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Short Communication

Effectiveness of *Curcuma domestica* leave extract in inhibiting the growth of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is one of the Gram-negative bacteria that causes nosocomial infection in patients admitted to the intensive care unit (ICU). The therapy provided could be antibiotics and the provision of therapy is considered difficult due to antibiotic resistance; therefore, an alternative is needed such as active ingredients from medicinal plants. Turmeric (*Curcuma domestica*) is believed to have compounds that have antibacterial activities. The aim of this study was to determine the antibacterial activities of ethanol extract from turmeric leaves against the growth of *Pseudomonas aeruginosa*. An experimental study was conducted using posttest-only design. Antibacterial activities were determined using disc diffusion method with concentration of 50%, 75%, and 100% *Curcuma domestica* extract. The positive and negative controls were ciprofloxacin and dimethyl sulfoxide (DMSO), respectively. The inhibition zone of 50%, 75%, and 100% extract groups against *Pseudomonas aeruginosa* were 8.9 mm, 10.6 mm, and 11.8 mm, respectively. There was no significant different of antibacterial activities between different concentrations of *Curcuma domestica* (50%, 75% and 100% of extracts). All groups of *Curcuma domestica* extract had lower antibacterial activities significantly than ciprofloxacin (positive control). This data indicated that the leave extract of *Curcuma domestica* had a weak inhibition against the growth of *Pseudomonas aeruginosa*.

Keywords: Antibacterial, *Curcuma domestica*, nosocomial infection, natural product, turmeric

Introduction

Nosocomial infection are infection that occur in patients during the treatment processes in health facilities who have been treated for approximately 72 hours [1]. This infection is the most common complication that can occur in hospitalized patients and is the fourth leading cause of death in the hospitals [2]. Nosocomial infections are divided into central line-associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections, and ventilator-related pneumonia [2]. The prevalence of nosocomial infections in developed countries varies between 3.5% and 12% and it is higher in developing countries such as Indonesia, from 6.1% to 16% [2]. According to data from the Indonesia Ministry of Health, nosocomial infections reached 15.74% in 2013, far above developed countries [3].

Pseudomonas aeruginosa is one of the Gram-negative bacteria that causes nosocomial infections that occur in patients with low immunity [4], and often occur in patients admitted to the intensive care unit (ICU) [5]. Transmission of *P. aeruginosa* can occur through the hands of

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health workers or contaminated hospital equipments [6]. *P. aeruginosa* is one of the bacteria are resistant to several antibiotics and therefore alternative antibacterial using active ingredients from plants could be important to overcome this issue and one plant that can be used as a potential antibacterial source is turmeric (*Curcuma domestica*) [7].

C. domestica leaves are believed to have a variety of benefits such as antiseptics, anticarcinogens, antidiabetic, antioxidants, antibacterial, antiviral, and anti-inflammatory [8-12]. Turmeric has compounds that function as antibacterial [13]. A study on the antimicrobial activity test of turmeric leaf extracts against Gram-negative bacteria such as *Escherichia coli* and *Shigella dysenteriae* has proven that the extracts inhibited the bacterial growth [13]. However, study on turmeric leaf extract against *P. aeruginosa* is still not available. The aim of this study was to determine the antibacterial activities of ethanol extract of turmeric leaves against *P. aeruginosa*.

Methods

Study design

A laboratory experimental study was conducted using a posttest-only control group. Three treatment groups with different concentrations of *C. domestica* extract, positive and negative control groups were used. The anti-bacterial effects of the extract were assessed by comparing the treatment, positive and control groups.

Curcuma domestica extraction

The samples of *C. domestica* were collected from Beringin, Lubuk Pakam, North Sumatra, Indonesia, and identification and confirmation of the *C. domestica* was conducted at the Medan Herbarium Laboratory, Universitas Sumatera Utara, Medan, Indonesia. Turmeric leaf extract was made by maceration method using ethanol solvent. The criteria for turmeric leaves used were fresh and without yellowish color on the leaves. A total of 3 kg of leaves were washed to remove impurities using clean running water and then were aerated for one day. The leaves were then dried using a drying cabinet with a 40-Watt incandescent lamp for six days. The dried turmeric leaves were then made into powder (720 g) and 12 liters of 96% ethanol solvent was added and macerated for five days. Filtrate was then separated by filtering with filter paper. The residue obtained from the filtering results was macerated again with the same type of solvent and the amount of solvent volume was half the amount of solvent volume in the first dilution for two days. The filtrates that have been obtained were evaporated with a rotary evaporator to obtain concentrated extract.

Phytochemical analysis

Qualitative phytochemical analysis was conducted at the Organic Chemistry Laboratory of Natural Materials, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara to determine the metabolite compounds of the *C. domestica*. The presence of flavonoid was tested with three reagents: $FeCl_3$, H_2SO_4, and Mg + HCl. Detection of alkaloid was conducted using two Bouchardart and Maeyer reagents. The presence of terpenoid and steroid were tested with Liebermann Bouchard and Salkowsky reagents; tannin was tested with $FeCl_3$ reagent. Saponin was tested by shaking until foam appeared, then HCl was added, and it was seen whether the foam disappeared after HCl was added.

Bacteria origin and bacterial suspension preparation

P. aeruginosa was obtained from the Pharmaceutical Microbiology Laboratory of Universitas Sumatera Utara and was rejuvenated before use. The 24-hour-old bacterial culture was suspended at 0.5 Mc Farland with a bacterial concentration of $1.5x10^8$ CFU/ml.

Antibacterial activity

A diffusion method was used to assess the antibacterial activity of the extract. A total of 10 ml of each extract concentration was prepared. Preparation of 50% and 75% extracts was made by adding 5 ml of concentrated extract with 5 ml of dimethyl sulfoxide (DMSO) and 7.5 ml of concentrated extract with 2.5 ml of DMSO, respectively. Each concentration of 50%, 75%, and

100% was dripped into the blank disc using 100 µl sterile filter tips; ciprofloxacin was used as positive control while DMSO as negative control. DMSO was used since it could dissolve polar and nonpolar compounds. The bacterial suspension was applied to the Mueller Hinton agar media and then five disks were placed (each containing three extract concentrations, positive control, and negative control). Five repetitions were conducted according to the calculation using Federer's formula. The plates were incubated for 18–24 hours at 37°C and the inhibition zone diameter was measured.

Statistical analysis

The normality and homogeneity of the data were tested using the Shapiro-Wilk normality and Levene's homogeneity test, respectively. Since the data did not distribute normally, Mann-Whitney test was used to determine the different between two groups. Statistical tests were conducted using SPSS Statistic program, version 26 (IBM, New York, USA).

Results

Phytochemical analysis of *Curcuma domestica*

C. domestica extracts were subjected to qualitative phytochemical screening tests to determine and confirm the chemical compounds and the results of the phytochemical screening test are presented in **Table 1**. The results suggested that some metabolites were positive in the extract including flavonoid, alkaloid, terpenoid, steroid, tannin, and saponin.

Table 1. Qualitative phytochemical analysis of *Curcuma domestica*

Metabolite compounds	Reagents	Result
Flavonoid	$FeCl_3$(aq)5%	Positive
	$H2SO_4$(p)	Positive
	Mg(s) + HCl(p)	Negative
Alkaloid	Bouchard	Positive
	Mayer	Positive
Terpenoid	Salkowski	Positive
	Liebermann Burchard	Positive
Steroid	Salkowski	Positive
	Liebermann Burchard	Positive
Tannin	$FeCl_3$(aq)5%	Positive
Saponin	Aquadest and alcohol 96% + HCl 2N	Positive

Antibacterial activity of *Curcuma domestica*

The results of the antibacterial activity test of *C. domestica* extracts against the growth of *P. aeruginosa* bacteria are presented in Table 2. The positive control, ciprofloxacin, had the greater mean inhibition zone compared to all *C. domestica* extract groups. Between the extract concentration groups, the higher the concentration, the greater the mean of inhibition zone. The negative control (DMSO) had no inhibition zone indicating there was no bias in the study.

Table 2. Antibacterial activities test of *Curcuma domestica* extract against *Pseudomonas aeruginosa*

Test repetition	Inhibition zone against *Pseudomonas aeruginosa*				
	C. domestica 50% (mm)	*C. domestica* 75% (mm)	*C. domestica* 100% (mm)	Ciprofloxacin (mm)	Dimethyl sulfoxide (mm)
1	7.0	8.0	10.0	25.0	0.0
2	8.0	10.5	9.5	28.0	0.0
3	10.0	12.0	13.5	30.0	0.0
4	9.7	11.4	12.0	35.0	0.0
5	9.8	11.5	14.0	32.0	0.0
Mean	8.9	10.7	11.8	30.0	0.0

Comparation of antibacterial activities between groups

To assess the different of antibacterial activities between groups, the Mann-Whitney test was used and the results are presented in **Table 3**. Our data indicated that there was no significant different of antibacterial activities between concentration groups of *C. domestica* against *P.*

aeruginosa with p=0.059 for 50% vs 75% extract group; p=0.059 for 50% vs 100% extract group; and p=0.402 for 75% vs 100% extract group. However, there was a significant different of antibacterial activities between each concentration group of *C. domestica* and ciprofloxacin; and between each concentration group of *C. domestica* and the control group.

Table 3. Comparation of antibacterial activities between concentrations of *Curcuma domestica* against *Pseudomonas aeruginosa*

Group	Comparation group	*p*-value
50% of *C. domestica* extract	75% of *C. domestica* extract	0.059
	100% of *C. domestica* extract	0.059
	Positive control	0.009*
	Negative control	0.005*
75% of *C. domestica* extract	50% of *C. domestica* extract	0.059
	100% of *C. domestica* extract	0.402
	Positive control	0.009*
	Negative control	0.005*
100% of *C. domestica* extract	50% of *C. domestica* extract	0.059
	75% of *C. domestica* extract	0.402
	Positive control	0.009*
	Negative control	0.005*

* Statistically significant at p=0.05

Discussion

Our data found that *C. domestica* contained secondary metabolites such as flavonoid, alkaloid, terpenoid, steroid, tannin, and saponin. Theoretically, those metabolites have antibacterial activities. Flavonoid could inhibit bacterial growth by denaturing proteins that cause the metabolic activity of bacterial cells to stop [14]. Flavonoid content also indicates the presence of antioxidant activity [14]. As antibacterial, flavonoid forms complex compounds with extracellular proteins and soluble proteins, causing damage to the bacterial cell membrane followed by the release of intracellular compounds [15]. Alkaloid has nitrogen-containing base groups which could react with amino acids of bacterial cell wall leading to change in amino acid composition and genetic balance, causing DNA damage [16]. The mechanism of action of terpenoid as an antibacterial is thought to involve lipophilic compounds in causing membrane damage [17]. Tannin as antibacterial is to inhibit the activity of enzymes that bind to bacterial substrates, indirectly inhibiting the oxidative phosphorylation mechanism and reducing important ions in bacterial metabolism [18]. Saponin could disrupt the bacterial work system by diffusing through the outer membrane or vulnerable cell walls, then binding to the cytoplasmic membrane and disrupting cell stability and osmosis from the cell [19]. However, the antibacterial activities of *C. domestica* extract against *P. aeruginosa* is significantly lower than ciprofloxacin. Another study also found that turmeric rhizome extract had a smaller inhibition zone than antibiotics against the growth of *Staphylococcus aureus* and *P. aeruginosa* [7].

Our data indicated that the inhibition zone of *C. domestica* extracts against *P. aeruginosa* increased with an increase in the concentration. This is in line with a previous study testing the activity of *C. domestica* extract against the growth of *Candida albicans* [20]. In the study, the inhibition zone at the concentration of 20%, 40% and 60% were 2.47 mm, 4.40 mm, and 7.47 mm, respectively [20]. A study indicated that the inhibition zone against *S. aureus,* Gram-positive bacteria, was greater than in *P. aeruginosa* which is Gram-negative bacteria [7]. This Gram-negative is more difficult to penetrate because it has more complex cell wall structure due to an outer layer of lipoproteins and lipopolysaccharides and an inner layer of peptidoglycan [7]. This could be one of the reasons that the antibacterial activities of *C. domestica* extract is not optimal.

In addition, the *C. domestica* leaves in this study were harvested in the afternoon and sent to the laboratory at the next day. This might cause the loss of biological activities of the turmeric leaves since the fresher turmeric plants the higher content of metabolite compounds [21]. In addition, several factors affect the growth and development of turmeric plants including internal factors such as genes and hormones and external factors such as nutrients, water, light, temperature, and humidity [22]. In this study, the turmeric plants were planted in the yard, were

not fertilized and had no special watering; therefore, they growth by relying solely on rainwater and sunlight. These conditions might also influent the activities of the metabolites.

Conclusion

Our data suggested that the leaves of *C. domestica* extract had weak activities to inhibit bacterial growth of *P. aeruginosa*. Although our data suggested the higher the concentration of the *C. domestica* extract, the greater the inhibition zone, there was no significant different of antibacterial activities between different concentration groups (50%, 75% and 100% extract) against *P. aeruginosa*.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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References

1. Joshi M, Kaur S, Kaur HP, *et al.* Nosocomial infection: Source and prevention. Int J Pharm Sci Res 2019;10(4):1613-1624.
2. Rahmawati SA, Dhamanti I. Infections prevention and control (IPC) programs in hospitals. J Heal Sci Prev 2021;5(1):23-32.
3. Irdan I. Faktor-faktor yang berhubungan dengan infeksi nosokomial (INOS) oleh perawat di IRNA bedah RSUD kayuagung kabupaten OKI Tahun 2017. Pros Semin Nas dan Disem Penelit Kesehat STIKes Bakti Tunas Husada Tasikmalaya. 2018;142-145:142-145.
4. Andayani N, Mahdani W, Nisyira M, *et al.* Distribution and antibacterial susceptibility pattern of isolated bacteria from endotracheal aspirates among ventilator-assisted pneumonia patients in Indonesia. Narra J. 2023;3(1):1-10.
5. Dharmayanti IGAMP, Sukrama DM. Karakteristik bakteri *Pseudomonas aeruginosa* dan pola kepekaanya terhadap antibiotik di intensive care unit (ICU) RSUP Sanglah pada bulan November 2014–Januari 2015. J Med Udayana 2019;8(4):1-9
6. Puspawati MD. Tropical skin infections. National Symposium of Dermatology and Venerology. Bali; 2016:31-50.
7. Fikayuniar L, Gunarti NS, Apriliani M. Uji aktivitas antibakteri ekstrak etanol rimpang kunyit (*Curcuma longa L.*) terhadap *Staphylococcus aureus* dan *Pseudomonas aeruginosa*. Pharma Xplore J Ilm Farm 2019;4(1):278–287.
8. Septiana E, Simanjuntak P. Aktivitas antimikroba dan antioksidan ekstrak beberapa bagian tanaman kunyit (*Curcuma longa*). 2015;5(1):31-40.
9. Puteri FD. Efek kurkumin pada kunyit (*Curcuma longa*) sebagai pengobatan kanker lambung. J Ilm Kesehat Sandi Husada 2020;12(2):860–864.

10. Ulina N, Turnip MB, Harahap U, *et al.* Phytochemical screening and antidiabetic test of ethanol extract of turmeric leaves (*Curcuma domestica Val* .) on decreasing blood glucose of diabetic rats. Int J Sci Technol Manag 2023;4(4):931-937.
11. Azhari LI, Rusmarilin H, Suryanto D, *et al.* Antimicrobial activity of turmeric leaf extract against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, and *Lactobacillus acidophilus*. IOP Conf Ser Earth Environ Sci 2018;205(1):1-8.
12. Celesta N, Sakthivel M, Senthil SP, *et al.* Review on *Curcuma longa* leaves exhibiting antioxidant property. IJAMSCR. 2021;9(2):283-287.
13. Apriliantisyah W, Haidir I, Rasfayanah, *et al.* Daya hambat ekstrak kunyit (*Curcucma domestica Val*) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. Fakumi Med J J Mhs Kedokt 2022;2(10):694-703.
14. Hasibuan NE, Azka A, Basri et al. Skrining fitokimia ekstrak etanol daun *Avicennia Marina* dari kawasan bandar bakau dumai. Aurelia J 2020;5(3):248-253.
15. Amalia A, Sari I, Nursanty R. Aktivitas antibakteri ekstrak etil asetat daun sembung (*Blumea balsamifera (L.) DC.*) terhadap pertumbuhan bakteri *Methicillin Resistant Staphylococcus aureus* (MRSA). J UIN Ar-Raniry 2017;5(1):387-391.
16. Fikri F, Rahmaningtyas IH, Prastiya RA, *et al.* Aktivitas antibakteri ekstrak daun sirsak (*Annona muricata L.*) terhadap pertumbuhan bakteri *Pseudomonas aeruginosa* secara in vitro (Antibacterial activity of soursop (*Annona muricata*) leaf extract on growth of bacteria *Pseudomonas aeruginosa* in vitro). J Vet 2019;20(3):384.
17. Wulansari ED, Lestari D, Khoirunissa MA. Kandungan terpenoid dalam daun ara (*Ficus carica* L.) sebagai agen antibakteri terhadap bakteri *Methicillin-Resistant Staphylococcus aureus*. Pharmacon 2020;9(2):219.
18. Saptowo A, Supriningrum R. Uji aktivitas antibakteri ekstrak kulit sekilang (*Embeliaborneensis Scheff*) terhadap bakteri *Propionibacterium acnes* dan *Staphylococcus epidermidis*. Al Ulum Sains dan Teknol 2022;7(2):93-97.
19. Anggraini W, Nisa SC, Da RR, *et al.* Antibacterial activity of 96 % ethanol extract cantaloupe fruit (*Cucumis melo l . Var . Cantalupensis*) against escherichia coli bacteria. Pharm J Indones. 2019;5(1):61–6.
20. Pulungan ASS. Aktivitas antijamur ekstrak etanol daun kunyit (*Curcuma longa LINN.*) terhadap jamur *Candida albicans*. Biolink 2017;3(2):124-128.
21. Kusbiantoro D, Purwaningrum Y. Pemanfaatan kandungan metabolit sekunder pada tanaman kunyit dalam mendukung peningkatan pendapatan masyarakat. Utilization of secondary metabolite in the turmeric plant to increase community income. J Kultiv 2018;17(1):544-949.
22. Cholilah S, Sudjino. Pengaruh ketersediaan air terhadap hasil dan kandungan kurkumin kunyit (*Curcuma domestica Valeton*). Bioma 2019;15(1):21-26.