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In vitro antibacterial activities of crude extracts of nine plants on multidrug resistance bacterial isolates of wound infections

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Abstract. Wound infections caused by multidrug resistance bacteria (MDR) have become serious health problems, and therefore, antibacterial agents from natural biological sources are necessary to overcome these problems. This study examines the antibacterial activities of nine plants (garlic, Solo garlic, Java plum (leaf), Java plum (fruit), lime, Kaffir lime, Siamese weed, mangosteen, and bitter melon) against MDR bacteria isolated from wounds. The antibacterial activities were evaluated using agar well diffusion assay to determine the inhibition zones, and microdilution method to determine the value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The best antibacterial activities were calculated as the most extensive inhibition zones with the smallest MIC and MBC values. Ethanol extracts from five plants (garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon) showed antibacterial activities against three MDR bacteria isolated from wounds. The bitter melon extract had the largest zones, 19.3 mm (methicillin-resistant *Staphylococcus aureus* [MRSA]), 10.6 mm (ESβL-producing *Escherichia coli*), and 13 mm (carbapenemase-resistant *Pseudomonas aeruginosa* [CRPA]) with the smallest MIC and MBC values against MRSA (3.12 and 25 mg/mL), ESβL- producing *E. coli* (12.25 and 50 mg/mL), and CRPA (6.25 and 25 mg/mL). This concludes that bitter melon has the potential to be developed as an antibacterial agent, particularly against MRSA strains, ESβL-producing *E. coli*, and CRPA that cause wound infections. Further in vivo research and the discovery of modes of action are needed to explain the antibacterial effects.

Keywords: In vitro antibacterial activities; wound infection; MRSA, ESβL-producing *Escherichia coli, CRPA*

Running title: antibacterial activities extracts of plants

INTRODUCTION

Skin is an important organ that protects the body from damage and invasion of pathogenic bacteria (Xu et al., 2015). When the skin is damaged, the wound that appears causes bacteria to easily infect and this condition affects health. The wound may be healed in a few days or will develop for a long time and become chronic. A chronic wound is one of the most serious and fatal human problems (Han and Ceilleey, 2017).

An infected wound will lead to longer recovery time and in some cases trigger death (Liang et al., 2019). *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are the bacteria commonly found in wounds (Pallavali et al., 2019). Antibiotics are commonly used to treat bacterial infections. However, the uncontrolled use of antibiotics contributes to the emergence of multidrug resistance (MDR) bacterial strains (Bologa et al., 2013). Patients infected with MDR bacteria may suffer from a prolonged disease that is difficult to treat and requires higher costs of treatment.

The Infectious Disease Society of America has considered the advent of several MDR bacteria, including those that are methicillin-resistant, extended-spectrum β-lactamase (ESβL) –producing-resistant and carbapenemase-resistant, as a distinct challenge in management (Boucher et al., 2009). The burn wound infections coused by MRSA (Chopra et al., 2016), MDR-*P.aeruginosa* (Nasser et al., 2020) and *E.coli* (Nasser et al., 2020) increase mortality and morbidity. The prevalence of infections caused by MRSA, MDR-*P.aeruginosa* and *E.coli* has increased in recent years.

Thus, new antibacterial agents from natural biological sources are require. Biological antibacterial agents can be obtained from honey (Panjaitan et al., 2018), mushrooms (Prastiyanto et al., 2020b, 2016), isolate bacteria from marine organisms (Asagabaldan et al., 2019), bacteriocins (Lestari et al., 2019), fruits (Prastiyanto et al., 2020d; Wahyuni et al., 2019), Latex (Prastiyanto et al., 2020c) and seeds (Ilvani et al., 2019; Prastiyanto et al., 2020a). Many studies in the medical field reported the importance of traditional medicinal plants as the alternatives of antimicrobial agents (Akhtar, 2015; Aumeeruddy-elalfi et al., 2015; Prastiyanto et al., 2021).

This study aims to investigate the antibacterial potentials of traditional plants. In this study, we used nine plants to measure the bacterial activities against resistant bacteria isolated from wounds, such as methicillin-resistant *S. aureus* (MRSA), (ESβL)-producing *E. coli*, and carbapenemase-resistant *P. aeruginosa* (CRPA). The nine plants were garlic, Solo garlic, Java plum (leaf), Java plum (fruit), lime, Kaffir lime, Siam weed, mangosteen, and bitter melon.

MATERIALS AND METHODS

Plant materials and Preparations of extracts

Nine samples of plants tested in the study were collected in the rainy season of December 2019. Different parts of the plants were washed with water to remove unnecessary materials, dried in the sun for seven days, ground and then stored in sterile airtight containers for further usages in the next processes. Plant extracts were prepared by maceration with 96% ethanol solvent. 200 g of ground plants were soaked in 600 mL of solvent for 24 hours at room temperature, protected from light and were later shaken. The solvent replacement was done every day until the solution was clear, in which no more active compounds were contained in the dry powder. The supernatant was filtered using Whatman No.1 filter paper. The maceration solutions were concentrated under reduced pressure using a rotary evaporator at 50 ^{0}C. The crude extracts were collected and allowed to dry at room temperature.

Isolation, identification of bacterial strains and antibiotic sensitivity test

MDR bacteria were directly isolated from wound samples obtained from patients in dr. Kariadi Hospital, Semarang, Central Java, Indonesia. All isolates were identified by biochemical tests using Vitek®MS (bioM´erieux, Marcy l'Etoile, France), following minimum inhibitory concentration (MIC) interpretive standards from the Clinical Laboratory Standard Institute M100-S25 (CLSI, 2019).

Antibacterial assay of plant extracts

Agar well diffusion assay

The antibacterial activities of various plant extracts were evaluated using a well-diffusion assay (Andleeb et al., 2020). MDR bacteria in a subculture on blood agar plate (BAP) media were incubated for 24 hours at (35 ± 2) ◦C. The MDR bacterial colonies were dissolved in a normal saline solution with a turbidity equivalent to the 0.5 McFarland standard. 100 µL of each MDR bacterium was inoculated in Muller Hilton agar (MHA) by spreading the bacterium on the surface of the agar using a sterilized glass spreader. After five minutes of inoculation, the wells were prepared using a sterilized steel *corkborer* (1cm in diameter). Four wells were made on each plate and loaded with each plant extract (250, 500, 750, and 1000 mg/mL). All plates were then incubated aerobically at 35 ± 2 ◦C for 16-20 hours. Dimethyl sulfoxide (DMSO) was used as a negative control. Vancomycin and oxacillin were applied as positive controls for MRSA, ampicillin and meropenem for ESBL-producing bacteria, and meropenem and tetracycline were for CR bacteria. Antibacterial activities of the extracts were determined by measuring the diameters of the inhibition zones in mm against the tested organism.

Determination of MIC and minimum bactericidal concentration (MBC) of the plant extracts

MIC values of plant extracts were determined in 12-well sterile microplates using the broth microdilution method (CLSI, 2018). Each test was carried out in triplicate. MHB (100 µL) was placed into the well and plant extract (100 µL) was put in the dilution series. 10 µL bacterial cell suspensions were placed in each well. Microplates were incubated aerobically at 35 ± 2 ^{0}C for 16-20 hours. Oxacillin was used as positive controls for MRSA, ampicillin was applied for ESBL-producing bacteria, while meropenem was utilized for CR bacteria.

MIC was determined by selecting the lowest concentration of plant extracts that inhibited bacterial growth and was detected by the naked eye without any assistance from a particular device. Then, wells were sub-cultured using a 10 µL inoculating loop on to a 5% sheep BAP at (35 ± 2) ^{0}C for 16–20 hours of incubation. The MBC was defined as the lowest concentration of the extract that did not show any growth (Yin et al., 2018).

RESULTS AND DISCUSSION

Extract yield

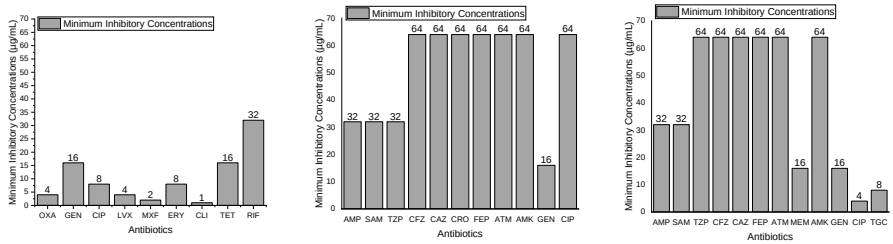
Ethanol extracts from nine plants were estimated to determine the extract yields (Table 1). Bitter melon showed the highest results, showing that its constituents were relatively polar.

Table 1. The extract yield

Plants	Scientific name	Part of plants	Yield (%)
Garlic	*Allium sativum Linn*	Tuber	1.11
Solo garlic	*Allium sativum*	Tuber	0.63
Java plum	*Syzygium cumini (L) Skeels*	Leaf	10.30
Java plum	*Syzygium cumini (L) Skeels*	Fruit	13.21
Lime	*Citrus aurantifolia*	Rind	11.20
Kaffir lime	*Citrus hystrix*	Rind	14.12
Siam weed	*Chromolaena odorata*	Leaf	9.50
Mangosteen	*Garcinia mangostana*	Rind	13.10
Bitter melon	*Momordica charantia*	Fruit	28.60

Tested microorganisms

The MDR bacteria were isolated from wounds, obtained from patients in Dr. Kariadi Hospital. The results of the identification and test of bacterial sensitivity to antibiotics are presented in Figure 1. The results reveal that the bacteria isolated from the wounds were *S. aureus*, *E. coli*, and *P. aeruginosa*, and they were resistant to several antibiotics. *S. aureus* is resistant to oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, tetracycline, and rifampicin. *E. coli* is resistant to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, amikacin, gentamicin, and ciprofloxacin. Whereas, *P. aeruginosa* is resistant to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefazolin, ceftazidime, cefepime, aztreonam, amikacin, gentamicin, ciprofloxacin, meropenem and tigecycline. The bacteria isolated from the wound samples were MRSA, (ESβL) -producing *E. coli* and carbapenemase-resistant *P. aeruginosa* (CRPA).



S.aureus ***E.coli*** ***P.aeruginosa***

Figure 1. The results of identification and sensitivity to bacterial antibiotics isolated from wounds. OXA: Oxacillin; GEN: Gentamicin; CIP: Ciprofloxacin; LVX: Levofloxacin; MXF: Moxifloxacin; ERY: Erythromycin; CLI: Clindamycin; TET: Tetracyclin; RIF: Rifampicin; AMP: Ampicillin; SAM: Ampicillin-sulbactam; TZP: Piperacillin-tazobactam; CFZ: Cefazolin; CAZ: Ceftazidime; CRO: Ceftriaxone; FEP: Cefepime; ATM: Aztreonam; AMK: Amikacin; MEM: Meropenem; TGC: Tigecycline

The antibacterial activities

Agar well diffusion assay

The antibacterial activities of the nine extracts were tested in vitro by agar well diffusion assay against three resistant bacteria causing wound infections. The antibacterial activities were determined by measuring the diameters of the inhibition zones in mm concerning MRSA, ESβL-producing *E. coli*, and CRPA (Figure 2).

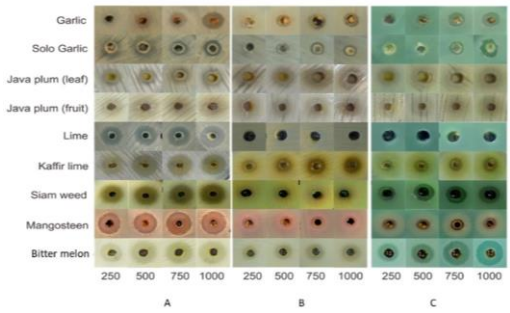


Figure 2. The inhibition zones of nine plants (250, 500, 750, and 1000 mg/mL) against MDR bacteria; A: MRSA; B: ESβL-producing *E. coli*; C: CRPA

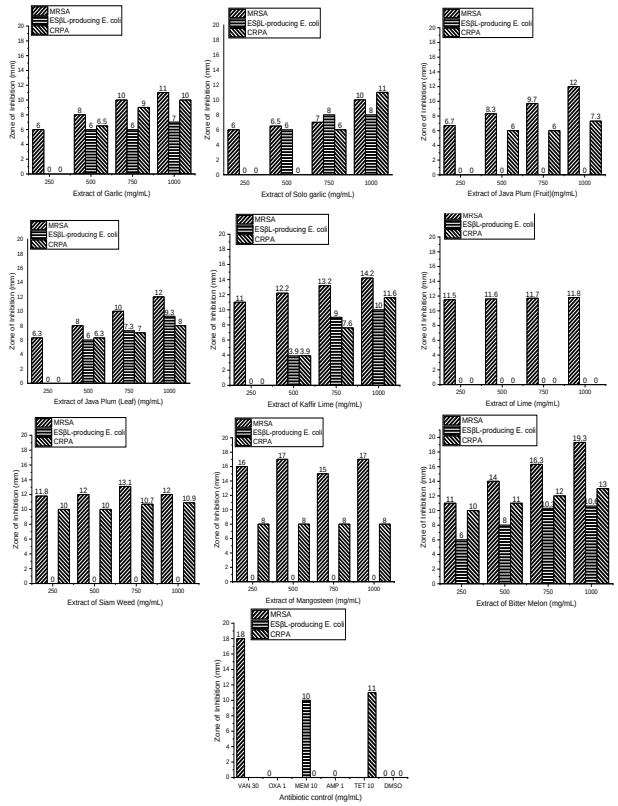


Figure 3. The diameters of the inhibition zones of nine plants. Vancomycin (VAN) and oxacillin (OXA) were used as positive controls for MRSA, ampicillin (AMP) and meropenem (MEM) for ESBL-producing *E. coli*, and meropenem (MEM) and tetracycline (TET) for CRPA.

Of the nine plant extracts at various concentrations (250, 500, 750, and 1000 mg/mL), the extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed inhibition zones in the three tested bacteria (Figure 3), and the bitter melon extract had the largest zone. The extracts of Java plum (fruit), Siam weed and mangosteen did not show any inhibition zones against ESβL-producing *E. coli*, but demonstrated inhibition zones on MRSA and CRPA. Meanwhile, lime extract only indicated an inhibition zone on MRSA. The inhibition zones of nine extracts of the three test bacteria disclosed inhibition zone diameters of 6-19.3 mm (MRSA), 3.9-10.6 mm (ESβL-producing *E. coli*), and 3.9-13 mm (CRPA). 1000 mg/mL bitter melon extract indicated the largest inhibition zone diameters of the three assessed bacteria of 19.3 mm (MRSA), 10.6 mm (ESβL-producing *E. coli*), and 13 mm (CRPA). The bitter melon extract also demonstrated a diameter of inhibition zone greater than the antibiotic control

MIC and MBC

MIC of nine extracts was tested in vitro by the microdilution method for three resistant bacteria isolated from wounds (Table 2). The extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed MIC values between 3.12 and 25 mg/mL for MRSA, ESβL-producing *E. coli*, and CRPA. Among the five extracts, bitter melon presented the lowest MIC values against MRSA (3.12 mg/mL), ESβL-producing *E. coli* (12.25 mg/mL), and CRPA (6.25 mg/mL). This result was lower than the value of antibiotic control.

MBC from nine extracts was tested in vitro by the microdilution method for three resistant bacteria isolated from wounds (Figure 4). The extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed MBC values for MRSA, ESβL-producing *E. coli*, and CRPA. The extracts of Java plum (fruit), Siam weed and mangosteen did not show any MBC values for ESβL-producing *E. coli*, but demonstrated inhibition on MRSA and CRPA. However, lime extract only presented MBC values on MRSA. The MBC values ranged from 25 to 50 mg/mL.

Table 2. The MIC values of nine plant extracts against MRSA, ESBL-producing *E. coli* and CRPA (mg/mL)

Extract and control	Tested bacteria		
	MRSA	ESBL-*E.coli*	CRPA
Garlic	12.5	25	12.5
Solo Garlic	25	25	12.5
Java Plum (Leaf)	25	25	12.5
Java Plum (Fruit)	25	-	25
Lime	25	-	-
Kaffir Lime	12.5	25	12.5
Siam Weed	25	-	25
Mangosteen	12.5	-	25
Bitter Melon	3.12	12.5	6.25
Oxacillin	4	-	-
Ampicillin	-	32	-
Meropenem	-	-	16

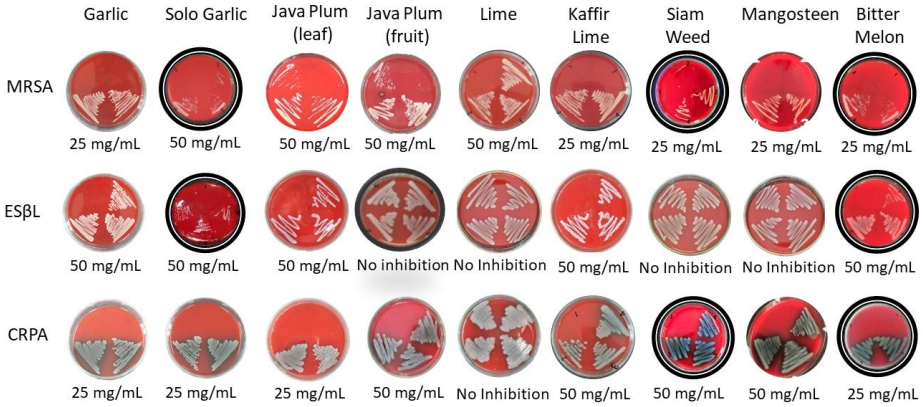


Figure 4. The MBC values of nine plant extract against MRSA, ESBL-producing *E. coli* and CRPA

Studies on antibacterial agents from natural ingredients are important efforts, particularly in recent times, due to the increasing level of antibiotic resistance among pathogenic bacteria. Abuse of antibiotics usage has been considered the major cause of the increasing antibiotic resistance against bacteria. The effort in this study focused on the use of widely available plants. Nine plants were used in this investigation to evaluate the antibacterial activities against MDR bacteria, including MRSA, ESBL-producing *E. coli* and CRPA, isolated from wounds. These results are consistent with the previous research reports that *S. aureus, E. coli*, and *P. aeruginosa* are the most common bacteria found in infected wounds (Manzuoerh et al., 2019; Petkovs˘ek et al., 2009).

The nine plant extracts appeared to have inhibition zone diameters ranging from 6 mm to 19.3 mm, with the most significant results are shown by the bitter melon extract. The bitter melon extract had the largest diameters of the inhibition zones in the three tested bacteria, 19.3 mm (MRSA), 10.6 mm (ESβL-producing *E. coli*), and 13 mm (CRPA), and greater diameters of inhibition zones than the antibiotic control.

The extract of bitter melon was proven to have lowest MIC and MBC values, against MRSA (3.12 and 25 mg/mL), ESβL-producing *E. coli* (12.25 and 50 mg/mL), and CRPA (6.25 and 25 mg/mL). This provides evidence that bitter melon ethanol extract shows antibacterial activities against MRSA strains, ESβL-producing E. coli, and CRPA. The extract displays broad-spectrum antimicrobial activities (Khan and Omoloso, 1998; Mwambete, 2009). Although testing of the groups contained in bitter melon was not performed in this research, some other studies have confirmed that bitter melon contains flavonoids, alkaloids, and terpenoids (Annapoorani and Manimegalai, 2013; Kumar et al., 2010; Leelaprakash et al., 2011).

The antibacterial activities of plants can be related to phytochemical compounds. Phytochemical compounds from plants protect the human body against infection. The most important phytochemicals are flavonoids, alkaloids, and terpenoids (Kumar et al., 2013). Flavonoids (Khalid et al., 2019) and terpenoids (Broniatowski and Mastalerz, 2015) have been recognized to show strong antibacterial activities. The mechanism of antibacterial activities of flavonoids, alkaloids, and terpenoids in bitter melon has not been identified. However, phytochemical compounds can inhibit bacterial growth by damaging bacterial cell walls (Abuga et al., 2020). Bitter melon is proven to be potentially developed as an antibacterial agent, especially for MDR strains from wounds. Further in vivo research and the investigation of modes of action are essential to explicate the antibacterial effects so that potential clinical drugs and health products can be advanced. This study can provide novel information about the benefits of bitter melon as a natural source of the antibacterial agent against MDR bacteria.

In conclusion, The bitter melon has the potential to be developed as an antibacterial agent, particularly against MRSA strains, ESβL-producing *E. coli*, and CRPA that cause wound infections. Further in vivo research and the discovery of modes of action are needed to explain the antibacterial effects.

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2 Bukti konfirmasi review dan hasil review pertama (9 Juni 2021)

Notifications

[biodiv] Editor Decision

2021-06-09 06:39 AM

Muhammad Evy Prastiyanto:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The In vitro antibacterial activities of crude extracts of nine plants on multidrug resistance bacterial isolates of wound infections: antibacterial activities extracts of plants".

Our decision is: Revisions Required

Reviewer A:
Recommendation: See Comments

In vitro antibacterial activities of crude extracts of nine plants on multidrug resistance bacterial isolates of wound infections

Abstract. Wound infections caused by bacteria is a become serious health problems, multidrug resistance bacteria (MDR) have increased this problem more severely, and therefore, antibacterial agents from natural biological sources are necessary to overcome these problems. This study examined the antibacterial activities of nine plants (garlic, Solo garlic, Java plum (leaf), Java plum (fruit), lime, Kaffir lime, Siamese weed, mangosteen, and bitter melon) against MDR bacteria isolated from wounds. The antibacterial activities were evaluated using agar well diffusion assay to determine the inhibition zones, and microdilution method to determine the value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The best antibacterial activities were calculated as the most extensive inhibition zones with the smallest MIC and MBC values. Ethanol extracts from five plants (garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon) showed antibacterial activities against three MDR bacteria isolated from wounds. The bitter melon extract had the largest zones, 19.3 mm (methicillin-resistant *Staphylococcus aureus* [MRSA]), 10.6 mm (ESβL-producing *Escherichia coli*), and 13 mm (carbapenemase-resistant *Pseudomonas aeruginosa* [CRPA]) with the smallest MIC and MBC values against MRSA (3.12 and 25 mg/mL), ESβL- producing *E. coli* (12.25 and 50 mg/mL), and CRPA (6.25 and 25 mg/mL). This concludes that bitter melon has the potential to be developed as an antibacterial agent, particularly against MRSA strains, ESβL-producing *E. coli*, and

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CRPA that cause wound infections. Further in vivo research and the discovery of modes of action are needed to explain the antibacterial effects.
Keywords: In vitro antibacterial activities; wound infection; MRSA, ESβL-producing *Escherichia coli, CRPA*
Running title: antibacterial activities extracts of plants

INTRODUCTION

Skin is an important organ that protects the body from damage and invasion of pathogenic bacteria (Xu et al., 2015). When the skin is damaged, the wound that exposes became prone to bacterial infection to easily infect and this condition affects health. The wound may be healed in a few days or will develop for a long time and become chronic. A chronic wound is one of the most serious and fatal human problems (Han and Ceilleey, 2017).

An infected wound may take longer time to recover, even may causes death some cases (Liang et al., 2019). *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are the bacteria commonly found in wounds (Pallavali et al., 2019). Antibiotics are commonly used to treat bacterial infections. However, the uncontrolled use of antibiotics contributes to the emergence of multidrug resistance (MDR) against many bacterial strains (Bologa et al., 2013). Patients infected with MDR bacteria may suffer from a prolonged disease that is difficult to treat and requires higher costs of treatment.

The Infectious Disease Society of America has considered the advent of several MDR bacteria, including those that are methicillin-resistant, extended-spectrum β-lactamase (ESβL) –producing-resistant and carbapenemase-resistant, as a distinct challenge in management (Boucher et al., 2009). The burn wound infections caused by MRSA (Chopra et al., 2016), MDR-*P.aeruginosa* (Nasser et al., 2020) and *E.coli* (Nasser et al., 2020) increase mortality and morbidity. The prevalence of infections caused by MRSA, MDR-*P.aeruginosa* and *E.coli* has increased in recent years.

Thus, new antibacterial agents from natural biological sources are require. Biological antibacterial agents can be obtained from honey (Panjaitan et al., 2018), mushrooms (Prastiyanto et al., 2020b, 2016), isolate bacteria from marine organisms (Asagabaldan et al., 2019), bacteriocins (Lestari et al., 2019), fruits (Prastiyanto et al., 2020d; Wahyuni et al., 2019), latex (Prastiyanto et al., 2020c) and seeds (Ilvani et al., 2019; Prastiyanto et al., 2020a). Many studies in the medical field reported the importance of traditional medicinal plants as the alternatives of antimicrobial agents (Akhtar, 2015; Aumeeruddy-elalfi et al., 2015; Prastiyanto et al., 2021).

This study aimed to investigate the antibacterial potentials of traditional plants. Nine plants examined to study their antibacterial activities against resistant bacteria isolated from wounds, such as methicillin-resistant *S. aureus* (MRSA), (ESβL)-producing *E. coli*, and carbapenemase-resistant *P. aeruginosa* (CRPA). The nine plants were garlic, Solo garlic, Java plum (leaf), Java plum (fruit), lime, Kaffir lime, Siam weed, mangosteen, and bitter melon.

MATERIALS AND METHODS

Plant materials and Preparations of extracts

Sampling of nine healthy plantswere carried out in the rainy season of December 2019. Different parts of the plants were washed with water to remove unnecessary materials, dried in the sun for seven days, ground and then stored in sterile airtight containers for further usages in the next processes. Plant extracts were prepared by maceration with 96% ethanol solvent. 200 g of grouned plant parts were soaked in 600 mL of solvent for 24 hours at room temperature, protected from light and were later shaken. The solvent replacement was done every day until the solution was clear, in which no more active compounds were contained in the dry powder. The supernatant was filtered using Whatman No.1 filter paper. The maceration solutions were concentrated under reduced

pressure using a rotary evaporator at 50 ˚C. The crude extracts were collected and allowed to dry at room temperature.

Isolation, identification of bacterial strains and antibiotic sensitivity test

MDR bacteria were directly isolated from wound samples obtained from patients in Dr. Kariadi Hospital, Semarang, Central Java, Indonesia. All isolates were identified by biochemical tests using Vitek®MS (bioM´erieux, Marcy l'Etoile, France), following minimum inhibitory concentration (MIC) interpretive standards from the Clinical Laboratory Standard Institute M100-S25 (CLSI, 2019).

Antibacterial assay of plant extracts

Agar well diffusion assay

The antibacterial activities of various plant extracts were evaluated using a well-diffusion assay (Andleeb et al., 2020). MDR bacteria in a subculture on blood agar plate (BAP) media were incubated for 24 hours at 35 ± 2°C. The MDR bacterial colonies were dissolved in a normal saline solution with a turbidity equivalent to the 0.5 McFarland standard. 100 µL of each MDR bacterium was inoculated in Muller Hilton agar (MHA) by spreading the bacterium on the surface of the agar using a sterilized glass spreader. After five minutes of inoculation, the wells were prepared using a sterilized steel *corkborer* (1cm in diameter). Four wells were made on each plate and loaded with each plant extract (250, 500, 750, and 1000 mg/mL). All plates were then incubated aerobically at 35 ± 2 °C for 16-20 hours. Dimethyl sulfoxide (DMSO) was used as a negative control. Vancomycin and oxacillin were applied as positive controls for MRSA, ampicillin and meropenem for ESBL-producing bacteria, and meropenem and tetracycline were for CR bacteria. Antibacterial activities of the extracts were determined by measuring the diameters of the inhibition zones in mm against the tested organism.

Determination of MIC and minimum bactericidal concentration (MBC) of the plant extracts

MIC values of plant extracts were determined in 12-well sterile microplates using the broth microdilution method (CLSI, 2018). Each test was carried out in triplicate. MHB (100 µL) was placed into the well and plant extract (100 µL) was put in the dilution series. 10 µL bacterial cell suspensions were placed in each well. Microplates were incubated aerobically at 35 ± 2 ˚C for 16-20 hours. Oxacillin was used as positive controls for MRSA, ampicillin was applied for ESBL-producing bacteria, while meropenem was utilized for CR bacteria.

MIC was determined by selecting the lowest concentration of plant extracts that inhibited bacterial growth and was detected by the naked eye without any assistance from a particular device. Then, wells were sub-cultured using a 10 µL inoculating loop on to a 5% sheep BAP at (35 ± 2) ˚C for 16–20 hours of incubation. The lowest concentration of the extract that did not show any growth was defined as MBC (Yin et al., 2018).

RESULTS AND DISCUSSION

Extract yield

Ethanol extracts from nine plants were estimated to determine the extract yields (Table 1). Bitter melon showed the highest results, showing that its constituents were relatively polar.

Table 1. The extract yield

Plants	Scientific name	Part of plants	Yield (%)
Garlic	*Allium sativum Linn*	Tuber	1.11
Solo garlic	*Allium sativum*	Tuber	0.63
Java plum	*Syzygium cumini (L) Skeels*	Leaf	10.30
Java plum	*Syzygium cumini (L) Skeels*	Fruit	13.21
Lime	*Citrus aurantifolia*	Rind	11.20
Kaffir lime	*Citrus hystrix*	Rind	14.12
Siam weed	*Chromolaena odorata*	Leaf	9.50
Mangosteen	*Garcinia mangostana*	Rind	13.10
Bitter melon	*Momordica charantia*	Fruit	28.60

Tested microorganisms

The MDR bacteria were isolated from wounds, obtained from patients in Dr. Kariadi Hospital. The results of the identification and test of bacterial sensitivity to antibiotics are presented in Figure 1. The results reveal that the bacteria isolated from the wounds were *S. aureus*, *E. coli*, and *P. aeruginosa*, and they were resistant to several antibiotics. *S. aureus* was found resistant to oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, tetracycline, and rifampicin. *E. coli* showed resistance against ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, amikacin, gentamicin, and ciprofloxacin. Whereas, *P. aeruginosa* was observed resistant to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefazolin, ceftazidime, cefepime, aztreonam, amikacin, gentamicin, ciprofloxacin, meropenem and tigecycline. The bacteria isolated from the wound samples were MRSA, (ESβL) -producing *E. coli* and carbapenemase-resistant *P. aeruginosa* (CRPA).

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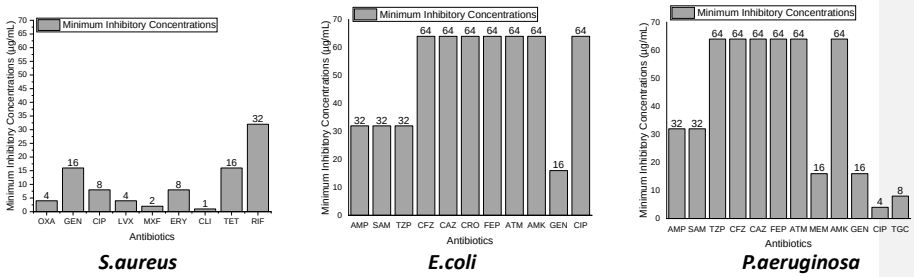

S.aureus ***E.coli*** ***P.aeruginosa***

Figure 1. The results of identification and sensitivity to bacterial antibiotics isolated from wounds. OXA: Oxacillin; GEN: Gentamicin; CIP: Ciprofloxacin; LVX: Levofloxacin; MXF: Moxifloxacin; ERY: Erythromycin; CLI: Clindamycin; TET: Tetracyclin; RIF: Rifampicin; AMP: Ampicillin; SAM: Ampicillin-sulbactam; TZP: Piperacillin-tazobactam; CFZ: Cefazolin; CAZ: Ceftazidime; CRO: Ceftriaxone; FEP: Cefepime; ATM: Aztreonam; AMK: Amikacin; MEM: Meropenem; TGC: Tigecycline

The antibacterial activities

Agar well diffusion assay

The antibacterial activities of the nine extracts were tested *in vitro* by agar well diffusion assay against three resistant bacteria causing wound infections. The antibacterial activities were determined by measuring the diameters of the inhibition zones in mm concerning MRSA, ESβL-producing *E. coli*, and CRPA (Figure 2).

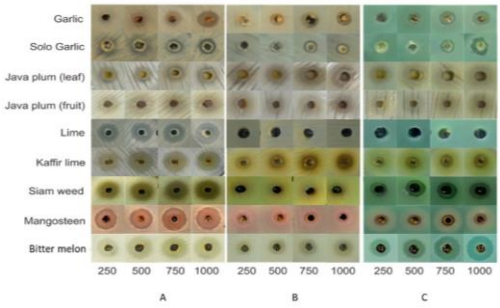

Figure 2. The inhibition zones of nine plants (250, 500, 750, and 1000 mg/mL) against MDR bacteria; A: MRSA; B: ESβL-producing *E. coli*; C: CRPA

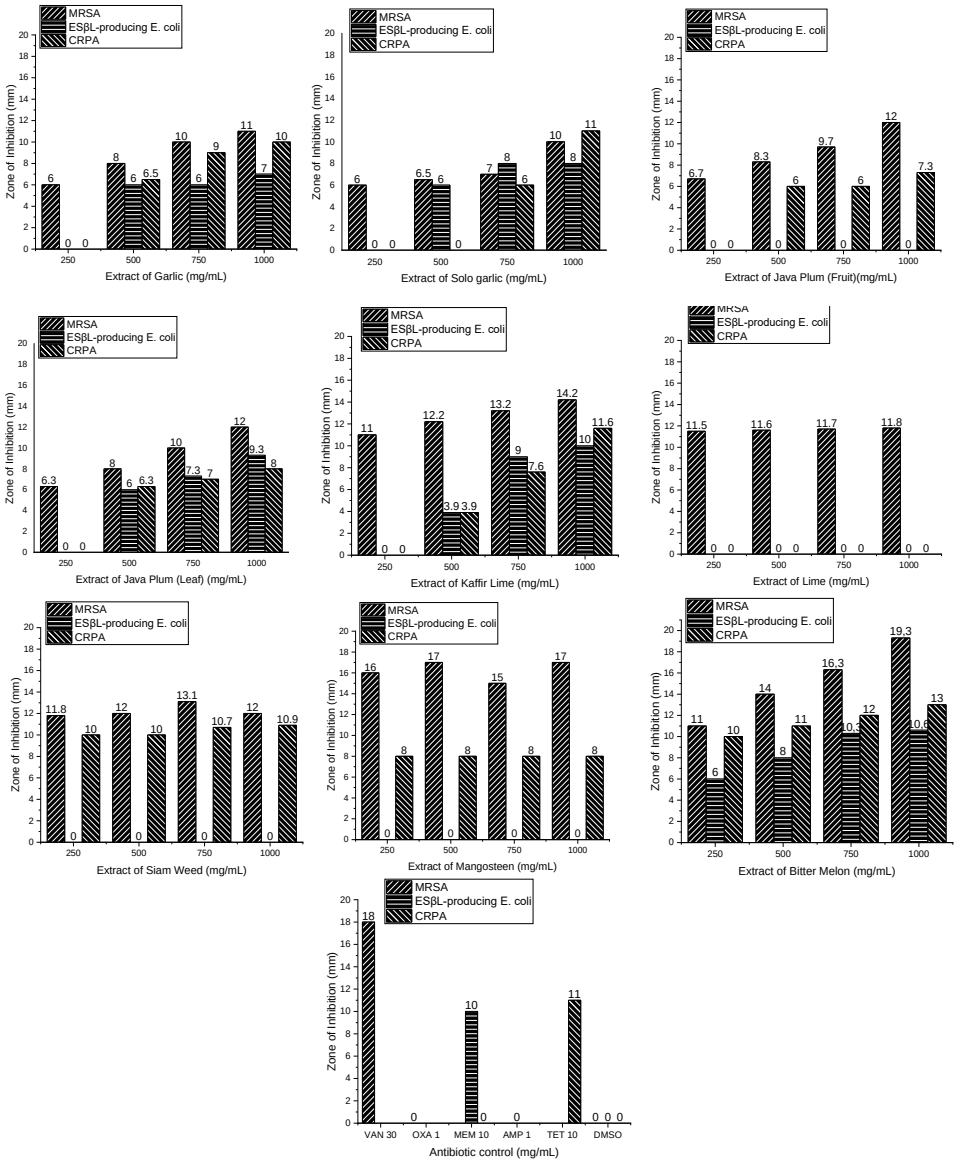

Figure 3. The diameters of the inhibition zones of nine plants. Vancomycin (VAN) and oxacillin (OXA) were used as positive controls for MRSA, ampicillin (AMP) and meropenem (MEM) for ESBL-producing *E. coli*, and meropenem (MEM) and tetracycline (TET) for CRPA.

Of the nine plant extracts at various concentrations (250, 500, 750, and 1000 mg/mL), the extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed inhibition zones in the three tested bacteria (Figure 3), and the bitter melon extract had the largest zone. The extracts of Java plum (fruit), Siam weed and mangosteen did not show any inhibition zones against ESβL-producing *E. coli*, but demonstrated inhibition zones on MRSA and CRPA. Meanwhile, lime extract only indicated an inhibition zone on MRSA. The inhibition zones of nine extracts of the three test bacteria disclosed inhibition zone diameters of 6-19.3 mm (MRSA), 3.9-10.6 mm (ESβL-producing *E. coli*), and 3.9-13 mm (CRPA). 1000 mg/mL bitter melon extract indicated the largest inhibition zone diameters of the three assessed bacteria of 19.3 mm (MRSA), 10.6 mm (ESβL-producing *E. coli*), and 13 mm (CRPA). The bitter melon extract also demonstrated a diameter of inhibition zone greater than the antibiotic control

MIC and MBC

MIC of nine extracts was tested *in vitro* by the microdilution method for three resistant bacteria isolated from wounds (Table 2). The extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed MIC values between 3.12 and 25 mg/mL for MRSA, ESβL-producing *E. coli*, and CRPA. Among the five extracts, bitter melon presented the lowest MIC values against MRSA (3.12 mg/mL), ESβL-producing *E. coli* (12.25 mg/mL), and CRPA (6.25 mg/mL). This result was lower than the value of antibiotic control.

MBC from nine extracts was tested *in vitro* by the microdilution method for three resistant bacteria isolated from wounds (Figure 4). The extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed MBC values for MRSA, ESβL-producing *E. coli*, and CRPA. The extracts of Java plum (fruit), Siam weed and mangosteen did not show any MBC values for ESβL-producing *E. coli*, but demonstrated inhibition on MRSA and CRPA. However, lime extract only presented MBC values on MRSA. The MBC values ranged from 25 to 50 mg/mL.

Table 2. The MIC values of nine plant extracts against MRSA, ESBL-producing *E. coli* and CRPA (mg/mL)

Extract and control	Tested bacteria		
	MRSA	ESBL-*E.coli*	CRPA
Garlic	12.5	25	12.5
Solo Garlic	25	25	12.5
Java Plum (Leaf)	25	25	12.5
Java Plum (Fruit)	25	-	25
Lime	25	-	-
Kaffir Lime	12.5	25	12.5
Siam Weed	25	-	25
Mangosteen	12.5	-	25
Bitter Melon	3.12	12.5	6.25
Oxacillin	4	-	-
Ampicillin	-	32	-
Meropenem	-	-	16

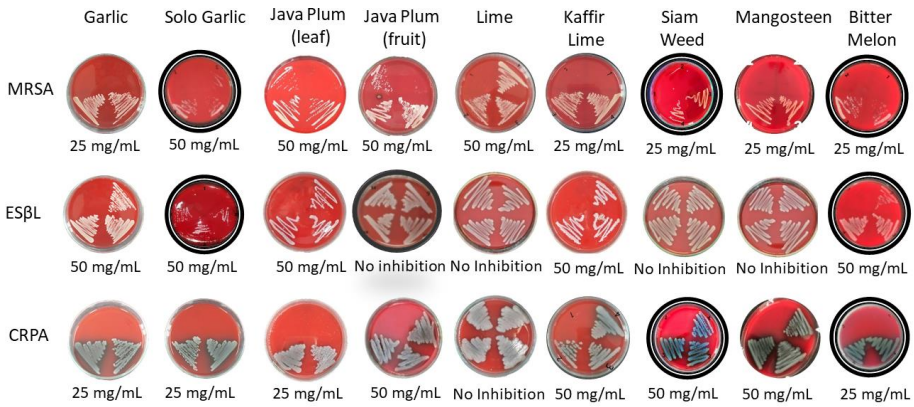

Figure 4. The MBC values of nine plant extract against MRSA, ESBL-producing *E. coli* and CRPA

Studies on antibacterial agents from natural ingredients are important efforts, particularly in recent times, due to the increasing level of antibiotic resistance among pathogenic bacteria. Abuse of antibiotics usage has been considered the major cause of the increasing antibiotic resistance against bacteria. The effort in this study focused on the use of widely available plants. Nine plants were used in this investigation to evaluate the antibacterial activities against MDR bacteria, including MRSA, ESBL-producing *E. coli* and CRPA, isolated from wounds. These results are consistent with the previous research reports that *S. aureus, E. coli*, and *P. aeruginosa* are the most common bacteria found in infected wounds (Manzuoerh et al., 2019; Petkovs˘ek et al., 2009).

The nine plant extracts appeared to have inhibition zone diameters ranging from 6 mm to 19.3 mm, with the most significant results were shown by the bitter melon extract. The bitter melon extract had the largest diameters of the inhibition zones in the three tested bacteria, 19.3 mm (MRSA), 10.6 mm (ESβL-producing *E. coli*), and 13 mm (CRPA), and greater diameters of inhibition zones than the antibiotic control.

The extract of bitter melon was proven to have lowest MIC and MBC values, against MRSA (3.12 and 25 mg/mL), ESβL-producing *E. coli* (12.25 and 50 mg/mL), and CRPA (6.25 and 25 mg/mL). This provides evidence that bitter melon ethanol extract shows antibacterial activities against MRSA strains, ESβL-producing *E. coli*, and CRPA. The extract displays broad-spectrum antimicrobial activities (Khan and Omoloso, 1998; Mwambete, 2009). Although, testing of the groups contained in bitter melon was not performed in this research, some other studies have confirmed that bitter melon contains flavonoids, alkaloids, and terpenoids (Annapoorani and Manimegalai, 2013; Kumar et al., 2010; Leelaprakash et al., 2011).

The antibacterial activities of plants can be related to phytochemical compoundswhich can protect the human body against microbial infection. The most important phytochemicals are flavonoids, alkaloids, and terpenoids (Kumar et al., 2013). Flavonoids (Khalid et al., 2019) and terpenoids (Broniatowski and Mastalerz, 2015) have been recognized to show strong antibacterial activities. The mechanism of antibacterial activities of flavonoids, alkaloids, and terpenoids in bitter melon has not been identified. However, phytochemical compounds can inhibit bacterial growth by damaging bacterial cell walls (Abuga et al., 2020). Bitter melon is proven to be potentially developed as an antibacterial agent, especially for MDR strains from wounds. Further *in vivo* research and the investigation of modes of action are essential to explicate the antibacterial effects so that potential

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clinical drugs and health products can be advanced. This study can provide novel information about the benefits of bitter melon as a natural source of the antibacterial agent against MDR bacteria.

In conclusion, the bitter melon has the potential to be developed as an antibacterial agent, particularly against MRSA strains, ESβL-producing *E. coli*, and CRPA that cause wound infections. Further, *in vivo* research and the discovery of modes of action are needed to explain the antibacterial effects.

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ACKNOWLEDGMENTS

The author would like to acknowledge Department of Medical Laboratory Technology, Universitas Muhammadiyah Semarang, Indonesia, for providing essential facilities for carrying out the study

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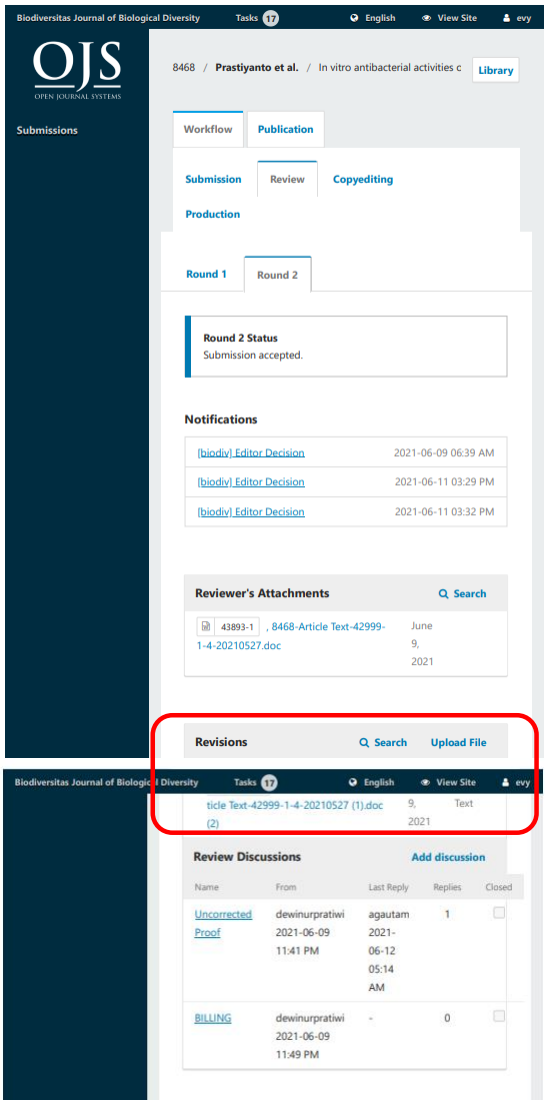

In vitro antibacterial activities of crude extracts of nine plants on multidrug resistance bacterial isolates of wound infections

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Abstract. Wound infections caused by bacteria is a become serious health problems, multidrug resistance bacteria (MDR) have increased this problem more severely, and therefore, antibacterial agents from natural biological sources are necessary to overcome these problems. This study examined the antibacterial activities of nine plants (garlic [*Allium sativum Linn*], Solo garlic [*Allium sativum*], Java plum (leaf) [*Syzygium cumini (L) Skeels*], Java plum (fruit) [*Syzygium cumini (L) Skeels*], lime [*Citrus aurantifolia*], Kaffir lime [*Citrus hystrix*], Siamese weed [*Chromolaena odorata*], mangosteen [*Garcinia mangostana*] and bitter melon [*Momordica charantia*)] against MDR bacteria isolated from wounds. The antibacterial activities were evaluated using agar well diffusion assay to determine the inhibition zones, and microdilution method to determine the value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The best antibacterial activities were calculated as the most extensive inhibition zones with the smallest MIC and MBC values. Ethanol extracts from five plants (garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon) showed antibacterial activities against three MDR bacteria isolated from wounds. The bitter melon extract had the largest zones, 19.3 mm (methicillin-resistant *Staphylococcus aureus* [MRSA]), 10.6 mm (ESβL-producing *Escherichia coli*), and 13 mm (carbapenemase-resistant *Pseudomonas aeruginosa* [CRPA]) with the smallest MIC and MBC values against MRSA (3.12 and 25 mg/mL), ESβL- producing *E. coli* (12.25 and 50 mg/mL), and CRPA (6.25 and 25 mg/mL). This concludes that bitter melon has the potential to be developed as an antibacterial agent, particularly against MRSA strains, ESβL-producing *E. coli*, and CRPA that cause wound infections. Further in vivo research and the discovery of modes of action are needed to explain the antibacterial effects.

Keywords: In vitro antibacterial activities; wound infection; MRSA, ESβL-producing *Escherichia coli, CRPA*

Running title: antibacterial activities extracts of plants

INTRODUCTION

Skin is an important organ that protects the body from damage and invasion of pathogenic bacteria (Xu et al., 2015). When the skin is damaged, the wound that exposes became prone to bacterial infection to easily infect and this condition affects health. The wound may be healed in a few days or will develop for a long time and become chronic. A chronic wound is one of the most serious and fatal human problems (Han and Ceilleey, 2017).

An infected wound may take longer time to recover, even may causes death some cases (Liang et al., 2019). *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are the bacteria commonly found in wounds (Pallavali et al., 2019). Antibiotics are commonly used to treat bacterial infections. However, the uncontrolled use of antibiotics contributes to the emergence of multidrug resistance (MDR) against many bacterial strains (Bologa et al., 2013). Patients infected with MDR bacteria may suffer from a prolonged disease that is difficult to treat and requires higher costs of treatment.

The Infectious Disease Society of America has considered the advent of several MDR bacteria, including those that are methicillin-resistant, extended-spectrum β-lactamase (ESβL) –producing-resistant and carbapenemase-resistant, as a distinct challenge in management (Boucher et al., 2009). The burn wound infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (Chopra et al., 2016), MDR-*P.aeruginosa* (Nasser et al., 2020) and *E.coli* (Nasser et al., 2020) increase mortality and morbidity. The prevalence of infections caused by MRSA, MDR-*P.aeruginosa* and *E.coli* has increased in recent years.

Thus, new antibacterial agents from natural biological sources are require. Biological antibacterial agents can be obtained from honey (Panjaitan et al., 2018), mushrooms (Prastiyanto et al., 2020b, 2016), isolate bacteria from marine organisms (Asagabaldan et al., 2019), bacteriocins (Lestari et al., 2019), fruits (Prastiyanto et al., 2020d; Wahyuni et al., 2019), latex (Prastiyanto et al., 2020c) and seeds (Ilvani et al., 2019; Prastiyanto et al., 2020a). Many studies in the medical field reported the importance of traditional medicinal plants as the alternatives of antimicrobial agents (Akhtar, 2015; Aumeeruddy-elalfi et al., 2015; Prastiyanto et al., 2021).

This study aimed to investigate the antibacterial potentials of traditional plants. Nine plants examined to study their antibacterial activities against resistant bacteria isolated from wounds, such as methicillin-resistant *S. aureus* (MRSA), (ESβL)-producing *E. coli*, and carbapenemase-resistant *P. aeruginosa* (CRPA). The nine plants were garlic, Solo garlic, Java plum (leaf), Java plum (fruit), lime, Kaffir lime, Siam weed, mangosteen, and bitter melon.

MATERIALS AND METHODS

Plant materials and Preparations of extracts

Sampling of nine healthy plantswere carried out in the rainy season of December 2019. Different parts of the plants were washed with water to remove unnecessary materials, dried in the sun for seven days, ground and then stored in sterile airtight containers for further usages in the next processes. Plant extracts were prepared by maceration with 96% ethanol solvent. 200 g of grouned plant parts were soaked in 600 mL of solvent for 24 hours at room temperature, protected from light and were later shaken. The solvent replacement was done every day until the solution was clear, in which no more active compounds were contained in the dry powder. The supernatant was filtered using Whatman No.1 filter paper. The maceration solutions were concentrated under reduced pressure using a rotary evaporator at 50 ˚C. The crude extracts were collected and allowed to dry at room temperature.

Isolation, identification of bacterial strains and antibiotic sensitivity test

MDR bacteria were directly isolated from wound samples obtained from patients in Dr. Kariadi Hospital, Semarang, Central Java, Indonesia. All isolates were identified by biochemical tests using Vitek®MS (bioM´erieux, Marcy l'Etoile, France), following minimum inhibitory concentration (MIC) interpretive standards from the Clinical Laboratory Standard Institute M100-S25 (CLSI, 2019).

Antibacterial assay of plant extracts

Agar well diffusion assay

The antibacterial activities of various plant extracts were evaluated using a well-diffusion assay (Andleeb et al., 2020). MDR bacteria in a subculture on blood agar plate (BAP) media were incubated for 24 hours at 35 ± 2°C. The MDR bacterial colonies were dissolved in a normal saline solution with a turbidity equivalent to the 0.5 McFarland standard. 100 µL of each MDR bacterium was inoculated in Muller Hilton agar (MHA) by spreading the bacterium on the surface of the agar using a sterilized glass spreader. After five minutes of inoculation, the wells were prepared using a sterilized steel *corkborer* (1cm in diameter). Four wells were made on each plate and loaded with each plant extract (250, 500, 750, and 1000 mg/mL). All plates were then incubated aerobically at 35 ± 2 °C for 16-20 hours. Dimethyl sulfoxide (DMSO) was used as a negative control. Vancomycin and oxacillin were applied as positive controls for MRSA, ampicillin and meropenem for ESBL-producing bacteria, and meropenem and tetracycline were for CR bacteria. Antibacterial activities of the extracts were determined by measuring the diameters of the inhibition zones in mm against the tested organism.

Determination of MIC and minimum bactericidal concentration (MBC) of the plant extracts

MIC values of plant extracts were determined in 12-well sterile microplates using the broth microdilution method (CLSI, 2018). Each test was carried out in triplicate. MHB (100 µL) was placed into the well and plant extract (100 µL) was put in the dilution series. 10 µL bacterial cell suspensions were placed in each well. Microplates were incubated aerobically at 35 ± 2 ˚C for 16-20 hours. Oxacillin was used as positive controls for MRSA, ampicillin was applied for ESBL-producing bacteria, while meropenem was utilized for CR bacteria.

MIC was determined by selecting the lowest concentration of plant extracts that inhibited bacterial growth and was detected by the naked eye without any assistance from a particular device. Then, wells were sub-cultured using a 10 µL inoculating loop on to a 5% sheep BAP at (35 ± 2) ˚C for 16–20 hours of incubation. The lowest concentration of the extract that did not show any growth was defined as MBC (Yin et al., 2018).

RESULTS AND DISCUSSION

Extract yield

Ethanol extracts from nine plants were estimated to determine the extract yields (Table 1). Bitter melon showed the highest results, showing that its constituents were relatively polar.

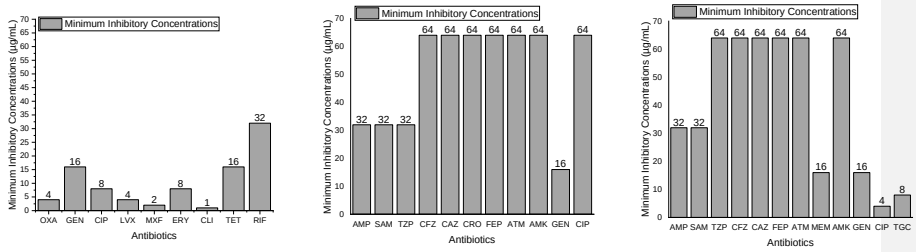
Table 1. The extract yield

Plants	Scientific name	Part of plants	Yield (%)
Garlic	*Allium sativum Linn*	Tuber	1.11
Solo garlic	*Allium sativum*	Tuber	0.63
Java plum	*Syzygium cumini (L) Skeels*	Leaf	10.30
Java plum	*Syzygium cumini (L) Skeels*	Fruit	13.21
Lime	*Citrus aurantifolia*	Rind	11.20

Kaffir lime	*Citrus hystrix*	Rind	14.12
Siam weed	*Chromolaena odorata*	Leaf	9.50
Mangosteen	*Garcinia mangostana*	Rind	13.10
Bitter melon	*Momordica charantia*	Fruit	28.60

Tested microorganisms

The results of the identification and test of bacterial sensitivity to antibiotics are presented in Figure 1. The results reveal that the bacteria isolated from the wounds were *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and they were resistant to several antibiotics. *S. aureus* was found resistant to oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, tetracycline, and rifampicin. *E. coli* showed resistance against ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, amikacin, gentamicin, and ciprofloxacin. Whereas, *P. aeruginosa* was observed resistant to ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefazolin, ceftazidime, cefepime, aztreonam, amikacin, gentamicin, ciprofloxacin, meropenem and tigecycline. The bacteria isolated from the wound samples were methicillin-resistant *St. aureus* MRSA, (ESβL)-producing *E. coli* and carbapenemase-resistant *P. aeruginosa* (CRPA).



S.aureus ***E.coli*** ***P.aeruginosa***

Figure 1. The results of identification and sensitivity to bacterial antibiotics isolated from wounds. OXA: Oxacillin; GEN: Gentamicin; CIP: Ciprofloxacin; LVX: Levofloxacin; MXF: Moxifloxacin; ERY: Erythromycin; CLI: Clindamycin; TET: Tetracyclin; RIF: Rifampicin; AMP: Ampicillin; SAM: Ampicillin-sulbactam; TZP: Piperacillin-tazobactam; CFZ: Cefazolin; CAZ: Ceftazidime; CRO: Ceftriaxone; FEP: Cefepime; ATM: Aztreonam; AMK: Amikacin; MEM: Meropenem; TGC: Tigecycline

The antibacterial activities

Agar well diffusion assay

The antibacterial activities of the nine extracts were tested *in vitro* by agar well diffusion assay against three resistant bacteria causing wound infections. The antibacterial activities were determined by measuring the diameters of the inhibition zones in mm concerning MRSA, ESβL-producing *E. coli*, and CRPA (Figure 2).

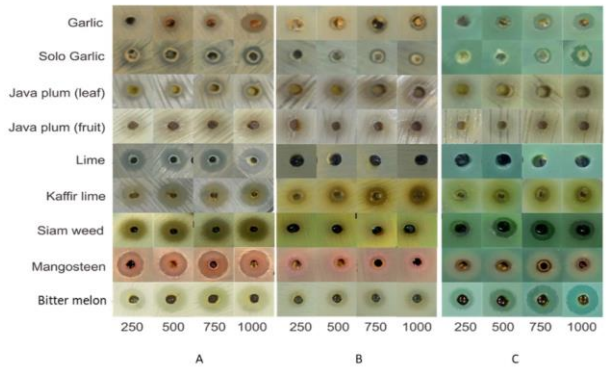


Figure 2. The inhibition zones of nine plants (250, 500, 750, and 1000 mg/mL) against MDR bacteria; A: MRSA; B: ESβL-producing *E. coli*; C: CRPA

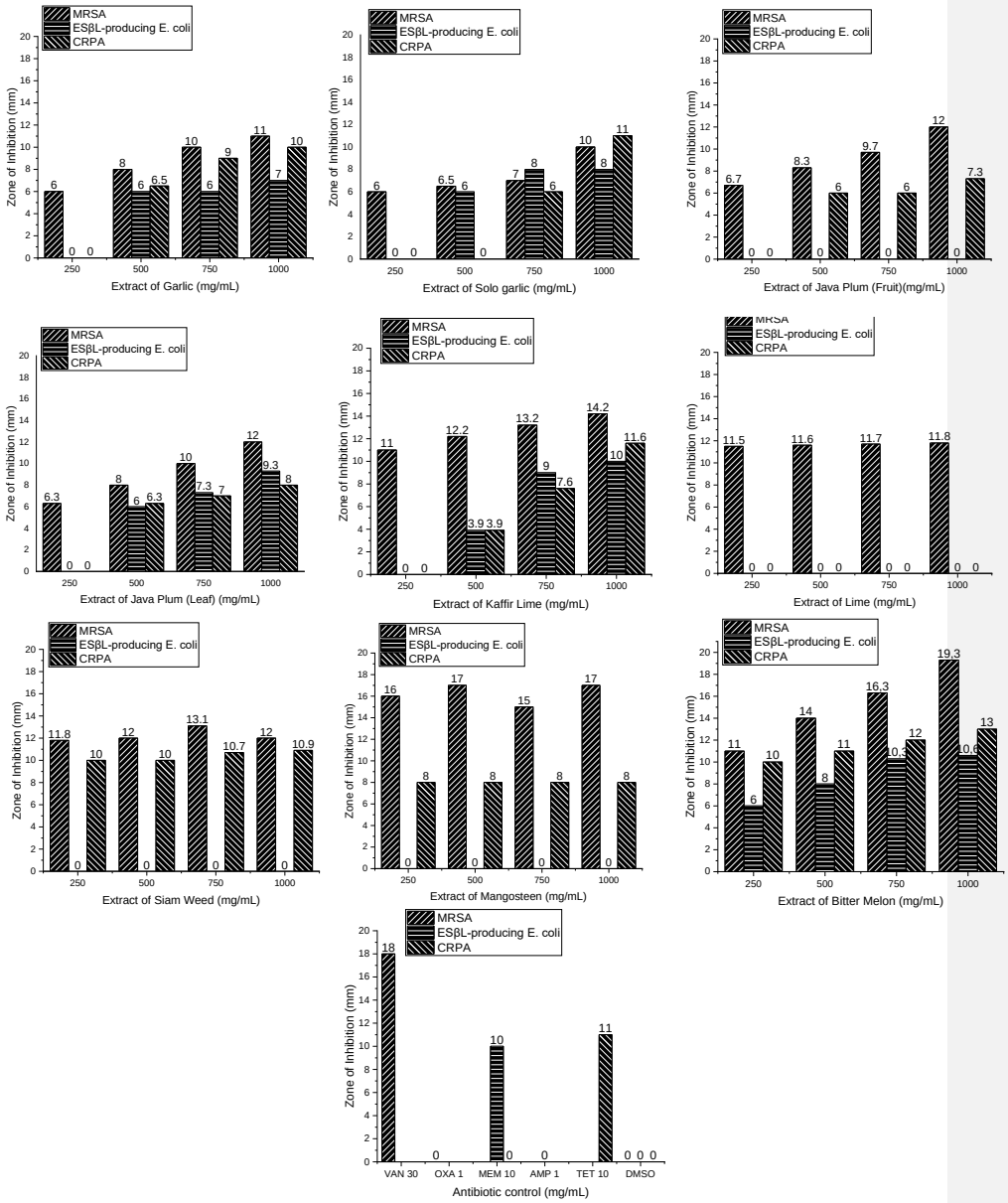


Figure 3. The diameters of the inhibition zones of nine plants. Vancomycin (VAN) and oxacillin (OXA) were used as positive controls for MRSA, ampicillin (AMP) and meropenem (MEM) for ESBL-producing *E. coli*, and meropenem (MEM) and tetracycline (TET) for CRPA.

Of the nine plant extracts at various concentrations (250, 500, 750, and 1000 mg/mL), the extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed inhibition zones in the three tested bacteria (Figure 3), and the bitter melon extract had the largest zone. The extracts of Java plum (fruit), Siam weed

and mangosteen did not show any inhibition zones against ESβL-producing *E. coli*, but demonstrated inhibition zones on MRSA and CRPA. Meanwhile, lime extract only indicated an inhibition zone on MRSA. The inhibition zones of nine extracts of the three test bacteria disclosed inhibition zone diameters of 6-19.3 mm (MRSA), 3.9-10.6 mm (ESβL-producing *E. coli*), and 3.9-13 mm (CRPA). 1000 mg/mL bitter melon extract indicated the largest inhibition zone diameters of the three assessed bacteria of 19.3 mm (MRSA), 10.6 mm (ESβL-producing *E. coli*), and 13 mm (CRPA). The bitter melon extract also demonstrated a diameter of inhibition zone greater than the antibiotic control

MIC and MBC

MIC of nine extracts was tested *in vitro* by the microdilution method for three resistant bacteria isolated from wounds (Table 2). The extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed MIC values between 3.12 and 25 mg/mL for MRSA, ESβL-producing *E. coli*, and CRPA. Among the five extracts, bitter melon presented the lowest MIC values against MRSA (3.12 mg/mL), ESβL-producing *E. coli* (12.25 mg/mL), and CRPA (6.25 mg/mL). This result was lower than the value of antibiotic control.

MBC from nine extracts was tested *in vitro* by the microdilution method for three resistant bacteria isolated from wounds (Figure 4). The extracts of garlic, Solo garlic, Java plum (leaf), Kaffir lime and bitter melon showed MBC values for MRSA, ESβL-producing *E. coli*, and CRPA. The extracts of Java plum (fruit), Siam weed and mangosteen did not show any MBC values for ESβL-producing *E. coli*, but demonstrated inhibition on MRSA and CRPA. However, lime extract only presented MBC values on MRSA. The MBC values ranged from 25 to 50 mg/mL.

Table 2. The MIC values of nine plant extracts against MRSA, ESBL-producing *E. coli* and CRPA (mg/mL)

Extract and control	Tested bacteria		
	MRSA	ESBL-*E.coli*	CRPA
Garlic	12.5	25	12.5
Solo Garlic	25	25	12.5
Java Plum (Leaf)	25	25	12.5
Java Plum (Fruit)	25	-	25
Lime	25	-	-
Kaffir Lime	12.5	25	12.5
Siam Weed	25	-	25
Mangosteen	12.5	-	25
Bitter Melon	3.12	12.5	6.25
Oxacillin	4	-	-
Ampicillin	-	32	-
Meropenem	-	-	16

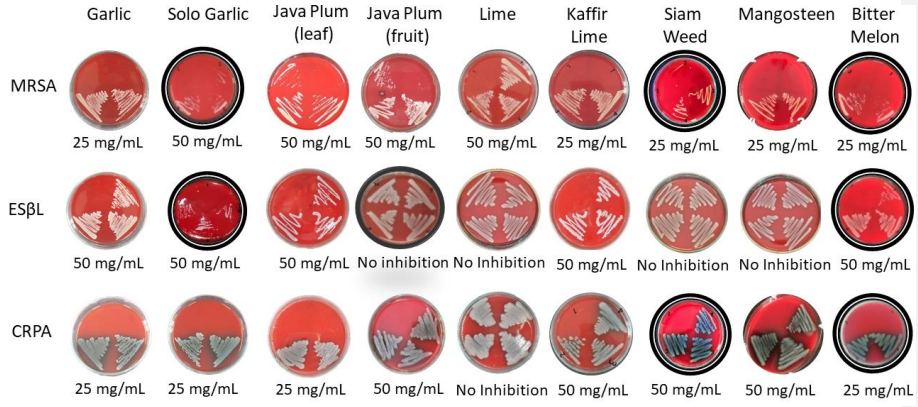


Figure 4. The MBC values of nine plant extract against MRSA, ESBL-producing *E. coli* and CRPA

Studies on antibacterial agents from natural ingredients are important efforts, particularly in recent times, due to the increasing level of antibiotic resistance among pathogenic bacteria. Abuse of antibiotics usage has been considered the major cause of the increasing antibiotic resistance against bacteria. The effort in this study focused

on the use of widely available plants. Nine plants were used in this investigation to evaluate the antibacterial activities against MDR bacteria, including methicillin-resistant *S.aureus*, ESBL-producing *E. coli* and CRPA, isolated from wounds. These results are consistent with the previous research reports that *S. aureus*, *E. coli*, and *P. aeruginosa* are the most common bacteria found in infected wounds (Manzuoerh et al., 2019; Petkovs˘ek et al., 2009).

The nine plant extracts appeared to have inhibition zone diameters ranging from 6 mm to 19.3 mm, with the most significant results were shown by the bitter melon extract. The bitter melon extract had the largest diameters of the inhibition zones in the three tested bacteria, 19.3 mm (methicillin-resistant *S.aureus*), 10.6 mm (ESβL-producing *E. coli*), and 13 mm (CRPA), and greater diameters of inhibition zones than the antibiotic control.

The extract of bitter melon was proven to have lowest MIC and MBC values, against MRSA (3.12 and 25 mg/mL), ESβL-producing *E. coli* (12.25 and 50 mg/mL), and CRPA (6.25 and 25 mg/mL). This provides evidence that bitter melon ethanol extract shows antibacterial activities against methicillin-resistant *S.aureus* strains, ESβL-producing *E. coli*, and CRPA. The extract displays broad-spectrum antimicrobial activities (Khan and Omoloso, 1998; Mwambete, 2009). Although, testing of the groups contained in bitter melon was not performed in this research, some other studies have confirmed that bitter melon contains flavonoids, alkaloids, and terpenoids (Annapoorani and Manimegalai, 2013; Kumar et al., 2010; Leelaprakash et al., 2011).

The antibacterial activities of plants can be related to phytochemical compoundswhich can protect the human body against microbial infection. The most important phytochemicals are flavonoids, alkaloids, and terpenoids (Kumar et al., 2013). Flavonoids (Khalid et al., 2019) and terpenoids (Broniatowski and Mastalerz, 2015) have been recognized to show strong antibacterial activities. The mechanism of antibacterial activities of flavonoids, alkaloids, and terpenoids in bitter melon has not been identified. However, phytochemical compounds can inhibit bacterial growth by damaging bacterial cell walls (Abuga et al., 2020). Bitter melon is proven to be potentially developed as an antibacterial agent, especially for MDR strains from wounds. Further *in vivo* research and the investigation of modes of action are essential to explicate the antibacterial effects so that potential clinical drugs and health products can be advanced. This study can provide novel information about the benefits of bitter melon as a natural source of the antibacterial agent against MDR bacteria.

In conclusion, the bitter melon has the potential to be developed as an antibacterial agent, particularly against methicillin-resistant *S.aureus* strains, ESβL-producing *E. coli*, and CRPA that cause wound infections. Further, *in vivo* research and the discovery of modes of action are needed to explain the antibacterial effects.

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4 Bukti konfirmasi artikel accepted (11 Juni 2021)

Notifications

[biodiv] Editor Decision

2021-06-11 03:29 PM

MUHAMMAD EVY PRASTIYANTO, NI MADE BUNGA ANGGELIA DEWI, TUSY DIAH PRATININGTIAS, NI MADE RAI PRATIWI, ANGGIS WINDAYANI, EKA WAHYUNENGSIH, ASTUTI, ELVIRA AMIR, FANDHI ADI WARDOYO :

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "In vitro antibacterial activities of crude extracts of nine plants on multidrug resistance bacterial isolates of wound infections".

Our decision is to: Accept Submission

Biodiversitas Journal of Biological Diversity

5 Bukti konfirmasi artikel published online (11 Juni 2021)

Notifications

[biodiv] Editor Decision

2021-06-11 03:32 PM

MUHAMMAD EVY PRASTIYANTO, NI MADE BUNGA ANGGELIA DEWI, TUSY DIAH PRATININGTIAS, NI MADE RAI PRATIWI, ANGGIS WINDAYANI, EKA WAHYUNENGSIH, ASTUTI, ELVIRA AMIR, FANDHI ADI WARDOYO :

The editing of your submission, "In vitro antibacterial activities of crude extracts of nine plants on multidrug resistance bacterial isolates of wound infections," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/8468

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