WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 14, 81-100. Research Article ISSN 2277– 7105

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY TEST OF BANDOTAN (*AGERATUM CONYZOIDES* L.) EXTRACT ON *STREPTOCOCCUS PYOGENES* BACTERIA

[1]*Yani Mulyani, [2]Syifa Rohmatika Nabhani and [3]Ika Kurnia Sukmawati

Faculty of Pharmacy, Universitas Bhakti Kencana, Bandung, Indonesia.

Article Received on
23 Sept. 2021,

Revised on 14 October 2021,
Accepted on 04 Nov. 2021

DOI: 10.20959/wjpr202114-22294

***Corresponding Author**
Dr. Yani Mulyani
Faculty of Pharmacy,
Universitas Bhakti Kencana,
Bandung, Indonesia.

ABSTRACT

Upper Respiratory Tract Infection is often word health problem that can be caused by *Streptococcus pyogenes* bacteria. The treatment can use antibiotics or medicinal plants as alternative medicine. Bandotan plant (Ageratum conyzoides L.) is a plant that has antioxidant and antibacterial activity in the presence of phenol and flavonoid compounds. The purpose of this study is to determine the antioxidant and antibacterial activity of bandotan extract with various solvents. Antioxidant testing was carried out using the DPPH method, the determination of total phenol and flavonoid levels was continued, while the antibacterial activity test using the microdilution method to determine the Minimum Inhibitory Concentration (MIC), continue by determination of the Minimum Kill Concentration (MKC). The results of antioxidant activity showed bandotan extract of various solvents (ethanol 70%, ethyl acetate and chloroform) antioxidant activity with IC50 values of 126.4871 g/mL, 90.2554 g/mL and 93.2538 g/mL, while the levels of total phenol and total flavonoid obtained were 28.85 ± 1.48% and 0.65 ± 0.03 %, 35.06 ± 1.14% and 9.67 ± 0.30%, 25.85 ± 0.29, respectively. % and 9.68 ± 0.38%. Meanwhile, the results of antibacterial activity showed bandotan extract with various solvents (70% ethanol, ethyl acetate, and chloroform) antibacterial activity with MIC of 256 ppm and MKC of 512 ppm.

KEYWORDS: Bandotan; antioxidant, antibacterial; Streptococcus pyogenes.

INTRODUCTION

Acute Respiratory Infection (ARI) is a major health problem in the world. From a global perspective, based on the Indonesian Basic Health Research (*Riskesdas*) 2007, the prevalence of ARI is 25.50%.[1] Acute respiratory infections are caused by bacteria and viruses.[2] One of

the bacteria that causes it is *Streptococcus pyrogenes*. *Streptococcus pyrogenes* are gram-positive which are group A *Streptococcus* bacteria found on the surface of the nasopharyngeal epithelium and skin.[3,4] *Streptococcus pyrogenes* is a bacterial pathogen that can cause various kinds of infections including pharyngitis, impetigo, acute rheumatic fever, and tonsillitis.[5]

Acute Respiratory Infections (ARI) must be treated immediately using antibiotics. However, the inappropriate use of antibiotics or the misuse of antibiotics causes resistance.[5] Antibiotic resistance can cause the disease to take longer to heal, the mortality rate or death to be higher, and the cost of the treatment process to increase.

In the treatment of Acute Respiratory Infections (ARI), besides using antibiotics, currently many medicinal plants are used as alternative treatments. One of the medicinal plants that have the potential of antibacterial is Bandotan (*Ageratum conyzoides* L.). Bandotan (*Agerantum conyzoides* L.) is a plant used by people in the tropics and subtropics in medicine as an anti-inflammatory, antinociceptive, antibacterial, burns, malaria, leprosy, dermatitis, asthma, and insecticide.[6] The secondary metabolites contained in Bandotan (*Agerantum conyzoides* L.) include flavonoids, coumarins, benzofurans, steroids, and terpenoids.[7]

Besides having antibacterial activity, Bandotan (*Agerantum conyzoides* L.) also has antioxidant activity. Bandotan plant (*Agerantum conyzoides* L.) contains phenolic compounds which function as antioxidants and antibacterials. The mechanism of the antibacterial action of phenolic compounds is to change membrane permeability and inhibit bacterial enzyme activity.[8] Based on a previous study, the Bandotan plant is proven to have antibacterial activity. The results of the study showed that Bandotan extract (*Ageratum conyzoides* L.) was proven to have antibacterial activity on *Helicobacter pylori* b testing the Minimum Inhibitory Concentration (MIC) of 10 ± 0.0 mg/ml.[9] Another study suggests that extract of Bandotan (*Ageratum conyzoides* L.) which was tested on *Streptococcus aureus* and *Escherichia coli* produce inhibition zones of 19.5 ± 2.4 mm and 8.1 ± 20.5 mm, respectively.[10] Therefore, in this study, the researchers tested the antioxidant and antibacterial activity of Bandotan extract (*Ageratum conyzoides* L.) with various solvents including chloroform, ethyl acetate, and ethanol on *Streptococcus pyogenes*.

MATERIALS AND METHOD

This study is an experimental study that included the making of Bandotan Simplicia, Bandotan

extract with various solvents (70% ethanol, ethyl acetate, and chloroform), characterization of simplicial and extract (drying shrinkage and extract yield), determination of antioxidant activity, and determination of total phenol and flavonoid levels, Gram staining, and determination of antibacterial activity on *Streptococcus pyogenes* bacteria.

Materials and Equipment

Equipment or tools used consisted of a rotary vacuum evaporator (Heidolph), water bath, oven (Memmert), autoclave, incubator (Memmert), microscope (Olympus), spectrophotometer (Shimazu), moisture balance (Denver instrument IR-30), analytical balance (Mettler Toledo), micropipette (Thermo Scientific), blender, laboratory glassware, wire loop, bunsen, volume pipette, glass jar, filter paper, spatel, test tube, tube clamp, water bath, object.

The materials used included Bandotan plant (*Ageratum conyzoides*) (Manoko Experimental Garden, Lembang), solvent (70% ethanol, ethyl acetate, and chloroform) (PT. Dwilab Mandiri), *Streptococcus pyogenes* bacteria (Universitas Indonesia), dimethyl sulfoxide (DMSO) (Merck), distilled water, sulfuric acid (Merck), Mayer reagent, Wagner reagent, dragendorff reagent, magnesium powder, amyl alcohol, hydraulic acid, $FeCl_3$ reagent, Liberman-Burchard reagent, DPPH, methanol, ascorbic acid, solution folin-ciocalteu, NaOH, filter paper, aluminum chloride, sodium acetate, crystal violet, iodine, safranin, *Mueller* Hinton Broth (MHB) (Oxoid), Blood Agar (Universitas Indonesia), and Mueller Hinton Agar (MHA) (Oxoid).

WORK PROCEDURES

Plant Determination

Bandotan plant (*Ageratum conyzoides* L.) was obtained from the Manoko Experimental Garden, Lembang. The determination was carried out at the Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjajaran Bandung.

Preparation of Simplicia

1 kg of fresh Bandotan (*Ageratum conyzoides* L.) was cleaned of dirt or other objects. Then, it was cleaned with water and dried by aerating at room temperature or by using an oven with a temperature of 50ºC. The drying process was carried out for approximately 6 days. The simplicial of Bandotan plant (*Ageratum conyzoides* L.) which had been made, was pollinated using a blender.[6,11]

Simplicia Drying Shrinkage Test

2 grams of Bandotan simplicial powder (*Ageratum conyzoides L.*) was weighed. Then, the simplicial powder was flattened in a weighing bottle to form a layer of approximately 5-10 mm. Then, it was put into the drying chamber with a drying temperature of 105℃. The test was repeated 3 times.[11]

Preparation of Bandotan Extract

The simplicial powder of Bandotan plant (*Ageratum conyzoides* L.) was weighed by ± 330 g (three times). Then, it was put into a different macerator. After that, three different solvents were added including 70% ethanol, ethyl acetate, and chloroform in each different macerator with a solvent ratio of 1:10 parts. The first 6 hours of immersion were carried out with occasional stirring and were left for 18 hours. After that, it was filtered to separate the macerate and extracted again by adding solvent with the same volume of solvent. The obtained macerate was concentrated using a rotary vacuum evaporator and evaporated using a water bath at a temperature of no more than 50^0C to become a thick extract.[6,11,12] The following formula was used to compute the extract yield:

$$\text{Yield} = \frac{\text{Boweight of extract obtained (g)}}{\text{Initial weight of Simplicia (g)}} \text{ x } 100\%$$

Phytochemical Screening

Alkaloids

1 mL of Bandotan extract (*Ageratum conyzoides* L.) was put in a test tube and 2N sulfuric acid was added and divided into two test tubes. 1 mL of Mayer and Drangendorff reagents were then added to the different test tubes. The tube with Mayer's reagent showed positive alkaloids with the formulation of a white or yellow precipitate. Meanwhile, the Dragendorff reagent showed a positive alkaloid with the formation of a red or orange-colored precipitate.[13,14]

Flavonoids

1 mL extract of Bandotan (*Ageratum conyzoides* L.) was put in a test tube added with 0.1 mg of Mg powder and 2 mL of amyl alcohol. The sample was then shaken and observed for color changes. Positive flavonoids were indicated by the formation of a red, yellow, or orange color on the amyl alcohol layer.[13]

Saponins

1 mL of Bandotan extract (*Ageratum conyzoides* L.) was put into a test tube and 1 mL of warm water was added. The mixture was stirred for 30 seconds. After observing the foam, the foam lasted for 10 minutes at a height of 1 cm and the foam did not disappear after adding a drop of 2N HCl, positive for saponins.[13,14]

Triterpenoids/steroids

1 mL of Bandotan extract (*Ageratum conyzoides* L.) was added 2 mL of chloroform and Liberman-Burchard in a test tube. Then, the observation showed that there is a color change formed. Bluish-green color indicates a positive steroid while red indicates positive for triterpenoids.[13,14]

Tannins

1 ml extract of Bandotan (*Ageratum conyzoides* L.) which was then added with $FeCl_3$ (10%) of 2 to 3 drops. The green or turquoise color indicates a positive for tannins.[13,15]

Antioxidant Activity Test

Testing was conducted in the following ways.[16–18]

DPPH Solution Preparation

A 50 ppm DPPH solution was made by dissolving 5 mg of DPPH and adding 100 mL of methanol in a volumetric flask.

Preparation of extract solution of Bandotan (*Ageratum conyzoides* L.)

Stock solution with a concentration of 500 ppm was prepared by weighing 5 mg Bandotan extract (*Ageratum conyzoides* L.). then, 10 mL of methanol was added. After that, concentration variations were made from 60 ppm, 100 ppm, 140 ppm, and 160 ppm for the 70% Bandotan ethanol extract (*Ageratum conyzoides* L.). Concentrations for ethyl acetate extract and chloroform Bandotan (*Ageratum conyzoides* L.) were 60 ppm, 80 ppm, 100 ppm, and 120 ppm.

Preparation of Comparison Solution (Ascorbic Acid)

The comparison was done with a concentration of 100 ppm made by weighing 1 mg of ascorbic acid. Then, 10 mL of methanol was added. After that, four variations of concentration were made including 1 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.

Blank in Antioxidant Activity Test

4 ml of DPPH solution was pipetted in a test tube. The solution was vortexed and incubated for 37^0C in a room without light. Absorbance measurements were made with a wavelength of 517 nm.

Antioxidant Activity Test

0.5 mL of Bandotan (*Ageratum conyzoides* L.) plant extract sample solution was pipetted with various concentrations. 3.5 mL of DPPH solution was added at different concentrations. After that, it was vortexed and incubated at 37^0C in a room without light. At a wavelength of 517 nm, the absorbance was measured.

Determination of Total Phenol Level

Total phenol level was determined in the following ways[11]**:**

Preparation of Extract Test Solution

Bandotan (*Ageratum conyzoides* L.) plant extract was weighed approximately 0.2 grams and put in Erlenmeyer. 25 mL of methanol was added and stirred with a magnetic stirrer for 30 minutes. Then, it was filtered on the volumetric flask, and methanol was added to the mark through a filter.

Preparation of Comparison Solution

10 mg of the comparison solution as weighed and put in a 25 mL measuring flask and dissolved in methanol to the mark. A series of dilutions of the comparison solution was carried out at the following concentrations of 100, 70, 50, 30, 15, and 5 µg/mL.

Total Phenol Level Test

1 mL was taken from the sample series and comparison solution. Dilute Folin-Ciocalteu LP was added and stored for 8 minutes and 4.0 mL of 1% NaOH was added and incubated for 1 hour. Each sample was measured at a wavelength of ± 730 nm. The blank was measured in the same way but without the addition of the test solution. After that, a calibration curve was made and the concentration of the test solution was calculated (Indonesian Ministry of Health, 2017).

Total Flavonoid Level Test

Flavonoid level was measured by[11]

Preparation of Test Solution

Extract of Bandotan (*Ageratum conyzoides* L.) was weighed approximately 0.2 grams and put in an Erlenmeyer flask. 25 mL of ethanol was added and stirred using a magnetic stirrer for 1 hour. Next, it was filtered in a 25 mL volumetric flask and added with ethanol to the mark.

Preparation of Comparison Solution

About 10 mg of the comparator was put into a 25 mL volumetric flask and dissolved with ethanol. A series of dilutions were carried out at concentrations of 250, 200, 150, 100, 80 g/mL.

Total Flavonoid Level Test

0.5 mL was pipetted separately from the sample and the comparison solution series. 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M sodium acetate, and 2.8 ml of water were added to each solution, mixed, and left at room temperature for about 30 minutes. Measurements were carried out at a maximum wavelength of 415 nm. After that, a calibration curve was made and the concentration of the test solution was calculated.

PREPARATION OF MEDIA

Preparation of Mueller Hinton Broth (MHB)

21 grams of Muller Hinton Broth (MHB) was weighed and dissolved in 1000 mL of distilled water. Then, it was heated until the medium dissolved, autoclave at 121^0C for 15 minutes.[19]

Preparation of Mueller Hinton Agar (MHA)

38 grams of Mueller Hinton Agar (MHA) was weighed and dissolved in 1000 mL of distilled water. The medium was heated until dissolved. It was sterilized by autoclaving at 121^0C for 15 minutes.[19]

Preparation of Blood Agar

40 grams of Blood Agar Base was weighed and dissolved into 1000 mL of distilled water. Then the Blood Agar Base media was heated until dissolved and then sterilized by autoclaving at 121^0C for 15 minutes. After sterilization, the media was left to warm or around 45℃ - 50℃. After the media was getting warm, 7% of sterile sheep blood was added.[20]

Gram Stain

About 1-2 drops of sterile distilled water were placed on the slide. One loop of bacterial colonies was placed in sterile diaquadest and spread evenly. Then, the slide was fixed on a

Bunsen flame. The slide was dripped with crystal violet and left for 1 minute. The slide was then washed with distilled water and let dry. After that, iodine was added and allowed to sit for one minute. It was then rinsed and dried with distilled water. Furthermore, it was dripped with 95% ethanol and allowed to stand for 30 seconds and washed with distilled water, and dried. Finally, safranin was added and left for 30 seconds. Next, it was washed with distilled water and dried. Bacteria were observed under a microscope with 100x magnification. If the observation results show that the bacteria are purple, they are Gram-positive, while if the color of the bacteria is red, the bacteria are Gram-negative.[21]

Preparation of Bacterial Suspension

1 mL of the results from the inoculum of *Streptococcus pyrogenes* was taken. Then, it was suspended in 0.9% physiological NaCl and vortexed until homogeneous. Then, the dilution was carried out to reach an absorbance of 0.08 – 0.10 using visible spectrophotometry at a wavelength of 625 nm.[18,19]

Preparation of Test Solution

Bandotan extract (*Ageratum conyzoides* L.) with various solvents including ethanol 70%, ethyl acetate, and chloroform, as well as a comparator (Amoxicillin) each weighed 102.4 mg. Then, it was dissolved in 10 mL of DMSO until the concentration became 10,240 ppm (Solution 1). After that, 1 mL of solution 1 was taken and dissolved by 10 mL of DMSO until the concentration was 1024 ppm.[18,19]

ANTIBACTERIAL ACTIVITY TESTING

Minimum Inhibitory Concentration Test (MIC)

100 µl Mueller Hinton Broth (MHB) was poured into holes 1-23 microplate. Then, 100 µl suspension of *Streptococcus pyrogenes* was taken and inserted into holes 2-12. Hole 1 contains Mueller Hinton Broth (MHB) as a negative control. 100 µl of Bandotan plant extract test solution (*Ageratum conyzoides* L.) was added with various solvents (ethanol, ethyl acetate, and chloroform) or a comparison drug solution (amoxicillin) in different rows (starting with the 12^{th} hole) and the dilution was carried out from the 12^{th} to the 3^{rd} hole until the concentrations obtained were 512 ppm, 256 ppm, 128 ppm, 64 ppm, 32 ppm, 16 ppm, 8 ppm, 4 ppm, 2 ppm, and 1 ppm. Subsequently, incubation at 37^0C was carried out for 24 hours. The turbidity in each microplate hole was observed visually. Any clear solution at the lowest concentration is called MIC.[18,19]

Minimum Bactericidal Concentration (MBC)

Sterile Petri dishes were prepared and Mueller Hinton Agar (MHA) was added. After that, 15µl of the test solution that looks clear (the result of the MIC test) was added. Incubation at 37^0C for 24 hours. Observing the results by determining the lowest concentration indicating bacterial death (no bacterial growth) is called the Minimum Bacterial Concentration (MBC).[18,22]

RESULTS AND DISCUSSION

Plant Determination

Bandotan plant (*Ageratum conyzoides* L.) is a plant obtained from the Manoko Experimental Garden, Lembang. Bandotan plant (*Ageratum conyzoides* L.) was determined at the Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjajaran, Bandung. The determination was done to determine the certainty of the identity of the plants used in the study in order to avoid mistakes in using the plants to be studied.[23]

Preparation of the Simplicia

Basically, all the processes for making Bandotan Simplicia (*Ageratum conyzoides* L.) are to remove impurities in the form of foreign objects, reduce the water content that affects the extraction results, and increase contact with the sample to diffuses more into the sample particles. Thus, the absorption process is more efficient.[24]

Drying Shrinkage Test

Determination of drying shrinkage in simplicial Bandotan (*Ageratum conyzoides* L.) is important to limit the water content in Simplicia (25). Based on the results, the water content obtained was 3.743% ± 0.066. the requirement for water content in Bandotan (*Ageratum conyzoides* L.) was not more than 10%. Therefore, the water content of Bandotan follows the requirements. If a large amount of water is present in simplicial, it might become a medium for bacterial growth which damages the compounds in simplicial Bandotan (*Ageratum conyzoides* L.).[11,25]

Preparation of Bandotan Extract

The preparation of extract of Bandotan (*Ageratum conyzoides* L.) using the maceration method with different solvents (ethanol 70%, ethyl acetate, and chloroform). The maceration method was chosen because it is the simplest method and protects the heating-resistant active compound found in leaves of Bandotan (*Ageratum conyzoides* L.).[26] Then, the leaves were soaked for the first 6 hours with occasional stirring and then left for 18 hours. The stirring

process was intended to accelerate the penetration of solvent through the cell wall into the cell cavity containing the active substance.[27] Re-maceration was carried out three times with the same amount of solvent to maximize the recovery of active and secondary metabolites in the extract. Furthermore, re-maceration was carried out to equalize the concentration of the solution outside and inside the cell.[28,29] The macerate was concentrated in a rotary vacuum evaporator and evaporated using a water bath at a temperature not exceeding 50°C until a concentrated extract was obtained. Then the yield of the extract was determined which aims to determine the amount of active substance that is interested.[27]

Based on the results, the yield of the extract obtained from Bandotan 70% ethanol extract was 10.574%, Bandotan ethyl acetate extract was 10.014%, while Bandotan chloroform extract was 10.387%. This indicates that the three Bandotan extracts (*Ageratum conyzoides* L.) meet the requirements of the extract yield, which is not less than 9.6%. The greater the yield of the extract produced, the more active substances are attracted to the extract of Bandotan (*Ageratum conyzoides* L.).[11,27]

Phytochemical Screening

Phytochemical screening was done by observing the color change visually using dye reagents. The test results are determined by the type of compound present in the plant.[28] The results of the phytochemical screening are presented in Table 1 below.

Table 1: Phytochemical Screening Results.

Sample	Compound Group				
	Alkaloids	**Flavonoids**	**Steroids/ Triterpenoid**	**Saponins**	**Tannins**
Bandotan 70% Ethanol Extract	-	+	+ Triterpenoids	+	-
Bandotan Ethyl Acetate Extract	-	+	+ Steroids	+	-
Bandotan Chloroform Extract	-	+	+ Steroids	+	-

Description: (+) contains compounds, (-) does not contain compounds

Table 1 presents the phytochemical screening results obtained according to the content of compounds in Bandotan containing flavonoid compounds (nobiletin), triterpenoids, steroids, and saponins.[11,29] The difference in the content of compounds in Bandotan (*Ageratum conyzoides* L.) is influenced by the height of planting, the type of soil and organic contained in the soil, soil pH, temperature, and air.[19,30]

Antioxidant Activity Testing

Antioxidant activity, especially natural antioxidants, has been widely studied for its ability to maintain the balance of antioxidant oxidation. The mechanism of action of natural antioxidants is to interfere with the propagation of oxidation reactions. Examples of natural plant antioxidant compounds such as polyphenolic compounds are flavonoids, cinnamic acid derivatives, and coumarins.[24] The method used in this stud was the DPPH method since it is a simple, easy method, with a small number of samples and a short testing time.[31] Based on the results of antioxidant testing of Bandotan extract (*Ageratum conyzoides* L.) the inhibition percentage and IC_{50} were obtained as presented in Table 2.

Table 2: Results of Absorbance Measurement, Inhibition Percentage, and IC_{50} of Bandotan Extract (Ageratum conyzoides L.) and Ascorbic Acid Comparison.

Sample	Concentration (ppm)	Absorbance	Inhibition %	IC_{50} (µg/mL)
Blank 1	0	0.929	0.000	
Bandotan 70% Ethanol Extract	60	0.669	27.987	126.4871
	100	0.542	41.658	
	140	0.421	54.682	
	160	0.364	60.818	
Blank 2	0	0.933	0.000	
Bandotan Ethyl Acetate Extract	60	0.615	34.084	90.2554
	80	0.511	45.230	
	100	0.412	55.841	
	120	0.327	64.952	
Blank 3	0	0.927	0.000	
Bandotan Chloroform Extract	60	0.632	31.823	93.2538
	80	0.525	43.366	
	100	0.419	54.800	
	120	0.341	63.215	
Blank 4	0	0.788	0.000	
Ascorbic Acid	2	0.667	15.355	6.6451
	4	0.533	32.360	
	6	0.402	48.985	
	8	0.321	59.264	
	10	0.219	72.208	

Table 2 showed that the antioxidant test for Bandotan extract (*Ageratum conyzoides* L.) produced different percentages of inhibition with IC_{50}. The IC_{50} value was defined as the concentration of antioxidant compounds that lost 50% of DPPH activity (2.2 diphenyl 1 picrylhydrazyl). The requirements for the IC_{50} value are if the IC_{50} value is < 50 µg/mL, it is said to be very strong as an antioxidant. Meanwhile, if the IC_{50} value is 50-100 µg/mL, the

antioxidant is strong, the antioxidant is said to be moderate if the IC_{50} value is 100-150 µg/mL and is said to be weak as an antioxidant if the IC_{50} is 150-200 µg/mL.[32]

Based on the results of the study, the antioxidant activity of 70% ethanol extract showed a moderate category. Meanwhile, Bandotan ethyl acetate extract and Bandotan chloroform extract showed strong antioxidant activity. The difference in antioxidant activity is caused by the large content of secondary metabolites contained in Bandotan (*Ageratum conyzoides* L.) and the polarity of the solvent to attract secondary metabolites that can function as antioxidants in each extract. The results of antioxidant activity in the comparison (ascorbic acid) showed very strong activity. This is because ascorbic acid is an antioxidant that has the ability to scavenge free radicals and is more stable. After all, it can donate hydrogen atoms to DPPH free radicals.[33] Thus, it can be said that Bandotan extract (*Ageratum conyzoides* L.) has been shown to have antioxidant activity.

Determination of Total Phenol and Flavonoid Levels

Phenol and flavonoids are secondary metabolites that are believed to have antioxidant activity. Phenolic compounds and flavonoids work with free radical scavenging mechanisms and reduce oxidative stress.[34] The process of testing the total phenol content was done using the Folin-Ciocalteu method.[11] For comparison, gallic acid was used. Gallic acid was used as a standard since it is very effective in forming complex compounds with Folin-Ciocalteu reagent. Meanwhile, in the total flavonoid test, quercetin was used as a comparison because compounds belonging to the flavonoid groups of flavonols with a ketone group on the C-4 atom are hydroxyl groups on adjacent C-3 and C-5 atoms.[34] Based on the results, the total phenol and total flavonoid levels are listed in Table below.

Table 3: Calculation Results of Total Phenol and Total Flavonoid Levels of Bandotan Extract (*Ageratum conyzoides* L.).

Sample	Total Phenol Level	Total Flavonoid Level
Bandotan 70% Ethanol Extract	28,.85 ± 1.48 %	0.65 ± 0.03 %
Bandotan Ethyl Acetate Extract	35.06 ± 1.14 %	9.67 ± 0.30 %
Bandotan Chloroform Extract	25.85 ± 0.29 %	9.68 ± 0.38 %

Table No. 3 presents the results obtained that the total phenol content was greater than the total flavonoid content of bandotan extract with various solvents (ethanol 70%, ethyl acetate, and chloroform). This is because flavonoids are a group of phenolic compounds.[26] The results showed that phenolic compounds and flavonoid aglycones were soluble in semipolar. Thus,

they were more attracted to semipolar solvents, namely ethyl acetate and chloroform. This caused the total flavonoid content to be higher than the bandotan extract with 70% ethanol. However, the total phenol content of Bandotan extract with chloroform has a lower content compared to those with 70% ethanol. This depends on the number of phenolic compounds attracted in each extract.[35]

Gram Stain

Gram staining is the most basic staining method used to determine the morphology or identification of a bacterium. The bacteria used in this study were *Streptococcus pyogenes*. Gram stain serves to determine Gram-positive and Gram-negative bacteria.[36] The results obtained on Gram staining of *Streptococcus pyogenes* bacteria were spherical bacteria with an arrangement of two or more chains. After the Gram staining process, *Streptococcus pyogenes* produced a purple color, which is a Gram-positive bacterium. Gram-positive bacteria have a thicker peptidoglycan layer, while the peptidoglycan layer of Gram-negative bacteria is thinner and contains lipids on the outside. Peptidoglycan is the main polysaccharide, consisting of two chemical subunits found only in the cell wall of bacteria. The subunits consist of N-acetyl glucosamine and N-acetyl carbamate.[36,37] The presence of a thick cell wall or rich in peptidoglycan in *Streptococcus pyogenes* causing the color of crystal violet to be retained on the bacteria. Thus, the color of the bacteria still gives a purple color, categorized as Gram-positive bacteria.[38]

Antibacterial Activity Testing

Testing the antibacterial activity of Bandotan extract (*Ageratum conyzoides* L.) on *Streptococcus pyogenes* bacteria was carried out using the microdilution method. The advantages of the microdilution method are that the number of samples used is small, it is more efficient, the results are easier to observe visually, the cost is lower when repeated, and easier to do.[39,40] Antibacterial testing was carried out by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) as presented in Table No. 4 below.

Table No. 4: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Bandotan Extract (Ageratum conyzoides L.) and comparison solution (Amoxicillin) on Streptococcus pyogenes.

Sample	*Streptococcus pyogenes*	
	Minimum Inhibitory Concentration (MIC)	**Minimum Bactericidal Concentration (MBC)**
Bandotan 70% Ethanol Extract	256 ppm	512 ppm
Bandotan Ethyl Acetate Extract	256 ppm	512 ppm
Bandotan Chloroform Extract	256 ppm	512 ppm
Amoxicillin	8 ppm	64 ppm

Table No. 4 presents the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) extract of Bandotan (*Ageratum conyzoides* L.) with various solvents (ethanol 70%, ethyl acetate, and chloroform) had the same concentration. Minimum Inhibitory Concentration (MIC) in the three extracts of Bandotan (*Ageratum conyzoides* L.) was 256 ppm. The MIC was determined based on the lowest concentration that prevented the growth of bacteria or looked clear compared to the positive control (media added with *Streptococcus pyogenes*). After that, the Minimum Bactericidal Concentration (MBC) was tested based on the results of MIC. The results of MBC obtained in the three extracts of Bandotan (*Ageratum conyzoides* L.) were 512 ppm. Determination of MBC was seen based on the concentration of Bandotan extract (*Ageratum conyzoides* L.) capable of killing *Streptococcus pyogenes.*[22] Meanwhile, the comparison (Amoxicillin) has a Minimum Inhibitory Concentration (MIC) of 8 ppm and a Minimum Bactericidal Concentration (MBC) of 64 ppm. The results of MIC and MBC on amoxicillin were greater than that of Bandotan extract. This is because amoxicillin is a broad-spectrum penicillin-derived antibiotic that is often used for its role in inhibiting the growth of gram-positive and gram-negative bacteria. The mechanism of action of amoxicillin is to inhibit bacterial cell wall synthesis.[41,42]

Previous studies mentioned that the antibacterial activity test of Bandotan (*Ageratum conyzoides* L.) ethanol extract has antibacterial activity on *Staphylococcus aureus* and *Escherichia coli.* Antibacterial activity was indicated by the value of the Minimum Inhibitory Concentration (MIC) of 12.5 ppm and 25 ppm. Furthermore, the water extract of Bandotan showed antibacterial activity on *Staphylococcus aureus* and *Escherichia coli*, with Minimum Inhibitory Concentration (MIC) of 50 ppm and 100 ppm, respectively.[43] Based on these results, Bandotan extract (*Ageratum conyzoides* L.) was shown to have antibacterial activity.

The ability of the extract to inhibit the growth of microorganisms is influenced by the

antimicrobial compounds contained in the extract. The flavonoid group of compounds works by killing bacteria by destroying the bacterial cell membrane.[44] Other compounds that work as antibacterial are saponins. The mechanism of saponins is by causing the permeability of the bacterial cell membrane to increase and causing leakage in the cell which allows compounds in the cell to come out. Moreover, steroid and triterpenoid compounds have the potential as antibacterial. Terpenoids are easily soluble in lipids and this property allows them to penetrate the cell walls of both Gram-positive and Gram-negative bacteria. The content of steroids and terpenoids in Bandotan (*Ageratum conyzoides* L.) works by destroying lipid membranes resulting in leakage of liposomes and bacterial cell death.[45]

CONCLUSION

Extract of Bandotan (*Ageratum conyzoides* L.) with various solvents (ethanol 70%, ethyl acetate, and chloroform) was proven to have antioxidant and antibacterial activity on *Streptococcus pyogenes.* The highest antioxidant activity was found in the ethyl acetate extract of Bandotan (*Ageratum conyzoides* L.) with an IC_{50} value of 90.2554 µg/mL, followed by phenol and total flavonoid content of 35.06 ± 1.14 % and 9.67 ± 0.30 %. The extract of Bandotan (*Ageratum conyzoides* L.) with various solvents has a Minimum Inhibitory Concentration (MIC) value of 256 ppm and a Minimum Bactericidal Concentration (MBC) value of 512 ppm.

SUGGESTION

It is necessary to investigate further antibacterial activity on other Acute Respiratory Infection (ARI) bacteria in order to strengthen the activity of Bandotan leaves (*Ageratum conyzoides* L.).

ACKNOWLEDGEMENT

The researchers would like to thank The Institution of Research and Community Services (Lembaga Penelitian dan Pengabdian Masyarakat, abbreviated as LPPM) Universitas Bhakti Kencana for research funding with a contract number: 015/14.LPPM/AMD/PE.I/UBK/2021 and thanks to the Faculty of Pharmacy, Universitas Bhakti Kencana, Laboratory of Food Technology, Universitas Pasundan, and MarkHerb Institut Teknologi Bandung for their assistance in testing, facilities, and infrastructure which enable this study to run well.

REFERENCES

1. Depkes. Laporan Nasional Riskesdas 2007. Badan Penelit dan Pengemb Kesehat Dep Kesehatan, Republik Indones Desember 2008 [Internet], 2008; 1–384. Available from:

http://kesga.kemkes.go.id/images/pedoman/Riskesdas 2007 Nasional.pdf

2. Antonelli M, Donelli D, Firenzuoli F. Ginseng integrative supplementation for seasonal acute upper respiratory infections: A systematic review and meta-analysis. Complement Ther Med [Internet], April, 2020; 52: 102457. Available from: https://doi.org/10.1016/j.ctim.2020.102457
3. Colineau L, Laabei M, Liu G, Ermert D, Lambris JD, Riesbeck K, et al. Interaction of Streptococcus pyogenes with extracellular matrix components resulting in immunomodulation and bacterial eradication. Matrix Biol Plus [Internet], 2020; 6–7: 100020. Available from: https://doi.org/10.1016/j.mbplus.2020.100020
4. Samir A, Abdel-Moein KA, Zaher HM. Emergence of penicillin-macrolide-resistant Streptococcus pyogenes among pet animals: An ongoing public health threat. Comp Immunol Microbiol Infect Dis [Internet], 2020; 68: 101390. Available from: https://doi.org/10.1016/j.cimid.2019.101390
5. Du GF, Le YJ, Sun X, Yang XY, He QY. Proteomic investigation into the action mechanism of berberine against Streptococcus pyogenes. J Proteomics [Internet], 2020; 215(October 2019): 103666. Available from: https://doi.org/10.1016/j.jprot.2020.103666
6. Faqueti LG, Brieudes V, Halabalaki M, Skaltsounis AL, Nascimento LF, Barros WM, et al. Antinociceptive and anti-inflammatory activities of standardized extract of polymethoxyflavones from Ageratum conyzoides. J Ethnopharmacol, 2016; 194: 369–77.
7. Subah S, Bogoda N, Glávits R, Venkatesh R, Murbach TS, Kolep-Csete K. Prenatal developmental toxicity study of an alkaloid-free Ageratum conyzoides extract powder in rats by oral administration. Regul Toxicol Pharmacol, 2020; 117.
8. Acharya S, Patra DK, Pradhan C, Mohapatra PK. Anti-bacterial, anti-fungal and anti-oxidative properties of different extracts of Bruguiera gymnorrhiza L. (Mangrove). Eur J Integr Med [Internet]., April, 2020; 36: 101140. Available from: https://doi.org/10.1016/j.eujim.2020.101140
9. Ngnameko CR, Njayou FN, Fowora M, Nemg FBS, Moundipa Fewou P, Smith SI. Inhibitory effect of medicinal plants from Cameroon on the growth and adhesion of Helicobacter pylori. Eur J Integr Med [Internet], January; 2019; 30: 100957. Available from: https://doi.org/10.1016/j.eujim.2019.100957
10. Akhtar N, Ihsan-ul-Haq, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arab J Chem [Internet], 2018; 11(8): 1223–35. Available from: https://doi.org/10.1016/j.arabjc.2015.01.013

11. Kemenkes RI. Farmakope Herbal Indonesia Edisi 2. In 2017; 561.
12. Fardiyah Q, Ersam T, Suyanta, Slamet A, Suprapto, Kurniawan F. New potential and characterization of Andrographis paniculata L. Ness plant extracts as photoprotective agent. Arab J Chem [Internet], 2020; 13(12): 8888–97. Available from: https://doi.org/10.1016/j.arabjc.2020.10.015
13. Kardinasari E, Devriany A. Phytochemical identification of bangka origin virgin green coconut oil: Anti-inflammatory and anti-bacterial potential. Enferm Clin [Internet], 2020; 30: 171–4. Available from: https://doi.org/10.1016/j.enfcli.2019.10.062
14. Nabi NG, Shrivastava M. Phytochemical Screening and Antioxidant Activity of Ethanol Extract of <I>Psoralea corylifolia</I>seeds. UK J Pharm Biosci., 2017; 5(2): 1.
15. Alqethami A, Aldhebiani AY. Medicinal plants used in Jeddah, Saudi Arabia: Phytochemical screening. Saudi J Biol Sci [Internet], 2020; (xxxx). Available from: https://doi.org/10.1016/j.sjbs.2020.11.013
16. Handayani V, Ahmad AR, Sudir M, Etlingera P, Sm RM. Uji Aktivitas Antioksidan Ekstrak Metanol Bunga dan Daun Patikala (Etlingera elatior (Jack) R. M. Sm) Menggunakan Abstrak. Pharm Sci Res., 2014; 1(2): 86–93.
17. Purwanto D, Bahri S, Ridhay A. Issn: 2477-5398 uji aktivitas antioksidan ekstrak buah purnajiwa (. KOVALEN J Ris Kim., April, 2017; 3: 24–32.
18. Mulyani Y, Tias PA, Sukmawati I. Test of Antioxidant and Antibacterial Activity of the Extract of SambungNyawa (Gynura procumbens [Lour .] Merr .) Against Streptococcus pyogenes Bacteria, 2021; 11(10): 1–11.
19. Sukmawati IK, Yuniarto A, Alighita W, Zam-zam J A. Antibacterial Activity of Extract and Fraction From Shitake Mushroom (Lentinula edodes) Against Acne Bacteria. Indones J Pharm Sci Technol, 2018; 6(1): 36.
20. Krihariyani, Dwi Woelansari, Evy Diah Kurniawan E. POLA PERTUMBUHAN Staphylococcus aureus PADA MEDIA AGAR DARAH MANUSIA GOLONGAN O, AB, DAN DARAH DOMBA SEBAGAI KONTROL. J Ilmu dan Teknol Kesehat, 2016; 3(2): 191–200.
21. 21. Nurhidayati S, Faturrahman F, Ghazali M. DETEKSI BAKTERI PATOGEN YANG BERASOSIASI DENGAN Kappaphycus alvarezii (Doty) BERGEJALA PENYAKIT ICE-ICE. J Sains Teknol Lingkung, 2015; 1(2): 24–30.
22. Penduka D, Mthembu W, Cele KH, Mosa RA, Zobolo AM, Opoku AR. Extracts of Ansellia africana and Platycarpha glomerata exhibit antibacterial activities against some respiratory tract, skin and soft tissue infections implicated bacteria. South African J Bot

[Internet], 2018; 116: 116–22. Available from: https://doi.org/10.1016/j.sajb.2018.02.403

23. Niah R, Febrianti DR, Ariani N. AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOL 96% DAUN SEPAT (Mitragynaspeciosa) DAN DAUN DADANGKAK (Hydrolea spinosa L.). J Insa Farm Indones, 2020; 3(2): 387–93.
24. Sari M, Ulfa RN, Marpaung MP, Purnama. Penentuan Aktivitas Antioksidan dan Kandungan Flavonoid Total Ekstrak Daun Papasan (Coccinia grandis L.) Berdasarkan Perbedaan Pelarut Polar. KOVALEN J Ris Kim, 2021; 7(1): 30–41.
25. Febriani D, Mulyanti D, Rismawati E. Karakterisasi Simplisia dan Ekstrak Etanol Daun Sirsak (Annona muricata Linn). Pros Penelit Spes Unisba, 2015; 475–80.
26. Zuraida Z, Sulistiyani S, Sajuthi D, Suparto IH. FENOL, FLAVONOID, DAN AKTIVITAS ANTIOKSIDAN PADA EKSTRAK KULIT BATANG PULAI (Alstonia scholaris R.Br). J Penelit Has Hutan, 2017; 35(3): 211–9.
27. Marpaung MP, Anggun S. Penentuan Parameter Spesifik Dan Nonspesifik Ekstrak Kental. J pharmacopolium, 2020; 3(2): 58–67.
28. Adjeng ANT, Hairah S, Herman S, Ruslin R, Fitrawan LOM, Sartinah A, et al. Skrining Fitokimia dan Evaluasi Sediaan Sabun Cair Ekstrak Etanol 96% Kulit Buah Salak Pondoh (Salacca zalacca (Gaertn.) Voss.) Sebagai Antioksidan. Pharmauho J Farm Sains, dan Kesehat, 2020; 5(2): 3–6.
29. Melissa, Muchtaridi M. Senyawa aktif dan manfaat farmakologis Ageratum conyzoides. Farmaka, 2017; 15(1): 200–2012.
30. Warsinah W, Baroroh HN. Isolation of compounds agents anti bacteria from Bandotan (Ageratum conyzoides L) plants using bioassay guided fractionation method. Ann Trop Med Public Heal., 2019; 22(11).
31. Wardaniati I, Taibah S. UJI AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOL BEE POLLEN LEBAH TRIGONA (Trigona itama). JOPS (Journal Pharm Sci., 2019; 3(1): 21–8.
32. Istiqomah N, Akuba J. FORMULASI EMULGEL DARI EKSTRAK DAUN KELOR (Moringa oleifera LAM) SERTA EVALUASI AKTIVITAS ANTIOKSIDAN DENGAN METODE DPPH. J Syifa Sci Clin Res., 2021; 3(1): 9–18.
33. Rohmah J, Saidi IA, Saidi IA, Rini CS, Rini CS, Masyitha DA, et al. AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOL, ETIL ASETAT, DAN n-HEKSANA BATANG TURI PUTIH (Sesbania grandiflora (L.) Pers.) DENGAN METODE DPPH (1,1-Diphenyl-2-picrylhydrazyl). J Kim Ris., 2020; 5(1): 67.
34. Hilma, Putri NA Della, Lely N. Jurnal Ilmiah Farmako Bahari DETERMINATION OF

TOTAL PHENOL AND TOTAL FLAVONOID CONTENT OF LONGAN (Dimoncarpus longan Lour) LEAF EXTRACT DAN TOTAL FLAVONOID EKSTRAK DAUN KELENGKENG (Dimoncarpus longan Lour), 2021; 80–7.

35. Nuri N, Puspitasari E, Hidayat MA, Ningsih IY, Triatmoko B, Dianasari D. Pengaruh Metode Ekstraksi terhadap Kadar Fenol dan Flavonoid Total, Aktivitas Antioksidan serta Antilipase Daun Jati Belanda (Guazuma ulmifolia). J Sains Farm Klin, 2020; 7(2): 143.
36. Nunki N, Titik Mutiarawati D, Prayekti E. PURPLE SWEET POTATO (Ipomoea batatas L.) PEELS EXTRACT AS AN ALTERNATIVE DYE FOR BACTERIA GRAM STAINING. Indones J Med Lab Sci Technol, 2020; 2(2): 76–84.
37. Silaban S, Marika DB, Simorangkir M. Isolation and characterization of amylase-producing amylolytic bacteria from rice soil samples. J Phys Conf Ser., 2020; 1485(1).
38. Fevria R, Hartanto I. Isolation and characterization of Lactic Acid Bacteria (Lactobacillus sp) from strawberry (Fragaria vesca). J Phys Conf Ser., 2019; 1317(1): 74–7.
39. Benkova M, Soukup O, Marek J. Antimicrobial susceptibility testing: currently used methods and devices and the near future in clinical practice. J Appl Microbiol, 2020; 129(4): 806–22.
40. Cordovana M, Ambretti S. Antibiotic susceptibility testing of anaerobic bacteria by broth microdilution method using the MICRONAUT-S Anaerobes MIC plates. Anaerobe [Internet], 2020; 63: 102217. Available from: https://doi.org/10.1016/j.anaerobe.2020.102217
41. Rupiniasih N nyoman, Indriani, Syamsuddin, Razak AR. AKTIVITAS ANTIBAKTERI FRAKSI n-HEKSAN, KLOROFORM, ETIL ASETAT BUNGA KAMBOJA (Plumeria alba) TERHADAP BAKTERI Staphylococcus aureus DAN Salmonella typhi. KOVALEN J Ris Kim, 2019; 5(2): 173–81.
42. Azizah R, Antarti AN. Uji Aktivitas Antibakteri Ekstrak Dan Getah Pelepah Serta Bonggol Pisang Kepok Kuning (Musa paradisiaca Linn.) Terhadap Bakteri Pseudomonas aeruginosa dan Klebsiella pneumoniae Dengan Metode Difusi Agar. JPSCR J Pharm Sci Clin Res., 2019; 4(1): 29.
43. Maulidya SAI, Nuari DA, Suryana S, Almarifah S. Antibacterial Activity of Bandotan (Ageratum conyzoides L) Leaves Extracts Against Methicillin-Resistant Staphylococcus aureus. Borneo J Pharm., 2020; 3(4): 243–8.
44. Rahmitasari RD, Suryani D, Hanifa NI. Aktivitas Antibakteri Ekstrak Etanolik Daun Juwet (Syzygium cumini (L.) Skeels) terhadap Bakteri Isolat Klinis Salmonella typhi

Antibacterial Activity of Ethanolic Extracts of Java Plum (Syzygium cumini (L) Skeels) Leaves against Clinical Isolate. J Farm Indones [Internet], 2020; 17(01): 138–48. Available from: http://jurnalnasional.ump.ac.id/ [Diakses pada 28 April 2021]

45. Aryani P, Kusdiyantini E, Suprihadi A. Isolasi Bakteri Endofit Daun Alang-Alang (Imperata cylindrica) dan Metabolit Sekundernya yang Berpotensi sebagai Antibakteri. J Akad Biol., 2020; 9(2): 20–8.