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HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW
KARYA ILMIAH : JURNAL NASIONAL TERAKREDITASI**

Judul Jurnal Ilmiah (Artikel)	: <i>Antibacterial Activity of Various Extracts of Averrhoa bilimbi against Multidrug Resistant Bacteria</i>				
Nama Penulis	: 1.Muhammad Evy Prastyianto, 2.Fandhi Adi Wardoyo, 3.Wildiani Wilson, 4.Sri Darmawati				
Jumlah Penulis	: 4 (empat) orang •				
Status Pengusul	: penulis pertama/penulis ke-4/penulis korespondensi **				
Identitas Jurnal Ilmiah	a. Nama Jurnal : Biosaintifika: Journal of Biology • & Biology Education, b. Nomor ISSN : EISSN :2333-87610 c. Volume, nomor, bulan, tahun : Vol 12, No.2, Agustus 2020 d. Akreditasi : SINTA 2/SK Dirjen Penguatan Riset dan Pengembangan No: 36/E/KPT/2019, 13 Desember 2019 e. Penerbit : Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Negeri Semarang f. DOI artikel (Jikaada) : https://doi.org/10.15294/biosaintifika.v12i2.23600				

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Total = (100%)	25	20	15	10	24
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- Kecukupan dan kemutahiran data/informasi dan metodologi:** Data-data hasil penelitian cukup menunjukkan ada kebaruan informasi. Terdapat 31 buah pustaka dari 32 yang kurang dari 10 th terakhir. Sebanyak 30 dari 32 pustaka berupa Jurnal . (ini menunjukkan proses review dan kecukupan pustakanya memenuhi (skor = 6,0).
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Semarang 10 November 2021
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Prof. Dr. Suworno Hadisusanto, SU

NIP/NIDN : 19541116 1983031002/0016115402
 Unit Kerja : Universitas Gadjah Mada Yogyakarta

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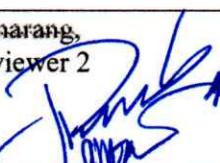
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Reviewer 2


Prof. Dr. Hermin Pancasakti Kusumaningrum, S.Si, M.Si
NIP/NIDN : 197002081994032001/0008027003
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NIP. 195912171984021001

Antibacterial activity of various extracts of averrhoa bilimbi against multidrug resistant bacteria

ME Prastyanto, FA Wardoyo, W Wilson... - ... : Journal of Biology & ..., 2020 - journal.unnes.ac.id

The multi-drug resistance (MDR) bacteria is a global health problem that causes high mortality every year. Therefore, novel antibacterial agents are needed from natural biological sources. This research aimed to investigate the antibacterial activities of various crude extracts of Averrhoa bilimbi against MDR bacteria. The antibacterial activity was calculated based on the use agar well diffusion assay and the minimum bactericidal concentration (MBC) using Mueller–Hinton broth in a microdilution method. Bacteria from ...

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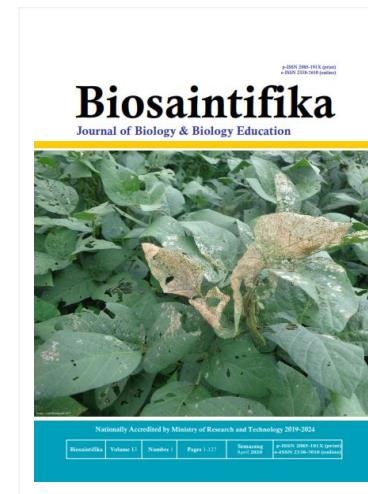
Vol 13, No 3 (2021): December 2021 Article-in-Press

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Agronomic Performance of Soybean Genotypes in Lowland Paddy Fields under Zero-tillage Condition

Mochammad Muchlish Adie*, Ayda Krisnawati, Rudi Iswanto

Indonesian Legume and Tuber Crops Research Institute, Indonesia

*Email: mm_adie@yahoo.com

Submitted: 16 March 2020. Revised: 26 April 2020. Accepted: 1 June 2020

Abstract. In Indonesia, soybean is mostly cultivated in lowland following the yearly planting pattern of paddy – paddy – soybean under zero-tillage condition. The research aim was to evaluate the agronomic performance of several soybean genotypes in lowland paddy fields under zero-tillage condition. A total of 12 soybean genotypes, including the check varieties of Wilis and Anjasmoro, were evaluated in lowland after rice planting in three locations (Klaten, Pasuruan, and Tabanan). A randomized block design with four replications was used in each location. The soybean yield is a complex character which determined by interrelated agronomic characters. The averages seed yield in Klaten, Pasuruan, and Tabanan were 2.97 t/ha, 3.02 t/ha, and 2.68 t/ha, respectively. Two genotypes produced equal yield with Anjasmoro, i.e. AT12-1062 (3.01 t/ha) and AT12-1037 (3.0 t/ha). Anjasmoro variety had the highest 100 seed weight (15.40 g), and only AT12-1035 showed the equal seed weight. The average days to maturity of 12 genotypes was 83 days. In addition to Anjasmoro variety, soybean genotypes AT12-1062 and AT12-1037 (medium maturity and medium seed size) as the new findings from this study were potential to be developed at lowland paddy fields under zero-tillage condition. The availability of the soybean genotypes adaptive to lowland paddy field under zero tillage condition is important to optimize the soybean productivity as well as the income of farmers in Indonesia.

Key words: Adaptability; Minimum Tillage; Wetland; Yield Productivity; Zero Tillage

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INTRODUCTION

In Indonesia, soybeans are mostly cultivated in lowland, according to a year planting pattern of paddy – paddy – soybean. The growing season of food crops in Indonesia consists of the rainy season (November/December), first dry season (February/March), and second dry season (June/July). Soybean in the paddy fields after the rice planting is usually cultivated in the second dry season and it has become the largest soybean area in Indonesia.

Research on Anjasmoro variety which treated by tillage and zero-tillage condition did not obtain the significant yield (Tarigan, 2015). The response of soybean variety of Wilis to three ways of soil processing (only processed around the planting hole, processed once, and processed twice) also showed a non-significant yield (Raintung, 2010). Other research using Grobogan variety revealed that the combination of maximum soil treatment and the weeding time at 24 and 44 days after planting produced the highest seed yield, lowest weed dry weight, highest number of pods per plant, and the highest number of seeds per plant (Akbar, 2012). Furthermore, Hosseini et al. (2016) reported a significant increase in yield of soybean planted in a no-tillage system compared to a conventional tillage system. However, Kiszonas (2010) concluded that no differences exist between

soybean grown in conventional tillage and no-tillage systems in Iowa and that locally adapted cultivars can be selected to maximize yield regardless of tillage system in Iowa. In Indonesia, soybean cultivation in the paddy field is a cultivation system that has been commonly used by farmers (Shurtleff & Aoyagi 2010). On the contrary, zero-tillage farming or minimum tillage is widely used in USA (Mathew et al., 2012; Islam & Reeder, 2014).

Various studies above showed that each soybean genotype responded differently to a certain environment, including zero-tillage environment. This is due to each genotype has different morphological and physiological characteristics. Even many studies found a significant interaction between genotype and environment (Yan & Rajcan, 2002; Pereira et al., 2009; Krisnawati et al., 2016; Krisnawati & Adie, 2018a) which indicates each genotype has a different adaptation to the specific environment. Soybean yield has been considered as a complex character, which not only determined by the adaptability to the environment but also determined by the interaction between the agronomic characters of yield components. Seed morphological characters, i.e. seed width and seed height were reported to play an important role in the yield and quality of the seed (El-Abady et al., 2012; Hu et al., 2013). Seed quality is often a prerequisite for industrial raw materials, including for indus-

Microbial Succession and Chemical Characteristics in Fermentation of *Ambonese arrack (Sopi)*, Traditional Beverage from Maluku

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Abstract. *Ambonese arrack* is one of the traditional fermented beverage product in Maluku, Indonesia. The microbiological research of this beverage that is processed using coconut sap as raw material has never been done before. The research aimed to analyze the microbial succession and chemical characteristics during fermentation of *Ambonese arrack*. The sample of coconut sap was taken from traditional producer in Mahia village, Ambon. The dominant microbes in the fermentation of *Ambonese arrack* were *Pichia polymorpha* and *Kloeckera javanica*. The highest numbers of these two microbes was obtained after 15 hours fermentation (9.6 log CFU/mL and 9.9 CFU/mL, respectively). The sugar content decreased from 593.3 mg/L to 474.3 mg/L, whereas ethanol content increased from 0.0018 g/L to 0.0100 g/L. The pH value decreased from 4.70 to 3.10. The research has isolated indigenous microbes in *Ambonese arrack* fermentation which was considered as novelty. The bacteria that play a role in fermentation can be used as a starter in the fermentation of various beverage products, especially *Ambonese arrack*. The results of this research can improve the quality of this fermentation product in the future.

Key words: *Ambonese Arrack*; Coconut Sap; Ethanol Content; Microbial Succession

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INTRODUCTION

Processing alcoholic beverages by fermentation is a tradition that has been going on for a long time in various parts of the world (Ohimain, 2016). Some regions in eastern Indonesia, such as Manado, Makassar and Maluku often consume this type of drink. One of the beverage product that is very popular in Maluku community is *Ambonese arrack* (locally named *sopi*). Besides being used as a drink to increase spirit for work, this drink is also used in various traditional ceremonies, so that it is considered as a symbol of friendship in the life of Maluku people (Gunawan, 2019). In other regions, people believe that the drink act to "strengthen of blood" and as an aphrodisiac (Chaves-Lopez et al., 2014)

Ambonese arrack is product of distillation of palm sap which has been fermented for more than a day. The palm sap (locally named *sageru*) is the sweet, oysterwhite coloured sap collected from the immature palm spadix (inflorescence) (Sudha et al., 2019). It is a rich source of simple sugars, such as sucrose, glucose, fructose and maltose (Law et al., 2011). The saps used in processing *Ambonese arrack* are from coconut (*Cocos nucifera* L), sugar palm (*Arenga pinnata* Merr) or *koli* (*Borassus sundaicus* Becc). The Maluku people more often use coconut sap to make

Ambonese arrack because coconut plants grow a lot in this area. *Ambonese arrack* processing using *koli* sap only by the people of Southwest Maluku (Sahusilawane et al., 2015). Besides being used to make *Ambonese arrack*, it could also be used as a yeast starter for bread making (Olowonibi, 2017). The palm sap that has been fermented can be distilled to produce *Ambonese Arrack*. If it is not distilled, the palm sap will produce *sagero vinegar*

The fermentation process of alcoholic beverages involves microbes (Escalante et al., 2008). Coconut sap used as a raw material contains yeast and bacteria. The composition of these microbes is largely determined by environmental conditions. Generally the dominant yeast in palm sap is *Saccharomyces* (Chandrasekhar et al., 2012), but in different places it is dominated by other species (Kalaiyarasi et al., 2013). Microbial composition is greatly influences the chemical characteristics which include sugar content, ethanol content, and acidity during palm sap fermentation. *Ambonese arrack* processing is still carried out on a household scale and it is not controlled. The equipment used in processing is also not aseptic, so it is possible to have microbial contaminants involved during fermentation. Contaminant microbes can reduce the quality of arrack flavour (Belda et al., 2017). This study aimed to analyze microbial succession and

Antibacterial Activity of Various Extracts of Averrhoa bilimbi against Multidrug Resistant Bacteria

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Antibacterial Activity of Various Extracts of *Averrhoa bilimbi* against Multidrug Resistant Bacteria

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Abstract. The multi-drug resistance (MDR) bacteria is a global health problem that causes high mortality every year. Therefore, novel antibacterial agents are needed from natural biological sources. This research aimed to investigate the antibacterial activities of various crude extracts of *Averrhoa bilimbi* against MDR bacteria. The antibacterial activity was calculated based on the use agar well diffusion assay and the minimum bactericidal concentration (MBC) using Mueller–Hinton broth in a microdilution method. Bacteria from wells were subcultured using inoculating loop onto a 5% sheep BAP. The best antibacterial activity, calculated as the most widely inhibitory zone and the smallest MBC values. The ethanolic extract showed antibacterial activity against all MDR bacterial test in the agar well diffusion assay (10–14.5 mm inhibition diameter). The MBC of water extract against ESBL + CR *Pseudomonas aeruginosa* showed the best antibacterial activity (12.5 mg/mL). The fruit of bilimbi was shown to be potentially developed as antibacterial agents, especially for MDR strains. Further in vivo research and discovery of action mode are needed to shed light on their antibacterial effects. This study can provide new information about the benefits of bilimbi as a source of natural antibacterial agents MDR-bacteria.

Key words: *Averrhoa bilimbi*; Antibacterial activity; Minimum Bactericidal Concentration; Resistance bacteria; Secondary metabolites

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INTRODUCTION

The antibiotic resistant bacterial strains have been a menace to public health globally. Particularly, multi-drug resistant (MDR) bacterial strains remain a serious cause of concern since they cause treatment failures and impose huge economic burdens especially in developing countries (Ballani and Babby, 2016). MDR bacteria are the main cause of severe complications in the therapies of contagious diseases and has become a serious problem that causes high mortality every year (Mekes et al., 2019). The MDR bacteria is a global health problem caused by inappropriate use of antibiotics. Patients with infections caused by MDR bacteria have a worse risk due to their difficult treatment and the likelihood of high mortality. Infections caused by MDR bacteria also consume more health-care resources than patients infected with non-resistant strains of the same bacteria (World Health Organization, 2018). So, novel antibacterial agents are needed from natural biological sources (Valle Jr et al., 2015). Biological antibacterial agents can be obtained from the mushrooms (Prastyanto et al., 2016), bacteria (Ryandini et al., 2018), bacteriocins (Lestari et al., 2019), and plant (Tillah et al., 2017; Wahyuni et al., 2019). There have been many reports found in the medical literature concerning the significance of traditional medicinal plants as alternatives to antibacterial agents (Akhtar, 2015; Aumeeruddy-elalfi et al., 2015).

Plants is a good source of antibacterial compounds, because of the variety and diversity of the chemical structures of the compounds contained therein (Ngameni et al., 2013). *Averrhoa bilimbi* Linn. (bilimbi) also known as belimbing wuluh in Indonesia is one of the medicinal plants that is found in tropical and subtropical countries and useful as a medicine, such as antidiabetic (Ahmad et al., 2019), hepatoprotective (Dnyaneshwar et al., 2010), anti-cancer, and antithrombotic agent (Ali et al., 2014).

Research related to antibacterial activity of bilimbi has been reported. The extracts of bilimbi fruit can inhibit bacteria *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Vibrio parahaemolyticus* ATCC 17802 (Seebaluck-sandoram et al., 2019). The fruits and roots extracts of bilimbi were also inhibit *Mycobacterium tuberculosis* (Mohamad et al., 2011). Research on antibacterial activity of extracts of bilimbi fruits against MDR bacteria have not been reported, so it is necessary to investigate the antibacterial potential of bilimbi fruit extract against MDR bacteria. In this study, we used five variations of bilimbi fruit extracts.

The study on antibacterial activity of bilimbi with various solvent (Methanol, Ethanol, Chloroform, N-Hexane and water) are expected to provide new information about the benefits of bilimbi. In addition, it also can support the bilimbi as a source of natural antibacterial agent againts MDR-bacteria. The re-

search aim was to investigate the antibacterial activities of fruit extracts of bilimbi with various solvents against MDR bacteria from clinical specimen.

METHODS

Plant materials

Fruits of bilimbi Figure 1 (green, not overly ripe) were freshly picked from a home garden in Kedungmundu, Semarang, Indonesia. The fruits were washed with water to remove all unwanted materials and then rinsed with sterilized distilled water, then dried under sunlight for two days. The dried bilimbi were then milled into fine powder using a milling machine and stored in a sterile airtight container until further use.



Figure 1. Bilimbi fruits (*Averrhoa bilimbi*)

Plant extract preparation

Bilimbi extracts were prepared using the maceration method with various solvents i.e.: methanol, eth-

anol, chloroform, N-Hexane and water. One hundred grams of powdered bilimbi was soaked in 300 mL of each solvent for 24 h at room temperature and protected from light with shaking. Solvent replacement was done every day. Replacement of solvent was done until the solution became clear with the assumption that there was no active compound contained in the dry powder. The supernatant was filtered through Whatman filter paper No.1 (Whatman). 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.

Bacterial preparation

The organisms used for in vitro antibacterial screening in this study is summarized in Table 1. MDR bacteria were isolated from patients of the dr. Kariadi Hospital, Semarang City, Indonesia. All isolates were identified and susceptibility patterns were obtained using Vitek®MS (bioMérieux, Marcy l'Etoile, France). The bacteria were subcultured overnight (24 h) at 35±2 °C on 5% sheep blood agar (BAP). The bacterial colonies were homogenized and adjusted to 0.5 McFarland standards (5×10^8 CFU/mL) using spectrophotometry.

Table 1. The organisms for in vitro antibacterial screening in this study

Species	Source	Antibiotic resistance pattern
ESβL, <i>K. pneumoniae</i> ssp <i>pneumoniae</i>	52 / male, swab wound	Ampicillin, Sulbactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Gentamicin, Sulfamethoxazole
ESβL, <i>E. coli</i> ,	29 / male, swab wound	Ampicillin, Sulbactam, Tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Gentamicin, Ciprofloxacin, Sulfamethoxazole
ESβL + CRE, <i>E. coli</i> ,	2 / male, pus	Ampicillin, Sulbactam, Tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Ertapenem, Meropenem, Ciprofloxacin, Sulfamethoxazole
ESβL + CRE, <i>K. pneumoniae</i> ssp <i>pneumoniae</i>	60 / female, bronkus	Ampicillin, Sulbactam, Tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Ertapenem, Meropenem, Gentamicin, Ciprofloxacin, Sulfamethoxazole
MRSA	53 / female, pleura	Benzylpenicillin, Oxacillin, Gentamicin, Ciprofloxacin, Levofloxacin, Erythromycin, Clindamycin, Tetracycline, Rifampicin, Sulfamethoxazole
ESβL + CR <i>Pseudomonas aeruginosa</i>	41 / female, sputum	Ampicillin, Sulbactam, Tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline, Nitrofurantoin, Sulfamethoxazole

ESβL: extended spectrum beta-lactamase, CRE: Carbapenem-resistant Enterobacteriaceae CR: Carbapenem-resistant, MRSA: Methicillin-resistant *Staphylococcus aureus*

Antibacterial susceptibility assays

The antibacterial activity from various extracts of bilimbi was evaluated using agar well diffusion assay (Andleeb et al., 2020). In this method, 100 µL of each test organism which was equivalent to a 0.5 McFar-

land standard was inoculated on the Muller Hilton Agar (MHA). Then it was spread onto the surface of the agar using a sterilized glass spreader. After 10 minutes of inoculation, the wells were prepared using sterilized steel cork borer (1cm diameter). Wells were

made in each plate, out of which five wells were loaded with each extracts (200 µg or 200 µL from 1000 µg/mL). All the plates were then incubated aerobically at 35 ± 2 °C for 24 h. Antibacterial activity of the extracts was determined by measuring the diameter of inhibition zone in mm against the test organism.

Minimum bactericidal concentration (MBC) of the bilimbi fruits extracts.

The MBC of bilimbi fruit extract was determined using Mueller–Hinton broth microdilution (CLSI, 2018). MBC determination was performed by a serial dilution technique using 12-well microtiter plates. Bilimbi fruit extract (100 mL) was placed into the well/plate. Then, 100 mL of bacterial cell suspensions (0.5 McFarland) were placed in each well/plate. Microplates were incubated for 24 h at 35 ± 2 °C. The MBC of bilimbi fruit extract was determined following the methods described by Parvez et al. (2019) with slight modifications. The bacteria in wells were subcultured using a 10 mL inoculating loop onto a 5% sheep BAP at (35±2) °C for 16–20 h incubation. The MBC was defined as the lowest concentration of the

extract that did not any growth of bacterial colony on 5% sheep BAP. Mueller–Hinton broth as negative control. Cefazolin and meropenem were used as positive controls for ESBL-producing bacteria. Cefazolin and tigecycline were used as positive controls for ESBL + Carbapenem resistance (CR), while Sulfa-methoxazole and vancomycin were used as positive controls for MRSA

RESULTS AND DISCUSSION

Antibacterial susceptibility assay

The antibacterial susceptibility assays were initially performed to determine the antibacterial activities of the various extracts of bilimbi fruit against MDR bacteria, namely, ESBL *K. pneumoniae* ssp *pneumonia*, ESBL *E. coli*, ESBL + CRE, *E. coli*, ESBL + CRE, *K. pneumoniae* ssp *pneumonia*, MRSA, ESBL + CR *P. aeruginosa*. The extract of bilimbi fruit with various solvents (methanol, ethanol, chloroform, n-Hexane and water) showed inhibition of bacterial growth against some or all of the test organisms (Figure 2 and Table 2).

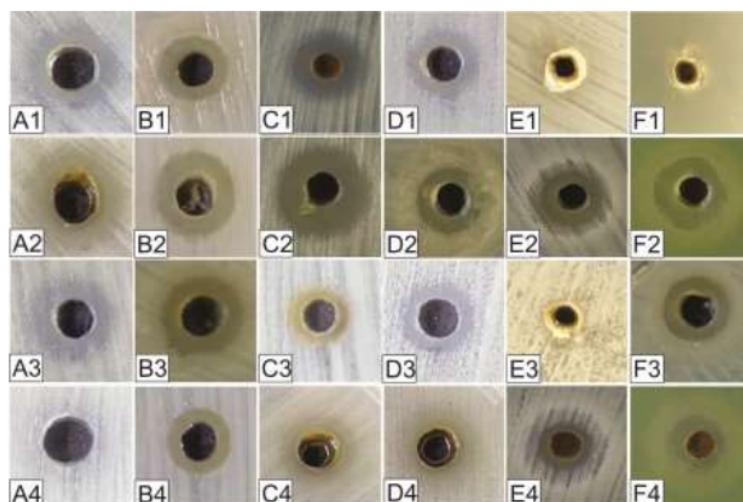


Figure 2. Inhibition zone of bilimbi extracts against MDR bacteria: (A) ESBL *K. pneumoniae* ssp *pneumonia*; (B) ESBL *E. coli*; (C) ESBL + CRE, *E. coli*; (D) ESBL + CRE, *K. pneumoniae* ssp *pneumonia*; (E) MRSA; (F) ESBL + CR *P. aeruginosa*, and solvent (1) chloroform; (2) ethanol; (3) methanol; (4) water

Table 2. Diameters of inhibition zone of bilimbi fruit extract (100 mg/mL) with various solvents against clinical isolates of MDR bacteria (mm)

Solvent	ESBL <i>K. pneumoniae</i> ssp. <i>pneumoniae</i>	ESBL <i>E. coli</i>	ESBL + CRE <i>E. coli</i>	ESBL + CRE <i>K. pneumoniae</i> ssp. <i>pneumonia</i>	MRSA	ESBL + CR <i>Pseu- domonas aerugino- sa</i>
Chloroform	6	10.5	11	8.5	-	-
Ethanol	10	10.5	14.5	10	11.5	13.5
Methanol	8	10.5	7	10	-	8.5
Water	-	9	9.5	8.5	10	11.5

The extracts exhibited inhibition zone ranging from 6 mm to 14.5 mm diameter, with the most noteworthy results shown by ethanol solvent. The ethanolic extract of the bilimbi fruit showed inhibition zones ranging from 10–14.5 mm against the clinical strains of all strain test. These results are in accordance with the research report by Valle-Jr et al. (2015) that, the ethanolic extract *P. betle* leaf demonstrated inhibition zones against MRSA, ESBL-Enterobacteriaceae and non-Enterobacteriaceae. The methanolic extract of bilimbi fruit demonstrated inhibition zones of 7–10.5 mm against the Gram-negative bacteria of all strain test, but not at MRSA (Gram positive). In other study, the methanolic extract of *Albizia adianthifolia*, *Alchornea laxiflora*, *Laportea ovalifolia* demonstrated inhibition zones against MDR Gram-negative bacteria (Tchinda et al., 2017). The water extract of bilimbi fruit demonstrated inhibition zones of 8.5–11.5 mm against all strain test, but not at *K. pneumonia*. The growths of *K. pneumoniae* were not inhibited by all the extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta* with water solvent that inhibit the other Enterobacteriaceae (Alo et al., 2012). The chloroform extract of Bilimbi fruit demonstrated inhibition zones of 6–11 mm against all of strain test, but not at *S. aureus* and *P. aeruginosa*. In other study, the chloroform extract of *Streptomyces* sp. strain Al-Dhabi-97 isolated from the marine could not inhibit *S. aureus* (Al-dhabi et al., 2020) and the chloroform extract of seed of *Callistemon lanceolatus* could not inhibit *P. aeruginosa* (Khavitha and Satish, 2014). Results of the antibacterial assays showed that the most commonly inhibited bacteria by the ethanolic plant extracts were the clinical ES β L + CRE, *E. coli*, ES β L + CR *P. aeruginosa*, MRSA, ES β L *E.*

coli, ES β L *K. pneumoniae* ssp *pneumonia*, ES β L + CRE, *K. pneumoniae* ssp *pneumonia*

Minimum bactericidal concentration (MBC) of the fruit extracts.

The antibacterial activity of the four extracts were assayed in vitro by the agar microdilution method against six resistant bacteria. The antibacterial activity against each bacterium was observed to be varied. Table 3 shows that the all bilimbi extracts exhibited inhibition of bacterial growth against MDR bacteria (12.5–50 mg/mL). Table 3 shows that all bilimbi extracts exhibited lower MBC values than Cefazolin against ESBL strains and lower than Sulfamethoxazole against MRSA. This showed that extract of bilimbi have the potential to be developed as antibacterial agents for MDR bacteria. The microdilution method was used in the present study because it is a quantitative reference method routinely used in clinical laboratories.

Furthermore, all extracts showed highly varying MBC values against MDR bacteria, but the lowest MBC value belonged to water extract (12.5 mg/mL) against ES β L + CR *P. aeruginosa*. Aqueous extract of the truffle *Terfezia claveryi* contains a potent antimicrobial agent toward *P. aeruginosa* (Janakat et al., 2005). In the present study, favorable antagonistic activities against various MDR bacteria were exhibited by the all extract of bilimbi. However, this study resolutely established the ethanolic fruit extract of bilimbi exhibiting wide zones of growth inhibition against MDR bacteria tested. Preliminary phytochemical studies of the fruit extracts using chemical methods and thin layer chromatography (TLC) revealed the presence of flavonoids, tannins, and terpenes (Hasanuzzaman et al., 2013; Patil et al., 2013).

Table 3. MBC of bilimbi fruit extract against clinical isolates of MDR bacteria (mg/mL).

Solvent	ES β L <i>K. pneumoniae</i> ssp <i>pneumoniae</i>	ES β L <i>E. coli</i>	ES β L + CRE, <i>E. coli</i>	ES β L + CRE <i>K. pneumoniae</i> ssp <i>pneumonia</i>	MRSA	ES β L + CR <i>Pseudomonas aeruginosa</i>
Chloroform	50	25	50	50	25	25
Ethanol	25	50	50	25	25	25
Methanol	50	50	50	50	25	25
Water	25	25	50	50	50	12.5
Cefazolin	64	64	64	64	-	64
Meropenem	0.25	0.25	-	-	-	-
Tigecycline	-	-	0.5	1	-	8
Sulfamethoxazole	-	-	-	-	320	-
Vancomycin	-	-	-	-	1	-

The antibacterial activity of the plant can be attributed to its phytochemical compounds. Phytochemical compounds act as shields against disease infec-

tions. The most important phytochemicals are tannins, flavonoids, alkaloids, and terpenes (Kumar et al., 2013). Flavonoids (Khalid et al., 2019), tannins

(Shahat and Marzouk, 2013), and terpenes (Broniatowski and Mastalerz, 2015) have been recognized to exhibit quite potent antibacterial activity. The mechanism of antibacterial activity of flavonoids, tannins, and terpenes compound in the bilimbi is unknown. However, according to (Abuga et al., 2020), phytochemical compounds can inhibit the growth of bacteria by destruction of the bacterial cell wall. The fruit of bilimbi was shown to be potentially developed as antibacterial agents, especially for MDR strains. Further in vivo research and discovery of action modes are needed to shed light on its antibacterial effects, so that potential clinical drug and health products could be developed. This study can provide new information about the benefits of bilimbi as a source of natural antibacterial against MDR bacteria.

CONCLUSION

The fruit of bilimbi was shown to be potentially developed as antibacterial agent, especially for MDR strains. The ethanolic extract showed the highest antibacterial activity against all MDR bacterial test in the agar well diffusion assay (10-14.5 mm inhibition diameter). The MBC of bilimbi water extract against ES β L + CR *Pseudomonas aeruginosa* showed the best antibacterial activity (12.5 mg/mL).

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