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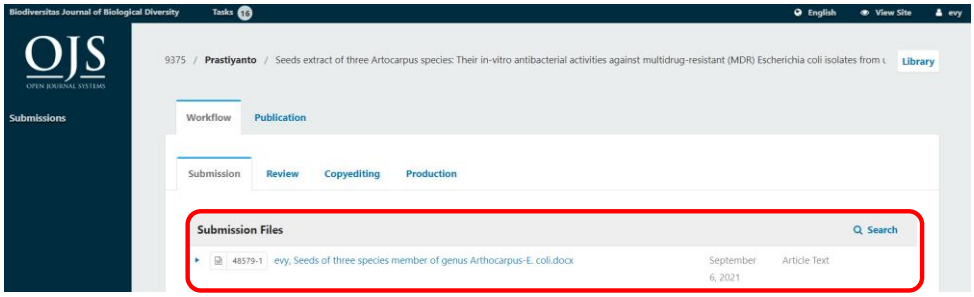

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Multidrug resistance (MDR)-*E. coli* is a major cause and a very serious problem of urinary tract infections (UTIs). As a result, it requires an antibacterial agent derived from biological materials. It has been reported that the seeds of *the Artocarpus* genus (*A. heterophyllous, A. champeden,* and *A. camansi*) have antibacterial properties against *Methicillin-Resistant Staphylococcus aureus* (MRSA). Indonesia has three *Artocarpus* species (*A. lanceipolius* [local name: *keledang*], *A. elasticus* [Local name: *Tarra*], and *A. odoratissimus* [Local name: *Terap*] whose antibacterial property has not been investigated. To minimize the research gap, this study aims to determine the antibacterial activity of seed extracts from *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* isolate of UTIs. In this manuscript, we show that *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seed extracts have the potential to be developed as antibacterial agents against UTI causing MDR-*E. coli*.

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Muhammad Evy Prastiyanto

Seeds Extract of three *Artocarpus* species: In vitro antibacterial activities against multidrug resistance (MDR) *Escherichia coli* isolates of urinary tract infections (UTIs)

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Abstract

Multidrug resistance (MDR)-*E. coli* is a major cause and a very serious problem of urinary tract infections (UTIs). As a result, it requires an antibacterial agent derived from biological materials. It has been reported that the seeds of *the Artocarpus* genus (*A. heterophyllous, A. champeden,* and *A. camansi*) have antibacterial properties against *Methicillin-Resistant Staphylococcus aureus* (MRSA). Indonesia has three *Artocarpus* species (*A. lanceipolius* [local name: *keledang*], *A. elasticus* [Local name: *Tarra*], and *A. odoratissimus* [Local name: *Terap*] whose antibacterial property has not been investigated. To minimize the research gap, this study aims to determine the antibacterial activity of seed extracts from *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* isolate of UTIs. Antibacterial activity was evaluated using the agar well diffusion assay method to determine the zone of inhibition. The microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The results have revealed that the seed extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* have the potential as antibacterial agents against MDR-*E. coli* isolate of UTIs. *A. elasticus* seed extract shows the largest zone of inhibition and the smallest MIC and MBC values, namely 7.0 ± 0.0 mm - 13.3 ± 0.4 mm with MIC value of 6.25-12.5 mg/mL and MBC of 12.5-25 mg/mL. In conclusion, *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seed extracts have the potential to be developed as antibacterial agents against UTI causing MDR-*E. coli*. Further in vivo research and the discovery of modes of action are needed to explain the antibacterial effect.

Keywords: Antibacterial activity, UTI, MDR-*E. coli*, *Artocarpus*, seeds

INTRODUCTION

Urinary tract infections (UTIs) are microbe-caused infections that are a major cause of morbidity in humans, particularly in children and newborns. Approximately 8% of girls and 2% of boys will experience at least one UTI between the ages of 1 month and 11 years (Simões e Silva et al., 2020). UTIs can affect the bladder, urethra, and kidneys, and it is estimated that approximately 150 million people worldwide suffer from UTIs each year (Zubair et al., 2019). In 2013, it would cost approximately $630 million for UTI medication and treatment in the United States (Millner and Becknell, 2019).

The most common pathogens that cause UTIs are gram-negative bacteria groups such as *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter*, *Proteus mirabilis*, *Proteus vulgaris, Citrobacter spp*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Mishra et al., 2013). On gram-positive such as *Enterococcus sp*, *Staphylococcus saprophyticus*, and *Staphylococcus aureus* (Millner and Becknell, 2019). Meanwhile, the fungi that cause UTIs include *Candida sp*. (such as *Candida albicans*, *Candida glabrata*, *Candida utilis*, *Candida kefyr*, *Candida guilliermondii*, and *Candida tropicalis*) and *Rhodotorula sp* (Behzadi et al., 2010). *E. coli* is the most common pathogen responsible for UTIs, accounting for approximately 80% to 90% of all UTI cases (Edlin et al., 2013).

Common antimicrobial agents used for UTIs are *β-lactams*, *aminoglycosides*, *quinolones*, and *trimethoprim-sulfamethoxazole* (Adwan et al., 2014). However, excessive and uncontrolled use of antimicrobial agents will cause multidrug resistance (MDR) strains of bacteria. *E. coli* strains that are resistant to extended-spectrum cephalosporins because they produce extended-spectrum b-lactamases (ESBLs) (Mukherjee et al., 2013) have recently emerged and are spreading rapidly throughout the world. This proves that the MDR-*E. coli* is now a serious public health issue that must be addressed and therefore, antibacterial agents from natural products are required.

Antibacterial agents derived from biological sources include mushrooms (Prastiyanto et al., 2016, 2020b), lactic acid bacteria (Lestari et al., 2019), latex (Prastiyanto et al., 2020c), fruits (Prastiyanto et al., 2020d) and seeds (Prastiyanto et al., 2020a). Many studies in the field of antimicrobial agents report the importance of plants as alternatives to antimicrobial agents (Prastiyanto et al., 2021a; Prastiyanto et al., 2021b). It has been reported that seeds of Artocarpus genus (*A. heterophyllus* [jackfruit], *A. champeden* [cempedak], and *A. camansi* [breadfruit]) have potential as antibacterial agents against Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Prastiyanto et al., 2020a). Aside from the three species of *Artocarpus* members, three more species are found throughout Indonesia, namely *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus*. There has never been any research reporting the antibacterial properties of the seeds of these species. This study aims to fill a research gap by determining the antibacterial potential of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seeds against UTI-causing MDR-*E. coli*.

MATERIALS AND METHODS

Seeds and extractions

The seeds of *A. lanceipolius* [local name: keledang], *A. elasticus* [Local name: Tarra], and *A. odoratissimus* [Local Name: Terap]), detailed in Figure 1, were collected in February 2021 from different regions in Indonesia. *A. lanceipolius* was collected from East Kutai Regency, East Kalimantan (1°05'31.9"N 116°55'00.9"E), *A. elasticus* was obtained from Luwu Regency, South Sulawesi (3°18'19.4"S 120°18'26.4"E), while *A. odoratissimus* was gathered from Tabalong Regency, South Kalimantan (2.198547°S 115.349065°E). The names of the seeds have been confirmed to the owners and local residents.

All seeds were washed under running water to remove dirt. The seeds were dried under the sun for seven days, then milled to obtain seed powder. Extraction was performed by maceration with a 96% ethanol solvent. 100g of each seed powder was extracted with 300 mL of ethanol for 24 hours at room temperature. The solvent replacement was carried out every 24 hours until the solution became clear, assuming that all active compounds in the powder had dissolved in ethanol. The supernatant was filtered with Whatman No.1 paper. The maceration results were concentrated using a rotary evaporator at a temperature of 40 °C to obtain crude extract.

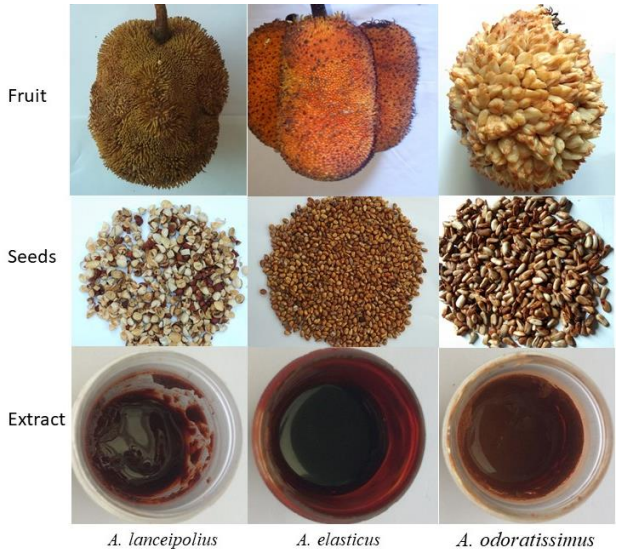

Figure 1. Photographs of *Artocarpus* (Photos were taken by Muhammad Evy Prastiyanto)

Isolation of *E.coli* from urine and antibiotic sensitivity tests

MDR-*E. coli* was isolated from urine samples collected from patients suffering from UTIs at RS. Dr. Kariadi Semarang in Central Java, Indonesia. All isolates were identified using MacConkey Agar (MCA) media, as well as biochemical tests with Vitek®MS and bacterial sensitivity tests using the Clinical Laboratory Standard Institute M100-S25 minimum inhibitory interpretation (MIC) standard (CLSI, 2019).

AntiMDR-*E.coli* of seed extracts

Agar well diffusion

The antibacterial activities of the seeds of three *Artocarpus* species were evaluated using a well-diffusion assay (Prastiyanto et al., 2020c). All MDR-*E. coli* bacterial isolates obtained from UTI patients were reinvigorated with blood agar plate media and incubated for 24 hours at 35±3 °C. All MDR-*Ecoli* isolates were standardized with 0.5 McFarland. Using a sterile cotton swab, each isolate was inoculated on Muller Hilton agar (MHA) media. After five minutes, the MHA media was perforated with a cork borer (0.5 cm in diameter) with four holes for each extract concentration (0.1 mg/mL, 1 mg/mL, 10 mg/mL, and 100 mg/mL) using *dimethyl sulfoxide* (DMSO) as a diluent. Each test was carried out four times. For each concentration, 100 L of the extract was added to each well. All treatments were incubated for 16-20 hours at 35±2 °C. Positive controls included *ampicillin*, *ceftriaxone*, *aztreonam*, *ciprofloxacin*, *gentamicin*, and *meropenem* (MRP), while negative controls included DMSO. The antibacterial activities of the seed extracts against MDR-*E. coli* were determined by measuring the inhibition zone diameter in mm. The broadest zone of inhibition was the best way to define antibacterial activity.

Determination of minimum inhibitory concentration (MIC) value

The MIC value of each extract was determined by the microdilution method using Muller Hilton broth (MHB) media (CLSI, 2018) on a microwell plate (Prastiyanto et al., 2020c). In this test, a slight modification was carried

out by adding 2,3,5-*Triphenyltetrazolium chloride* 0.05% to the MHB medium. 100 µL of MHB was added to each well, and 100 µL of seed extract was added to the first well, followed by a series of dilutions until reaching well 12. 10 µL of MDR-*E. coli* bacterial suspension was added for each treatment. The MIC value was determined by selecting the lowest concentration of seed extract that inhibited the growth of the MDR-*E. coli* bacteria by observing the color change on the microwell plate and compared to the control. The best antibacterial activity was indicated by the lowest MIC value.

Determination of minimum bactericidal concentration (MBC) value

MBC was the continuation of MIC. MIC wells were sub-cultured to a 5% sheep BAP at (35 ± 2) °C for 16–20 hours of incubation. The MBC value was determined by considering the growth of bacteria on BAP media. The value of MBC is defined as the lowest concentration of seed extract which shows that MDR-*E. coli* bacteria cannot grow (Yin et al., 2018). The best antibacterial activity was specified by the lowest MBC value.

Phytochemical Screening of Extract

Phytochemical analysis (tannins and flavonoids) of the crude extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seeds was carried out with using previously described methods (Eve et al., 2020)

RESULTS AND DISCUSSION

Extract yield

The results of extraction with ethanol solvent of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seeds are presented in Table 1. *A. elasticus* showed higher yield values than the other two species. This shows that *A. elasticus* seeds have more active substances. Ethanol solvents produced extracts with better antibacterial potential than the other solvents (Prastiyanto et al., 2020d). These results are in line with the results of previous research on ethanol extract in *A. heterophyllus* seeds which produced higher solubility of the active substances than the hexanes (Eve et al., 2020)

Table 1. The extract yield

Scientific name	Local name	Part of plants	Yield (%)
A. lanceipolius	*Keledang*	seeds	8.80
A. elasticus	*Tarra*	seeds	21.00
A. odoratissimus	*Terap*	seeds	16.80

MDR-*E. coli* isolates of urinary tract infections (UTIs)

MDR-*E. coli* was isolated from urine samples of people with UTIs. The results of the bacterial sensitivity test to antibiotics are detailed in Table 2. The results of the identification and sensitivity test of bacteria to antibiotics show that *E. coli* from UTIs sufferers was an MDR strain because it was resistant to at least three classes of antibiotics. MDR-*E. coli* strain #1 was resistant to *β* lactam (*penicillin* and *cephalosporin*), monobactam (*aztreonam*), aminoglicosides (*gentamicin*), and fluroquinolone (*ciprofloxacine*). MDR-*E. coli* strain #2 was resistant to *β* lactam antibiotics, aminoglicosides and fluroquinolone. MDR-*E. coli* strain #3 was resistant to *β* lactam antibiotics, monobactam, and fluroquinolone. MDR-E. coli strain #4 was resistant to *β* lactam antibiotics, monobactam, and aminoglicosides. Meanwhile, the MDR-E. coli strain #5 was resistant to *β* lactam, monobactam, carbapemen, and fluroquinolone groups. *E. coli* bacterium is the major cause of UTIs in the world. The results of this study have revealed that the five *E. coli* isolates were extended-spectrum beta-lactamase (ESBL)-producing *E. coli* because the five isolates were resistant to penicillin, cephalosporin, and monobactam antibiotics. Isolate #5 was an ESBL-producing *E. coli* and an isolate that was resistant to the carbapenem group. According to Mazzariol *et al*. (2017), ESBL-producing *E. coli* has become the main cause of MDR *E. coli* cases that trigger UTIs (Mazzariol et al., 2017).

Table 2. The organisms for in vitro antibacterial screening in this study

Species	Source	Antibiotic resistance pattern
MDR-*E. coli #1*	34 years, female, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, gentamicin, ciprofloxacine*
MDR-*E. coli #2*	29 years, male, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, gentamicin, ciprofloxacine, sulfamethoxazole*
MDR-*E. coli #3*	9 months, male, urine	*Ampicillin, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, ciprofloxacin nitrofurantoin, sulfamethoxazole*
MDR-*E. coli #4*	2 years, male, urine	*Ampicillin, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, gentamicin, sulfamethoxazole*
MDR-*E. coli #5*	2 years, male, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, ciprofloxacine, sulfamethoxazole*

The antibacterial activities of extracts against MDR-*E. coli*

Agar well diffusion assay

The antibacterial activities of seeds *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* were evaluated using agar well diffusion assay against MDR-*E. coli* bacteria from UTI patients. The antibacterial activities of the seed extracts at concentrations of 0.1 mg/mL, 1 mg/mL, 10 mg/mL, and 100 mg/mL were determined by measuring the inhibition zone diameter in mm against five MDR-*E. coli* isolates, as seen in Table 3.

The results of this study show that the three extracts had antibacterial activities against MDR-*E. coli*, as indicated by the presence of an inhibition zone. The inhibition zone formed from *A. lanceipolius* seed extract was 0.0 ± 0.0 - 11.8 ± 0.8 mm, while the inhibition zone of standard bacteria of ATCC 25922 had a larger diameter, 7.3 ± 0.4 mm - 13.5 ± 0.5 mm. The inhibition zone formed from *A. elasticus* seed extract was 7.0 ± 0.0 mm - 13.3 ± 0.4 mm, while the inhibition zone of standard bacteria of ATCC 25922 appeared to have a larger diameter, 10.3 ± 1.1 mm -14.8 ± 0.4 mm. The inhibition zone diameter of *A. odoratissimus* seed extract was 6.0 ± 0.0 mm - 12.5 ± 0.5 mm, while the inhibition zone diameter of bacteria ATCC 25922 was 9.0 ± 0.7 mm - 14.0 ± 0.7 mm. The findings also depict that the seed extracts of three Artocarpus species' had a larger inhibition zone than the control antibiotics. In addition, the inhibition zone of standard bacteria was greater than that of the MDR bacteria. *A. elasticus* seed extract appeared to show better antibacterial activities than *A. lanceipolius* and *A. odoratissimus* extracts because it had a larger inhibition zone than the other two extracts.

This study concluded the potential of the seed ethanol extract of three species of the *Artocarpus* genus, including *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* as antibacterial agents against MDR-*E. coli* because previous research (Eve, *et al*., 2020) reported that the ethanol extract of *A. heterophyllus* seeds which is a member of the *Artocarpus* genus did not have antibacterial activities against MDR-*E. coli*.

Table 3. The diameters of the inhibition zones of seeds of *Artocarpus* (with *ampicillin* (AMP), *ceftriaxone* (CTR), *aztreonam* (ATM), *ciprofloxacin* (CIP), *gentamicin* (GEN), and *meropenem* (MRP) as the positive controls)

Seed extract	Concentration (mg/mL)	The inhibition zones of seeds of *Artocarpus (mm)*					
		MDR-*E. coli*					*E.coli standard*
		1	**2**	**3**	**4**	**5**	ATCC 25922
A. lanceipolius	0.1	6.3± 0.4	7.3± 0.4	7.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	7.3 ± 0.4
	1	7.0± 0.7	8.3 ± 0.4	7.3 ± 0.4	9.0 ± 0.8	7.5 ± 0.5	8.8± 0.8
	10	9.0± 0.4	10.5 ± 0.5	10.3 ± 0.8	10.0 ± 0.7	9.3 ± 0.4	10.3 ± 0.4
	100	10.0± 0.5	11.8 ± 0.8	10.5 ± 0.9	10.5 ± 0.5	10.8 ± 0.4	13.5 ± 0.5
A. elasticus	0.1	9.0 ± 0.7	7.0 ± 0.0	11.3 ± 0.8	9.5 ± 0.5	7.0 ± 0.0	10.3 ± 1.1
	1	9.8 ± 0.4	10.0 ± 0.7	11.8 ± 0.4	10.0 ± 0	10.5 ± 0.5	14.8 ± 0.4

	10	10.8 ± 0.4	9.3 ± 0.4	12.3 ± 0.4	11.0 ± 0	12.3 ± 0.4	10.3 ± 0.4
	100	11.8 ± 0.4	12.0 ± 0.0	12.8 ± 0.4	11.5 ± 0.5	13.3 ± 0.4	14.3 ± 0.4
A. odoratissimus	0.1	7.3 ± 0.4	7.0 ± 0.0	7.3 ± 0.4	6.0 ± 0.0	6.0 ± 0.0	9.0 ± 0.7
	1	7.8 ± 0.4	7.5 ± 0.5	8.5 ± 0.5	8.0 ± 1.0	7.3 ± 0.4	10.5 ± 0.5
	10	9.5 ± 0.5	9.3 ± 0.4	11.5 ± 0.5	10.5 ± 0.5	11.8 ± 0.4	10.3 ± 0.5
	100	11.3 ± 0.4	11.5 ± 0.5	12.5 ± 0.5	10.8 ± 0.8	12.5 ± 0.5	14.0 ± 0.7
Antibiotic	AMP 10 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	CTR 30 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.0 ± 0.0
	ATM 30 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	23.0 ± 0.0
	CIP 5 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	35.0 ± 0.0
	GEN 10 µg	9.0 ± 0.0	7.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	1.0 ± 0.0	19.0 ± 0.0
	MRP 10 µg	9.0 ± 0.0	10.0 ± 0.0	9.0± 0.0	11.0 ± 0.0	0.0 ± 0.0	33.0 ± 0.0
DMSO	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

MIC and MBC

MIC values of the seed ethanol extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* from UTIs patients were decided by the microdilution method using a microwell plate, as shown in Figure 2. The results revealed that the MIC values of the three extracts ranged from 12.5 mg/mL to 6.25 mg/mL. The *A. elasticus* seed extract showed better results than the other two extracts because it had a lower MIC value of 6.25 mg/mL for almost all MDR-*E. coli*. MDR-E coli #5 was the only isolate indicating a MIC value of 25 mg/mL. MDR-*E. coli* #5 is (ESBL)-producing *E. coli* + carbapenem-resistant, making this isolate possible to be more resistant to antibacterial agents.

The results also showed a better value than some previous studies. Seed extracts of *A. heterophyllus*, *A. champeden*, and *A. camansi* against methicillin-resistant *Staphylococcus aureus* (MRSA) had MIC values of 15.62 mg/mL(Prastiyanto et al., 2020a). *A. heterophyllus* seed extract had a MIC value of 125 mg/mL against MDR-*Pseudomonas aeruginosa* (Eve et al., 2020)

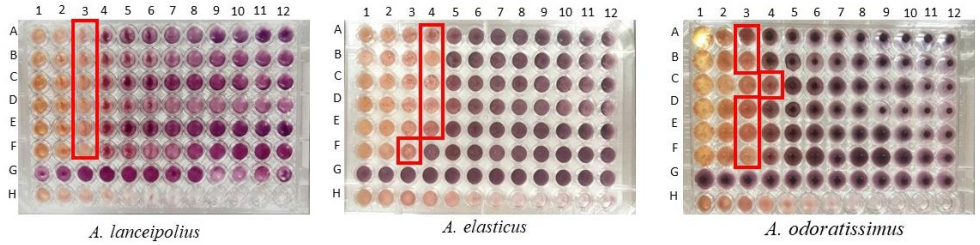

Figure 2. MIC values of *A. lanceipolius*, *A. elasticus* and *A. odoratissimus* seed extracts against MDR-*E. coli:* A. MDR-*E. coli* #1, B. MDR-*E. coli* #2, C. MDR-*E. coli* #3, D. MDR-*E. coli* #4, E. MDR-*E. coli* #5, F. *E. coli* ATCC 25922. At concentrations: 1). 50 mg/mL, 2). 25 mg/mL, 3). 12.5 mg/mL, 4). 6.25 mg/mL, 5). 3.13 mg/mL, 6). 1.56 mg/mL, 7). 0.78 mg/mL, 8). 039 mg/mL, 9) 0.19 mg/mL, 10) 0.09 mg/mL, 11). 0.04 mg/mL, 12). 0.02 mg/mL (Photos were taken by Muhammad Evy Prastiyanto)

The MBC values of the seed ethanol extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* were determined by the microdilution method using a microwell plate from the results of the MIC cultured on BAP media, as presented in Figure 3. The results exhibited that the three extracts had MBC values ranging from 25 to 12.5 mg/mL. *A. elasticus* seed extract showed better results than the other two extracts

because it had a lower MBC value of 12.5 mg/mL for almost all MDR and only MDR-*E. coli* #5, which was (ESBL)-producing *E. coli* + carbapenem-resistant, showed an MBC value of 25 mg/mL. The outcomes of this study presented better values of MBC than previous studies. Seed extracts of *A. heterophyllus* (MBC: 62.25 mg/mL), *A. champeden* (MBC: 31.25 mg/mL) and *A. camansi* (MBC: 250 mg/mL) had the antibacterial activities against MRSA (Prastiyanto et al., 2020a). Another study reported that *A. heterophyllus* leaf extracts did not have antibacterial activities against *E. coli*, as indicated by 0 MIC and 0 MBC (Mishra and Padhy, 2013)

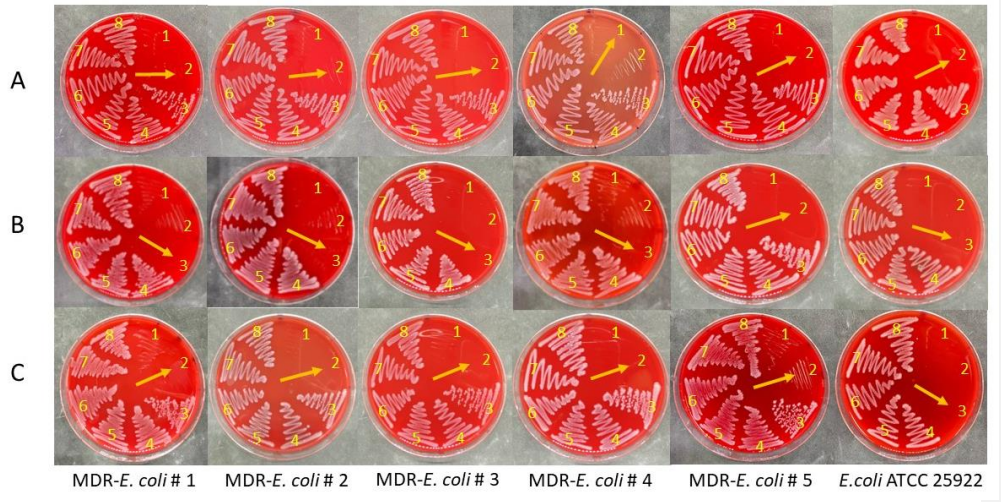

Figure 3. MBC values of *A. lanceipolius*, *A. elasticus* and *A. odoratissimus* seeds against MDR-*E. coli*: A) *A. lanceipolius,* B) *A. elasticus,* C). *A. Odoratissimus.* Concentrations: 1). 50 mg/mL, 2). 25 mg/mL, 3). 12.5 mg/mL, 4). 6.25 mg/mL, 5). 3.13 mg/mL, 6). 1.56 mg/mL, 7). 0.78 mg/mL, 8). 039 mg/mL, : MBC values. (Photos were taken by Muhammad Evy Prastiyanto)

Phytochemical Screening of Extracts

Qualitative screening of phytochemical contents, including flavonoids and tannins, was carried out on all extracts. The phytochemical contents are demonstrated in Table 3.

Table 4. The results of the phytochemical analysis of seed extracts

Seed extract	Phytochemicals content	
	Flavonoids	Tannins
A. lanceipolius	+	+
A. elasticus	+	+
A. odoratissimus	+	+

The results disclosed that flavonoids and tannins were present in all seed extracts. Flavonoids are secondary metabolites of *2-phenyl-benzyl-γ-pyrone* derivatives and these compounds are most commonly discovered in plants (Buer et al., 2010) because flavonoids are compounds that are known to be synthesized by plants in response to infection. According to Cushnie & Lamb (2005), many compounds from flavonoids have potential as antibacterial agents (Cushnie and Lamb, 2005). In this study, the mechanism of MDR-*E.coli* inhibition by flavonoids from *Artocarpus* seed extracts was not identified. However, *quercetin*, *apigenin*, and *3,6,7,3 ', 4'– pentahydroxyflavone* were reported as *gyrase* DNA-inhibiting flavonoids from *E. coli* (Ohemeng et al., 1993).

Tannins were also contained in all *Artocarpus* seed extracts. Tannins are the most abundant polyphenols in edible plants (Chung et al., 1998). Many studies reported the potential of tannins as antibacterial agents. A previous study (Dabbaghi et al., 2019) reported the antibacterial activities of tannins against *E. coli* dependent on the content of *phenolic hydroxyl* groups. This present study did not report the inhibition mechanism of tannins in *Artocarpus* seed extracts. However, several research reports suggested that tannins interfere with cell metabolism (Belhaoues et al., 2020).

In conclusion, the seed extracts of three Artocarpus species are potential to be developed as antibacterial agents against UTI-causing MDR-*E. coli*. *A. elasticus* seed extract has better potential than the other two seed extracts. Further in vivo research and the discovery of modes of action are required to explain the antibacterial effects.

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2 Bukti konfirmasi review dan hasil review 21 September 2021

Notifications

[biodiv] Editor Decision

2021-09-21 06:22 AM

Muhammad Evy Prastiyanto:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Seeds extract of three Artocarpus species: In vitro antibacterial activities against multidrug resistance (MDR) Escherichia coli isolates of urinary tract infections (UTIs)".

Our decision is: Revisions Required

Reviewer A:
Recommendation: Revisions Required

Seeds extract of three *Artocarpus* species: Their in-vitro antibacterial activities against multidrug-resistant (MDR) *Escherichia coli* isolates from urinary tract infections (UTIs)

Abstract. Multidrug-resistant (MDR)-*E. coli* is a major cause and has become a very serious problem in urinary tract infections (UTIs). As a result, it requires an antibacterial agent derived from biological materials. It has been reported that the seeds of three species of *Artocarpus* (*A. heterophyllous*, *A. champeden*, and *A. camansi*) have antibacterial properties against *Methicillin-Resistant Staphylococcus aureus* (MRSA). However, there are three other *Artocarpus* species in Indonesia, i.e., keledang (*A. lanceipolius*), tarra (*A. elasticus*), and terap (*A. Odoratissimus*) whose antibacterial property has not been investigated. To minimize the research gap, this study aims to determine the antibacterial activity of seed extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* isolates of UTIs. Antibacterial activity was evaluated using the agar well diffusion assay. The microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The results revealed that the seed extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* have the potential as antibacterial agents against MDR-*E. coli* isolate of UTIs. *A. elasticus* seed extract shows the widest zone of inhibition in the range of 7.0-13.3 mm and the smallest MIC and MBC values of 6.25-12.5 mg/mL and 12.5-25 mg/mL, respectively. In conclusion, *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus*

seed extracts have the potential to be developed as antibacterial agents against UTI-causing MDR-*E. coli*. Further in vivo research and determining the mode of action of antibacterial activity are needed.

Keywords: Antibacterial activity, UTI, MDR-*E. coli*, *Artocarpus*, seeds

INTRODUCTION

Urinary tract infections (UTIs) are microbe-caused infections that are a major cause of morbidity in humans, particularly in children and newborns. Approximately 8% of girls and 2% of boys experience at least one UTI between the ages of 1 month and 11 years (Simões e Silva et al., 2020). UTIs can affect the bladder, urethra, and kidneys, and it is estimated that approximately 150 million people worldwide suffer from UTIs each year (Zubair et al., 2019). In 2013, it cost approximately $630 million for UTI medication and treatment in the United States (Millner and Becknell, 2019).

The most common pathogens that cause UTIs are Gram-negative bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter*, *Proteus mirabilis*, *Proteus vulgaris, Citrobacter* spp., *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Mishra et al., 2013), Gram-positive such as *Enterococcus* sp., *Staphylococcus saprophyticus*, and *Staphylococcus aureus* (Millner and Becknell, 2019). Meanwhile, the fungi that cause UTIs include *Candida* sp. (such as *Candida albicans*, *Candida glabrata*, *Candida utilis*, *Candida kefyr*, *Candida guilliermondii*, and *Candida tropicalis*) and *Rhodotorula* sp. (Behzadi et al., 2010). *E. coli* is the most common pathogen responsible for UTIs, accounting for approximately 80% to 90% of UTI cases (Edlin et al., 2013).

Commonly used antimicrobial agents for UTIs are *β-lactams*, *aminoglycosides*, *quinolones*, and *trimethoprim-sulfamethoxazole* (Adwan et al., 2014). However, excessive and uncontrolled use of antimicrobial agents causes bacterial strains to become multidrug-resistant (MDR). *E. coli* strains that are resistant to extended-spectrum cephalosporins producing extended-spectrum β-lactamases (ESBLs) (Mukherjee et al., 2013) emerged recently and are spreading worldwide rapidly. This proves that the MDR-*E. coli* is now a serious public health issue that must be addressed and therefore, antibacterial agents from natural products are required to overcome bacterial resistance.

Antibacterial agents derived from biological sources include mushrooms (Prastiyanto et al., 2016, 2020b), lactic acid bacteria (Lestari et al., 2019), latex (Prastiyanto et al., 2020c), fruits (Prastiyanto et al., 2020d), and seeds (Prastiyanto et al., 2020a). Many studies on antimicrobial agents report the importance of plants as alternative antimicrobial agents (Prastiyanto et al., 2021a; Prastiyanto et al., 2021b). It has been reported that seeds of three species of *Artocarpus*, namely jackfruit (*A. heterophyllus*), cempedak (*A. champeden*), and breadfruit (*A. camansi*) have the potential as antibacterial agents against Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Prastiyanto et al., 2020a). Aside from the three species of *Artocarpus*, other species of Artocarpus are found throughout Indonesia, namely *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* but no study has reported the antibacterial properties of the seeds of these species. This study aims to fill the research gap by determining the antibacterial potential of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seeds against MDR-*E. Coli* causing UTIs.

MATERIALS AND METHODS

Seed Collection and extractions

The seeds of keledang (*A. lanceipolius*), tarra (*A. elasticus*), and terap (*A. odoratissimus*) (Figure 1.) were collected in February 2021 from different regions in Indonesia. *A. lanceipolius* was collected from East Kutai Regency, East Kalimantan (1°05'31.9"N 116°55'00.9"E), *A. elasticus* was obtained from Luwu Regency, South Sulawesi (3°18'19.4"S 120°18'26.4"E), while *A. odoratissimus* was gathered from Tabalong Regency, South

Kalimantan (2.198547°S 115.349065°E). The local names of the plants have been confirmed by the owners and residents.

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The collected seeds were washed under running water to remove dirt. The seeds were dried under the sun for seven days, then ground to obtain seed powder. Extraction was performed by maceration with a 96% ethanol solvent. One hundred grams of each seed powder was extracted with 300 mL of ethanol for 24 hours at room temperature. The solvent replacement was carried out every 24 hours until the solution became clear, assuming that all active compounds in the powder had dissolved in ethanol. The supernatant was filtered with Whatman No.1 paper. Filtrates were concentrated using a rotary evaporator at a temperature of 40 °C to obtain crude extract.

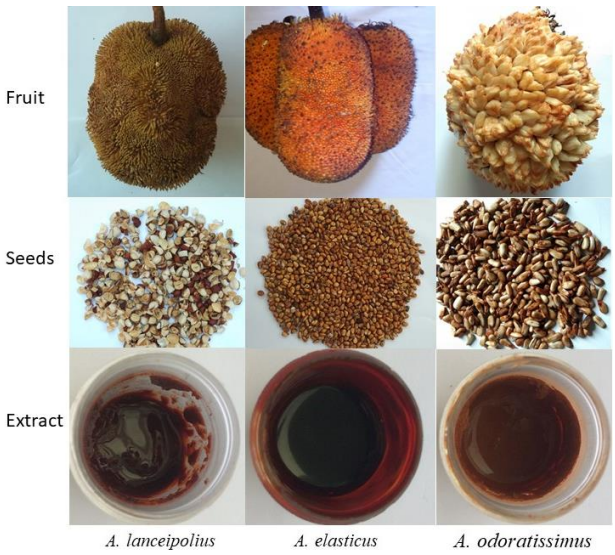

Figure 1. Fruits, seeds, and extracts of three species of *Artocarpus* (Photos were taken by Muhammad Evy Prastiyanto)

Isolation of *E.coli* from urine and the antibiotic sensitivity tests

MDR-*E. coli* was isolated from urine samples collected from patients suffering from UTIs in RS. Dr. Kariadi Semarang, Central Java, Indonesia. All isolates were identified using MacConkey Agar (MCA) media, as well as biochemical tests with Vitek®MS and bacterial sensitivity tests using the Clinical Laboratory Standard Institute M100-S25 for minimum inhibitory concentration (MIC) interpretation (CLSI, 2019).

Antibacterial activity of seed extracts against MDR-*E.coli*

Agar well diffusion

The antibacterial activity of the seed extract of three *Artocarpus* species was evaluated using a well-diffusion assay (Prastiyanto et al., 2020c). All isolates of MDR-*E. coli* obtained from UTI patients were cultured on blood agar plate media and incubated for 24 hours at 35±3°C. All MDR-*E. coli* isolates were standardized with 0.5 McFarland. Each isolate was inoculated on Muller Hilton Agar (MHA) media using a sterile cotton swab. After five minutes, the MHA media was perforated with a cork borer (0.5 cm in diameter). Four holes for each extract concentration: 0.1 mg/ml, 1 mg/ml, 10 mg/ml, and 100 mg/ml). The extract was dissolved with dimethyl sulfoxide (DMSO). Each test was carried out in four replicates. One hundred µL of each concentration of the extract was added to each well and then incubated for 16-20 hours at 35±2°C. Positive controls included ampicillin, ceftriaxone, aztreonam, ciprofloxacin, gentamicin, and meropenem (MRP), while DMSO was used as a negative control. Antibacterial activity of seed extract against MDR-*E. coli* was determined by measuring the diameter of the inhibition zone (mm). The widest zone of inhibition is the best antibacterial activity.

Determination of minimum inhibitory concentration (MIC)

The MIC value of each extract was determined by the microdilution method using Muller Hilton broth (MHB) media (CLSI, 2018) on a microwell plate (Prastiyanto et al., 2020c). In this test, a slight modification was carried out by adding 2,3,5-*Triphenyltetrazolium chloride* 0.05% to the MHB medium. 100 µL of MHB was added to each well, and 100 µL of seed extract was added to the first well, followed by a series of dilutions until reaching well 12th. After finish diluting, 10 µL of MDR-*E. coli* bacterial suspension (.....CFU/ml) was added to each well except……. The MIC value was determined by observing the lowest concentration of seed extract that inhibited the growth of MDR-*E. coli* bacteria. It was indicated by the color change on the microwell plate and compared to the control. The best antibacterial activity of the extract was indicated by the lowest MIC value.

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Determination of minimum bactericidal concentration (MBC)

MBC was the continuation of MIC. The mixture of MIC well was sub-cultured on a 5% sheep BAP at (35 ± 2)°C and incubated for 16–20 hours. The MBC value was determined by observing the growth of bacteria on BAP media. The value of MBC is defined as the lowest concentration of seed extract which shows that MDR-*E. coli* bacteria cannot grow (Yin et al., 2018). The best antibacterial activity was specified by the lowest MBC value.

Phytochemical Screening of Extract

Phytochemical analysis (tannins and flavonoids) of the crude extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seeds was carried out using previously described methods (Eve et al., 2020)

RESULTS AND DISCUSSION

Extract yield

The results of seed extract of three species of *Artocarpus* (*A. lanceipolius, A. elasticus, and A. odoratissimus*) with ethanol as a solvent are presented in Table 1. *A. elasticus* showed a higher yield than the other two species. This shows that *A. elasticus* seeds have higher chemical substances that can be extracted in ethanol. Ethanol solvents produced extracts with better antibacterial potential than the other solvents (Prastiyanto et

al., 2020d). These results are in line with the results of previous research on an ethanol extract of *A. heterophyllus* seeds which produced higher solubility of the active substances than the hexanes (Eve et al., 2020).

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Table 1. The extract yield of three species of *Artocarpus*

Scientific name	Local name	Part of plants	Yield (%)
A. lanceipolius	*Keledang*	seeds	8.80
A. elasticus	*Tarra*	seeds	21.00
A. odoratissimus	*Terap*	seeds	16.80

MDR-*E. coli* isolates from urinary tract infections (UTIs)

MDR-*E. coli* was isolated from urine samples of patients with UTIs. The results of the bacterial sensitivity test to antibiotics are presented in Table 2. The results of the identification and sensitivity test of bacteria to antibiotics show that *E. coli* from UTI patients was an MDR strain because it was resistant to at least three classes of antibiotics. MDR-*E. coli* strain #1 was resistant to *β*-lactams (*penicillin* and *cephalosporin*), monobactams (*aztreonam*), aminoglicosides (*gentamicin*), and fluoroquinolones (*ciprofloxacine*). MDR-*E. coli* strain #2 was resistant to *β*-lactam antibiotics, aminoglicosides and fluroquinolone. MDR-*E. coli* strain #3 was resistant to *β*-lactam antibiotics, monobactams, and fluoroquinolones. MDR-*E. coli* strain #4 was resistant to *β*-lactam antibiotics, monobactams, and aminoglicosides. Meanwhile, the MDR-*E. coli* strain #5 was resistant to *β* lactams, monobactams, carbapenem, and fluoroquinolones groups. *E. coli* bacterium is the major cause of UTIs in the world. The results of this study revealed that five isolates of *E. coli* collected from urine samples of UTI patients were *E.coli* producing extended-spectrum beta-lactamase (ESBL)- because all of these isolates were resistant to penicillin, cephalosporin, and monobactam antibiotics. Isolate #5 was an ESBL-producing *E. coli* and an isolate that was resistant to the carbapenem group. According to Mazzariol *et al.* (2017), ESBL-producing *E. coli* is the main cause of MDR *E. coli* cases that trigger UTIs (Mazzariol et al., 2017).

Table 2. Resistance screening of *E,coli* isolated from urine samples of UT I patients to several antibiotics

Species	Source	Antibiotic resistance pattern
MDR-*E. coli #1*	34 years, female, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, gentamicin, ciprofloxacine*
MDR-*E. coli #2*	29 years, male, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, gentamicin, ciprofloxacine, sulfamethoxazole*
MDR-*E. coli #3*	9 months, male, urine	*Ampicillin, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, ciprofloxacin nitrofurantoin, sulfamethoxazole*
MDR-*E. coli #4*	2 years, male, urine	*Ampicillin, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, gentamicin, sulfamethoxazole*
MDR-*E. coli #5*	2 years, male, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, ciprofloxacine, sulfamethoxazole*

The antibacterial activities of extracts against MDR-*E. coli*

Agar well diffusion assay

The antibacterial activities of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seed extracts were evaluated using agar well diffusion assay against MDR-*E. coli* bacteria from UTI patients. The antibacterial activities of the seed extracts at concentrations of 0.1 mg/ml, 1 mg/ml, 10 mg/ml, and 100 mg/mL were determined by measuring the inhibition zone diameter (mm) against five isolates of MDR-*E. coli* (Table 3.)

The results of this study show that three extracts of *Artocarpus* seeds had antibacterial activities against MDR-*E. coli*, as indicated by the presence of an inhibition zone. The inhibition zone of *A. lanceipolius* seed extract against MDR-*E.coli* was from 0.0 ± 0.0 to 11.8 ± 0.8 mm, while its inhibition zone against *E.coli* ATCC 25922 was wider, i.e., from 7.3 ± 0.4 mm to 13.5 ± 0.5 mm. The inhibition zone of *A. elasticus* seed extract against MDR *E.coli* was from 7.0 ± 0.0 mm to 13.3 ± 0.4 mm, while its inhibition zone against standard *E.coli* ATCC 25922 was wider, i.e., 10.3 ± 1.1 mm to 14.8 ± 0.4 mm. The inhibition zone of *A. odoratissimus* seed extract against MDR *E.coli* was 6.0 ± 0.0 mm - 12.5 ± 0.5 mm, while the inhibition zone against *E.coli* ATCC 25922 was 9.0 ± 0.7 mm - 14.0 ± 0.7 mm. The findings showed that the seed extracts of three Artocarpus species' had a wider inhibition zone than the control antibiotics. In addition, the inhibition zone against the *E,coli* standard was wider than that of the MDR- *E.coli*. *A. elasticus* seed extract had better antibacterial activities than *A. lanceipolius* and *A. odoratissimus* extracts because it had a wider inhibition zone than the other two extracts.

The results of this study can be concluded that the seed ethanol extract of three species of *Artocarpus,* namely *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* has the potential as antibacterial agents against MDR-*E. coli*. However, previous research by Eve *et al*. (2020) showed that the ethanol extract of *A. heterophyllus* seeds which is a member of the genus *Artocarpus* did not have antibacterial activities against MDR-*E. coli*.

Table 3. Diameter of inhibition zones of seed extract of three species of *Artocarpus* and several antibiotics standard as the positive control against MDR-*E.coli* and *E.coli* ATCC 25922

Seed extract	Concentration (mg/mL)	The inhibition zones (mm) against					
		MDR-*E. coli*					*E.coli standard*
		1	**2**	**3**	**4**	**5**	ATCC 25922
A. lanceipolius	0.1	6.3± 0.4	7.3± 0.4	7.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	7.3 ± 0.4
	1	7.0± 0.7	8.3 ± 0.4	7.3 ± 0.4	9.0 ± 0.8	7.5 ± 0.5	8.8± 0.8
	10	9.0± 0.4	10.5 ± 0.5	10.3 ± 0.8	10.0 ± 0.7	9.3 ± 0.4	10.3 ± 0.4
	100	10.0± 0.5	11.8 ± 0.8	10.5 ± 0.9	10.5 ± 0.5	10.8 ± 0.4	13.5 ± 0.5
A. elasticus	0.1	9.0 ± 0.7	7.0 ± 0.0	11.3 ± 0.8	9.5 ± 0.5	7.0 ± 0.0	10.3 ± 1.1
	1	9.8 ± 0.4	10.0 ± 0.7	11.8 ± 0.4	10.0 ± 0	10.5 ± 0.5	14.8 ± 0.4
	10	10.8 ± 0.4	9.3 ± 0.4	12.3 ± 0.4	11.0 ± 0	12.3 ± 0.4	10.3 ± 0.4
	100	11.8 ± 0.4	12.0 ± 0.0	12.8 ± 0.4	11.5 ± 0.5	13.3 ± 0.4	14.3 ± 0.4
A. odoratissimus	0.1	7.3 ± 0.4	7.0 ± 0.0	7.3 ± 0.4	6.0 ± 0.0	6.0 ± 0.0	9.0 ± 0.7
	1	7.8 ± 0.4	7.5 ± 0.5	8.5 ± 0.5	8.0 ± 1.0	7.3 ± 0.4	10.5 ± 0.5
	10	9.5 ± 0.5	9.3 ± 0.4	11.5 ± 0.5	10.5 ± 0.5	11.8 ± 0.4	10.3 ± 0.5
	100	11.3 ± 0.4	11.5 ± 0.5	12.5 ± 0.5	10.8 ± 0.8	12.5 ± 0.5	14.0 ± 0.7
Antibiotic	AMP 10 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

	CTR 30 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.0 ± 0.0
	ATM 30 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	23.0 ± 0.0
	CIP 5 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	35.0 ± 0.0
	GEN 10 µg	9.0 ± 0.0	7.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	1.0 ± 0.0	19.0 ± 0.0
	MRP 10 µg	9.0 ± 0.0	10.0 ± 0.0	9.0± 0.0	11.0 ± 0.0	0.0 ± 0.0	33.0 ± 0.0
DMSO	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Determination of MIC and MBC value

The MIC value of the seed ethanol extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* from UTI patients were performed by the microdilution method using a microwell plate, as shown in Figure 2. The results revealed that the MIC values of the three extracts ranged from 12.5 mg/mL to 6.25 mg/mL. The *A. elasticus* seed extract showed better antibacterial activity than the other two extracts because it had a lower MIC value of 6.25 mg/mL for almost all MDR-*E. coli*. MDR-E coli #5 was the only isolate with a MIC value of 25 mg/mL. MDR-*E. coli* #5 is (ESBL)-which is an *E. coli* + carbapenem-resistant producer, so this isolate might be more resistant to antibacterial agents.

The results of this study also showed better MIC value than some previous studies. Seed extracts of *A. heterophyllus*, *A. champeden*, and *A. camansi* against methicillin-resistant *Staphylococcus aureus* (MRSA) had MIC values of 15.62 mg/mL(Prastiyanto et al., 2020a). *A. heterophyllus* seed extract had a MIC value of 125 mg/mL against MDR-*Pseudomonas aeruginosa* (Eve et al., 2020)

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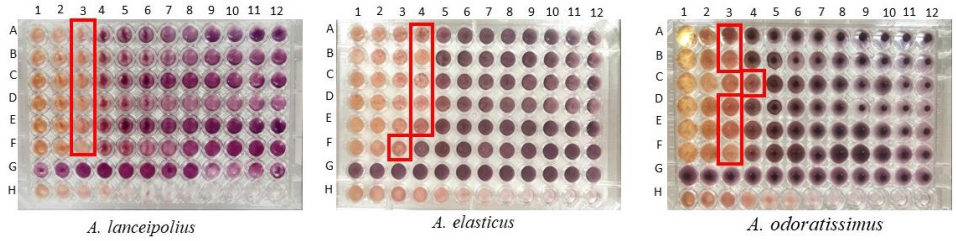

Figure 2. MIC values of *A. lanceipolius*, *A. elasticus* and *A. odoratissimus* seed extracts against MDR-*E. coli:* A. MDR-*E. coli* #1, B. MDR-*E. coli* #2, C. MDR-*E. coli* #3, D. MDR-*E. coli* #4, E. MDR-*E. coli* #5, F. *E. coli* ATCC 25922. At concentration of: 1). 50 mg/mL, 2). 25 mg/mL, 3). 12.5 mg/mL, 4). 6.25 mg/mL, 5). 3.13 mg/mL, 6). 1.56 mg/mL, 7). 0.78 mg/mL, 8). 039 mg/mL, 9) 0.19 mg/mL, 10) 0.09 mg/mL, 11). 0.04 mg/mL, 12). 0.02 mg/mL (Photos were taken by Muhammad Evy Prastiyanto)

The MBC value of the seed ethanol extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* was determined by the microdilution method using a mixture of MIC cultures in a microwell plate and cultured on BAP media, as presented in Figure 3. The results exhibited that three extracts had MBC values ranging from 25 to 12.5 mg/mL. *A. elasticus* seed extract showed better results than the other two extracts because it had a lower MBC value of 12.5 mg/mL for almost all MDR and only MDR-*E. coli* #5, which was

(ESBL)- *E. coli* + carbapenem-resistant producer, showed an MBC value of 25 mg/mL. The results of this study presented better values of MBC than previous studies. The MBC values of seed extracts of *A. heterophyllus*, *A. champeden,* and *A. camansi* against MRSA were 62.25 mg/ml, 31.25 mg/ml, and 250 mg/ml, respectively.) (Prastiyanto et al., 2020a). Another study reported that *A. heterophyllus* leaf extracts did not have antibacterial activities against *E. coli*, as indicated by MIC and MBC values of 0 (Mishra and Padhy, 2013).

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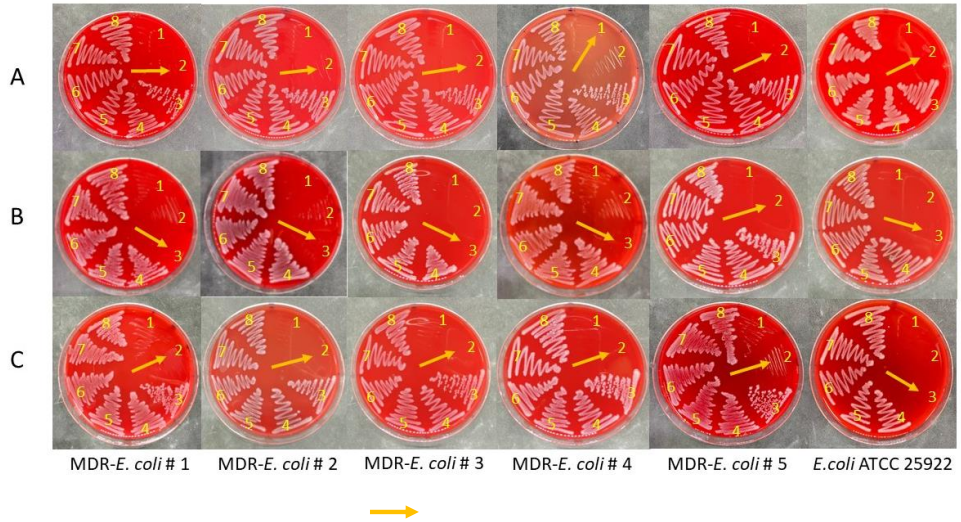

Figure 3. MBC values of *A. lanceipolius*, *A. elasticus* and *A. odoratissimus* seed extract against MDR-*E. coli*: A) *A. lanceipolius,* B) *A. elasticus,* C). *A. Odoratissimus.* Concentrations: 1). 50 mg/mL, 2). 25 mg/mL, 3). 12.5 mg/mL, 4). 6.25 mg/mL, 5). 3.13 mg/mL, 6). 1.56 mg/mL, 7). 0.78 mg/mL, 8). 039 mg/mL, : MBC values. (Photos were taken by Muhammad Evy Prastiyanto)

Phytochemical Screening of Extracts

Qualitative screening of phytochemical contents, i.e., flavonoids and tannins, were carried out on all extracts (Table 3.)

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Table 4. The results of the phytochemical analysis of seed extracts

Seed extract	Phytochemicals content	
	Flavonoids	Tannins
A. lanceipolius	+	+
A. elasticus	+	+
A. odoratissimus	+	+

The results revealed that all seed extracts contain flavonoids and tannins. Flavonoids are secondary metabolites of *2-phenyl-benzyl-γ-pyrone* derivatives and these compounds are most commonly found in plants (Buer et al., 2010) because flavonoids are compounds that are known to be synthesized by plants in response to infection. According to Cushnie & Lamb (2005), many compounds of flavonoids have the potential as antibacterial agents (Cushnie and Lamb, 2005). In this study, the mechanism of inhibition of MDR-*E.coli* by flavonoids from *Artocarpus* seed extracts was not known yet. However, *quercetin*, *apigenin*, and *3,6,7,3 ', 4'–pentahydroxyflavone* were reported as *gyrase* DNA-inhibiting flavonoids of *E. coli* (Ohemeng et al., 1993).

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Tannins were also contained in all *Artocarpus* seed extracts. Tannins are the most abundant polyphenols in edible plants (Chung et al., 1998). Many studies reported the potential of tannins as antibacterial agents. A previous study (Dabbaghi et al., 2019) reported the antibacterial activities of tannins against *E. coli* was depend on the content of *phenolic hydroxyl* groups. However, the inhibition mechanism of tannins in *Artocarpus* seed extracts was not performed in this study. Several previous research suggested that tannins interfere with cell metabolism (Belhaoues et al., 2020).

In conclusion, the seed extracts of three *Artocarpus* species are potential to be developed as an antibacterial against UTI-causing MDR-*E. coli*. *A. elasticus* seed extract has better potency than the other two seed extracts. Further in vivo research and study regarding the mode of action are necessary to be carried out.

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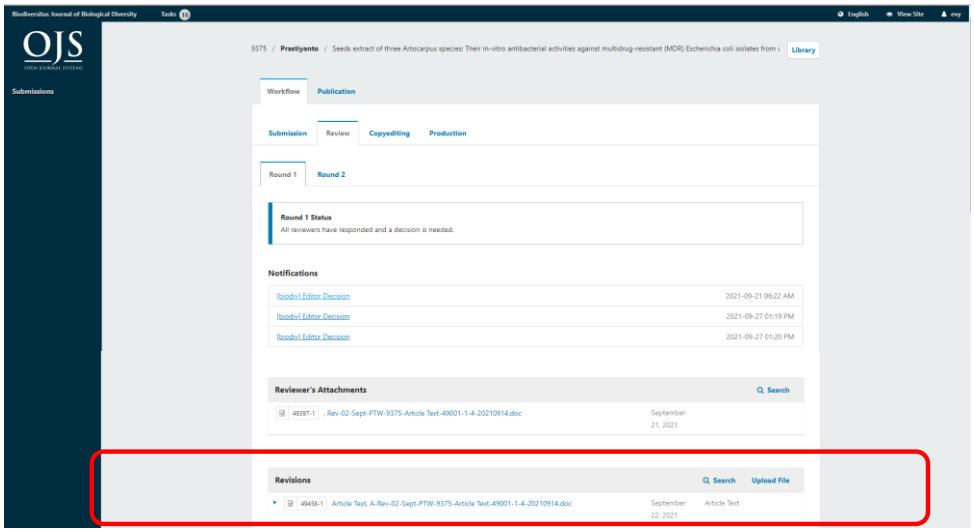
3 Bukti konfirmasi submit revisi dan artikel yang diresubmit (22 September 2021)



Seeds extract of three *Artocarpus* species: Their in-vitro antibacterial activities against multidrug-resistant (MDR) *Escherichia coli* isolates from urinary tract infections (UTIs)

Abstract. Multidrug-resistant (MDR)-*E. coli* is a major cause and has become a very serious problem in urinary tract infections (UTIs). As a result, it requires an antibacterial agent derived from biological materials. It has been reported that the seeds of three species of *Artocarpus* (*A. heterophyllous*, *A. champeden*, and *A. camansi*) have antibacterial properties against *Methicillin-Resistant Staphylococcus aureus* (MRSA). However, there are three other *Artocarpus* species in Indonesia, i.e., keledang (*A. lanceipolius*), tarra (*A. elasticus*), and terap (*A. Odoratissimus*) whose antibacterial property has not been investigated. To minimize the research gap, this study aims to determine the antibacterial activity of seed extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* isolates of UTIs. Antibacterial activity was evaluated using the agar well diffusion assay. The microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The results revealed that the seed extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* have the potential as antibacterial agents against MDR-*E. coli* isolate of UTIs. *A. elasticus* seed extract shows the widest zone of inhibition in the range of 7.0-13.3 mm and the smallest MIC and MBC values of 6.25-12.5 mg/mL and 12.5-25 mg/mL, respectively. In conclusion, *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seed extracts have the potential to be developed as antibacterial agents against UTI-causing MDR-*E. coli*. Further in vivo research and determining the mode of action of antibacterial activity are needed.

Keywords: Antibacterial activity, UTI, MDR-*E. coli*, *Artocarpus*, seeds

INTRODUCTION

Urinary tract infections (UTIs) are microbe-caused infections that are a major cause of morbidity in humans, particularly in children and newborns. Approximately 8% of girls and 2% of boys experience at least one UTI between the ages of 1 month and 11 years (Simões e Silva et al., 2020). UTIs can affect the bladder, urethra, and kidneys, and it is estimated that approximately 150 million people worldwide suffer from UTIs each year (Zubair et al., 2019). In 2013, it cost approximately $630 million for UTI medication and treatment in the United States (Millner and Becknell, 2019).

The most common pathogens that cause UTIs are Gram-negative bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter*, *Proteus mirabilis*, *Proteus vulgaris, Citrobacter* spp., *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Mishra et al., 2013), Gram-positive such as *Enterococcus* sp., *Staphylococcus saprophyticus*, and *Staphylococcus aureus* (Millner and Becknell, 2019). Meanwhile, the fungi that cause UTIs include *Candida* sp. (such as *Candida albicans*, *Candida glabrata*, *Candida utilis*, *Candida kefyr*, *Candida guilliermondii*, and *Candida tropicalis*) and *Rhodotorula* sp. (Behzadi et al., 2010). *E. coli* is the most common pathogen responsible for UTIs, accounting for approximately 80% to 90% of UTI cases (Edlin et al., 2013).

Commonly used antimicrobial agents for UTIs are *β-lactams*, *aminoglycosides*, *quinolones*, and *trimethoprim-sulfamethoxazole* (Adwan et al., 2014). However, excessive and uncontrolled use of antimicrobial agents causes bacterial strains to become multidrug-resistant (MDR). *E. coli* strains that are resistant to extended-spectrum cephalosporins producing extended-spectrum β-lactamases (ESBLs) (Mukherjee et al., 2013) emerged recently and are spreading worldwide rapidly. This proves that the MDR-*E. coli* is now a serious public health issue that must be addressed and therefore, antibacterial agents from natural products are required to overcome bacterial resistance.

Antibacterial agents derived from biological sources include mushrooms (Prastiyanto et al., 2016, 2020b), lactic acid bacteria (Lestari et al., 2019), latex (Prastiyanto et al., 2020c), fruits (Prastiyanto et al., 2020d), and seeds (Prastiyanto et al., 2020a). Many studies on antimicrobial agents report the importance of plants as alternative antimicrobial agents (Prastiyanto et al., 2021a; Prastiyanto et al., 2021b). It has been reported that seeds of three species of *Artocarpus*, namely jackfruit (*A. heterophyllus*), cempedak (*A. champeden*), and breadfruit (*A. camansi*) have the potential as antibacterial agents against Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Prastiyanto et al., 2020a). Aside from the three species of *Artocarpus*, other species of Artocarpus are found throughout Indonesia, namely *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* but no study has reported the antibacterial properties of the seeds of these species. This study aims to fill the research gap by determining the antibacterial potential of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seeds against MDR-*E. Coli* causing UTIs.

MATERIALS AND METHODS

Seed Collection and extractions

The seeds of keledang (*A. lanceipolius*), tarra (*A. elasticus*), and terap (*A. odoratissimus*) (Figure 1.) were collected in February 2021 from different regions in Indonesia. *A. lanceipolius* was collected from East Kutai Regency, East Kalimantan (1°05'31.9"N 116°55'00.9"E), *A. elasticus* was obtained from Luwu Regency, South Sulawesi (3°18'19.4"S 120°18'26.4"E), while *A. odoratissimus* was gathered from Tabalong Regency, South Kalimantan (2.198547°S 115.349065°E). The local names of the plants have been confirmed by the owners and the scientific names of the plants were identified by Department of Biology, Universitas Negeri Semarang

The collected seeds were washed under running water to remove dirt. The seeds were dried under the sun for seven days, then ground to obtain seed powder. Extraction was performed by maceration with a 96% ethanol

solvent. One hundred grams of each seed powder was extracted with 300 mL of ethanol for 24 hours at room temperature. The solvent replacement was carried out every 24 hours until the solution became clear, assuming that all active compounds in the powder had dissolved in ethanol. The supernatant was filtered with Whatman No.1 paper. Filtrates were concentrated using a rotary evaporator at a temperature of 40 °C to obtain crude extract.

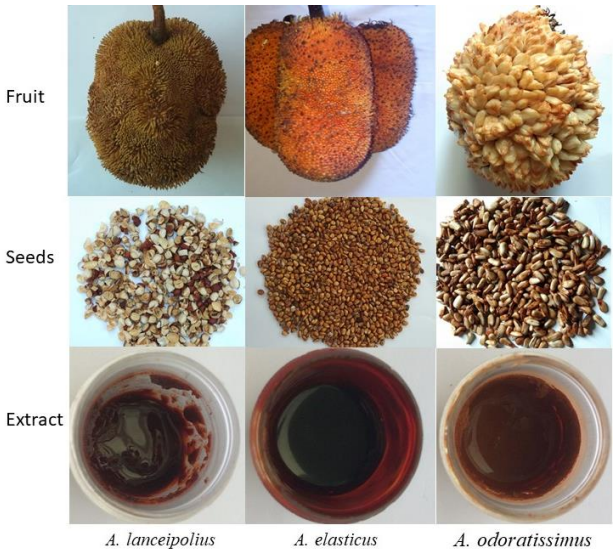


Figure 1. Fruits, seeds, and extracts of three species of *Artocarpus* (Photos were taken by Muhammad Evy Prastiyanto)

Isolation of *E.coli* from urine and the antibiotic sensitivity tests

MDR-*E. coli* was isolated from urine samples collected from patients suffering from UTIs in RS. Dr. Kariadi Semarang, Central Java, Indonesia. All isolates were identified using MacConkey Agar (MCA) media, as well as biochemical tests with Vitek®MS and bacterial sensitivity tests using the Clinical Laboratory Standard Institute M100-S25 for minimum inhibitory concentration (MIC) interpretation (CLSI, 2019).

Antibacterial activity of seed extracts against MDR-*E.coli*

Agar well diffusion

The antibacterial activity of the seed extract of three *Artocarpus* species was evaluated using a well-diffusion assay (Prastiyanto et al., 2020c). All isolates of MDR-*E. coli* obtained from UTI patients were cultured on blood agar plate media and incubated for 24 hours at 35±3°C. All MDR-*E. coli* isolates were standardized with 0.5

McFarland. Each isolate was inoculated on Muller Hilton Agar (MHA) media using a sterile cotton swab. After five minutes, the MHA media was perforated with a cork borer (0.5 cm in diameter). Four holes for each extract concentration: 0.1 mg/ml, 1 mg/ml, 10 mg/ml, and 100 mg/ml). The extract was dissolved with dimethyl sulfoxide (DMSO). Each test was carried out in four replicates. One hundred µL of each concentration of the extract was added to each well and then incubated for 16-20 hours at 35±2°C. Positive controls included ampicillin, ceftriaxone, aztreonam, ciprofloxacin, gentamicin, and meropenem (MRP), while DMSO was used as a negative control. Antibacterial activity of seed extract against MDR-*E. coli* was determined by measuring the diameter of the inhibition zone (mm). The widest zone of inhibition is the best antibacterial activity.

Determination of minimum inhibitory concentration (MIC)

The MIC value of each extract was determined by the microdilution method using Muller Hilton broth (MHB) media (CLSI, 2018) on a microwell plate (Prastiyanto et al., 2020c). In this test, a slight modification was carried out by adding 2,3,5-*Triphenyltetrazolium chloride* 0.05% to the MHB medium. 100 µL of MHB was added to each well, and 100 µL of seed extract was added to the first well, followed by a series of dilutions until reaching well 12th. After finish diluting, 10 µL of MDR-*E. coli* bacterial suspension 0.5 McFarland standards (1.5×10^8CFU/mL) was added to each well except……. The MIC value was determined by observing the lowest concentration of seed extract that inhibited the growth of MDR-*E. coli* bacteria. It was indicated by the color change on the microwell plate and compared to the control. The best antibacterial activity of the extract was indicated by the lowest MIC value.

Determination of minimum bactericidal concentration (MBC)

MBC was the continuation of MIC. The mixture of MIC well was sub-cultured on a 5% sheep BAP at (35 ± 2)°C and incubated for 16–20 hours. The MBC value was determined by observing the growth of bacteria on BAP media. The value of MBC is defined as the lowest concentration of seed extract which shows that MDR-*E. coli* bacteria cannot grow (Yin et al., 2018). The best antibacterial activity was specified by the lowest MBC value.

Phytochemical Screening of Extract

Phytochemical analysis (tannins and flavonoids) of the crude extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seeds was carried out using previously described methods (Eve et al., 2020)

RESULTS AND DISCUSSION

Extract yield

The results of seed extract of three species of *Artocarpus* (*A. lanceipolius, A. elasticus, and A. odoratissimus*) with ethanol as a solvent are presented in Table 1. *A. elasticus* showed a higher yield than the other two species. This shows that *A. elasticus* seeds have higher chemical substances that can be extracted in ethanol. Ethanol solvents produced extracts with better antibacterial potential than the other solvents (Prastiyanto et al., 2020d). These results are in line with the results of previous research on an ethanol extract of *A. heterophyllus* seeds which produced higher solubility of the active substances than the hexanes (Eve et al., 2020). This can be attributed to the higher solubility of the seed phytochemicals in ethanol than in hexane. The high polarity of ethanol causes strong interactions with most of the polar phytochemicals from the seed

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T. Tsukatani *et al. 2009*
Colorimetric microbial viability assay based on reduction of water-soluble tetrazolium salts for antimicrobial susceptibility testing and screening of antimicrobial substances

Anal. Biochem.

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extract, so it has a better extraction potential when compared to hexane which extracts most of the nonpolar phytochemical compounds (Sbihi et al., 2018)

Table 1. The extract yield of three species of *Artocarpus*

Scientific name	Local name	Part of plants	Yield (%)
A. lanceipolius	*Keledang*	seeds	8.80
A. elasticus	*Tarra*	seeds	21.00
A. odoratissimus	*Terap*	seeds	16.80

MDR-*E. coli* isolates from urinary tract infections (UTIs)

MDR-*E. coli* was isolated from urine samples of patients with UTIs. The results of the bacterial sensitivity test to antibiotics are presented in Table 2. The results of the identification and sensitivity test of bacteria to antibiotics show that *E. coli* from UTI patients was an MDR strain because it was resistant to at least three classes of antibiotics. MDR-*E. coli* strain #1 was resistant to *β*-lactams (*penicillin* and *cephalosporin*), monobactams (*aztreonam*), aminoglicosides (*gentamicin*), and fluoroquinolones (*ciprofloxacine*). MDR-*E. coli* strain #2 was resistant to *β*-lactam antibiotics, aminoglicosides and fluroquinolone. MDR-*E. coli* strain #3 was resistant to *β*-lactam antibiotics, monobactams, and fluoroquinolones. MDR-*E. coli* strain #4 was resistant to *β*-lactam antibiotics, monobactams, and aminoglicosides. Meanwhile, the MDR-*E. coli* strain #5 was resistant to *β* lactams, monobactams, carbapenem, and fluoroquinolones groups. *E. coli* bacterium is the major cause of UTIs in the world. The results of this study revealed that five isolates of *E. coli* collected from urine samples of UTI patients were *E.coli* producing extended-spectrum beta-lactamase (ESBL)- because all of these isolates were resistant to penicillin, cephalosporin, and monobactam antibiotics. Isolate #5 was an ESBL-producing *E. coli* and an isolate that was resistant to the carbapenem group. According to Mazzariol *et al*. (2017), ESBL-producing *E. coli* is the main cause of MDR *E. coli* cases that trigger UTIs (Mazzariol et al., 2017).

Table 2. Resistance screening of *E,coli* isolated from urine samples of UT I patients to several antibiotics

Species	Source	Antibiotic resistance pattern
MDR-*E. coli #1*	34 years, female, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, gentamicin, ciprofloxacine*
MDR-*E. coli #2*	29 years, male, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, gentamicin, ciprofloxacine, sulfamethoxazole*
MDR-*E. coli #3*	9 months, male, urine	*Ampicillin, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, ciprofloxacin nitrofurantoin, sulfamethoxazole*
MDR-*E. coli #4*	2 years, male, urine	*Ampicillin, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, gentamicin, sulfamethoxazole*
MDR-*E. coli #5*	2 years, male, urine	*Ampicillin, sulbactam, tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, ciprofloxacine, sulfamethoxazole*

The antibacterial activities of extracts against MDR-*E. coli*

Agar well diffusion assay

The antibacterial activities of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* seed extracts were evaluated using agar well diffusion assay against MDR-*E. coli* bacteria from UTI patients. The antibacterial activities of the

seed extracts at concentrations of 0.1 mg/ml, 1 mg/ml, 10 mg/ml, and 100 mg/mL were determined by measuring the inhibition zone diameter (mm) against five isolates of MDR-*E. coli* (Table 3.)

The results of this study show that three extracts of *Artocarpus* seeds had antibacterial activities against MDR-*E. coli*, as indicated by the presence of an inhibition zone. The inhibition zone of *A. lanceipolius* seed extract against MDR-*E.coli* was from 0.0 ± 0.0 to 11.8 ± 0.8 mm, while its inhibition zone against *E.coli* ATCC 25922 was wider, i.e., from 7.3 ± 0.4 mm to 13.5 ± 0.5 mm. The inhibition zone of *A. elasticus* seed extract against MDR *E.coli* was from 7.0 ± 0.0 mm to 13.3 ± 0.4 mm, while its inhibition zone against standard *E.coli* ATCC 25922 was wider, i.e., 10.3 ± 1.1 mm to 14.8 ± 0.4 mm. The inhibition zone of *A. odoratissimus* seed extract against MDR *E.coli* was 6.0 ± 0.0 mm - 12.5 ± 0.5 mm, while the inhibition zone against *E.coli* ATCC 25922 was 9.0 ± 0.7 mm - 14.0 ± 0.7 mm. The findings showed that the seed extracts of three Artocarpus species' had a wider inhibition zone than the control antibiotics. In addition, the inhibition zone against the *E,coli* standard was wider than that of the MDR- *E.coli*. *A. elasticus* seed extract had better antibacterial activities than *A. lanceipolius* and *A. odoratissimus* extracts because it had a wider inhibition zone than the other two extracts.

The results of this study can be concluded that the seed ethanol extract of three species of *Artocarpus,* namely *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* has the potential as antibacterial agents against MDR-*E. coli*. However, previous research by Eve *et al*. (2020) showed that the ethanol extract of *A. heterophyllus* seeds which is a member of the genus *Artocarpus* did not have antibacterial activities against MDR-*E. coli*.

Table 3. Diameter of inhibition zones of seed extract of three species of *Artocarpus* and several antibiotics standard as the positive control against MDR-*E.coli* and *E.coli* ATCC 25922

Seed extract	Concentration (mg/mL)	The inhibition zones (mm) against					
		MDR-*E. coli*					*E.coli standard*
		1	**2**	**3**	**4**	**5**	ATCC 25922
A. lanceipolius	0.1	6.3± 0.4	7.3± 0.4	7.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	7.3 ± 0.4
	1	7.0± 0.7	8.3 ± 0.4	7.3 ± 0.4	9.0 ± 0.8	7.5 ± 0.5	8.8± 0.8
	10	9.0± 0.4	10.5 ± 0.5	10.3 ± 0.8	10.0 ± 0.7	9.3 ± 0.4	10.3 ± 0.4
	100	10.0± 0.5	11.8 ± 0.8	10.5 ± 0.9	10.5 ± 0.5	10.8 ± 0.4	13.5 ± 0.5
A. elasticus	0.1	9.0 ± 0.7	7.0 ± 0.0	11.3 ± 0.8	9.5 ± 0.5	7.0 ± 0.0	10.3 ± 1.1
	1	9.8 ± 0.4	10.0 ± 0.7	11.8 ± 0.4	10.0 ± 0	10.5 ± 0.5	14.8 ± 0.4
	10	10.8 ± 0.4	9.3 ± 0.4	12.3 ± 0.4	11.0 ± 0	12.3 ± 0.4	10.3 ± 0.4
	100	11.8 ± 0.4	12.0 ± 0.0	12.8 ± 0.4	11.5 ± 0.5	13.3 ± 0.4	14.3 ± 0.4
A. odoratissimus	0.1	7.3 ± 0.4	7.0 ± 0.0	7.3 ± 0.4	6.0 ± 0.0	6.0 ± 0.0	9.0 ± 0.7
	1	7.8 ± 0.4	7.5 ± 0.5	8.5 ± 0.5	8.0 ± 1.0	7.3 ± 0.4	10.5 ± 0.5
	10	9.5 ± 0.5	9.3 ± 0.4	11.5 ± 0.5	10.5 ± 0.5	11.8 ± 0.4	10.3 ± 0.5
	100	11.3 ± 0.4	11.5 ± 0.5	12.5 ± 0.5	10.8 ± 0.8	12.5 ± 0.5	14.0 ± 0.7
Antibiotic	AMP 10 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	CTR 30 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.0 ± 0.0

	ATM 30 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	23.0 ± 0.0
	CIP 5 µg	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	35.0 ± 0.0
	GEN 10 µg	9.0 ± 0.0	7.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	1.0 ± 0.0	19.0 ± 0.0
	MRP 10 µg	9.0 ± 0.0	10.0 ± 0.0	9.0± 0.0	11.0 ± 0.0	0.0 ± 0.0	33.0 ± 0.0
DMSO	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Determination of MIC and MBC value

The MIC value of the seed ethanol extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* from UTI patients were performed by the microdilution method using a microwell plate, as shown in Figure 2. The results revealed that the MIC values of the three extracts ranged from 12.5 mg/mL to 6.25 mg/mL. The *A. elasticus* seed extract showed better antibacterial activity than the other two extracts because it had a lower MIC value of 6.25 mg/mL for almost all MDR-*E. coli*. MDR-E coli #5 was the only isolate with a MIC value of 25 mg/mL. MDR-*E. coli* #5 is (ESBL)-which is an *E. coli* + carbapenem-resistant producer, so this isolate might be more resistant to antibacterial agents.

The results of this study also showed better MIC value than some previous studies. Seed extracts of *A. heterophyllus*, *A. champeden*, and *A. camansi* against methicillin-resistant *Staphylococcus aureus* (MRSA) had MIC values of 15.62 mg/mL(Prastiyanto et al., 2020a). *A. heterophyllus* seed extract had a MIC value of 125 mg/mL against MDR-*Pseudomonas aeruginosa* (Eve et al., 2020)

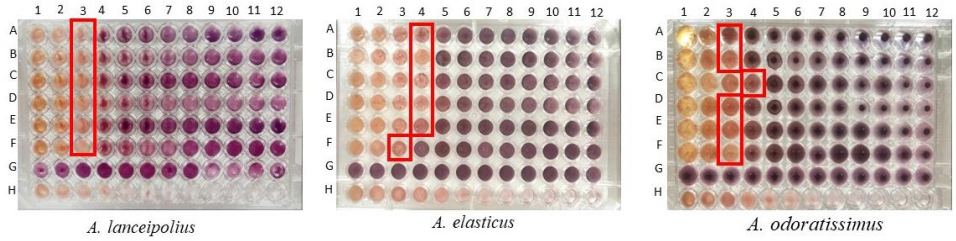

Figure 2. MIC values of *A. lanceipolius*, *A. elasticus* and *A. odoratissimus* seed extracts against MDR-*E. coli:* A. MDR-*E. coli* #1, B. MDR-*E. coli* #2, C. MDR-*E. coli* #3, D. MDR-*E. coli* #4, E. MDR-*E. coli* #5, F. *E. coli* ATCC 25922. At concentration of: 1). 50 mg/mL, 2). 25 mg/mL, 3). 12.5 mg/mL, 4). 6.25 mg/mL, 5). 3.13 mg/mL, 6). 1.56 mg/mL, 7). 0.78 mg/mL, 8). 039 mg/mL, 9) 0.19 mg/mL, 10) 0.09 mg/mL, 11). 0.04 mg/mL, 12). 0.02 mg/mL (Photos were taken by Muhammad Evy Prastiyanto)

The MBC value of the seed ethanol extracts of *A. lanceipolius*, *A. elasticus*, and *A. odoratissimus* against MDR-*E. coli* was determined by the microdilution method using a mixture of MIC cultures in a microwell plate and cultured on BAP media, as presented in Figure 3. The results exhibited that three extracts had MBC values ranging from 25 to 12.5 mg/mL. *A. elasticus* seed extract showed better results than the other two extracts because it had a lower MBC value of 12.5 mg/mL for almost all MDR and only MDR-*E. coli* #5, which was (ESBL)- *E. coli* + carbapenem-resistant producer, showed an MBC value of 25 mg/mL. The results of this study

presented better values of MBC than previous studies. The MBC values of seed extracts of *A. heterophyllus*, *A. champeden,* and *A. camansi* against MRSA were 62.25 mg/ml, 31.25 mg/ml, and 250 mg/ml, respectively.) (Prastiyanto et al., 2020a). Another study reported that *A. heterophyllus* leaf extracts did not have antibacterial activities against *E. coli*, as indicated by MIC and MBC values of 0 (Mishra and Padhy, 2013).

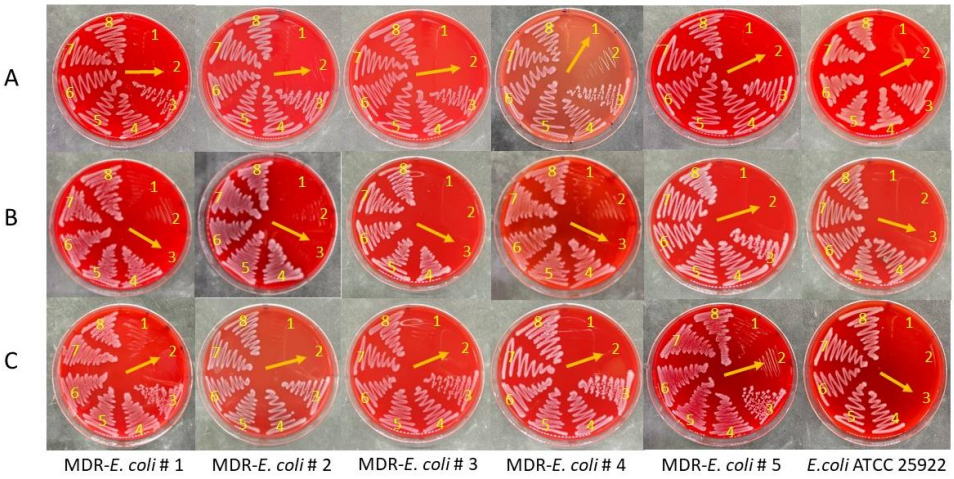

Figure 3. MBC values of *A. lanceipolius*, *A. elasticus* and *A. odoratissimus* seed extract against MDR-*E. coli*: A) *A. lanceipolius,* B) *A. elasticus,* C). *A. Odoratissimus.* Concentrations: 1). 50 mg/mL, 2). 25 mg/mL, 3). 12.5 mg/mL, 4). 6.25 mg/mL, 5). 3.13 mg/mL, 6). 1.56 mg/mL, 7). 0.78 mg/mL, 8). 039 mg/mL, : MBC values. (Photos were taken by Muhammad Evy Prastiyanto)

Phytochemical Screening of Extracts

Qualitative screening of phytochemical contents, i.e., flavonoids and tannins, were carried out on all extracts (Table 3.)

Table 4. The results of the phytochemical analysis of seed extracts

Seed extract	Phytochemicals content	
	Flavonoids	Tannins
A. lanceipolius	+	+
A. elasticus	+	+
A. odoratissimus	+	+

The results revealed that all seed extracts contain flavonoids and tannins. Flavonoids are secondary metabolites of *2-phenyl-benzyl-γ-pyrone* derivatives and these compounds are most commonly found in plants (Buer et al., 2010) because flavonoids are compounds that are known to be synthesized by plants in response to infection (Panche et al., 2016). According to Cushnie & Lamb (2005), many compounds of flavonoids have the potential as antibacterial agents (Cushnie and Lamb, 2005). In this study, the mechanism of inhibition of MDR-*E.coli* by flavonoids from *Artocarpus* seed extracts was not known yet. However, *quercetin*, *apigenin*, and *3,6,7,3 ', 4'– pentahydroxyflavone* were reported as *gyrase* DNA-inhibiting flavonoids of *E. coli* (Ohemeng et al., 1993).

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Tannins were also contained in all *Artocarpus* seed extracts. Tannins are the most abundant polyphenols in edible plants (Chung et al., 1998). Many studies reported the potential of tannins as antibacterial agents. A previous study (Dabbaghi et al., 2019) reported the antibacterial activities of tannins against *E. coli* was depend on the content of *phenolic hydroxyl* groups. However, the inhibition mechanism of tannins in *Artocarpus* seed extracts was not performed in this study. Several previous research suggested that tannins interfere with cell metabolism (Belhaoues et al., 2020).

In conclusion, the seed extracts of three *Artocarpus* species are potential to be developed as an antibacterial against UTI-causing MDR-*E. coli*. *A. elasticus* seed extract has better potency than the other two seed extracts. Further in vivo research and study regarding the mode of action are necessary to be carried out.

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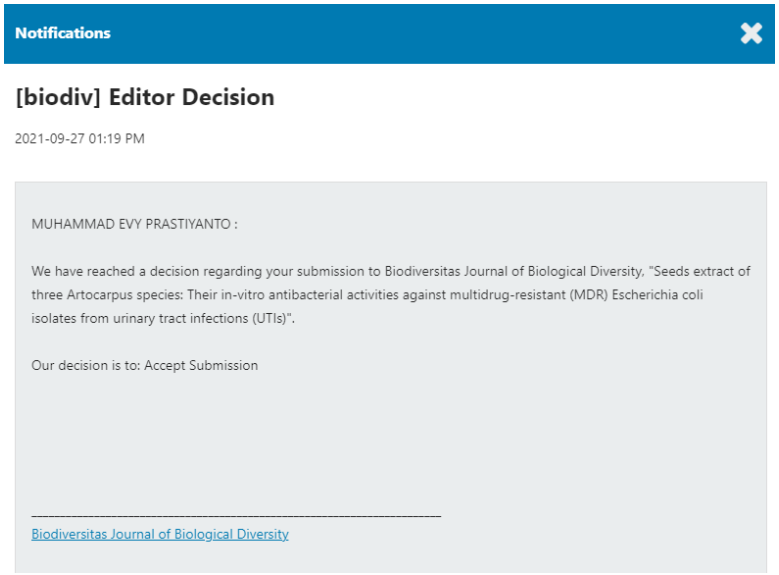


Notifications

[biodiv] Editor Decision

2021-09-27 01:19 PM

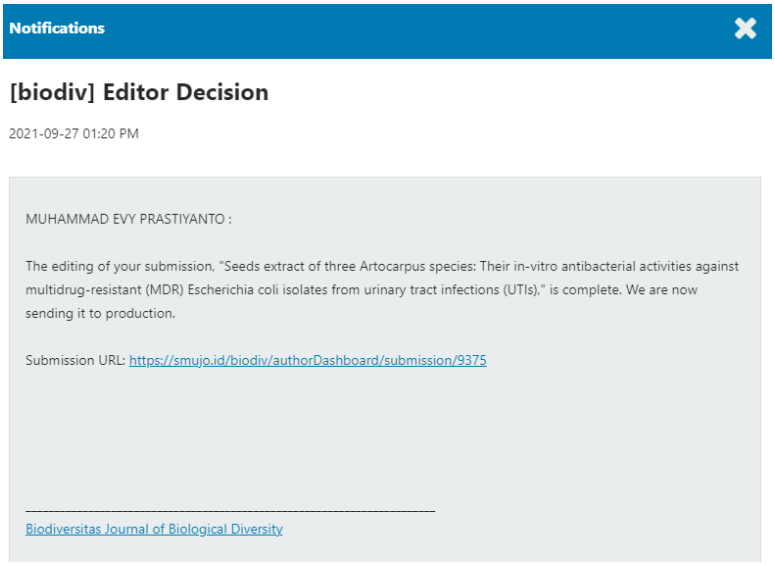
MUHAMMAD EVY PRASTIYANTO :

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Seeds extract of three Artocarpus species: Their in-vitro antibacterial activities against multidrug-resistant (MDR) Escherichia coli isolates from urinary tract infections (UTIs)".

Our decision is to: Accept Submission

Biodiversitas Journal of Biological Diversity

5 Bukti konfirmasi artikel published online 27 Sepetember 2021



Notifications

[biodiv] Editor Decision

2021-09-27 01:20 PM

MUHAMMAD EVY PRASTIYANTO :

The editing of your submission, "Seeds extract of three Artocarpus species: Their in-vitro antibacterial activities against multidrug-resistant (MDR) Escherichia coli isolates from urinary tract infections (UTIs)," is complete. We are now sending it to production.

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