

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

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

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28 **tract.** Prastiyanto ME. 2021. Seeds extract of three *Artocarpus* species: Their in-vitro antibacterial activities against multidrug-resistant (MDR) *Escherichia coli* isolates from urinary tract infections (UTIs). *Biodiversitas* 22: 4362-4368. 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. heterophyllus*, *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*), 15 a (*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 extra 9 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. 34 *asticus* 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 4 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, *Artocarpus*, MDR-*E. coli*, seeds, UTI

### 5 INTRODUCTION

Urinary tract 4 ections (UTIs) are microbe-caused infections that are a major cause of morbidity in humans, particularly in children and 23 yborns. 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 af 5 t 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 Bec 51 :il 2019).

5 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* 10) 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  $\beta$ -lactams, aminoglycosides 22 inolones, and trimethoprim-sulfamethoxazole (Adwan et al. 2014). However, excessive and uncontrolled use of 39 timicrobial agents causes bacterial strains to become multidrug-resistant (MDR). *E. coli* strains t 47 are resistant to extended-spectrum cephalosporins producing extended-spectrum  $\beta$ -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 (F 46 iyanto 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 38 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*) 44 d 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**

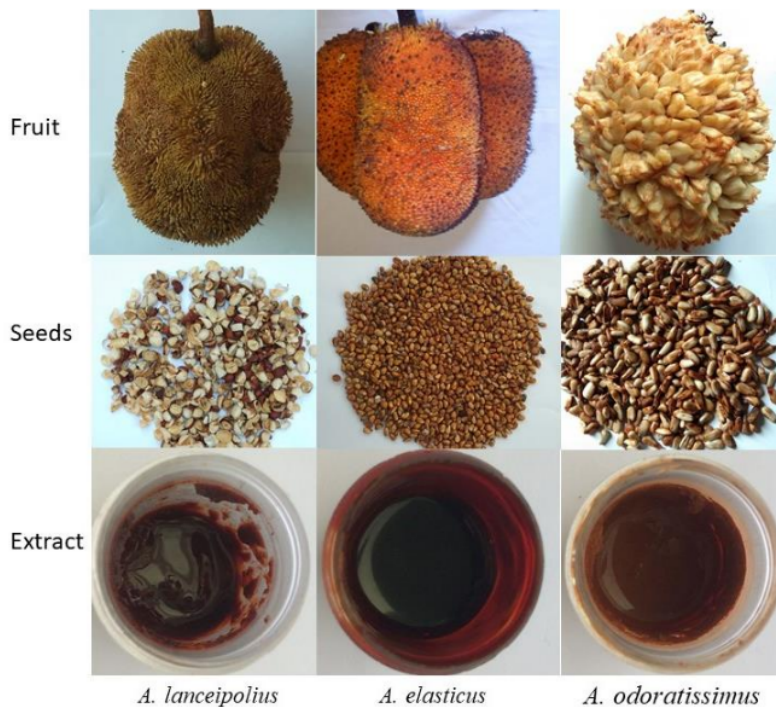
**Seed collection and extractions**

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 District, East Kalimantan (1°05'31.9"N 116°55'00.9"E), *A. elasticus* was obtained from Luwu District, South Sulawesi (3°18'19.4"S 120°18'26.4"E), while *A. odoratissimus* was gathered from Tabalong District, 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 in the 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.

**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).



**Figure 1.** Fruits, seeds, and extracts of three species of *Artocarpus*

### 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). Ten 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 (Tsukatani et al. 2009). 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 fivefold diluting, 10 µL of MDR-*E. coli* bacterial suspension 0.5 McFarland standards (1.5 × 10<sup>8</sup> CFU/mL) was added to each well except negative control. The microwell plate was incubated for 18 hours at 37°C. After incubation, 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 were carried out using previously described methods (Eve et al. 2020).

## RESULTS AND DISCUSSION

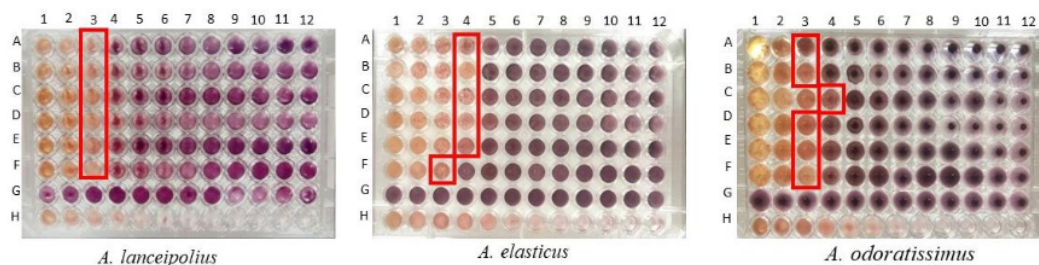
### Extract yield

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 phytochemical compounds of the seeds in ethanol than in hexane. The high polarity of ethanol causes strong interactions with most of the polar phytochemical compounds from the seed extract, so it has a better extraction potential when compared to hexane which extracts nonpolar phytochemical compounds (Sbihi et al. 2018).

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

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), aminoglycosides (gentamicin), and fluoroquinolones (ciprofloxacin). MDR-*E. coli* strain #2 was resistant to β-lactam antibiotics, aminoglycosides and fluoroquinolone. 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 aminoglycosides. 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 meropenem 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).





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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* #2, 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). 0.39 mg/mL, 9). 0.19 mg/mL, 10) 0.09 mg/mL, 11). 0.04 mg/mL, 12). 0.02 mg/mL

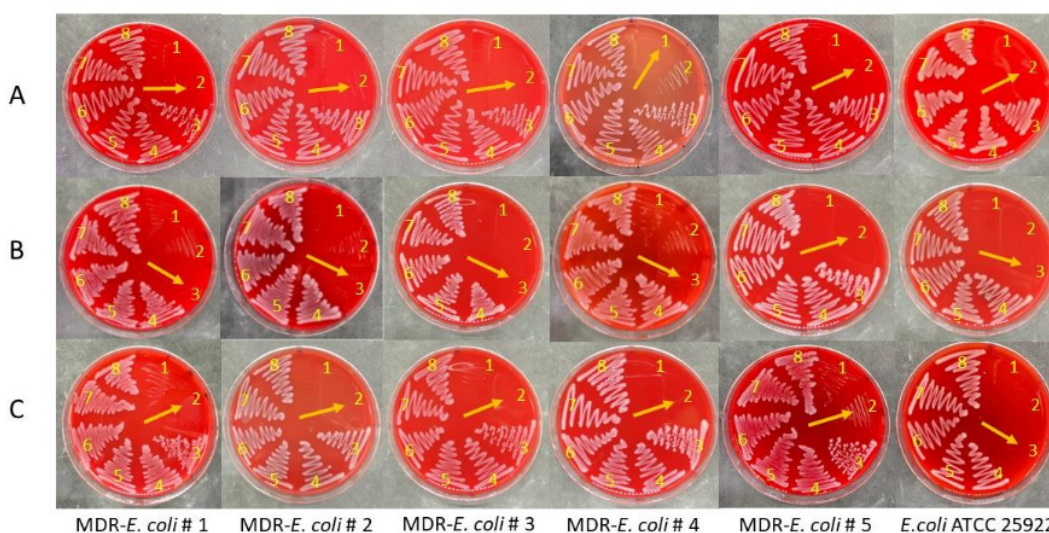


Figure 3. MBC values of *A. lanceipolius*, *A. elasticus* and *A. odoratissimus* seed extract against MDR-*E. coli*: A) *A. lanceipolius*, B) *A. elasticus*, C). *A. Odoratissimus*. Concentrations: 1). 50 mg/mL, 2). 25 mg/mL, 3). 12.5 mg/mL, 4). 6.25 mg/mL, 5). 3.13 mg/mL, 6). 1.56 mg/mL, 7). 0.78 mg/mL, 8). 0.39 mg/mL, → : MBC values

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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*.

#### 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

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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 15.625 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).

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. champpe*, 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).

**Phytochemical screening of extracts**

Qualitative screening of phytochemical contents, i.e., flavonoids and tannins, were carried out on all extracts (Table 4).

The results revealed that all seed extracts contain flavonoids and tannins. Flavonoids are secondary metabolites of 2-phenyl-benzyl- $\gamma$ -pyrone derivatives and these compounds are most commonly found in plants because flavonoids are compounds that are known to be synthesized by plants in response to infection (Panche et al. 2016). According to Cushnie and 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 are reported as gyrase DNA-inhibiting flavonoids of *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* 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).

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

Seed extract	Phytochemicals content	
	Flavonoids	Tannins
<i>A. lanceipolius</i>	+	+
<i>A. elasticus</i>	+	+
<i>A. odoratissimus</i>	+	+

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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