10.61 ± 1.25 mm to 16.85 ± 1.94 mm at a concentration of 0.625 mg/disc to 5.00 mg/disc, with a MIC value of 0.47 ± 0.00 mg/disc and MBC value of 1.83 ± 0.79 mg/mL (Jiamboonsri et al. 2011). These results revealed that the antibacterial activity of mango seed extract was more effective against Gram-positive (MRSA) than Gram-negative (MDR-*P. aeruginosa*). These results align with studies by Mutua et al. (2017) that mango kernel extract has better growth inhibition against *S. aureus* than *Escherichia coli*. It relates to tannins and flavonoid content in the extract. The differences in the ability to inhibit the growth of Gram-negative and Gram-positive bacteria are due to the differences in their cell walls. According to Huang et al. (2018), the low susceptibility of Gram-negative toward antibacterial agents is due to the presence of lipopolysaccharide in the membrane, which makes it more resistant.

The qualitative phytochemical screening of the seed extract of seven mango cultivars

The phytochemical screening of the ethanol extract of seven mango cultivars includes the contents of triterpenoids, steroids, flavonoids, alkaloids, phenolics, tannins, and saponins (Table 3). The results revealed that all seven mango cultivars' seed extracts contained flavonoids, alkaloids, phenolics, tannins, and saponins, but no steroids were detected in the extracts. Furthermore, triterpenoid compounds were only found in Kweni cultivars. A study by Somkuwar and Kamble (2013) showed the ethanol extract of mango seed kernels contained alkaloids, saponins, tannins, and flavonoids. Meanwhile, another study by El-gied et al. 2012) showed that the ethanol extracts of mango seeds contained saponins, triterpenes, and steroids but did not contain tannins or alkaloids. The geographic location of growth strongly influences differences in the phytochemical content of mango seed extracts. Yadav et al. (2022) highlighted that the geographical location of growth affects its phytochemical content.

This study did not evaluate the antibacterial activity of any phytochemical content of the extract against the test bacteria. However, based on previous studies, several phytochemicals have Antibacterial activity. For example, flavonoid and phenolic compounds have potent antibacterial properties. The flavonoid compounds inhibit the formation of bacterial cell walls and cause cell lysis (Royani et al. 2022). Mango seeds have been reported to have a large number of phenolic families. Polyphenols found in mango seeds include cyanidin, mangiferin gallate, homomangiferin, isomangiferin, isomangiferin gallate, and rhamnetin 3-0 galactoside/glucoside (Tirado-kulieva et al. 2021).

This study revealed that mango seed extract contains tannins; the most abundant tannin derivatives in mango are gallotanins (Kim et al. 2021). Galotannins extracted from mango seed kernels have antibacterial activity against *S. aureus* and *E. coli* (Engels et al. 2009). The compounds trap proteins on the bacterial surface, causing cell dysfunction (Luís et al. 2014). Alkaloids are one of the largest and most diverse phytochemical groups with antibacterial properties. Plants with high alkaloid content exhibit effective antibacterial properties. Alkaloids extracted from *Callistemon citrinus* leaves showed antibacterial activity against *P. aeruginosa* (ATCC 27853). The mode of action of alkaloids as antibacterial was by inhibiting ATP-dependent transport of compounds across bacterial cell membranes (Mabhiza et al. 2016). Saponins were also found in all mango cultivars. The mechanism of saponins as antibacterials is to lower the surface tension of bacterial cells, which results in increased permeability and cell leakage, which cause the release of intracellular compounds (Khan et al. 2018).

Terpenoids belong to the class of lipid compounds. Mangoes have been reported to contain several terpenoids, including terpinolene, ocimene, careen, myrcene, or limonene (Hernández-Sánchez et al. 2001). Of the seven cultivars of mangoes, Kweni showed positive results of terpenoid compounds. According to Lalel et al. (2003), terpenoid compounds are responsible for the aroma of mangoes. Therefore, it was identified that the Kweni cultivar had a strong aroma among the tested mango cultivars. The study did not identify the inhibition mechanism of terpenoid derivatives against pathogenic bacteria. However, previous studies reported that terpenoids could inhibit two important processes for bacterial survival and hinder oxygen uptake and oxidative phosphorylation of bacteria (Griffin et al. 1999). Because *P. aeruginosa* is an obligately aerobic bacterium, oxygen absorption is critical for producing energy for growth. Furthermore, oxidative phosphorylation is the process that causes cellular respiration in the cytoplasmic membrane. Thus, the interaction of terpenoids alters cellular respiration, resulting in the release of oxidative phosphorylation in bacteria (Zengin and Baysal 2014).

Table 3. Phytochemical content of seven mango cultivars from Indonesia

Seed Extract	Phytochemical test						
	Triterpenoids	Steroids	Flavonoids	Alkaloids	Phenolics	Tannins	Saponins
Cengkir	-	-	+	+	+	+	+
Kopyor	-	-	+	+	+	+	+
Golek	-	-	+	+	+	+	+
Kweni	+	-	+	+	+	+	+
Avocado	-	-	+	+	+	+	+
Arumanis	-	-	+	+	+	+	+
Manalagi	-	-	+	+	+	+	+

In conclusion, the results of the susceptibility test of *P. aeruginosa* bacteria from wound samples to antibiotics have ascertained that all of the bacteria are MDR-*P. aeruginosa* strains. The antibacterial activity in-vitro results against MDR-*P. aeruginosa* isolated from wounds showed evidence that all seven mango cultivars' seed kernel extracts have Antibacterial activity against MDR-*P. aeruginosa*. The seed extracts of the Kweni mango cultivar demonstrated greater antibacterial activity than the other six cultivars tested. Thus, seed kernel extracts of Kweni mango cultivars might be the most effective sources of antibacterials.

ACKNOWLEDGMENTS

We acknowledge the support of the Ministry of Education, Culture, Research, and Technology for Penelitian Desentralisasi (Penelitian Produk Vokasi Unggulan Perguruan Tinggi) Number 15/D4/RA.02.02/2022. We also thank Universitas Muhammadiyah Semarang, Indonesia, for facilitating this research.

REFERENCES

Bassetti M, Vena A, Croxatto A, Righi E, Guery B. 2018. How to manage *Pseudomonas aeruginosa* infections? Risk factors for antimicrobial resistance in *P. aeruginosa*. Drugs Context 7: 1-18. DOI: 10.7573/dic.212527.

Branski LK, Al-mousawi A, Rivero H, Jeschke MG, Sanford AP, Herndon DN. 2009. Emerging Infections in Burns. Surg Infect 10 (5): 389-397. DOI: 10.1089/sur.2009.024.

CLSI. 2020. M100 Performance Standards for Antimicrobial. Wayne.

Dzotam JK, Kuete V. 2017. Antibacterial and Antibiotic-Modifying Activity of Methanol Extracts from Six Cameroonian Food Plants against Multidrug-Resistant Enteric Bacteria. Biomed Res Intl 2017: 19. DOI: 10.1155/2017/1583510.

El-gied AAA, Joseph MRP, Mahmoud IM, Abdelkareem AM, Hakami AMAl, Hamid ME. 2012. Antimicrobial Activities of Seed Extracts of Mango (*Mangifera indica* L .). Adv Microbiol 2012: 571-576.

Engels C, Knödler M, Zhao Y, Carle R, Gänzle MG. 2009. Antimicrobial activity of gallotannins isolated from mango (*Mangifera indica* L.) kernels. J Agric Food Chem 57: 196558. DOI: 10.1021/jf901621m

Estrela C, Rodrigues C, Estrela DA. 2001. Two Methods to Evaluate the Antimicrobial Action of Calcium Hydroxide Paste. J Endod 27: 12-15. DOI: 10.1097/00004770-200112000-00002.

Eve A, Aliero AA, Nalubiri D, Adeyemo RO, Akinola SA, [illegible]s T, Nabaasa S, Nabukeera S, Alkali B, Ntulume I. 2020. In vitro antibacterial activity of crude extracts of *Artocarpus heterophyllus* seeds against selected diarrhoea-causing superbug bacteria. Sci World J 2020. DOI: 10.1155/2020/9813970.

Everts R. 2017. How to Treat Prevention and treatment. The Health Media Ltd, New Zealand.

Ghosh B, Majumder S, Acharyya S, Ghosh A, Saha S, Sarkar S, Chakraborty S, Bhattacharya M. 2022. Comparative phytochemical analysis of mature mango leaves from nineteen cultivars of Murshidabad district, India. Asian J Nat Prod Biochem 20: 48-55. DOI: 10.13057/biofar/f200202.

Griffin SG, Wyllie SG, Markham JL, Leach DN. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity †. Flavour Fragr J 14: 4-5. DOI: 10.1002/(SICI)1099-1026(199909/10)14:5<322::AID-FFJ837>3.0.CO;2-4.

Hernández-Sánchez, Sanz-Berzosa, Casaña-Giner, Primo-Yúfera, 2001. Attractiveness for *Ceratitis capitata* (Wiedemann) (Dipt., Tephritidae) of mango (*Mangifera indica*, cv. Tommy Atkins) airborne terpenes. J Appl Entomol 125: 10-11. DOI: 10.1046/j.1439-0418.2001.00510.x.

Huang C, Kuo C, Wu C, Kuan A, Guo H, Lin Y, Wang P. 2018. Free radical-scavenging, anti-inflammatory, and antibacterial activities of water and ethanol extracts prepared from commercial-puffing pretreated mango (*Mangifera indica* L.) peels. J Food Qual 2018. DOI: 10.1155/2018/1025387.

Jiamboonsri P, Pithayanukul P, Bavovada R, Chomnawang MT. 2011. The inhibitory potential of thai mango seed kernel extract against Methicillin-Resistant *Staphylococcus Aureus*. Molecules 16: 6255-6270. DOI: 10.3390/molecules16086255.

Kaur J, Rathinam X, Nasi M, Leng KM, Ayyalu R, Kathiresan S, Subramaniam S. 2010. Preliminary investigation on the antibacterial activity of mango (*Mangifera* indica L: *Anacardiaceae*) seed kernel. Asian Pac J Trop Med 3: 707-710. DOI: 10.1016/S1995-7645(10)60170-8.

Khan MI, Ahhmed A, Shin JH, Baek JS, Kim MY, Kim JD. 2018. Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study in vitro and in vivo. Evidence-Based Complement Altern Med 12: 5486106. DOI: 10.1155/2018/3486106.

Kim H, Castellon-chicas MJ, Arbiser S, Talcott ST, Drury NL, Smith S, Mertens-Talcott SU. 2021. Intestinal microbial health benefits, and associated mechanisms of actions. Molecules 26: 2732. DOI: 10.3390/molecules26092732.

Kumar M, Saurabh V, Tomar M, Hasan M, Changan S, Sasi M, Maheshwari C. 2021. Mango (*Mangifera indica* L.) Leaves nutritional composition, phytochemical profile, and health-promoting bioactivities enhanced reader. Antioxidants 10: 299. DOI: 10.3390/antiox10020299.

Lalel HJD, Singh Z, Chye S. 2003. Aroma volatiles production during fruit ripening of 'Kensington Pride mango. Postharvest Biol Technol 27: 323-336. DOI: 10.1016/S0925-5214(02)00117-5.

Lestari SD, Sadiq ALO, Safitri WA, Dewi SS, Prastiyanto ME. 2019. The antibacterial activities of bacteriocin *Pediococcus acidilactici* of breast milk isolate against methicillin-resistant *Staphylococcus aureus*. J Phys Conf Ser 1375: 012021. DOI: 10.1088/1742-6596/1374/1/012021.

Luís Â, Silva F, Sousa S, Duarte AP. 2014. Antistaphylococcal and biofilm inhibitory activities of gallic, caffeic, and chlorogenic acids. J Biofouling 30 (1): 69-79. DOI: 10.1080/08927014.2013.845878.

Ma L, Conover M, Lu H, Parsek MR, Bayles K, Wozniak DJ. 2009. Assembly and Development of the Pseudomonas aeruginosa Biofilm Matrix. PLoS Pathog 5: e1000354. DOI: 10.1371/journal.ppat.1000354.

Mabhiza D, Chitemerere T, Mukanganyama S. 2016. Antibacterial Properties of Alkaloid Extracts from *Callistemon citrinus* and *Vernonia adonis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Intl J Med Chem 2016. DOI: 10.1155/2016/6304163.

Moradali MF, Ghods S, Rehm BHA. 2017. *Pseudomonas aeruginosa* Lifestyle: A paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol 7: 39. DOI: 10.3389/fcimb.2017.00039.

Mutua JK, Imathiu S, Owino W. 2017. Evaluation of the proximate composition, antioxidant potential, and antimicrobial activity of mango seed kernel extracts. Food Sci Nutr 5: 349-357. DOI: 10.1002/fsn3.399.

Nagoba BS, Selkar SP, Wadher BJ, Gandhi RC. 2013. Acetic acid treatment of pseudomonal wound infections A review. J Infect Public Health 6: 410-415. DOI: 10.1016/j.jiph.2013.05.005.

Nasser M, Ogali M, Kharat AS. 2018. Prevalence of MDR *Pseudomonas aeruginosa* of war-related wound and burn ward infections from some conflict areas of Western Yemen. Wound Med 20: 58-61. DOI: 10.1016/j.wndm.2018.02.001.

Prastiyanto ME, Dewi N, Pratiningtias T, Pratiwi N, Indayani A, Wahyunengsih E, Astuti, Amir E, Wardoyo F. 2021. In vitro antibacterial activities of crude extracts of nine plants on multi-drug resistance bacterial isolates of wound infections. Biodiversitas 22: 2641-2647. DOI: 10.13057/biodiv/d220712.

Prastiyanto ME. 2021. Seeds extract of three *Artocarpus* species: Their in-vitro antibacterial activities against multidrug-resistant (MDR) *Escherichia coli* isolates from urinary tract infections (UTIs). Biodiversitas 22: 4356-4362. DOI: 10.13057/biodiv/d221028.

Prastiyanto ME, Rukmana RM, Saraswati DK, Darmawati S, Maharani ETW, Tursinawati Y. 2020a. Anticancer potential of methanolic extracts from *Pleurotus* species on Raji cells and antibacterial activity against Methicillin-Resistant *Staphylococcus aureus*. Biodiversitas 21: 5644-5649. DOI: 10.13057/biodiv/d211221.

Prastiyanto ME, Tama PD, Ananda N, Wilson W, Mukaromah AH. 2020b. Antibacterial Potential of *Jatropha* sp. Latex against Multidrug-Resistant Bacteria. Intl J Microbiol 2020. DOI: DOI: 10.1155/2020/8509650.

Prastiyanto ME, Wardoyo FA, Wilson W, Darmawati S. 2020c. Antibacterial activity of various extracts of *Averrhoa bilimbi* against Multidrug Resistant Bacteria. Biosaintifika 12: 163-168. DOI: 10.15294/biosaintifika.v12i2.23600.

Prastiyanto ME, Kartika AI, Darmawati S. 2022. Bioprospecting of bacterial symbionts of sponge *Spongia officinalis* from Savu Sea, Indonesia, for antibacterial potential against multi-drug-resistant bacteria. Biodiversitas 23: 1118-1124. DOI: 10.13057/biodiv/d230256.

Puca V, Marulli RZ, Grande R, Vitale I, Niro A. 2021. Microbial Species Isolated from Infected Wounds and Antimicrobial Resistance Analysis: Data Emerging from a Three-Years Retrospective Study. Antibiotics 10 (10): 1162. DOI: 10.3390/antibiotics10101162.

Rahim K, Saleha S, Zhu X, Huo L, Basit A. 2016. Bacterial Contribution in Chronicity of Wounds. Microb Ecol 73 (3): 710-721. DOI: 10.1007/s00248-016-0867-9.

Royani A, Hanafi M, Julistiono H, Manaf A. 2022. Materials Today : Proceedings The total phenolic and flavonoid contents of *Aloe vera* and *Morinda citrifolia* extracts as antibacterial material against *Pseudomonas aeruginosa*. Mater Today: Proc. DOI: 10.1016/j.matpr.2022.06.466.

Smolle C, Cambiaso-Daniel J, Forbes AA, Wurzer P, Branski LK, Huss F, Kamolz L, Surgery R, Surgery M, Surgery P. 2018. Recent Trends in Burn Epidemiology Worldwide: A Systematic Review. HHS Public Access 43: 249-257. DOI: 10.1016/j.burns.2016.08.013.

Somkuwar D, Kamble V. 2013. Phytochemical screening of ethanolic extracts of stem, leaves, flower and seed kernel of *Mangifera indica* L. Pharmacognosy 4: 383-389.

Tirado-kulieva V, Atoche-Dioses S, Hernández-Martínez E. 2021. Phenolic compounds of mango (*Mangifera indica*) by-products: Antioxidant and antimicrobial potential , use in disease prevention and food industry. Sci Agropecu 12: 283-293. DOI: 10.17268/sci.agropecu.2021.031.

Tzaneva V, Irena M, Galina T, Dimitar P. 2016. Antibiotic treatment and resistance in chronic wounds of vascular origin. Clujul Med 89: 365-370. DOI: 10.15386/cjmed-647.

Ullah F, Akbar S, Ahmed J. 2009. Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan. Burns 35: 1020-1025. DOI: 10.1016/j.burns.2009.01.005.

Yadav D, Pal AK, Singh SP, Sati K. 2022. Phytochemicals in mango (*Mangifera indica*) parts and their bioactivities: A Review. Crop Res 57: 79-95. DOI: 10.31830/2454-1761.2022.012.

Zengin H, Baysal AH. 2014. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecules 19 (11): 17773-17798. DOI: 10.3390/molecules191117773.

Antibacterial activity of seed kernel extracts of seven mangoes (Mangifera indica) cultivars native to Indonesia against MDR-Pseudomonas aeruginosa isolated from wounds

ORIGINALITY REPORT

19%	12%	11%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Calamvale Community College Student Paper	<1%
2	Submitted to Caritas Institute of Higher Education Student Paper	<1%
3	bmcbiotechnol.biomedcentral.com Internet Source	<1%
4	esajournals.onlinelibrary.wiley.com Internet Source	<1%
5	ftp.academicjournals.org Internet Source	<1%
6	www.apjtcm.com Internet Source	<1%
7	www.screwworm.ars.usda.gov Internet Source	<1%
8	Thomas Trunk, Hawzeen S. Khalil, Jack C. Leo. "Bacterial autoaggregation", AIMS	<1%

	Microbiology, 2018 Publication	
9	escientificpublishers.com Internet Source	<1 %
10	frl.publisso.de Internet Source	<1 %
11	medwelljournals.com Internet Source	<1 %
12	old.sjournals.com Internet Source	<1 %
13	Lihui Zhu, Rongrong Liao, Weilong Tu, Yonghong Lu, Xuan Cai. "Pyrodextrin enhances intestinal function through changing the intestinal microbiota composition and metabolism in early weaned piglets", Applied Microbiology and Biotechnology, 2020 Publication	<1 %
14	Nithitanakool, Saruth, Pimolpan Pithayanukul, Sandrine Bourgeois, Hatem Fessi, and Rapepol Bavovada. "The Development, Physicochemical Characterisation and in Vitro Drug Release Studies of Pectinate Gel Beads Containing Thai Mango Seed Kernel Extract", Molecules, 2013. Publication	<1 %
	www.rsisinternational.org	

15	Internet Source	<1%
16	Submitted to Anglo-Chinese School (Independent) Student Paper	<1%
17	revistabiociencias.uan.edu.mx Internet Source	<1%
18	alicia.concytec.gob.pe Internet Source	<1%
19	es.slideshare.net Internet Source	<1%
20	jglobal.jst.go.jp Internet Source	<1%
21	Submitted to University of Cape Town Student Paper	<1%
22	Submitted to University of Teesside Student Paper	<1%
23	html.rhhz.net Internet Source	<1%
24	Submitted to Florida International University Student Paper	<1%
25	Lacroix, M.. "The influence of MAP condition and active compounds on the radiosensitization of Escherichia coli and	<1%

Salmonella typhi present in chicken breast", Radiation Physics and Chemistry, 200409/10
Publication

26	Marina Silalahi. "Pemanfaatan dan Bioaktivitas Bilimbing Wuluh (Averrhoa bilimbi L.)", Titian Ilmu: Jurnal Ilmiah Multi Sciences, 2021 Publication	<1 %
27	Satriya Pranata, Shu-Fang Vivienne Wu, Javad Alizargar, Ju-Han Liu, Shu-Yuan Liang, Yu-Ying Lu. "Precision Health Care Elements, Definitions, and Strategies for Patients with Diabetes: A Literature Review", International Journal of Environmental Research and Public Health, 2021 Publication	<1 %
28	bmcinfectdis.biomedcentral.com Internet Source	<1 %
29	lermagazine.com Internet Source	<1 %
30	reader.repository.unimus.ac.id Internet Source	<1 %
31	Pitchaon Maisuthisakul, Michael H. Gordon. "Characterization and storage stability of the extract of Thai mango (Mangifera indica Linn. Cultivar Chok-Anan) seed kernels", Journal of Food Science and Technology, 2012 Publication	<1 %

32	apm.amegroups.com Internet Source	<1 %
33	archive.org Internet Source	<1 %
34	www.saiie.co.za Internet Source	<1 %
35	ChunJiang Zhang, HongYu Li, Tian Yun, YongHong Fu, ChunMei Liu, Bu Gong, BenJia Neng. " Chemical composition, antimicrobial and antioxidant activities of the essential oil of Tibetan herbal medicine Benth ", Natural Product Research, 2008 Publication	<1 %
36	Xiaozhen Wu, Jiayi Li, Zhe Zhou, Ziqiu Lin, Shimei Pang, Pankaj Bhatt, Sandhya Mishra, Shaohua Chen. "Environmental Occurrence, Toxicity Concerns, and Degradation of Diazinon Using a Microbial System", Frontiers in Microbiology, 2021 Publication	<1 %
37	Submitted to Ateneo de Davao University Student Paper	<1 %
38	Submitted to University of Adelaide Student Paper	<1 %
39	acikbilim.yok.gov.tr Internet Source	<1 %

40	datospdf.com Internet Source	<1%
41	Submitted to Macquarie University Student Paper	<1%
42	Mahmoud Emam, Doaa R. Abdel-Haleem, Shaimaa M. Farag, Mohamed A. El-Ansari, Mansour Sobeh. "Larvicidal Activity of Pentagalloyl Glucose and Mangiferin Isolated from the Waste of Mango Kernel Against Culex pipiens L.", Waste and Biomass Valorization, 2021 Publication	<1%
43	sciforum.net Internet Source	<1%
44	Justine Keathley, Michèle Kearney, Véronique Garneau, Juan de Toro-Martín et al. "Changes in systolic blood pressure, postprandial glucose, and gut microbial composition following mango consumption in individuals with overweight and obesity", Applied Physiology, Nutrition, and Metabolism, 2022 Publication	<1%
45	Li, Bin, Yong Yi, Qi Wang, Patrick C. Y. Woo, Lin Tan, Hua Jing, George F. Gao, and Cui Hua Liu. "Analysis of Drug Resistance Determinants in Klebsiella pneumoniae Isolates from a	<1%

Tertiary-Care Hospital in Beijing, China", PLoS ONE, 2012.
Publication

46	Paula P. Amador, Ruben M. Fernandes, Maria C. Prudêncio, Mário P. Barreto, Isabel M. Duarte. " Antibiotic resistance in wastewater: Occurrence and fate of producers of Class A and Class C β-lactamases ", Journal of Environmental Science and Health, Part A, 2014 Publication	<1 %
47	Sanjai Saxena, Charu Gomber. " Antimicrobial Potential of . ", Pharmaceutical Biology, 2008 Publication	<1 %
48	biomaterialsres.biomedcentral.com Internet Source	<1 %
49	epdf.pub Internet Source	<1 %
50	eprints.ulm.ac.id Internet Source	<1 %
51	Mohamad Khalil, Soukayna Hayek, Nour Khalil, Nadia Serale, Laura Vergani, Maria Calasso, Maria De Angelis, Piero Portincasa. "Role of Sumac (Rhus coriaria L.) in the management of metabolic syndrome and related disorders: Focus on NAFLD-	<1 %

atherosclerosis interplay", Journal of Functional Foods, 2021
Publication

---

52 Muhammad Ali Rajput, Rafeeq Alam Khan. "Phytochemical screening, acute toxicity, anxiolytic and antidepressant activities of the Nelumbo nucifera fruit", Metabolic Brain Disease, 2017 <1%
Publication

---

53 Soumya Majumder, Arindam Ghosh, Sourav Chakraborty, Malay Bhattacharya. "Brewing and biochemical characterization of Camellia japonica petal wine with comprehensive discussion on metabolomics", Food Production, Processing and Nutrition, 2022 <1%
Publication

---

54 en.wikipedia.org <1%
Internet Source

---

55 Arnida Arnida, Maulidia Maulidia, Amalia Khairunnisa, Sutomo Sutomo, Faisal Faisal. "Standardization of Simplicia and Ethanol Extract of Purun Danau (Lepironia articulata (Retz.) Domin) Rhizome", Borneo Journal of Pharmacy, 2021 <1%
Publication

---

56 Dayang Fredalina Basri, Radhiah Khairon. " Pharmacodynamic Interaction of Galls Extract in Combination with Vancomycin against <1%

MRSA Using Microdilution Checkerboard and Time-Kill Assay ", Evidence-Based Complementary and Alternative Medicine, 2012
Publication

57	jcdr.in Internet Source	<1%
58	Alfi Sophian, Ratna Purwaningsih, Muindar Muindar, Eka Putri Juniarti Igirisa, Muhammad Luthfi Amirullah. "Detection of Salmonella typhimurium ATCC 14028 in Powder Prepared Traditional Medicines Using Real-Time PCR", Borneo Journal of Pharmacy, 2021 Publication	<1%
59	Submitted to American Public University System Student Paper	<1%
60	Doaa Hamad, Heba El-Sayed, Wafaa Ahmed, Hana Sonbol, Mohammed Abdel Halim Ramadan. "GC-MS Analysis of Potentially Volatile Compounds of Pleurotus ostreatus Polar Extract: In vitro Antimicrobial, Cytotoxic, Immunomodulatory, and Antioxidant Activities", Frontiers in Microbiology, 2022 Publication	<1%
61	Elsayed Aboulmagd. "In vitro synergistic activity of amikacin combined with subinhibitory concentration of tigecycline	<1%

against extended spectrum -lactamase-producing Klebsiella pneumoniae", Journal of Microbiology and Antimicrobials, 2013
Publication

---

62 Karthik Sambanthamoorthy, Chunyuan Luo, Nagarajan Pattabiraman, Xiarong Feng et al. "Identification of small molecules inhibiting diguanylate cyclases to control bacterial biofilm development", Biofouling, 2013 — <1%
Publication

---

63 Submitted to London School of Hygiene and Tropical Medicine — <1%
Student Paper

---

64 Roberto Machado Cruz, Sergio Valmor Barbosa. "Histologic evaluation of periradicular tissues in dogs treated with calcium hydroxide in combination with HCT20 and camphorated P-chlorophenol", Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 2005 — <1%
Publication

---

65 ann-clinmicrob.biomedcentral.com — <1%
Internet Source

---

66 fvhe.vfu.cz — <1%
Internet Source

---

67 innovareacademics.in — <1%
Internet Source

68	umpir.ump.edu.my Internet Source	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		