Mar'ie et al (2022)
Vol 10(1): 5462
https://doi.org/10.20884/1.api.2022.10.1.5462

Acta Pharmaciae Indonesia:
Acta Pharm Indo
E-ISSN 2621-4520

RESEARCH ARTICLE Open Access

Antibacterial activity of *Cassia alata* stem ethanol extract against *Staphylococcus aureus*

Ahmad Mahdi Mar'ie, Irfan Zamzani*, Siti Nashihah

ABSTRACT

Background: *Cassia alata* leaves, also known as petai cina or gelinggang in Indonesia, are commonly used to treat skin ailments.

Objective: The purpose of this study is to test whether stem of *C. alata* has antibacterial activity against *Staphylococcus aureus*.

Methods: *C. alata* stems were extracted using an ultrasound-assisted extraction method. For antibacterial activity, disc diffusion was used with extract concentrations of 25%, 50%, 75%, 100%.

Results: The inhibition zones of the ethanol extract of *C. alata* stems at 25%, 50%, and 75% concentrations were 17.6 mm, 21 mm, and 22.6 mm, respectively, with the highest inhibition zone at 100% concentration at 25 mm.

Conclusion: The ethanolic extract of *C. alata* stems has a strong inhibitory effect on *Staphylococcus aureus*.

Keywords: antibacterial activity, Cassia alata, ultrasound-assisted extraction, Staphylococcus aureus

Introduction

Skin disease remains a public health issue in both developed and developing countries. Bacteria, viruses, fungi, and protozoa are among the microorganisms that can cause infection. *Staphylococcus aureus* is a pathogenic bacteria that causes infections and skin problems [1].

Staphylococcus aureus frequently infects the skin, causing itching and even inflammation of the affected area [2]. The Dayak community has utilized *C. alata* as a medication to treat skin disorders. The plant part employed is the leaves, which are pounded before being adhered or rubbed on itchy skin [3]. Antibacterial activity of *C. alata* leaf extract against *Staphylococcus aureus* has been demonstrated in some studies [3].

C. alata is a plant that grows in humid environments. *C. alata* belongs to the Magnoliophyta division and is easily found in tropical and subtropical areas [4]. *C. alata* has been used as a traditional medicine to treat bacterial infections like syphilis and bronchitis, as well as fungal infections like tinea versicolor, ringworm, eczema, as well as parasitic infections such as malaria. *C. alata* contains alkaloids, saponins, tannins, steroids, anthraquinones, flavonoids, and carbohydrates. Flavonoids in *C. alata* have anti-inflammatory, anti-allergic, antimicrobial, and antioxidant properties. Flavonoids in *C. alata* have anti-inflammatory, anti-allergic, antimicrobial, and antioxidant properties and are effective against a variety of fungus and bacteria. *C. alata* extract's antibacterial activity has been demonstrated in several studies [5,6].

C. alata leaves ethanol extract can inhibit the activity of the *Tinea pedis* [7]. *C. alata* leaves ethanol extract can inhibit the activity of the *Trichophyton Sp* [8]. The ethanol extract of *C. alata* leaves can inhibit the activity of *Ralstonia solanacearum* and *Streotococcous sobrinos* [4]. *C. alata* leaves ethanol extract can inhibit the activity of *E. coli* and *Staphylococcus aureus* [3]. *C. alata* flower methanol extract can inhibit the activity of *Staphylococcus epidermidis* [5].

C. alata stems are still understudied, despite their potential as an antibacterial to treat itching caused by

Department of Pharmacy, Universitas Muhammadiyah Banjarmasin, South Kalimantan, Indonesia

*Corresponding author: Jl. Gubernur Sarkawi, Lingkar Utara, Handil Bakti, Barito Kuala regency, South Kalimantan, Indonesia. E-mail: irfan.zamzani@umbjm.ac.id

the *Staphylococcus aureus* on the skin. Therefore, we are interested in exploring the antibacterial activity of *C. alata* stem against the *Staphylococcus aureus*.

Methods

Production and evaluation the quality of dried form (simplicia) *C. alata* stem

C. alata stems were collected in Banjarmasin, South Kalimantan, Indonesia, in the Pramuka street area. The stems were then sorted, rinsed, cut, and dried. Furthermore, a rod milling machine was used to create the powder form. Following that, the powder's quality was assessed using the procedures listed below.

Water content. The toluene distillation method was used to determine the water content. The toluene layer was extracted after 200 mL of toluene was saturated with 4 mL of distilled water for 1x24 hours. After that, 5 g of sample powder was heated for 15 minutes in a flask filled with toluene. The distillation speed was increased until the toluene totally separated [9].

Drying weight reduction. 1 g of simplicia was carefully weighed and placed in a covered porcelain crucible that had been thawed and heated at 105°C for 30 minutes. In a porcelain crucible, the simplicia was flattened by shaking the crucible until evenly dispersed. The simplicia was heated in an oven at 100-105°C, weighed, then reheated until it reached a constant weight [10].

Water solubility. 5 g of simplicia powder was macerated with 100 ml of chloroform P (2.5 ml of chloroform in 1000 ml of distilled water) for 24 hours using a flask while shaking occasionally for the first 6 hours, then allowed to stand. A mixture was quickly filtered, and 20 ml of the filtrate was evaporated in a flat bottomed shallow dish over a water bath to dryness, and the remainder was heated at 105°C until the weight remained constant. The content was calculated in the percent of the material that has been dried in the air; the water-soluble content requirement was not less than 24% [9].

Ethanol solubility. 5 g simplicia powder was macerated in 100 ml ethanol for 24 hours as directed in the monograph, using a flask and occasionally shaking for the first 6 hours, then left to stand. The mixture was immediately filtered, 20 ml of the filtrate was evaporated in a shallow dish. The requirement for ethanol-soluble extract content was not less than 6% [9].

Ultrasound-assisted extraction of *Cassia alata*

C. alata extract was prepared using the ultrasound-assisted extraction (UAE) method. *C. alata* stem powder was placed in an Erlenmeyer and poured with a 1:4 ratio of 96% ethanol solvent Covered erlenmeyer was placed in an ultrasonic cleaning bath and extracted for 30 minutes at a temperature of 40°C with a wave frequency of 40 kHz [11]. The 40 kHz wave was chosen because it was more effective than frequencies lower than 25 kHz or higher than 60 kHz. After that, it was left to sit for 30 minutes before being filtered to separate the filtrate from the dregs [12]. The extract was then evaporated to obtain a thick extract, and the yield was calculated.

Phytochemical screening

Alkaloids. In a test tube, 500 mg ethanol extract of C. alata stem was placed. 1 mL 2N HCl and 9 mL distilled water were mixed together and heated for 2 minutes in a water bath, then cooled and filtered. The filtrate was divided into two test tubes, each containing 0.5 ml, and Meyer and Dragendorff reagents were added. A Meyer's positive result was represented by the formation of a white precipitate, while the addition of Dragendorff's reagent produced a red-orange precipitate [13].

Flavonoids. A total of 100 mg of sample from 96% ethanol extract of *C. alata* stem was placed in a test tube. After adding 10 mL of ethanol and dissolving it, a few drops of H_2SO_4 were added to the test tube. The formation of a yellow to dark brown color indicates positive flavonoid findings [14].

Saponins. 100 mg of the *C. alata* stem ethanol extract was placed in a beaker glass, mixed with 10 ml of hot water, and then boiled for 5 minutes. It was also filtered, with the filtrate used as a test solution. The filtrate was placed in a sealed test tube, agitated for 10 seconds, and then set aside for 10 minutes. Following that, 1 ml of 2 M HCl was added. The presence of saponins is seen by the formation of a stable foam [13].

Tannins. 100 mg ethanol *C. alata* stem extract was mixed with 10 ml hot water, boiled for 5 minutes, and then filtered. Some of the filtrate obtained was added with a 1% $FeCl_3$ solution. A positive result is indicated by the formation of a blackish green color [13].

Antibacterial activity test

The concentrations of the extracts that were evaluated were 25%, 50%, 75%, and 100%. 3 g of NA

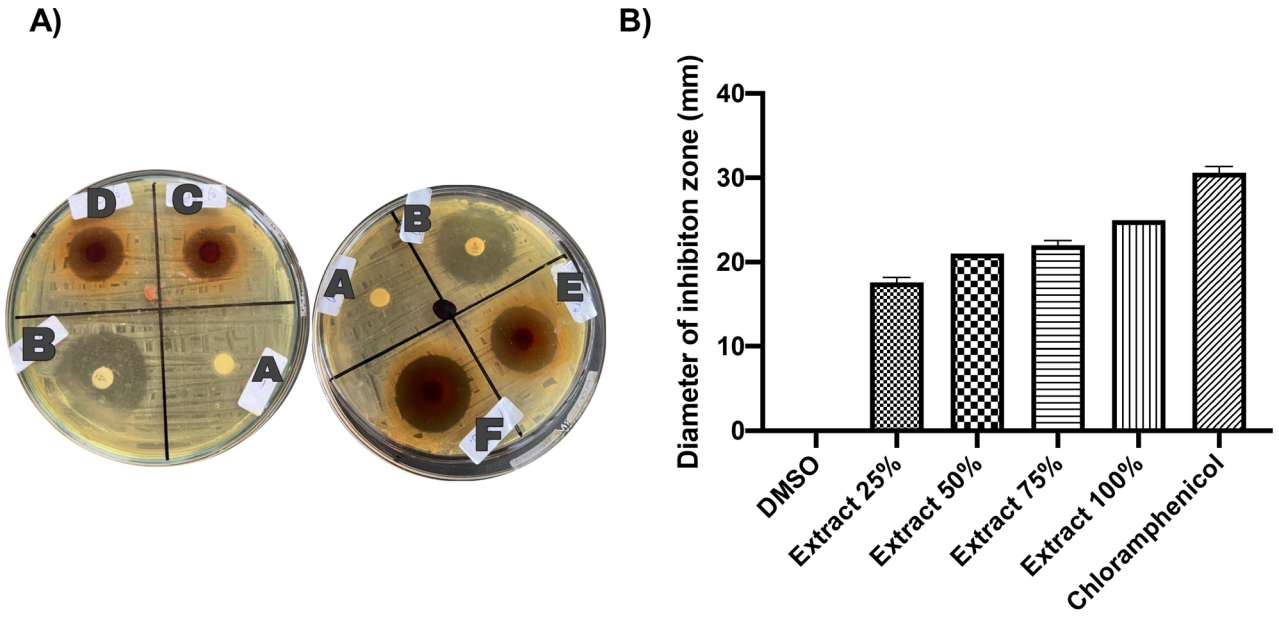

Figure 1. Antibacterial activity of ethanol *C. alata* stem extract. A). Antibacterial activity test results using the diffusion method: A (DMSO 1%, negative control), B (chloramphenicol positive control), C (extract concentration 25%), D (extract concentration 50%), E (extract concentration 75%), E (extract concentration 100%). B) The measurement of the inhibition zone ethanol extract of *C. alata* stem against *Staphylococcus aureus*

was mixed with 1 L of distilled water, homogenized, and then heated to boiling to make NA media. The media was sterilized in an autoclave for up to 15 minutes at 116°C - 121°C. Media were then placed in petri dish and wait for it to turn into agar.

Inoculated *Staphylococcus aureus* was taken with sterile wire and suspended in a tube containing 2 ml of 0.9% NaCl solution until the turbidity was equal to Mc. Farland's standard turbidity. A total of six petri dishes containing agar media were prepared. The bacteria were then cultivated in the agar media using a sterile loop on the slanted agar medium before being dipped into the Mc Farland solution. A sterilized cotton swab dipped in Mc. Farland was used to scratch in a zig-zag pattern. The discs with the tested extract concentrations of 25%, 50%, 75%, and 100% were then inserted. The test treatment was carried out three times. Chloramphenicol was used as a positive control, while 1% DMSO was used as a negative control. The plates were incubated for 24 hours at 37°C in an incubator [15].

Results

Simplicia quality evaluation results

A total of 5 kg of wet *C. alata* stems produced 1900 g of simplicia dry powder. The percentage value obtained during the production of simplicia was 38%. Simplicia quality evaluation of *C. alata* stems included four tests: water content, drying weight reduction, water solubility, and ethanol solubility, with values of 4.5%, 7.5%, 34%, and 13%, respectively (Table 1). These four parameters indicate that the quality of simplicia has met the requirements of simplicia according to monograph [10].

Extraction and phytochemical screening test

The thick extract obtained was 20.2 g, with a yield of 2.89%. The extract showed reddish black color. Identification of phytochemical compounds showed that 96% ethanol extract of *C. alata* stems contained alkaloids, flavonoids, saponins, and tannins (Table 2).

Antibacterial activity test

The antibacterial activity was represented by the inhibition zone. At a concentration of 25%, the inhibition zone was 17.6 mm, indicating a relatively strong inhibitory property. At concentrations of 50% and 75%, the average inhibition zone was 21 mm and 22.6 mm, showing a very strong inhibition. At a concentration of 100%, it has a large inhibition zone (25 mm) and is classified as a very strong inhibition property (Figure 1).

According to the results, the 1% DMSO negative control produced no inhibition, indicating that DMSO

Table 1. Simplicia quality evaluation

Test	Standard	Results
Water content	<10%	4,5%
Drying weight reduction	<10%	7,5%
Water solubility	>24%	34%
Ethanol solubility	>6%	13%

Table 2. Phytochemical screening test

Test	Reagent	Result	Note
Flavonoids	H_2SO_4	(+)	Dark brown
Alkaloids	Mayer	(+)	White precipitate
	Dragendorf	(+)	Orange precipitate
Saponins	HCl 2N	(+)	Stable foam formation up to 2 cm
Tannins	$FeCl_3$	(+)	Dark green

had no effect on the test results for the extract concentration, whereas the chloramphenicol treatment produced an inhibition zone of 30.6 mm, indicating a very strong inhibition property.

Discussion

Antibacterial extract at a concentrations of 50% and 75% showed a very strong inhibitory. The increase in the inhibitory zone corresponded to an increase in the extract concentration. It could be because the extract of *C. alata* stems contains a higher active substance. The increasing concentration of the extract aligned with an increase in the compound's concentration.

The total compounds extracted using ultrasound-assisted extraction are higher than those extracted by maceration. From maceration and ultrasound-assisted extraction, total flavonoid levels were 0.42% and 0.75%, respectively [12]. Because the compound obtained by ultrasound-assisted extraction is higher than that obtained from conventional extraction, the increased chemical content is effective for reducing bacterial activity by destroying cell walls [4].

The leaves of the *C. alata* plant were used in the majority of studies. The ethanol extract of *C. alata* leaves inhibited S. aureus less effectively than the 96% ethanol extract of *C. alata* stems. The diameter zone inhibition was 5 mm, 7.2 mm, 7.6 mm, 12.2 mm, and 12.5 mm, respectively, at 10%, 30%, 50%, 70%, and 90% ethanol extract of *C. alata* stem treatment [16].

In this study, all concentrations of *C. alata* stem extract showed an inhibitory effect against *Staphylococcus aureus*. Secondary metabolites such as flavonoids, tannins, saponins, and alkaloids may be responsible for the inhibitory activity [17]. Flavonoids attack bacteria's cell walls, which are made up of lipids and amino acids [18]. Flavonoids can also interfere with nucleic acid production, cell membrane function, and energy metabolism [19].

Alkaloids interfere with bacterial growth by inhibiting cell metabolism [20]. Alkaloids have antibacterial properties, which are mediated by interfering with peptidoglycan components in bacterial cells, causing the cell wall layer to not fully form, resulting in cell death [21]. Because tannins have a target on the polypeptide wall of the bacterial cell, they exert antibacterial activity by causing *Staphylococcus aureus* cells to lyse. Tannins can also inhibit the transport of proteins through the inner layer of cells by inactivating bacterial enzymes [22]. Saponins work against bacteria by causing *Staphylococcus aureus* proteins and enzymes to leak out. Saponins are active chemicals that can cause hemolysis in cells by increasing membrane permeability. Bacteria are broken down or lysed when saponins contact with them [23].

Conclusion

Antibacterial activity tests of *C. alata* ethanol extracted by ultrasound-assisted extraction revealed that extract concentrations of 25%, 50%, 75%, and 100% demonstrated antibacterial activity against *Staphylococcus aureus*, yielding zones of inhibition of 17.6 mm, 21 mm, 22.6 mm, and 25 mm, respectively.

Acknowledgment

We thank to Dr. Sarmoko for editing and visualization the graph as well as Agil Patria Putra for translating and proofreading this manuscript.

Funding

None.

Author contributions

AMM designed the research and collecting data; AMM and IZ wrote the first manuscript; IZ and SN analyzed the data; all authors interpretated the data and agreed to the final version of the manuscript.

Received: 7 February 2022
Revised: 19 May 2022
Accepted: 22 May 2022
Published online: 1 June 2022

References

1. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28: 603–661. https://doi.org/10.1128/CMR.00134-14
2. Taylor TA, Unakal CG. Staphylococcus Aureus. StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
3. Oktavia KN, Aryati F, Herman H. Uji Aktivitas Antibakteri Ekstrak Daun Gelinggang (Cassia alata L). Proc Mul Pharm Conf. 2021;14: 160–165. https://doi.org/10.25026/mpc.v14i1.561
4. Egra S, Mardiana M, Kurnia A, Kartina K, Murtilaksono A, Kuspradini H. Uji potensi ekstrak daun tanaman ketepeng (Cassia alata L)dalam menghambat pertumbuhan bakteri Ralstonia solanacearum dan Streptococcussobrinus. Ulin J Hut Trop. 2019;3: 25–31. https://doi.org/10.32522/ujht.v3i1.2059
5. Fatmawati S, Yuliana, Purnomo AS, Abu Bakar MF. Chemical constituents, usage and pharmacological activity of Cassia alata. Heliyon. 2020;6: e04396. https://doi.org/10.1016/j.heliyon.2020.e04396
6. Angelina M, Mardhiyah A, Dewi RT, Fajriah S, Muthiah N, Ekapratiwi Y, et al. Physicochemical and phytochemical standardization, and antibacterial evaluation of Cassia alata leaves from different locations in Indonesia. PHAR. 2021;68: 947–956. https://doi.org/10.3897/pharmacia.68.e76835
7. Anwar AND. Manfaat Daun Ketepeng Cina (Cassia alata L.) sebagai Antifungi pada Tinea Pedis. Jurnal Agromedicine. 2015;
8. Asmah N, Halimatussakdiah H, Amna U. Analisa Kandungan Senyawa Metabolit Sekunder Ekstrak Daun Ketepeng Cina (Cassia alata L.) dari Bireum Bayeun, Aceh Timur. JQ. 2020;2: 7–10. https://doi.org/10.33059/jq.v2i2.2646
9. Depkes RI. Materia Medika Indonesia. Depkes RI; 1989.
10. Depkes RI. Parameter Standar Umum Ekstrak Tumbuhan Obat. Depkes RI; 2000.
11. Buanasari, Febrianto Y, Cholifah, Chakim A. Potensi metode ultrasonic-assisted extraction (UAE) dalam mengekstrak senyawa aktif dari bahan alam. Jurnal Farmasi & Sains Indonesia. 2019;2: 106–112.
12. Utami NF, Sutanto S, Nurdayanty SM, Suhendar U. Pengaruh berbagai metode ekstraksi pada penentuan kadarflavonoid ekstrak etanol daun iler (Plectranthus scutellarioides). fitofarmaka JIF. 2020;10: 76–83. https://doi.org/10.33751/jf.v10i1.2069
13. Nugrahani R, Andayani Y, Hakim A. Skrining fitokimia dari ekstrak buah buncis (Phaseolus vulgaris L.) dalam sediaan serbuk. JPPIPA. 2016;2: 96–103. https://doi.org/10.29303/jppipa.v2i1.38
14. Tjitda PJP, Nitbani FO. Skrining fitokimia ekstrak metanol, kloroform dan n-heksan daun flamboyan. JSTK. 2019;13: 70. https://doi.org/10.20527/jstk.v13i2.5949
15. Mpila D, Fatimawali F, Wiyono W. Uji aktivitas antibakteri ekstrak etanol daun mayana (Coleusatro purpureus [L] Benth) terhadap Staphylococcus aureus, Escherichia coli dan Pseudomonas aeruginosa secara in-vitro. Pharmacon. 2012;
16. Lathifah QA, Turista DDR, Puspitasari E. Daya Antibakteri Ketepeng Cina (Cassia alata L.) Terhadap Staphylococcus aureus, Pseudomonas aerugenosa, dan Klebsiella pneumonia. jak. 2021;10: 29. https://doi.org/10.26630/jak.v10i1.2718
17. Fajri M, Marfu'ah N, Artanti LO. Aktivitas antifungi daun ketepeng cina (Cassia alata L.) fraksi etanol, n-heksan, dan kloroform terhadap jamur Microsporium canis. pharmasipha. 2018;2: 1–8. doi:10.21111/pharmasipha.v2i1.2134
18. Zamzani I, Triadisti N. Antibacterial Activity of Extract and Fraction of Baccaurea macrocarpa Leaf on Escherichia coli and Bacillus cereus. Proceedings ISETH. 2019; 551–556.
19. Pendit PACD, Zubaidah E, Sriherfyna FH. Karakteristik fisik-kimia dan aktivitas antibakteri ekstrak daun belimbing wuluh (Averrhoa bilimbi L.). Jurnal Pangan dan Agroindustri. 2016;4: 400–409.
20. Haryati NA, Saleh C, Erwin. Uji toksisitas dan aktivitas antibakteri ekstrak daun merah tanaman pucuk merah (Syzygium myrtifolium Walp.) terhadap bakteri Staphylococcus aureus dan Escherichia coli. Jurnal Kimia Mulawarman. 2016;13: 35–40.
21. Marfuah I, Dewi EN, Rianingsih L. Kajian potensi ekstrak anggur laut (Caulerpa racemosa)sebagai antibakteri terhadap bakteri Escherichia coli dan Staphylococcus aureus. Jurnal Pengolahan dan Bioteknologi Hasil Perikanan. 2018;7: 7–14.
22. Ngajow M, Abidjulu J, Kamu VS. Pengaruh Antibakteri Ekstrak Kulit Batang Matoa (Pometia pinnata) terhadap Bakteri Staphylococcus aureus secara In vitro. JM. 2013;2: 128. https://doi.org/10.35799/jm.2.2.2013.3121
23. Sapara TU. Efektivitas antibakteri ekstrak daun pacar air (Impatiens balsamina L.) terhadap pertumbuhan Porphyromonas gingivalis. Pharmacon. 2016.