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Research

Inhibitive Properties of *Anredera Cordifolia* Extract on *Escherichia Coli* Swimming Motility

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Abstract

Background *Escherichia coli*, a Gram-negative bacterium, is one of the main etiology of urinary tract infection (UTI). An important pathogenicity factor influencing biofilm production is its motility, swimming, or swarming. *Anredera cordifolia* (AC) leaves have been proven to have an antibacterial effect on various Gram-positive and Gram-negative bacteria, including *E. coli*.

Objective To determine the effect of AC leaves extract on swimming motility property of *E. coli* derived from UTI patients. **Methods** AC leaves were extracted with the maceration method. *E. coli* was identified and isolated from UTI patients. There are 4 diluting concentrations of AC leaves extract (from 875 μg per ml to 7000 μg per ml) and one negative control. The preparation underwent incubation, inoculation, and re-incubation. Swimming motility was measured manually, and the result was tested for significant difference between each dose with one-way ANOVA (limit of significance is 0.05)

Results On AC leaves extract concentration of 1750 μg per ml, the swimming motility has been inhibited significantly compared to negative control and lower concentration of 875 μg per ml (0.543 cm vs 1.673 cm and 1.358 cm, respectively; $p < 0.001$). Increasing concentration (3500 μg per ml and 7000 μg per ml) did not show a significant difference in inhibition compared to 1750 μg per ml (0.539 cm and 0.530 vs 0.543).

Conclusion AC leaves extract with a concentration at least 1750 μg per ml would inhibit the swimming motility of *E. Coli* derived from UTI patients.

Keywords: *Escherichia coli*; Anredera cordifolia; swimming motility; urinary tract infections

INTRODUCTION

Escherichia coli (*E. coli*), a Gram-negative bacterium, generates biofilm through endotoxin production (e.g., hemolysin, cytotoxic necrotizing factor 1 [CNF-1], and cytolethal distending toxin [CDT]), flagellar motility, and the fimbriae. Apart from motility function, *E. coli*'s flagella help avoid the human immune response and assist in biofilm production. Protected by the biofilm, *E. coli* further translocates its protein into host cells and triggers the host's pro-inflammatory response through toll-like receptor 5 (TLR-5) signaling.[1,2] These pathogenicity properties make *E. coli* an important cause of urinary tract infections in outpatient, inpatient, and population settings in Indonesia.[3,4]

Anredera cordifolia (AC), known as 'binahong' in Indonesia, has been traditionally used for fever, gout, and gastrointestinal problems. AC leaves contain oleanolic acid, saponins, flavonoids, alkaloids, tannins, and essential oils.[5,6] These active phytochemical contents have been associated with antibacterial activity. The previous study has proven that AC leaves extract poses an antibacterial property against both Gram-positive (e.g., *Bacillus pumilus*, and *Staphylococcus aureus*) and Gram-negative bacteria (e.g., *Enterobacter cloacae*, *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*).[5,7-9] Further study is needed to describe specific biofilm inhibition property of AC extract on *E. coli* pathogenicity, mainly its flagellar swimming motility.

METHODS

AC leaves extract preparation

AC leaves extract was derived from dehydrated AC leaves preparation (CV Merapi Farma, Yogyakarta, Indonesia) at Organic Chemistry Laboratory, Universitas Kristen Duta Wacana, Yogyakarta, Indonesia. The maceration technique was performed to produce the ethanol extract of AC leaves. One hundred grams of AC leaves were powdered and dissolved in 96% ethanol solution (ratio 1:3) for 3 days and homogenized manually every 24 hours. Macerated preparations were filtered twice with calico fabrics and 5 times with filter paper until no trace of leave powder was left, then evaporated with a rotary evaporator for 90 minutes at 40°C. The final paste form was stored in an amber glass bottle, refrigerated at 4°C.

Preparation of each dose was started with diluting 7 mg paste with 1 ml dimethylsulfoxide (DMSO) and filtered with sterile membrane filters (Sartorius, Germany) to create a 7000 μg per ml extract solution. Subsequently, we dilute the solution serially into four doses (875 μg per ml, 1750 μg per ml, 3500 μg per ml, and 7000 μg per ml) using the serial dilution method.

Specimen processing

The *E. coli* specimen was derived from specimen collection at Microbiology Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia (previously identified with the semiautomatic method). Further preparation was performed at Microbiology Laboratory, Universitas Kristen Duta Wacana, Yogyakarta, Indonesia. Two hundred ul of isolated *E. coli* specimen was cultured in a 5 ml brain heart infusion (BHI) medium (Oxoid, ThermoFisher, Hampshire, United Kingdom), with an incubation period of 24 hours on 37 ^{0}C. The specimen was subsequently centrifuged for 15 minutes at 3000 rpm. After discarding the supernatant, we resuspended with bacteriological peptone until the turbidity was similar with standardized 0.5 MacFarland solution (equal to 1.5 x 10^{8} colony-forming unit [CFU] per ml).

Bacterial motility tests

For each treatment arm, we prepared four tubes (Eppendorf™ Tubes; Eppendorf, Hamburg, Germany) containing 450 μl BHI medium, 25 μl AC extract (with different doses), and 25 μl of *E. coli* specimen; and for the negative control, we prepared one tube containing 475 μl BHI media and 25 μl of *E. coli* specimen into (labeled A, B, C, D, and E). Afterward, the specimens were incubated at 37 ^{0}C for 24 hours. After incubation, we put the specimens (0.5 μl each) from treatment and control tubes on petri dish containing semisolid BBL™ motility test medium (BD, Berkshire, United Kingdom), three media for each tube hence in total 15 petri dishes. The specimens underwent further incubation at 37 ^{0}C for 24 hours.

Measurement of surface motility

Among five types of surface motility (swarming, twitching, swimming, sliding, and gliding), this study measured swimming motility. Preparation of semisolid growth medium (using BBL™) ensured that *E. coli* could only move with swimming motility, thus the visual distance on the petri dishes would be the measurement of swimming motility.[10] Swimming motility is measured with the distance of bacterial growth away from the inoculation point. The distance for each sample was measured by finding an average of the sum of two close points, two midpoints, and two furthest points using a sliding caliper. The measurement of each sample was replicated three times in the same petri dishes.

Statistical analysis

The measurement from each AC dose and negative control is presented with mean and standard deviation. The difference in motility between each test arm was tested with one-way ANOVA (limit of significance is 0.05) if the measurement followed Normal distribution.

RESULT

Figure 1 shows the different motility of *E. coli* between negative control and various concentration of AC leaves extract. Dish C, D, and E (AC leaves extract of 1750 μg per ml, 3500 μg per ml, and 7000 μg per ml, respectively) show smaller growth area compared to dish A (negative control), and dish B (AC leaves extract of 875 μg per ml).

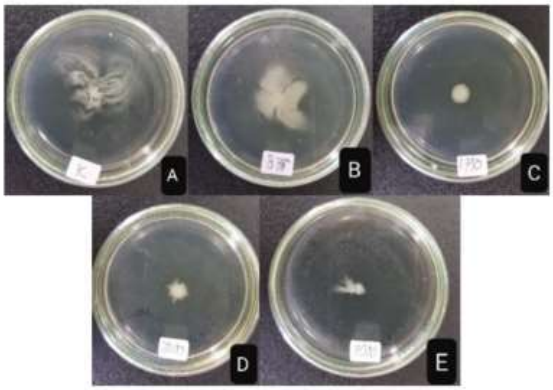

Figure 1. *Inhibition of E. coli motility after exposure to AC leaves extract. A. negative control, B-E. AC leaves concentration of 875 μg per ml, 1750 μg per ml, 3500 μg per ml, and 7000 μg per ml, respectively.*

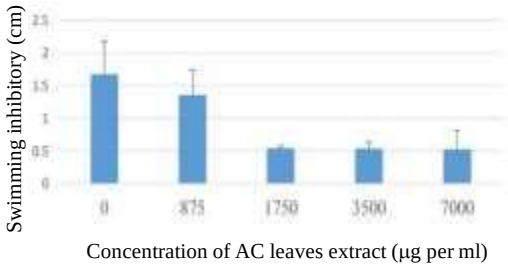

Figure 2. *Effects of AC leaves extract on E. coli swimming motility.*

The mean distance of negative control and treatment arms (875 μg per ml, 1750 μg per ml, 3500 μg per ml, and 7000 μg per ml) are 1.673 cm, 1.358 cm, 0.543 cm, 0.539 cm, and 0.530 cm (Figure 2). Overall ANOVA test comparing each dose showed a significant result $p < 0{,}001$. Further post-hoc analysis showed a significant difference between negative control or the lowest dose and extract with ≥ 1750 μg per ml, and no significant difference between each dose ≥ 1750 μg per ml (Table1).

Table 1. Post-hoc analysis of the difference of motility between negative control and each dose of AC extract

Table 1. Post-hoc analysis of the difference of motility between negative control and each dose of AC extract.**Base**	**Comparison**	**Difference (95% CI) in cm**	**P-Value**
Negative control	AC extract 875 µg per ml	0.317 (-0.2765 to 0.9098)	0.545
	AC extract 1750 µg per ml	1.130 (0.5369 to 1.7231)	< 0.001
	AC extract 3500 µg per ml	1.134 (0.5410 to 1.7273)	< 0.001
	AC extract 7000 µg per ml	1.143 (0.5502 to 1.7365)	< 0.001
AC extract 875 µg per ml	AC extract 1750 µg per ml	0.813 (0.2202 to 1.4065)	0.003
	AC extract 3500 µg per ml	0.818 (0.2244 to 1.4106)	0.003
	AC extract 7000 µg per ml	0.827 (0.2335 to 1.4198)	0.003
AC extract 1750 µg per ml	AC extract 3500 µg per ml	0.004 (-0.5890 to 0.5973)	1.000
	AC extract 7000 µg per ml	0.013 (-0.5798 to 0.6065)	1.000
AC extract 3500 µg per ml	AC extract 7000 µg per ml	0.009 (-0.5840 to 0.6023)	1.000

DISCUSSION

Anredera cordifolia leaves extract showed potential inhibitory activity on E. coli swimming motility in this study. A previous study with comparable methodology showed similar inhibition of AC leaves extract against *E. coli* and other Gram-negative and Gram-positive bacteria.[7–9] Darsana et al performed a growth inhibition using the disc diffusion method and discovered similar results, but the inhibitory effect was predictably stronger with a higher concentration of AC leaves extract.[11] This effect is probably related to the active contents of AC leaves (polyphenols, flavonoids, alkaloids, saponins, and tannins). Polyphenols might damage the bacterial cell walls and their synthesis, flavonoids induce lysis of cell walls and membrane, alkaloid compounds impair the peptidoglycan part of bacterial cells, phenols and saponins denaturize proteins and dissolve fatty acid which destroys carbon chains of bacterial cell membranes, tannins induce acidic environment and coagulation of cell walls protein. Every component of AC leaves extract mutually inhibits swimming motility through inhibition of bacterial cell wall synthesis, cell membrane function, protein synthesis, and bacterial cell growth.[5,6] However, compared to other species, AC leaves might possess weaker antibacterial activity.[12] The optimal concentration of AC leaves extract discovered in this study is 1750 µg per ml, where a higher concentration did not result in a shorter distance. Although generally other plant extracts showed similar effects against *E. coli*, some studies reported different optimal concentrations. *Lonicera caerulae* extract and grape seed extract had an optimum concentration against *E. coli*, while *Acacia senegal* leaves extract showed possible higher doses to inhibit flagellar motility.[13,14] This might be caused by dissimilar methodology and choice of concentration. However, this study is lacking in comparison with the positive control, such as common antibiotics in which the specimen is susceptible. In summary, AC leaves extract has a potential inhibitory effect against *E. coli* biofilm formation, specifically on its flagellar motility.

CONCLUSION

AC leaves are among many important indigenous plants that need to be explored for their benefit, not only against non-communicable diseases, but also infectious diseases including urinary tract infections caused by *E. coli*. This study demonstrated that AC leaves might be used to prevent biofilm formation of *E. coli*, specifically in the urinary tract. Further studies are needed to determine the toxicity level, before progressing to in vivo studies and clinical trials.

CONFLICT OF INTEREST FUNDING RESOURCES

There is no conflict of interest, and this research is privately funded.

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