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The activity antibiofilm of betadine leaf (*Jatropha multifida* Linn) juice on urinary catheters

Rada Tania[1], Wani Devita Gunardi[2]*, Susana Elya Sudrajat[3]

[1]Student of Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, Jakarta, [2]Department of Microbiology, Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, Jakarta, [3]Department of Pharmacology, Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, Jakarta

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ABSTRACT

Biofilm formation can reduce the efficacy of antibiotics against pathogenic microorganisms. Flavonoids are proven to have antibiofilm activity. Betadine leaf (*Jatrophas multifida* Linn) contains abundant flavonoids. This study aimed to evaluate the biofilm activity of the betadine leaf juice on urinary catheters. Biofilm forming *Escherichia coli* was isolated from a urinary catheter. The betadine leaf juice was prepared by harvesting, washing, drying, juicing, centrifuging, and sterilizing the juice. Phytochemical analysis was performed to identify the presence of steroids, alkaloids, flavonoids, and phenols. Biofilm and antibiofilm assays were conducted by incubating thioglycolate medium with varying concentrations of the betadine leaf juice and *E. coli* suspension, with bacterial growth and biofilm formation assessed based on turbidity observations. Alkaloids, steroids, flavonoids, and phenolic compounds have been found in the sterilized betadine leaf juice. Bacterial growth was observed in all tubes containing different dilutions of the betadine leaf juice. However, turbidity levels increased as the juice concentration decreased. Additionally, the weight of the urinary catheter was significantly greater in tubes with lower juice concentrations (p=0.02). In conclusion, the *J. multifida* Linn leaf juice has potential antibiofilm activity against *E. coli* in urinary catheters.

Keywords:
antibiofilm;
betadine leaves;
flavonoid;
phenolic compound;
microbial resistance

ABSTRAK

Pembentukan biofilm dapat mengurangi efikasi antibiotik terhadap mikroorganisme patogen. Flavonoid terbukti memiliki aktivitas antibiofilm. Daun betadine (*Jatrophas multifida* Linn) mengandung flavonoid yang melimpah. Penelitian ini bertujuan untuk mengevaluasi aktivitas biofilm dari jus daun betadin pada kateter urin. *Escherichia coli* pembentuk biofilm diisolasi dari kateter urin. Jus daun betadin dibuat dengan cara memanen, mencuci, mengeringkan, membuat jus, menyentrifugasi, dan mensterilkan jus. Analisis fitokimia dilakukan untuk mengidentifikasi keberadaan steroid, alkaloid, flavonoid, dan fenol. Uji biofilm dan antibiofilm dilakukan dengan menginkubasi media tioglikolat dengan berbagai konsentrasi jus daun betadin dan suspensi *E. coli*, dengan pertumbuhan bakteri dan pembentukan biofilm dinilai berdasarkan pengamatan kekeruhan. Alkaloid, steroid, flavonoid, dan senyawa fenolik telah ditemukan dalam jus daun betadin yang disterilkan. Pertumbuhan bakteri diamati di semua tabung yang berisi pengenceran jus daun betadin yang berbeda. Namun, tingkat kekeruhan meningkat seiring dengan penurunan konsentrasi jus. Selain itu, berat kateter urin secara signifikan lebih besar dalam tabung dengan konsentrasi jus yang lebih rendah (p=0,02). Sebagai kesimpulan, jus daun *J. multifida* Linn memiliki potensi aktivitas antibiofilm terhadap *E. coli* dalam kateter urin.

*corresponding author: wani.gunardi@ukrida.ac.id

INTRODUCTION

Biofilm formation by microorganisms has been proven to increase pathogenicity and microbial resistance to antibiotics.[1–3] A previous study has identified various biofilm-forming bacteria isolated from urinary catheters. *Escherichia coli* is a biofilm forming bacteria consisting of a bacterial colony embedded in a matrix of extracellular polymeric substances (EPS) which protects the microbes from adverse environmental conditions and results in antibiotics resistance.[1]

Betadine leaves, also known as iodine plants (*Jatropha multifida* Linn) are widely grown in Indonesia, particularly in Lubuk Linggau, South Sumatra.[4] The local people use betadine leaves for the treatment of wounds.[5] Additionally, betadine leaves are believed by the community to have numerous health benefits, including as antimicrobial and several other important benefits.[6,7] These benefits may be attributed to the presence of several active compounds in the leaves. Phytochemical identification, betadine leaves contain phenolic compounds, tannins, and flavonoids.[4,8]

Flavonoids are widely distributed in nature, and biomedically significant due to their biological activities such as antibacterial, antifungal, and antiprotozoal activities.[9,10] Meanwhile, phenolic compounds have the ability to form complexes with proteins through hydrogen bond formation, allowing them to disrupt bacterial cell membranes.[11,12] Recent studies have identified the potential of flavonoids as quorum sensing inhibitors as well as antibiofilm agents.[9,13] Leticia *et al.*[10] reported that the use of flavonoids quercetin, myricetin, and scutellarein can reduce biofilm formation by *Staphylococcus aureus* in both *in vitro* and *in vivo* models on urinary catheters.[10] Skogman *et al.*[14] also reported the effectiveness of flavonoids as a class of quorum sensing inhibitors in *E. coli* and *Pseudomonas aeruginosa*.[14] Hakiki *et al.*[15] also reported that flavonoids can inhibit biofilm formation in *Enterococcus faecalis* bacteria. Moreover, the active fraction of betadine leaves showed activity against *S. aureus* ATCC 25923.[16]

This study aimed to evaluate antibiofilm activity of betadine leaf juice against biofilm forming *E. coli* on urinary catheters.

MATERIAL AND METHODS

Bacterial isolate

Isolate biofilm forming *E. coli* was obtained from urinary catheter collected by Gunardi *et.al.* in the previous study.[1]

Preparation of leaf juice

Jatropha multifida Linn was obtained from Lubuk Linggau, South Sumatra (FIGURE 1). Betadine leaf plants grow in durian plantation areas in the city of Lubuk Linggau, South Sumatra. The plantation area is at an altitude of approximately 130 m above sea level with an average humidity of 80-90%. The fresh old green leaves were harvested, each stem had one leaf and it was separated from the stems, then washed to remove any foreign materials. After air-drying, the leaves were processed using a slow juicer. The juice and pulp were separated, with the liquid collected in a tube for centrifugation at 10.000 rpm for 5 min. The liquid was then filtered, and the resulting extract was sterilized in an autoclave.

FIGURE 1. *Jatropha multifida* Linn plants.[17]

Phytochemical testing

Phytochemical testing was conducted to detect several compounds, including steroids, alkaloids, flavonoids, and phenols. One milliliter of *J. multifida* Linn leaf juice was taken, and placed into a tube, then dissolved with 0.5 mL of chloroform. After that, 3-5 drops of concentrated H_2SO_4 were added along the sides of the tube. A green or blue color formation indicated a positive result for steroids. Alkaloids were tested by placing 1 mL of the juice into a test tube and adding 3-5 drops of Dragendorff's reagent. A positive result was indicated by the formation of a brown or orange precipitate. For flavonoid testing, 1 mL of the juice was placed into a test tube, and then 1-2 mL of hot methanol was added, followed by magnesium powder and 0.5 mL of concentrated hydrochloric acid. A positive result was indicated by the formation of a red or orange color. For phenol testing, 1 mL of the juice was placed into a test tube and 10 drops of 1% $FeCl_3$ solution were added. A positive result was indicated by the development of a green, red, purple, blue, or dark black color.

Biofilm inhibition testing

For the biofilm inhibition test using *J. multifida* Linn leaf juice, six test tubes were prepared as follows: Tube (-) contained 5 mL of thioglycolate medium and 5 mL of sterilized the juice; Tube (+) contained 5 mL of thioglycolate medium and 1 mL of the *E. coli* suspension; Tube 1 contained 5 mL of thioglycolate medium, 1/2 dilution of the juice, and 1 mL of the *E. coli* suspension; Tube 2 contained 5 mL of thioglycolate medium, 1/4 dilution of the juice, and 1 mL of the *E. coli* suspension; Tube 3 contained 5 mL of thioglycolate medium, 1/8 dilution of the juice, and 1 mL of the *E. coli* suspension; Tube 4 contained 5 mL of thioglycolate medium, 1/16 dilution of the juice, and 1 mL of the *E. coli* suspension. The selection of the 1/2 concentration for initial testing was based on preliminary trials utilizing the well and disc diffusion methods, which demonstrated that this concentration achieved an optimal balance between efficacy and minimal inhibition. This finding suggested that further dilutions could be explored for a more comprehensive assessment. All tubes were incubated at 37°C for 24 hr to monitor bacterial growth, indicated by turbidity. The turbidity levels were assessed using the McFarland standard and compared to the positive control. Each step of the experiment was conducted in triplicate.

Antibiofilm testing

For the antibiofilm test on urinary catheters using *J. multifida* Linn leaf juice, six test tubes were prepared as follows: Tube (-) contained 5 mL of thioglycollate medium, 5 mL of the sterilized juice, and a 2 cm segment of urinary catheter; Tube (+) contained 5 mL of thioglycolate medium, 1 mL of the *E. coli* suspension, and a 2 cm segment of urinary catheter; Tube 1 contained 5 mL of thioglycolate medium, 1/2 dilution of the juice, 1 mL of the *E. coli* suspension, and a 2 cm segment of urinary catheter; Tube 2 contained 5 mL of thioglycolate

medium, 1/4 dilution of the juice, 1 mL of the *E. coli* suspension, and a 2 cm segment of urinary catheter; Tube 3 contained 5 mL of thioglycolate medium, 1/8 dilution of the juice, 1 mL of the *E. coli* suspension, and a 2 cm segment of urinary catheter; Tube 4 contained 5 mL of thioglycolate medium, 1/16 dilution of the juice, 1 mL of the *E. coli* suspension, and a 2 cm segment of urinary catheter. The sterilized thioglycolate medium was incubated at 37°C for 72 hr to observe bacterial growth, which would be indicated by turbidity. All this step has been done triplicate.

Bacterial biofilm from urinary catheter weighing

The urinary catheter was weighed after conducting an antibiofilm test using the *J. multifida* Linn leaf juice. The urinary catheter was immersed in a tube containing 5 mL of distilled water and vortex to mix and dislodge bacterial biofilms adhering to the catheter. The mixture was then filtered using Whatman filter paper with a glass vacuum filtration set. Finally, the biofilm from catheter was weighed using a digital analytical balance.

Data analysis

Normality data from the bacterial biofilm weight measurements on the catheter was analyzed using Shapiro Wilk test. Kruskal-Wallis test was used to determine the differences of the bacterial biofilm weight across categories of group and t-test was used to compare each group with the positive control group. A p value <0.05 was considered significant.

RESULTS

Phytochemical tests on non-sterilized betadine leaf juice revealed the presence of steroids, flavonoids, and phenolic compounds, while alkaloids were not detected in this juice. In contrast, the phytochemical test results for sterilized betadine leaf juice showed the presence of alkaloids, steroids, flavonoids, and phenolic compounds (TABLE 1).

The antibiofilm activity was assessed by observing the turbidity of the samples. The level of turbidity was determined by McFarland testing. In the experiment, betadine leaf juice was used in Series 1, 2, 3 and 4, which were sterilized. The results showed that the negative control tube remained clear, indicating no bacterial growth. However, turbidity was observed in the positive control tube and in Tubes 1, 2, 3, and 4, indicating bacterial growth. Although all tubes showed turbidity, the levels varied: Tube 1 had a turbidity level of +1, Tube 2 had +2, Tube 3 had +3, and Tube 4 had +4 (TABEL 2 and FIGURE 2).

However, there were differences in turbidity levels, with the tube containing a higher concentration of the betadine leaf juice showing the lowest turbidity (TABLE 2). This suggests that the active compounds in betadine leaves have potential in inhibiting bacterial growth, but the extraction method needs optimization.

TABLE 1. Active compound identifies in betadine leaf juice

Active compound	Without sterilization	Sterilization
Alkaloid	-	+
Flavonoid	+	+
Steroid	+	+
Phenols	+	+

FIGURE 2. Biofilm inhibition test of *E. coli* using *J. multifida* Linn leaf juice. NC: negative control group (thioglicolate broth), PC: positive control group (thioglicolate broth with *E. coli* whithout *J. multifida* Linn), Tube 1: ½ concentration of *J. multifida* Linn, Tube 2:1/4 concentration of *J. multifida* Linn, Tube 3: 1/8 concentration of *J. multifida* Linn, and Tube 4: 1/16 concentration of *J. multifida* Linn. Negative (-), positive (+) 1, 2, 3, 4, 5 are the level of turbidity, +1 (<0,5 McF), +2 (0,5 McF), +3 (>0,5 McF - <1 McF), +4 (>1 McF – 1,5 McF), +5 (> 2 McF)

TABLE 2. Biofilm inhibition test of E. coli using J. multifida Linn leaf juice

Tube	Series 1	Series 2	Series 3	Turbidity level
Control (-)	-	-	-	0
Control (+)	+++++	+++++	+++++	5
Tube 1	+	+	+	1
Tube 2	++	++	++	2
Tube 3	+++	+++	+++	3
Tube 4	++++	++++	++++	4

The next step in testing the antibiofilm activity of betadine leaf juice involved assessing biofilm formation on urinary catheters. This was conducted by weighing the urinary catheters after treatment and incubation for 3 d (FIGURE 3). The average weight of the urinary catheters was 0 g in the negative control tube; 0.073 g in the positive control tube; 0.031 g in tube 1; 0.0493 g in tube 2; 0.059 g in tube 4; and 0.071 g in tube 4 (TABLE 3). The statistical analysis showed that the weight of bacterial biofilms is not same across categories of group ($p = 0.02$). The curve of means plot also showed that the less concentration of the *J. multifida* Linn leaf juice increase the weight of bacterial biofilms (FIGURE 4).

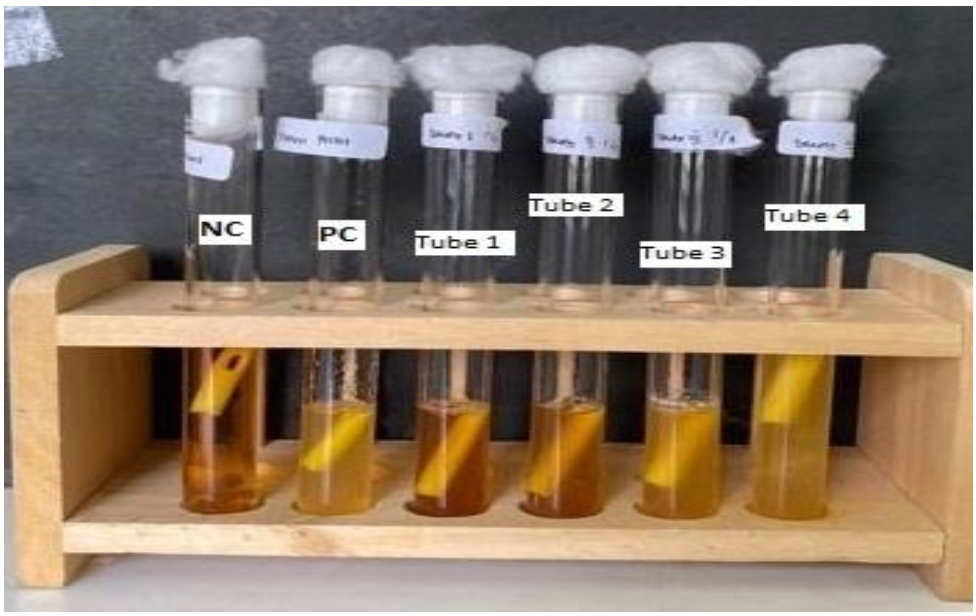

FIGURE 3. Results of the antibiofilm test on urinary catheters using *J. multifida* Linn leaf Juice in 3rd d. NC: negative control group (thioglicolate broth), PC: positive control group (thioglicolate broth with E. coli whithout *J. multifida* Linn), Tube 1: ½ concentration of *J. multifida* Linn, Tube 2:1/4 concentration of *J. multifida* Linn, Tube 3: 1/8 concentration of *J. multifida* Linn, and Tube 4: 1/16 concentration of J. *multifida* Linn.

TABLE 3. The weight of bacterial biofilm from urinary catheter after the experiment

Tube	Series 1 (g)	Series 2 (g)	Series 3 (g)	Mean ± SD (g)
Control (-)	0.000	0.000	0.000	0.00
Control (+)	0.094	0.062	0.064	0.073±0.018
Tube 1	0.034	0.029	0.030	0.031±0.003
Tube 2	0.038	0.055	0.055	0.049±0.010
Tube 3	0.052	0.065	0.060	0.059±0.007
Tube 4	0.067	0.080	0.066	0.071±0.008

DISCUSSION

The active compound identified in this research has similarities with the previous research. Harliananda *et al.*,[8] found alkaloids, steroid and phenol in dry leaves and found alkaloids, flavonoid, steroid, and phenol in wet leaves that were extracted by maceration. Senou *et al.*,[18] also reported the presence of flavonoids, tannins, alkaloids, and steroid in the leaves of *J. multifida* Linn that was extracted by maceration.

The turbidity observed in the samples treated with betadine leaf juice is likely due to the active compounds in the juice being less than optimal. Previous studies reported that the extraction technique used significantly affects the amount of active compounds that can

be extracted from natural materials.[19–21] This study used a slow juicer technique to extract the active compounds, which may be less effective in extracting the active compounds from betadine leaves. Noranizan *et al.*,[22] reported that phenolic compounds are better retained using a blender than a slow juicer. As a result, turbidity persisted in the samples treated with the juice. Moreover, previous research also reported that betadine leaves was inactive against strains of *E. coli.*[18]

The use of urinary catheters is one of the primary causes of nosocomial urinary tract infections that often caused by *E. coli* (UPEC).[1,23] This pathogen can produce biofilms, leading to persistent and recurrent infections. In *E. coli*, the presence of virulence genes *fim A* and *pap C* is closely related to biofilm formation.[1,24,25] According to this study, in Tube 1, the catheter's weight was lower compared to Tubes 2, 3, and 4, as Tube 1 contained a higher concentration of *J. multifida* Linn leaf juice. This leaf juice contains flavonoid compounds with antimicrobial properties that can form complexes with dissolved extracellular proteins and microbial cell walls.[9,10] Additionally, phenolic compounds in the leaf juice also play a role by forming complexes with proteins through hydrogen bonds, which can damage bacterial cell membranes.[11,12] In a previous study, it was also reported that the stem of the betadine plant exhibited antibiofilm activity, as indicated by its IC_{50} values of 0.3 mg/mL and 0.76 mg/mL against *S. aureus* and MRSA.[26] Although the extraction methods, plant parts, and test bacteria used were different, this indicates that the Betadine plant has potential as an antibiofilm agent. However, the biofilm formation observed in urinary catheters treated with *J. multifida* Linn leaf juice suggests that the extraction process was not yet optimal. Therefore, further research is needed using alternative extraction techniques that can more effectively extract the active compounds from the betadine leaves. This would better determine the antibiofilm efficacy of the active compound.

CONCLUSION

In conclusion, the *J. multifida* Linn leaf juice has antibiofilm activity against *E. coli* in urinary catheters by inhibit biofilm formation. Further study is needed to isolate the active compounds from the *J. multifida* Linn leaf that have antibiofilm activity.

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