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Original Research Article

Antibacterial effectiveness test and characterization of ethanol extract of bandotan herba against *Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*

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ABSTRACT

Background: The bandotan plant has been officially recognized by the world health organization (WHO) as a traditional medicinal medicine. The Chinese government has officially recognized the bandotan plant as having health-promoting and longevity-enhancing properties. The content of several secondary metabolites of this bandotan plant, such as alkaloids, tannins, flavonoids, glycosides, and saponins, makes this plant have significant potential as an alternative to traditional therapy. This research aims to evaluate the bactericidal efficacy of the ethanol extract derived from the bandotan herb against *Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa* bacteria.
Methods: The test was conducted using agar diffusion and streaking methods, with the test sample concentration being taken into account. The test results demonstrated that the ethanol extract of herba bandotan, at a dosage of 300 mg/mL, possesses inhibitory effects on the development of *Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa bacteria*.
Results: The concentration required to kill *Staphylococcus aureus bacteria* by 50 mg/mL is achieved with a percent reduction of 98.27%. Similarly, a concentration of 75 mg/mL is needed to kill *Pseudomonas aeruginosa bacteria* with a percent reduction of 96.36%. Lastly, a concentration of 100 mg/mL can effectively eliminate *Staphylococcus aureus bacteria* with a percent reduction of 99.24%.
Conclusions: The study's findings indicate that the ethanol extract derived from the bandotan plant had bactericidal and bacteriostatic properties against *Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa bacteria.*

Keywords: *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa,* MIC and inhibitory zone

INTRODUCTION

Antibacterial testing is a substitute examination performed to ascertain the efficacy of a sample treatment in inhibiting bacterial growth.[1] Bacteria are the etiological agents of infectious illnesses. Infectious diseases provide a significant challenge to public health. The community must prioritize health as it directly impacts work productivity. Therefore, maintaining good health is crucial. Infectious diseases are illnesses that result from the presence and activity of harmful microorganisms, including bacteria, viruses, parasites, and fungus.[2] Most

infectious illnesses primarily target the skin. At now, the management of infectious diseases entails the utilization of antibiotics, despite the recognized fact that prolonged antibiotic usage might result in development of resistance.[3]

Skin illness is a type of infection caused by bacteria. *Staphylococcus epidermidis, Staphylococcus aureus, and Pseudomonas aeruginosa* are examples of bacteria that can cause skin infections. *Staphylococcus epidermidis* is a gram-positive-bacteria that mostly causes opportunistic infections on the skin, particularly in those with compromised immune systems. *Staphylococcus epidermidis* is a type of bacteria that colonizes the surface of the skin.[4,5]

Staphylococcus aureus is a spherical, gram-positive, and anaerobic bacteria. *Staphylococcus aureus* is a pathogenic bacterium that specifically targets the skin, resulting in the development of boils, cellulitis, and *Staphylococcal* scalded skin syndrome (SSSS). *Pseudomonas aeruginosa* is a pathogenic bacterium responsible for causing a significant number of infectious disorders, accounting for approximately 10-15% of annual cases. A bacterial infection known as urinary tract infection, which is prevalent in numerous healthcare facilities, is caused by this bacterium.[6]

Antibiotics are employed to conquer infection. Research consistently demonstrates that prolonged antibiotic treatment can result in adverse consequences that can be harmful to patients. Antibiotic usage can lead to the development of resistance, characterized by the antibiotics' failure to eradicate microorganisms. Hence, there is a requirement for alternative therapies that exhibit low adverse reactions and can effectively combat the proliferation of naturally occurring germs.[7]

Antibiotics are employed to overcome infection. The location where the research was conducted the bandotan plant, which is native to Indonesia, has undergone extensive testing. Bandotan plants have been officially recognized by the WHO as traditional medical remedies. The Chinese government has officially recognized the bandotan plant as having health-promoting and longevity-enhancing properties. The bandotan plant possesses numerous secondary metabolites, including alkaloids, tannins, flavonoids, glycosides, and saponins, which contribute to its significant potential as a substitute for conventional treatment. Given the aforementioned context, this research aims to evaluate the efficacy of bandotan plants in suppressing the proliferation of *Staphylococcus epidermidis, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria*. This study suggests that prolonged antibiotic usage may lead to adverse effects that can be harmful to patients. Antibiotics can lead to the development of resistance, characterized by the ineffectiveness of antibiotics un eradicating germs. Hence, there is a requirement for alternative therapies that exhibit low adverse reactions and can effectively combat the proliferation of naturally occurring germs.[8,9]

Based on the background above, this research aims to see the ability of natural ingredients from the bandotan plant as an alternative natural treatment to inhibit bacterial growth.

METHODS

This study methodology employs an experimental approach. This research was conducted from November 2023 to January 2024

Equipment and resources

This research uses laboratory glass equipment such as beakers, erlenmeyers, autoclaves, ovens, laminar air flow (LAF), calipers, micro pipettes, petri dishes and evaporating dishes. The further study materials utilized encompass distilled water, dimethylsulfoxide (DMSO), *Stahphylococcus aureus* (ATCC® 6538), *Staphylococcus epidermidis* (ATCC® 12228TM), *Pseudomonas aeruginosa* (ATCC® 15442TM), buffer, Muller-Hinton agar (MHA), nutrient agar (NA), and nutrient broth (NB).

Preparation of the sample

The specimens utilized in this investigation were bandotan plants obtained from the Medanense herbarium laboratory, which is part of the department of biology in the faculty of mathematics and natural sciences at the universitas Sumatera Utara. The herb bandotan was gathered, thereafter cleansed, drained, and desiccated in a drying cabinet. The symplisia was subsequently pulverized using a blender to decrease the size of the sample.[10]

Bandotan herb extraction

The powdered form of the bandotan herb was subjected to maceration using a solvent of 96% ethanol for a duration of 5 days. The resulting mixture was then filtered, and the filtrate was concentrated using a rotary evaporator until a dense extract was achieved.

Characterization and phytochemical screening of herba bandotan (Ageratum conyzoides) simplisia

The conducted tests for symbilical characterization encompass the analysis of water content, total ash content, acid-insoluble ash content, water-soluble juice content, and ethanol-soluble juice content. The conducted phytochemical screening tests encompassed identification tests for alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids.

Preparation of nutrient agar media (NA)

Preparation of nutritional agar medium the nutrient agar powder, weighing 23 g, was measured and placed into an Erlenmeyer flask. Water was gradually added to the flask until it reached a total volume of 1000 ml. The mixture was then heated and stirred until the powder completely

dissolved and became clear. Next, the flask was covered with cotton that was coated with aluminum foil. The entire setup was sterilized in an autoclave at a temperature of 121^0 C for a duration of 15 minutes.[11]

Preparation of muller Hinton agar (MHA) media

The MHA powder, weighing approximately 38 g, was placed in an Erlenmeyer flask and gradually mixed with distilled water until a total volume of 1000 ml was reached. The mixture was then heated to boiling while continuously stirring until the powder entirely dissolved and became transparent. Subsequently, the Erlenmeyer flask was sealed with aluminum foil-wrapped cotton and subjected to sterilization in an autoclave at a temperature of 121°C for a duration of 15 minutes.[7]

Antibacterial activity test of herba bandotan ethanol extract against S. aureus, S. epidermidis, and Pseudomonas aeruginosa by Agar diffusion method

The ethanol extract of the bandotan plant was subjected to antibacterial activity tests at different doses. The experiment was performed with the agar diffusion technique on paper plates. 0.1 ml of bacterial inoculum was added to a sterile petri plate, followed by pouring 15 ml of nutritional agar media at a temperature of $45\text{-}50^0$ C. The petri dish was thereafter homogenized on a flat surface to ensure thorough mixing of the media and bacterial suspension. Subsequently, multiple paper plates, saturated with ethanol extracts of bandotan herb at different concentrations and dimethyl sulfoxide (DMSO) as a control, are placed closely together on a media. These plates are then incubated at a temperature of 37 °C for a duration of 20 hours. Following incubation, the diameter of the surrounding area where inhibition occurs is measured using a caliper.[12]

Antibacterial activity test of herba bandotan ethanol extract against S. aureus, S. epidermidis, and Pseudomonas aeruginosa by streaking method

Following the determination of the MIC value, the subsequent antibacterial activity test is conducted using the streaking method. The purpose of this test is to ascertain the least lethal concentration (KBM) of the ethanol extract from the bandotan plant against the microorganisms *Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa.*

Pseudomonas aeruginosa: Prepare a test tube containing 2 ml of Mueller-Hinton broth (MHB). Use a sterile cotton swab to streak the inhibition zone formed at each concentration. Dip the swab into each test tube and transfer the contents onto a labeled sterile petri dish. Measure 15 ml of plate count agar (PCA) media and pour it into a petri dish. Homogenize the media by making a figure 8 movement. Incubate all petri dishes in an incubator at 37°C for 24 hours. After 24 hours, count the colonies formed using a colony counter (Interscience Scan300). The counter can detect colonies as small as 0.1 mm and count up to a maximum of 1000. The minimum kill concentration value is defined as the lowest concentration that can decrease bacterial colonies by 98-99% of the initial number.[13]

RESULTS

Results of the characteristic examination of herba bandotan (Ageratum conyzoides) simplisia

Characterization testing of simplisia is conducted to assess the quality of the simplisia being examined. The outcomes of simplisia characterisation test are presented in Table 1.

Table 1: Results of the characteristic features of bandotan (*Ageratum conyzoides*) herba simplisia.

Characteristic testing	Result (%)
Water content	5.95
Water soluble juice content	17.12
Ethanol soluble juice content	20.83
Total ash content	8.05
Acid insoluble ash content	3.0

From the test results it can be concluded that this bandotan herb simplicia meets the characteristics sample testing standards in accordance with the national formulary.[14]

Phytochemical screening results of ethanol extract of herba bandotan (Ageratum conyzoides)

Phytochemical screening tests are conducted to ascertain the presence and quantity of secondary metabolites in a given sample. The findings from phytochemical screening analysis of bandotan herb are presented in Table 2.

Phytochemical screening tests on bandotan herbs for simplicia and extracts gave the same results.

Table 2: Analysis of the attributes of the Bandotan herb simplisia (*Ageratum conyzoides*).

Secondary metabolites	Reagents	Simplisia	Extracts
Alkaloids	Dragendroff, Bouchardart Mayer	+	+
Flavonoids	Mg powder + amyl alcohol + HCl (p)	+	+
Glycosides	Molish + H_2SO_4	+	+
Saponins	Hot water	+	+
Tannins	$FeCl_3$	+	+
Triterpenoids/ steroids	Lieberman-Bourchat	+	+

Description: (+) = contains metabolite compounds.

Antibacterial activity test results of herba bandotan ethanol extract against Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa bacteria

The test was conducted using the agar diffusion method. The antibacterial activity test was conducted on herba bandotan ethanol extract to evaluate its effectiveness against *Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa bacteria*.

The findings of this test may be seen in Table 3, while Figure 1 illustrates the impact of different concentrations of herba bandotan extract.

The results of the potable kill concentration test on the growth of *Pseudomonas aeruginosa, Staphylococcus epidermidis, and Staphylococcus aureus bacteria* can be seen in the table below.

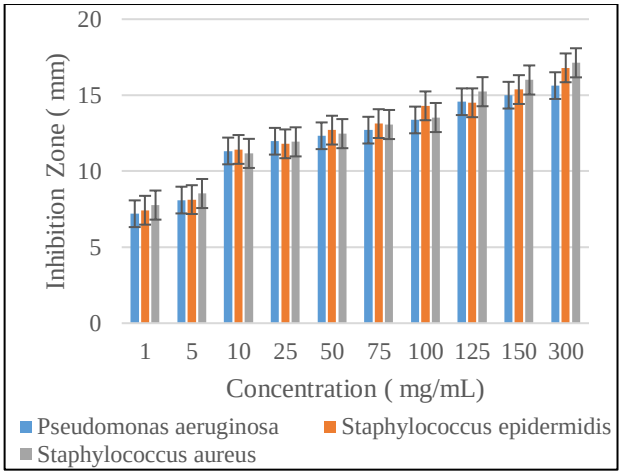

Figure 1: Effect of concentration of herba bandotan ethanol extract on the inhibition of *Pseudomonas aeruginosa, S. epidermidis, and S. aureus* bacteria.

Table 3: Diameter of the zone of inhibition for the growth of *Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa* bacteria when exposed to an ethanol extract of herba bandotan.

Concentration (mg/mL)	*Pseudomonas aeruginosa*	*S. epidermidis*	*S. aureus*
300	15.63±0.25	16.80±0.10	17.13±0.15
150	15.00±0.20	15.37±0.31	16.00±0.20
125	14.57±0.25	14.50±0.30	15.23±0.15
100	13.37±0.25	14.30±0.30	13.53±0.35
75	12.70±0.10	13.13±0.32	13.07±0.21
50	12.33±0.21	12.70±0.36	12.47±0.38
25	11.97±0.21	11.80±0.20	11.93±0.25
10	11.33±0.25	11.43±0.25	11.17±0.21
5	8.10±0.10	8.13±0.15	8.53±0.15
1	7.20±0.10	7.43±0.21	7.77±0.23

Table 4: Results of the minimum kill concentration test against the growth of *Staphylococcus aureus* bacteria.

Concentration	*Staphylococcus aureus*			
	Count	**Difference**	**% reduction**	**Log reduction**
K-	1969	0	0.00	0.00
1	1785	184	9.34	0.97
5	709	1260	63.99	1.81
10	422	1547	78.57	1.90
25	43	1926	97.82	1.99
50	34	1935	98.27	1.99
75	33	1936	98.62	1.99
100	15	1954	99.24	2.00
125	8	1961	99.59	2.00
150	5	1964	99.75	2.00
300	0	1969	100.00	2.00

Table 5: Results of the minimum kill concentration test against the growth of *Staphylococcus epidermidis* bacteria.

Concentration	*Staphylococcus epidermidis*			
	Count	**Difference**	**% reduction**	**Log reduction**
K-	2151	0	0.00	0.00
1	1734	417	13.93	1.29
5	657	1494	69.46	1.84
10	600	1551	72.11	1.86

Continued.

Concentration	*Staphylococcus epidermidis*			
	Count	**Difference**	**% reduction**	**Log reduction**
25	426	1725	80.20	1.90
50	107	2044	95.03	1.98
75	17	2134	98.37	2.00
100	15	2136	99.49	2.00
125	14	2137	99.52	2.00
150	5	2146	99.88	2.00
300	0	2151	100.00	2.00

Table 6: Results of the minimum kill concentration test on the growth of *Pseudomonas aeruginosa* bacteria.

Concentration	*Pseudomonas aeruginosa*			
	Count	**Difference**	**% reduction**	**Log reduction**
K-	3328	0	0.00	0.00
1	1822	1506	45.25	1.66
5	1821	1507	45.28	1.66
10	727	2601	78.16	1.89
25	454	2874	86.36	1.94
50	111	3217	96.66	1.99
75	21	3307	98.21	2.00
100	17	3311	99.35	2.00
125	16	3312	99.30	2.00
150	4	3324	99.77	2.00
300	0	3328	100.00	2.00

DISCUSSION

Characteristic testing of herba bandotan (Ageratum conyzoides) simplisia

Table 1 shows characterization test results, indicating that water content test of 5.95% satisfies test requirements, which specify a maximum of 10%.[15] Water content analysis is conducted to uphold the quality of simplisia due to its correlation with the proliferation of mold. When water content surpasses 10%, it becomes a favorable environment for microbial proliferation, leading to a decline in quality of simplisia.[16] An analysis is conducted to determine the quantity of substances that can dissolve in water and ethanol by testing the content of a juice that is soluble in both liquids. Ethanol soluble juice percentage has been determined to be 20.83%, whereas water-soluble juice content is 17.12%. This occurs due to the extraction of both polar and non-polar secondary metabolites from ethanol juice. Ash content analysis is conducted to ascertain the presence of internal minerals (physiological ash). Endogenous minerals originate from the plant's own tissue.[17] The acid insoluble ash content determination seeks to quantify the presence of silicates, such as sand, in the simplisia by dissolving the entire ash in hydrochloric acid. The ash percentage in the simplisia was determined to be 8.05%, with an acid-insoluble ash content of 3%.

Phytochemical screening of bandotan herbs (Ageratum conyzoides)

Table 2 reveals the presence of many secondary metabolite components in bandotan plants, including alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids, as determined by the phytochemical screening test. The bandotan herbs include a high concentration of flavonoids, terpenoids, and steroids in their compound composition. On the other hand, the leaves of the bandotan herbs are mostly composed of alkaloids, tannins, flavonoids, terpenoids, and steroids.[18] This is consistent with prior research findings. Bandotan plants are recognized for their alkaloid compounds, flavonoids, tannins, saponins, cardiac glycosides, anthraquinones, minerals, vitamins, and other substances that have antibacterial properties in their leaves and root.[19]

Antibacterial activity test results of herba bandotan ethanol extract against Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa bacteria

According to Table 3, the test results indicate that the antibacterial activity of the ethanol extract of the bandotan herb increases proportionally with the concentration. Consequently, the diameter of the inhibitory zone formed also increases. The diameter of the inhibition zone is influenced by the concentration of the extract. Higher concentrations result in larger inhibition zones, although even the smallest concentration of 1 mg/mL still exhibits antibacterial action, albeit categorized as mild inhibition.[20] The strength of an antibacterial is determined by examining the diameter of the inhibition zone formed. This measurement can be classified into different categories: an inhibition zone less than 9 mm is considered weak, 9-12 mm is considered moderate, 13-18 mm is considered strong, and above 18 mm is considered very strong.[21]

Previous research showed that bandotan extract was effective against *Pseudomonas aeruginosa* bacteria at concentrations >50 mg/mL with a KHM value of 75.86 mg/mL.[22] The bandotan leaf ethanol extract exhibits a MIC of 12.5 mg/mL against *Staphlococcus aureus* bacteria.[23]

Tables 4-6 indicate that at a concentration of 300 mg/ml, the ethanol extract of bandotan herb does not allow the formation of colonies of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* bacteria in the minimum kill concentration test. The ethanol extract of bandotan herb at a concentration of 50 mg/ml has a KBM value, or minimum kill concentration, that can effectively kill *Staphylococcus aureus* bacteria with a reduction percentage of 98.27%, *Staphylococcus epidermidis* with a reduction percentage of 95.03%, and *Pseudomonas aeruginosa bacteria* with a reduction percentage of 96.36%. At a higher concentration of 75 mg/ml, the bandotan herb ethanol extract can kill *Staphylococcus aureus bacteria* with a reduction percentage of 98.62%, *Staphylococcus epidermidis* with a reduction percentage of 99.49%, and *Pseudomonas aeruginosa bacteria* with a reduction percentage of 99.37%. Furthermore, at a concentration of 100 mg/ml, the bandotan herb ethanol extract can effectively kill *Staphylococcus aureus bacteria* with a reduction percentage of 99.24%, *Staphylococcus epidermidis* with a reduction percentage of 99.49%, *and Pseudomonas aeruginosa bacteria* with a reduction percentage of 99.3%. The KBM value is established by identifying the minimum concentration of extracts that can decrease the number of colonies by 98%-99% compared to the initial colonies (negative control).[10,24]

CONCLUSION

The ethanol extract of bandotan herb exhibits in vitro antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis, and Pseudomonas aeruginosa*, which are known to cause infections. The antibacterial activity is observed at a concentration of 50 mg/ml, with a diameter of inhibition zones measuring 12.33 mm on *Pseudomonas aeruginosa*, 12.70 mm on *Staphylococcus epidermidis*, and 12.47 mm on *Staphylococcus aureus.*

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Ethical approval: The study was approved by the Institutional Ethics Committee

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