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RESEARCH ARTICLE

Potential of ethanolic extracts from seed kernel Indonesian cultivars mangoes as an anti-multidrug-resistant (MDR) *Escherichia coli*

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Abstract

Escherichia coli is a type of bacteria that often found in wound infections. The use of antibiotics to treat these uncontrolled infections has resulted in the emergence of multi-drug resistant (MDR) strains of pathogenic bacteria. This rapid increase in MDR *E. coli* strains has led to significant morbidity and mortality in human populations. Consequently, the exploration of natural antibacterial agents is urgently needed. One promising resource is mango extract, which is recognised for its antibacterial properties and is obtained through solvent extraction using 96% ethanol. This study aimed to determine the antibacterial activity of seed extracts from seven different mango cultivars (*Mangifera indica* L.), against MDR *E. coli.* The study focused on determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using dilution method. The concentrations examined for both MIC and MBC were 5, 10, 50, and 100 mg/mL. The results indicate that the mango seed extract exhibit significant antibacterial activity against MDR-*E. coli.*, with the kweni cultivar showing the largest inhibition zone measuring 23.25 mm. The ethanol extract from the kweni seeds yielded the most favorable results, with an MIC value of ≥0.078 mg/mL and an MBC value that ranged from 1.25-10 mg/mL. In conclusion, mango seed extract, particularly from the kweni cultivar, has substantial potential to be developed as an effective antibacterial agent against MDR-*E. coli*.

1. INTRODUCTION

In underdeveloped nations, 1-2 percent of the population is estimated to sustain chronic injuries (1). Infection is one of the most common reasons for delayed wound healing is. If infections are overlooked, they can progress from localized to systemic, potentially resulting in multiple organ dysfunction syndrome, sepsis, and potentially fatal outcomes. Gram-negative *Escherichia coli* (*E. coli*) bacteria often proliferate on wound dressings (2). These bacteria are rod-shaped or

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bacilli, facultatively anaerobic, and belong to the *Enterobacteriaceae* family. Although *E. coli* is part of the normal flora in the human intestine and rarely cause infection in healthy individuals, some pathogenic strains can cause diarrhoea (3).

E. coli is commonly found in the of gastrointestinal tract of poultry, animals, and humans (4). The excessive use of antibiotics is considered the primary cause of antibiotic resistance. Resistance can develop through horizontal gene transfer or gene mutations. Multidrug-resistant (MDR) bacteria typically carry several genes that confer drug resistant. The rapid emergence of MDR *E. coli* strains has resulted in significant morbidity and mortality in humans (5).

The treatment of wound infections must be approached carefully. Bacterial infections are usually treated with antibiotics; however, uncontrolled antibiotic use can lead to resistance against one or more drug classes, resulting in MDR strains of *E. coli* can exhibit resistance to broad-spectrum cephalosporin, which produces broad-spectrum beta-lactamase (6). Studies have shown that *E. coli* exhibits considerable variation in its resistance patterns. This bacterium acts as a reservoir for antibiotic resistance genes that can be transferred to other pathogenic bacteria. Such resistance may extend to three or more classes of antibiotics, leading to the development of multidrug-resistant (7). *E. coli* may also show resistance to specific antibiotics, such as *co-trimoxazole*, *ampicillin*, *ciprofloxacin*, *levofloxacin*, and *nitrofurantoin* (8). Utilising natural ingredients as antibacterial agents presents a promising alternative treatment strategy. Various antimicrobial compounds with extensive medical benefits have been identified in previous studies (9). Antibacterial agents can be sourced from different sources, including honey (10), lactic acid bacteria (11), fungi (12,13), sea (14-16), plants (17), and fruits (18-21). The mango plant (*Mangifera indica* L.) is a notable natural antibacterial agent is (6). Commonly found in tropical regions, mangoes belong to the Anarcardiaceae family within the order Sapindales. Mango seeds are rich in bioactive substances, such as polyphenols, flavonoids, and tannins, which provide antibacterial and antioxidant properties (22,23). Mango seeds are a common by-product of mango consumption and have important nutritional and therapeutic benefits. Mango seed extracts may help lower blood sugar, prevent bacterial infections, and reduce inflammation. Additionally, the seeds contain dietary fiber, calcium, magnesium, potassium, and essential fatty acids that promote overall health and wellness. However, comprehensive data on the efficacy of various Indonesian mango seed extracts against clinical MDR *E. coli* isolates are still lacking. Nevertheless, further research is necessary to fully explore and understand the potential uses of mango seeds in medicine, nutrition, and cosmetics (22).

This study aimed to analyse the secondary metabolites found in mango seeds and evaluate their potential as natural ingredients to be used as antibacterial agents. The goal of this study is to explore their use as traditional medicines for treating infections caused by *E. coli*.

2. MATERIALS AND METHODS

The objective of this study to investigate multi-drug resistant (MDR) *E. coli*, which was assessed using the dilution method. The antibacterial potential of mango seeds was evaluated using the minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods. The concentrations tested in both the MIC and MBC methods were 5 mg/mL, 10 mg/mL, 50 mg/mL and 100 mg/mL. Confirmation tests for MIC and MBC were performed using the streak plate method. The dilution method involves preparing various concentrations of the test solution, which are then added to a fungal suspension in the media. Bacteria or fungi are inoculated onto the medium once it solidifies.

The MDR *E. coli* used in this study was obtained from the Microbiology Laboratory at the Health Laboratory, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, Semarang, Indonesia. The research materials consisted of seven types of mango seeds: Cengkir Mango, Kopyor Mango, Golek Mango, Kweni Mango, Avocado Mango, Arumanis Mango, Manalagi Mango along with *E. coli* isolates. The media used included MacConkey Agar (CM0115, OXOID, United Kingdom), Muller Hinton Agar (CM0337, OXOID, United Kingdom), Muller Hinton Broth (CM0405, OXOID, United Kingdom), and Blood Agar Plate (CM0271, OXOID, United Kingdom). The other materials involved were 96% ethanol solvent (EMSURE®, 159010, MERCK, Germany), physiological saline (0.85% NaCl), paper filters, and antibiotics such as ampicillin, ceftriaxone, aztreonam, ciprofloxacin, gentamicin, meropenem. Additional chemicals included Bate Smith-Metcalfe NaOH (10%), Mayer's reagent, $FeCl_3$, Aquadest, 2N HCl (EMSURE® 100312, MERCK, Germany), and Lieberman – Burchard reagent.

2.1. Seed Collection and Extraction

In November 2021, seven varieties of mango seeds were collected from various locations in Indonesia. These mango seeds were gathered from Central Java, specifically from the following locations: the Demak Regency Agriculture & Food Office in Central Java, which produces Golek mango seeds; Heritage Mango Park in Pasuruan, East Java, which produces avocado mango seeds; and Agro Cepoko Regency Garden in Semarang, Central Java, which produces Arumanis and Manalagi mango seeds. Botanists identified all mango seeds as suitable for study. The collected seeds were cleaned under running water to remove dirt. The seeds were then sun-dried for seven days to prepare them for extraction into mango powder. To achieve a consistent particle size, the dried seeds were crushed into a fine powder using a professional blender and then sieved using a 40-mesh (≈0.4 mm) sieve. A maceration method using a 96% ethanol solvent was employed for extraction. Three hundred millilitres of ethanol was used to extract 100 grams of each seed powder, which was left to sit at room

temperature for 24 h. The solvent was changed every 24 h at room temperature until the solution became clear and the powder was believed to have no more active ingredients. A Whatman No. 1 paper filter was used to remove the supernatant and the filtrate was concentrated at 50 °C using a rotary evaporator to produce a viscous extract. The yield of the ethanolic extract was calculated as (weight of extract / weight of dry powder) × 100.

2.2. Bacterial Strain Isolation, Identification, and Antibiotic Sensitivity Testing

E. coli isolates were obtained from wound samples collected from patients at Tugurejo Hospital in Semarang, Indonesia. Bacteria were cultivated on MacConkey agar media and incubated for 24 h at 35°C. Positive catalase and oxidase tests were performed to identify non-lactose fermenters. The Vitek®MS (Automated Microbial Identification System, bioMérieux, Marcy-l'Étoile, France) was employed for bacterial identification and susceptibility testing (24).

2.3. Antibacterial Efficacy of Mango Seed Extract Against MDR *E. coli*

The antibacterial activity of mango seed extract was assessed using diffusion testing (25). Each MDR *E. coli* isolate recovered from infected patients was grown on MacConkey medium and incubated at 37°C for 24 h. Test-inoculated bacteria were collected using a sterile loop, and once the turbidity reached the industry standard of 0.5 McFarland by visual comparison with a McFarland standard tube, resulting in a suspension of approximately 1.5×10^8 CFU/mL the bacteria were suspended in 5 mL of 0.85% physiological NaCl in a test tube. Using a sterile cotton swab, each isolate was then spread onto Muller Hinton Agar (MHA). After 5 min, a cork borer was used to make four holes in the MHA medium. The extracts were dissolved in dimethyl sulfoxide (DMSO) to create stock solutions of known concentration (5, 10, 50, and 100 mg/mL). Then, 100 µL of these stock solutions were added to the wells. Each test was repeated four times. Each well received 100 µL of each concentration, which was then incubated for 24 hours at 37°C. Control methods included antibiotics such as ampicillin, ceftriaxone, aztreonam, ciprofloxacin, gentamicin, and meropenem. Antibacterial activity was determined by measuring the inhibitory zone diameter in millimetres.

2.4. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of each extract was determined using a microwell plate dilution method with Muller Hinton Broth (MHB) medium. Each well received 100 µL of MHB and 100 µL of extract in the first well, and subsequent dilutions were performed to the twelfth well. After dilution, 10 µL of MDR *E. coli* bacteria suspended in physiological NaCl was added to each well according to the McFarland 0.5 standard, except for the negative controls. The microwells were incubated for 18 h at 37°C. The turbidity in the microwells compared to the control determined the MIC value; the extract with the lowest MIC value indicated the best antibacterial action.

2.5. Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by culturing the MIC wells on BAP medium for 24 hours at 37°C. No growth was observed on the BAP medium, which indicated the MBC values. The lowest concentration of seed extract at which MDR *E. coli* could not grow was recorded as the MBC value. The minimum bactericidal concentration (MBC) was determined by subculturing aliquots from wells showing no visible growth onto fresh agar plates. The MBC was defined as the lowest extract concentration of that resulted in ≥99.9% reduction of the initial inoculum, or practically, the lowest concentration at which no visible bacterial growth was observed after subculture. The lowest MBC value indicated the best antibacterial action.

2.6. Phytochemical Screening

Phytochemical tests were conducted using reagents specific to each compound, and the results are indicated by colour changes (Table 1). The phytochemical screening of the ethanol extract from the seven mango cultivars assessed the presence of flavonoids, alkaloids, phenolics, tannins, saponins, and triterpenoids. All seven mango seed extracts contained flavonoids, alkaloids, phenolics, tannins, saponins, and triterpenoids. The results of the research were analysed by calculating the MBC and MIC from the extracts of the seven cultivars of mango seeds.

Table 1. Phytochemical Test

Phytochemical Test	Reagent	Color Change
Flavonoids	Bate Smith-Metcalfe NaOH 10%	Red
Alkaloids	Mayer	Orange, Yellowish-white precipitate
Phenolics	$FeCl_3$	Black
Tannins	$FeCl_3$	Blackish Blue
Saponins	Aquadest, heated, shaken, + HCl 2N	Embossed foam
Triterpenoids	Lieberman - Burchard	Red Purple

2.7. Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean values ± standard deviation (SD). Data were processed using IBM SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA). No further statistical comparisons were conducted, as the primary aim of the study was to descriptively evaluate the antibacterial activity (MIC and MBC values) across mango cultivars.

3. RESULTS AND DISCUSSION

3.1. Extract Results

Figure 1 shows the extracts from seven types of mango seeds (Cengkir, Kopyor, Golek, Kweni, Avocado, Arumanis, Manalagi (*Mangifera indica L.*)) using ethanol as a solvent. The yield of ethanol extracted from these seven types of mango seeds is shown as a percentage in Table 2.

According to Table 2, the extract yields from Avocado mangoes and certain mango seeds are higher than those of other six mango varieties, indicating that avocado and mango seeds contain a greater number of chemical compounds that can be effectively extracted using ethanol. The higher yield from avocado mango seed extracts can be attributed to several factors. The Avocado mango seeds likely contain elevated levels of bioactive compounds, such as flavonoids, polyphenols, and tannins, which are soluble in ethanol, thereby enhancing the extraction efficiency. Additionally, these seeds may have a higher concentration of natural fats and oils that can be effectively extracted by ethanol . The physical structure of the seeds, such as softer tissues or larger pores, may also allow for better solvent penetration, leading to improved extraction. Furthermore, the lower moisture and fibere content in avocado mango seeds might reduce resistance during the extraction process, allowing ethanol to efficiently dissolve the desired compounds . Overall, these factors contribute to the higher extract yield, indicating that avocado mango seeds contain more chemical components that are easily extracted with ethanol.

3.2. MDR *Escherichia coli*

The study focused on MDR *E. coli*, which was measured using the dilution method. This method involves diluting the test solution to achieve several concentrations, each of which is then added to a fungal suspension in the media. For solid dilution, each concentration was mixed with agar media. The media is inoculated with bacteria after solidification. The MDR *E. coli* used in this study was isolated from various samples, including *E. coli* 1E and 4E from urine samples, *E. coli* 5E from blood samples, *E. coli* 7E from pus samples, and an ATCC sample 14028 derived from American national standards. The *E. coli* strains are resistant to one or more antibiotic classes, as indicated by the results of tests conducted to identify and assess their antibiotic sensitivity. Previous research has shown that *E. coli* from rectal samples exhibited significant resistance to various antibiotic classes, including trimethoprim-sulfamethoxazole (49.5%) and ampicillin (76%) (26).

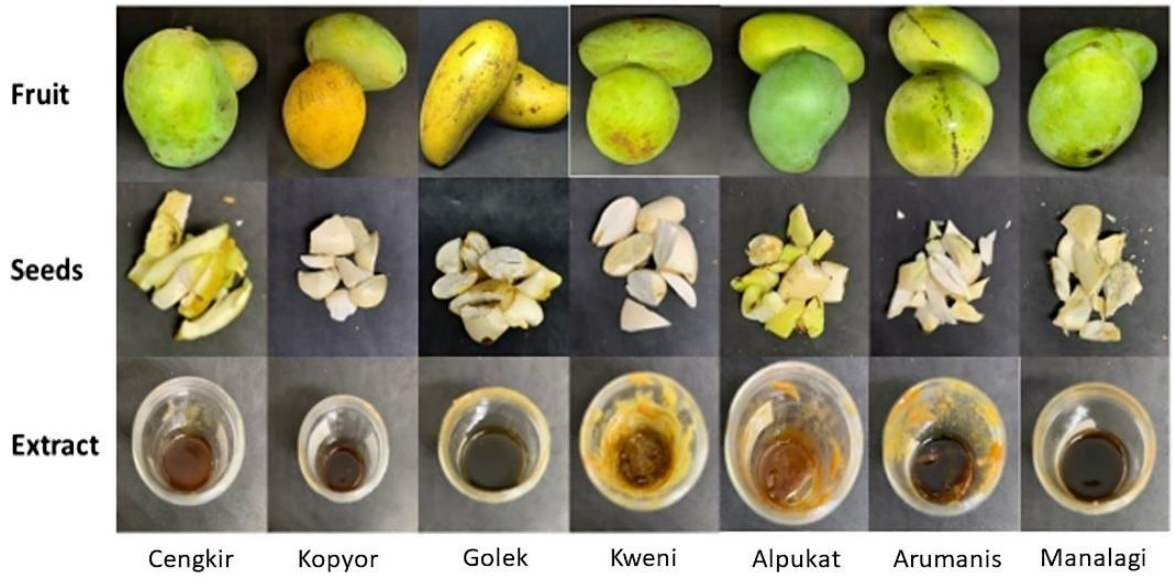

Figure 1. Extract results of seven mango cultivars

Table 2. Results of Seven Mango Seed Extracts

Cultivars	Percentage (%)
Cengkir	25.35
Kopyor (*Mangifera laurina* B)	11.86
Golek	28.31
Kweni (*Mangifera odorata* G)	12.31
Avocado	40.91
Arumanis (*Mangifera indica* L.)	23.00
Manalagi	29.44

3.3. Antimicrobial Efficacy of Seed Extract Against MDR *E. coli*: Agar Well Diffusion Test

The antibacterial activity of seven mango seed extracts against MDR *E. coli* was assessed using the agar well diffusion assay. This was performed by measuring the inhibitory zone diameter (in mm) against five isolates of *E. coli* at various concentrations: 5, 10, 50, and 100 mg/mL. The antibacterial efficacy of the seven mango seed extracts against MDR *E. coli* was demonstrated (Table 3). The *Cengkir* mango seed extract exhibited an inhibitory zone ranging from 9.25 ± 0.8 mm to 19.25 ± 0.8 mm for MDR *E. coli* and from 9.25 ± 0.8 mm to 14.5 ± 1.5 mm for *E. coli* ATCC 14028. The *Kopyor* mango seed extract had an inhibitory zone of 9 ± 0.7 mm to 20.5 ± 1.5 mm for MDR *E. coli* and 7.75 ± 1.3 mm to 15.5 ± 2.2 mm for *E. coli* ATCC 14028. The *Golek* mango seed extract showed an inhibition zone of 10.5 ± 1.1 mm to 21 ± 0.7 mm for MDR *E. coli* and 11 ± 1.0 mm to 15 ± 1.2 mm for *E. coli* ATCC 14028. The inhibition zone of *Kweni* mango seed extract was 12± 3 mm to 23.25 ± 1.9 mm for MDR *E. coli*, whereas that of *E. coli* ATCC 14028 ranged from 9.25 ± 0.8 mm to 19.25 ± 1.8 mm. The Avocado mango seed extract showed an inhibition zone of 12.5 ± 0.8 mm to 19.75 ± 0.4 mm against MDR *E. coli* and 11.75 ± 0.4 mm to 15.75 ± 0.8 mm against *E. coli* ATCC 14028. For the *Arumanis* mango seed extract, the inhibition zone against MDR *E. coli* was 6.25 ± 0.4 to 16.25 ± 1.9 mm, with *E. coli* ATCC 14028 measuring 7.25 ± 1.0 to 11.25 ± 0.4 mm. The inhibition zone of *Manalagi* mango seed extract for MDR *E. coli* was 6.5 ± 0.5 mm to 15.5 ± 0.5mm, whereas that for *E. coli* ATCC 14028 was 6.25 ± 0.4 mm to 12.25 ± 0.4 mm. These results indicate that all seven mango seed extracts had wider inhibition zones than the antibiotic controls. The presence and abundance of bioactive secondary metabolites in *Mangifera odorata* (Kweni) are responsible for its improved performance compared with other mango types. Although quantitative trends indicate that Kweni contains these metabolites at higher levels, the presence of flavonoids, tannins, and phenolic compounds was verified by qualitative phytochemical tests. While tannins aid in microbial cell membranes rupture and protein precipitation, flavonoids and phenolics are widely known for their potent antioxidant and antibacterial qualities. The increased bioactivity of Kweni extracts is probably due to the synergistic action of these chemicals. This suggests that Kweni's superiority is due to its concentration and bioactive potency as well as its chemical variety, which together offer a molecular foundation for its increased pharmacological potential.

3.4. Calculation of Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC)

The MIC analysis revealed that the *Cengkir* and Avocado mango seed extracts had an MIC value of ≥ 0.312. The *Kopyor*, *Golek,* and *Arumanis* mango seed extracts had the same MIC value of ≥0.625. The *Manalagi* mango seed extract showed an MIC value of ≥0.156, while the Kweni mango seed extract had the lowest MIC value of ≥0.078 (Figure 2). The MBC results indicated that all seven mango cultivars showed MBC values at concentrations of ≥10 mg/mL (Figure 3).

In terms of extraction yield, avocado mangoes showed the highest extraction yield among the seed extracts of other mangoes. This suggests that the chemical content of Avocado mango seeds has polarity properties that facilitate optimal extraction with ethanol. Previous studies have indicated that ethanol solvents produce better extracts than other solvents (25). The high polarity of ethanol allows for strong interactions with polar phytochemical compounds, which are pharmacologically significant and effective in reducing free radical activity (27). This finding contrasts with a study conducted by (28), who suggested a 50% ethanol-water mixture for optimal extraction of bioactive substances from mango seeds. The viscous extracts were then prepared at concentrations of 5, 10, 50, and 100 mg/mL to measure antibacterial activity using the well diffusion method, as shown in Table 3.

The inhibition zone based on the agar well diffusion test using seven types of mango seed extract, ranged from 6.25 ± 0.4 mm to 23.25 ± 1.9 mm (Table 3). The largest inhibitory zone was produced by the *Kweni* mango seed extract, with a diameter ranging from 12 ± 3 mm to 23.25 ± 1.9 mm against MDR *E. coli*. The study results exceeded those of previous studies, which reported an inhibitory rate obtained from mango seed extract against *E. coli MTCC* 46 bacteria was 8.2 ± 0.3 mm. In a study conducted by (23), the antibacterial activity of the mango seed extract with a methanol solvent at a concentration of 100 mg/dL resulted in an inhibition zone of 0.64 ± 0.25 cm and at 200 mg/dL resulted in an inhibitory zone of 1.0 ± 0.23 cm (23). Another study showed that the use of ethanol as a solvent is preferred due to its lower toxicity

compared with methanol (15). Unlike prior studies that utilised standard strain bacterial strains, this study focused on the MDR strain of *E. coli*.

Antibacterial activity was quantitatively measured using the MIC method with microdilution techniques in Mueller Hinton broth (29) The results indicated that among the seven mango cultivars tested, all exhibited antibacterial potential against both multidrug-resistant (MDR) E. coli and E. coli ATCC when 100 mL of each cultivar was subject to serial dilution across twelve microplates. Notably, the *Kweni* mango seed extract demonstrated the best MIC value of ≥0.078 (Figure 2). These findings are significantly better than those of previous studies that reported MIC values exceeding 1 mg/ml against *E. coli* (30).

The presence of various phytochemicals, including alkaloids, flavonoids, phenolics, triterpenoids, tannins, and saponins, can be attributed to the inhibition of bacterial growth by the extracts (Table 4). Previous study has shown that ethanol extracst from mango seeds contain a diverse array of phytochemicals, similar to those identified in this study (25,31). The variation in the phytochemical composition of the extracts is likely influenced by the geographical location of the samples, which affects the growth conditions of the plants. Although the antibacterial efficacy of each specific phytochemical compound found in the mango seed extract was not evaluated in this study, earlier studies have indicated that the observed antibacterial activity is linked to these compounds (23). Specifically, flavonoids have been shown to exhibit antibacterial properties through three primary mechanisms: directly killing bacteria, enhancing the effectiveness of antibiotics in synergy, and reducing bacterial pathogenicity. This research aligns with research indicating that flavonoid and phenolic compounds possess strong antibacterial properties, capable of causing bacterial cell walls lysis (32).

Table 3. Diameter of the inhibition zones of mango seed extract of seven species *of Mangifera indica* L alongside several standard antibiotics used as positive control against MDR *E. coli* and *E. coli* ATCC 14028

Seed extract	Concentration (mg/mL)	Inhibition zones (mm) against Multidrag-Resistent *Escherichia coli* strain Mean ± SD				
		***E. coli* 1E**	***E. coli* 4E**	***E. coli* 5E**	***E. coli* 7E**	**ATCC 14028**
Cengkir	5	14.75 ± 0.8	14.25 ± 0.4	10.75 ± 1	9.25 ± 0.8	9.25 ± 0.8
	10	15.75 ± 0.8	12.25 ± 1.5	10.5 ± 1.2	10 ± 0.7	10 ± 0.7
	50	17.5 ± 1.6	16.5 ± 0.8	12.75 ± 1	11.5 ± 1.1	12 ± 0
	100	19.25 ± 0.8	18.5 ± 0.5	14.25 ± 0.8	13.5 ± 1.5	14.5 ± 1.5
Kopyor	5	11.5 ± 2.5	9 ± 1.2	8.25 ± 0.4	7.5 ±0.9	7.75 ± 1.3
	10	12.75 ± 2.5	9 ± 0.7	10.25 ± 1.8	8.25 ± 1.3	10.25 ± 1.0
	50	17.75 ± 1.0	11.5 ± 1.7	12 ± 0.7	9.75 ± 0.4	11.5 ± 2.3
	100	20.5 ± 1.5	14.75 ± 1.9	13.5 ± 0.5	11.5 ± 0.5	15.5 ± 2.2
Golek	5	10.5 ± 1.1	16 ± 0.7	14.75 ± 0.8	11.75 ± 1.6	11 ± 1.0
	10	12.25 ± 1.3	17.75 ± 0.8	15.75 ± 0.4	13 ± 3.5	11.75 ± 0.8
	50	16.5 ± 1.5	19.25 ± 0.4	17.75 ± 1.9	11.75 ± 2.8	13.5 ± 1.1
	100	19 ± 2.1	21 ± 0.7	20.75 ± 1.0	13 ± 2.9	15 ± 1.2
Kweni	5	17.5 ± 0.5	15 ± 0.8	15.5 ± 0.6	15.5 ± 0.6	9.25 ± 0.8
	10	19 ± 0.7	17.5 ± 1.1	16.5 ± 1.6	12± 3	11 ± 0.7
	50	22.75 ± 0.8	20 ± 1.4	20 ± 1.2	`15 ±3.6	15.75 ± 0.8
	100	23.25 ± 1.9	22 ± 1.5	22.25 ± 0.8	18 ±3.4	19.25 ± 1.8
Avocado	5	14.75 ± 0.8	14.75 ± 0.4	14.25 ± 0.4	12.5 ± 0.8	11.75 ± 0.4
	10	15.75 ± 0.8	15.75 ± 0.4	15 ± 0	14 ±0.7	13 ± 0.7
	50	17.5 ± 1.6	18 ± 0.7	16.5 ± 0.5	13 ± 1	14.25 ± 1.0
	100	19.25 ± 0.8	19.75 ± 0.4	18.75 ± 0.4	14.75 ± 0.4	15.75 ± 0.8
Arumanis	5	7.75 ± 0.8	9.25 ± 2.8	7.75 ± 1.3	6.25 ± 0.4	7.25 ± 1.0
	10	9 ± 1.2	9.75± 2.6	8.5± 1.1	7.25 ± 0.4	8 ± 0
	50	12.5 ± 1.5	10.5 ± 1.1	12 ± 0.7	9 ± 0.7	10 ± 0
	100	16.25 ± 1.9	13.25 ± 2.7	11.5 ± 0.8	11 ± 0.7	11.25 ± 0.4
Manalagi	5	7.25 ± 0.4	6.25 ± 0.4	6.5 ± 0.5	6.5 ± 0.5	6.25 ± 0.4
	10	8 ± 0	7.25 ± 0.8	8 ± 0.7	7.75 ± 0.4	8 ± 0
	50	13 ± 1.2	9.25 ± 1.5	9.75 ± 0.4	12 ± 4	10 ± 0.7
	100	15.5 ± 0.5	12 ± 0.7	11.25 ± 0.4	10.75 ± 0.4	12.25 ± 0.4
Antibiotics	AMP 10 µg	0 ± 0.0	0 ± 0.0	0 ± 0.0	8 ± 0.0	34 ± 0.0
	CTR 30 µg	0 ± 0.0	7 ± 0.0	0 ± 0.0	0 ± 0.0	23 ± 0.0
	ATM 30 µg	0 ± 0.0	6 ± 0.0	0 ± 0.0	9 ± 0.0	0 ± 0.0
	CIP 5µg	0 ± 0.0	0 ± 0.0	28 ± 0.0	0 ± 0.0	36 ± 0.0
	GEN 10 µg	10 ± 0.0	20 ± 0.0	0 ± 0.0	20 ± 0.0	33 ± 0.0
	MRP 10 µg	18 ± 0.0	20 ± 0.0	29 ± 0.0	11 ± 0.0	45 ± 0.0
DMSO	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 4. Results of phytochemical analysis of mango seeds ethanol extract

Seed kernel extract	Phytochemical analysis						
	Flavonoid	Alkaloid	Fenolat	Triterpenoid	Tanin	Saponin	Steroid
Cengkir	+	+	+	+	+	+	-
Kopyor	+	+	+	+	+	+	-
Golek	+	+	+	+	+	+	-
Kweni	+	+	+	+	+	+	-
Alpukat	+	+	+	+	-	+	-
Arumanis	+	+	+	+	+	+	-
Manalagi	+	+	+	+	+	+	-

The phytochemical of tests conducted in this study revealed tannin content in the extract of seven mango seeds cultivars. Tannins possess anticancer, antibacterial, and antifungal properties, making them potential candidates for use as alternative drugs (33). This aligns with study conducted by (34). who suggested that tannin compounds interact with proteins on the surface of bacteria, leading to cell dysfunction. Additionally, alkaloids exhibited strong antibacterial properties. Past research has shown that alkaloids inspired the development of several antibacterial drugs, where the synthesis of quinine serendipitously led to the production of quinolones, structural modifications of azomycin resulted in metronidazole, and utilisations of quinoline scaffolds contributed to the creation of bedaquiline. Saponins present in all mango cultivars examined, also play a role in antibacterial activity by reducing the surface tension of bacterial cells. This reduction increases cell permeability and causes leakage, thereby facilitating the release of intracellular compounds. Moreover, triterpenoids identified in the extracts also possess antibacterial (34). Previous studies have addressed the antibacterial mechanism of triterpenoids, including the inhibition of oxygen absorption and oxidative phosphorylation. In this study, the MBC values of the seed extract from the seven mango cultivars ranged from 1.25 to 10 mg/mL, as illustrated in Figure 3.

Multidrug-resistant (MDR) bacteria acquire resistance through a variety of processes that reduce the effectiveness of antibiotics, such as active efflux of antibiotics via efflux pumps, enzymatic drug degradation or modification, modification of drug target sites, and decreased cell membrane permeability. Many traditional antibiotics become ineffective because of these modifications, which together reduce drug accumulation and target interaction within bacterial cells. Phytochemical compounds in ethanolic extracts of mango seed kernel, such as flavonoids, tannins, and phenolic acids, counteract these resistance mechanisms by targeting bacteria through multifaceted modes of action. They can disrupt bacterial cell membrane integrity, inhibit efflux pumps function, interfere with enzyme systems essential for bacterial survival, and prevent biofilm formation. This multitargeted approach simultaneously hinders multiple resistance pathways, reducing the likelihood of resistance development and making these phytochemicals effective against MDR strains.

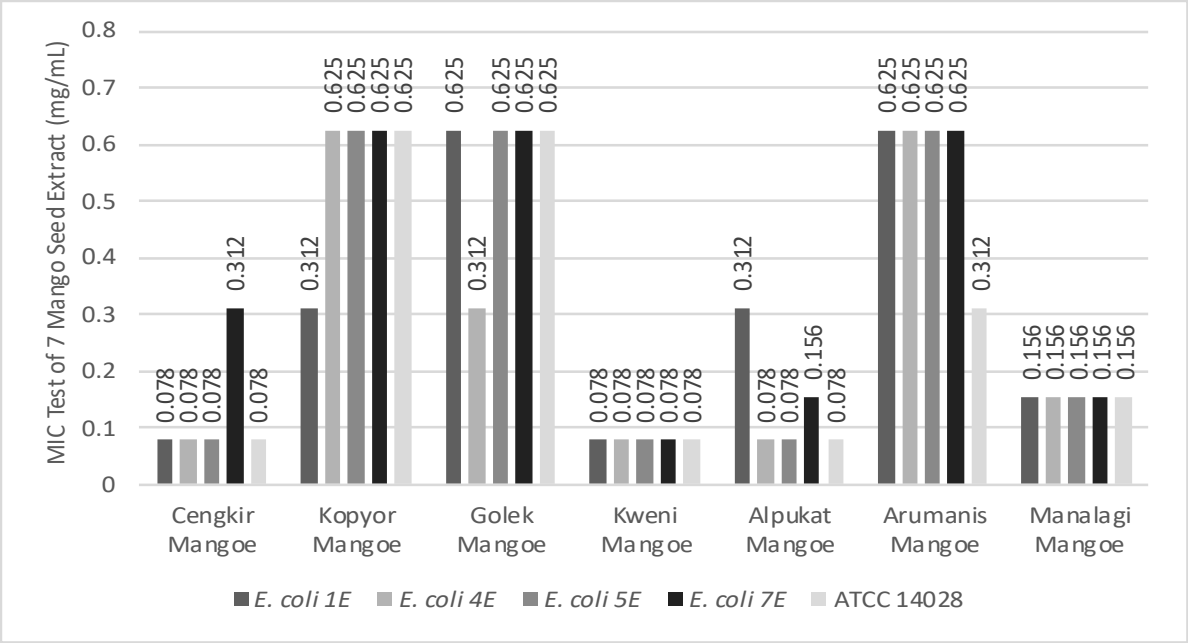

Figure 2. Graph of the MIC Test of the Seven Mango Seed Extract (mg/mL)

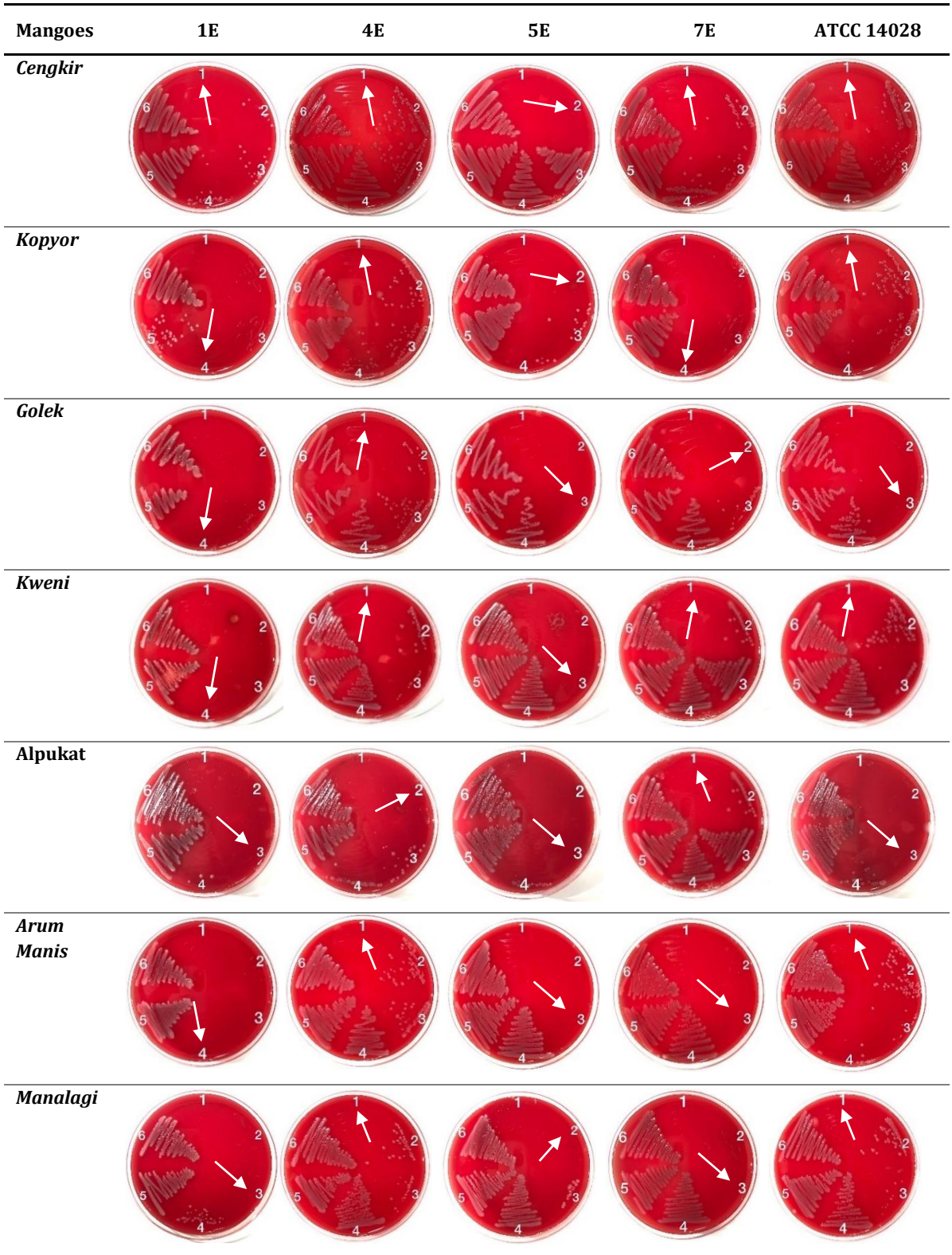

Figure 3. MBC value of the seven-mango seed extract: A) *Cengkir* mango. (B) *Kopyor* mango. (C) *Golek* mango. (D) *Kweni* mango. (E) Avocado mango. (F) *Arumanis* mango. (G) *Manalagi* mango. Where at concentration 1) 10 mg/mL. 2) 5 mg/mL; 3) 2.5 mg/mL; 4) 1.25 mg/mL; 5) 0.625 mg/mL; 6) 0.3125 mg/mL.

The observed MBC values of ≥10 mg/mL are notably better than those reported in previous studies on mango seed extract against *E. coli* (Figure 3), where the MBC was 100 mg/mL (15). These findings suggest that mango seed extract holds significant potential as an antibacterial alternative against MDR *E. coli.*

The potential of ethanolic extracts from the seed kernels of Kweni and other Indonesian mango cultivars as alternative therapeutic agents in clinical and wound treatment settings is highlighted by their encouraging in-vitro antibacterial activity against MDR bacteria. Such extracts could be turned into topical formulations for the treatment of infected wounds, particularly those containing MDR bacteria, in areas where antibiotic resistance and healthcare resources are scarce. This provide a practical and affordable solution. Additionally, their progression into preclinical and clinical trials is supported by the low minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) that have been shown, as well as the very low toxicity in early animal investigations. These actions are essential to completely clarify their pharmacodynamics, safety profiles, and ideal delivery systems.

4. CONCLUSIONS

This study indicates that seed extracts from several Mangifera cultivars exhibit antibacterial action against MDR *E. coli.* The efficacy of the extracts was verified by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), which show dose-dependent antibacterial effects. With lower MIC and MBC values than other cultivars, *Mangifera odorata* (Kweni) demonstrated the highest potency among the evaluated cultivars. These findings reveal that extracts from mango seeds, especially Kweni, are a promising natural antibacterial source that may be investigated further to create complementary or alternative approaches to treat MDR bacterial infections.

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