EKSAKTA
Journal of Sciences and Data Analysis

E-ISSN: 2720-9326
P-ISSN: 2716-0459

DOI :10.20885/EKSAKTA.vol5.iss2.art10

Research Article

Biochemical Test and Antimicrobial Potentials of Bandotan *(Ageratum conyzoides)* Leaves against Pathogenic bacteria in the Water

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Received: 6 August 2024; Accepted: 29 October 2024; Published: 31 October 2024

Abstract: Water purification technology using coagulation has been carried out like using plants that have potential source of natural coagulants namely Bandotan (*Ageratum conyzoides*) but using bandotan leaves has not been done. Eventhough, Bandotan leaves containing a lot of protein. Each type of protein has an isoelectric point at a different pH like isoelectric point, the protein will have a neutral charge. The calculation results of the number of colonies in samples were B1, B2, and B3 with dilutions of 266. 10^5, 9. 10^7 and 104. 10^9 CFU/mL. The results of Gram staining identification showed that the B1, B2 and B3 isolates had the shape are bacillus, bacillus and coccus with Gram positive. The bacteria thrive in different habitats, so bacteria from the sample have been very cloudy to pH of 6.0 and 8.0 because neutralophiles properties. The B3 isolate sample have high clear zone for *S. aureus* of 13 mm more than *E. coli* of 11 mm. So, the bandotan is able to inhibit the growth of pathogenic bacteria in the water.

Keywords: Morphological, water, antimicrobial, bandotan, *ageratum*

Introduction

Water quality is influenced by turbidity, because turbidiy can indicate the presence of solid particles in clean water so that the water is suitable for consumption [1]. Technologies that are able to remove solid particles in water are the processes of coagulation, flocculation and sedimentation[2]. Coagulation is the first stage of the particle aggregation process which is carried out by adding a coagulant. The addition of coagulants functions to speed up the process of binding solid particles contained in water and their settling.

Water purification technology using coagulation has been carried out like using plants that have potential source of natural coagulants namely Bandotan (*Ageratum conyzoides*) but using bandotan leaves has not been done. Eventhough, Bandotan leaves containing a lot of protein (4.42%) and carbohydrates (3.10%) per grams[3]. It's also contains various chemical compounds such as alkaloids, flavonoids, chromene, benzopyrans and terpenoids, saponins, tannins, and phenolics which can be used as antibacterials to kill pathogenic bacteria in the water.

Based on the results of national socio-economic survey carried out by the central statistics agency in 2019 that the percentage of drinking water was below the national average of 57.60% from 89.27% [4]. Plants that have the potential to be a source of natural coagulants are plants that contain a lot of protein. Each type of protein has an isoelectric point at a different pH like isoelectric point, the protein will have a neutral charge. This will affect its interaction with compounds in the water. The presence of bivalence cations in water also affect the effectiveness of the coagulation process [5] which is free from pathogenic bacteria like *Escherichia coli* or *Staphyloccocus aureus*.So, bandotan is rich source of macromolecules and chemical compounds, it's able to kill all water

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phatogenic bacteria [6]. Therefore, problem of water pollution caused by pathogenic bacteria can be overcome [7].

Materials and Methods

Materials

The research was conducted in the integrated and environmental health laboratory D-III sanitation politeknik kementerian kesehatan Bengkulu and chemistry research laboratory of Universitas Islam Indonesia for 5 months. The materials used in this research are bandotan (*Ageratum conyzoides*) obtained from Yogyakarta, *Escherichia coli, Staphyloccus aureus*, distilled water, antibiotic disc (erythromycin), nutrient agar (Oxoid), nutrient broth (Oxoid), mueller hilton broth (merck), mueller hilton agar (merck) peptone water (Oxoid), HCl 37%, alcohol 70%, phenol solid Na_2SO_3, NaOH 2 M, Ka-Na Tartrate 40%, starch, glucose, Gram staining solution, wrap, aluminium foil, universal pH, ethyl acetate p.a, paper disc

Preparation and Isolation of Bandotan

The bandotan sample through a maceration process for 24 h at 37 °C. The maceration product obtained then carried out in a multi-stage dilution process using peptone water media and then that it was grown in nutrient agar [6]. After that, morphological identification with macroscopic and microscopic identification by observing the shape and size of the colony, elevation of bacteria which is cancave or convex, the shape of edge from colony is circular or irregular and color from colony by Gram staining test [7] and then, make observations with microscope at 100X magnification [8].

pH Tolerance of Isolated Bacteria

The hydrogen ion concentration tolerance of the isolated bacteria was tested at pH (4, 6, 7, 8, and 10), the pH was adjusted by HCl 37% and NaOH. It's incubated for 24 h at 37 °C. the turbidity level of nutrient broth was observed with indicates that bacteria have growth from the medium [9]. Turbidity at OD600 nm indicates whether there are many or few bacteria living in the water.

Antimicrobial Activity Test

Antimicrobial activity was grown in nutrient broth of 9.0 mL and enriched for pathogenic bacteria, and then carried out using agar Mueller Hinton Broth (MHB) of 0.76 g and 2.0 g nutrient agar on which pathogen bacteria had been applied. Then the antibiotic (Erythromycin) disc on the medium. The sample isolates grown on test medium using paper disc and observed for 24 h at 37 °C. The clear zone formed is measured [10].

Results and Discussions

Preparation and Isolation of Bandotan

Bandotan or wedusan (Ageratum conyzoides) is a plant that is often found in Indonesia because of its unique ingredients and smells like goats, but it has many bioactive compound which are very beneficial for humans, especially in the water purification process. Apart from these plants, bandotan is plants containing tannins and chemical compounds in the bandotan plant such as essential oils, organic acids, coumarins, ageratochromene, friedelin, s-sitosterol, stigmasterol, pottasium chloride, sulfur and α- sitosterol. Leaves and flower contain saponins, flavonoids and polyphenols. Bandotan plants obtained from the center of essential oil studies (CEOS) garden in Candibinangun area, Pakem, sleman Regency, Special Region of Yogyakarta. The bandotan leaves as a sample in the research cleaned and macerated using nutrient broth as a liquid medium for 24 h.

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Figure 1. Bandotan leaves

Maceration is the process of soaking a sample using an organic solvent at a certain temperature at 37 ^{o}C. This process is very beneficial in isolating natural compounds because by soaking plant samples, cell walls and membranes will break down due to the pressure difference between inside and outside the cell, so that secondary metabolite compounds in the plant cell cytoplasm will be dissolved in organic solvent and compound extraction will be perfect because the soaking process is carried out for 24 h. Maceration methods can be adapted to extract a variety of molecules by combining different solvents, temperatures, and agitation to promote more selective and effective mass transfer of high-value chemicals from bimass. So, the choice of solvent for the maceration process will provide high effectiveness by paying attention to the solubility of natural compound in the solvent. The process of maceration and isolation of bandotan compounds uses nutrient broth which is used as a growth medium and breaks down compound in plant cells.

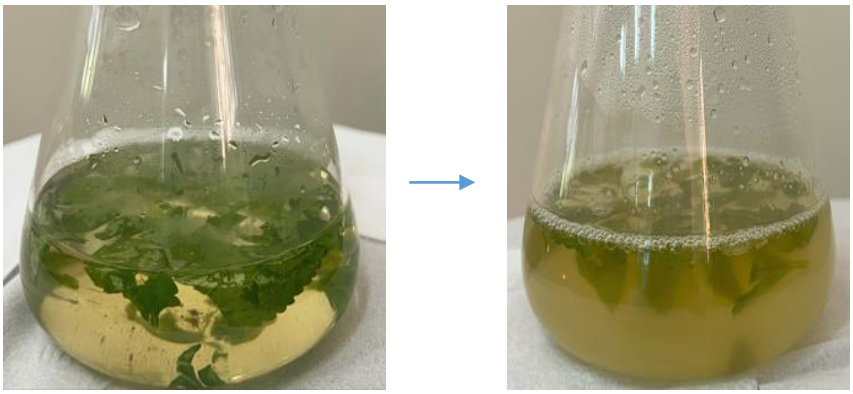

Figure 2. Maceration of bandotan leaves

The maceration using nutrient broth because nutrient broth is a standard medium that can be used to grow various types of microorganisms. After that its carried out multilevel dilution uses peptone water medium. The multilevel dilutions were carried out up to 10^{9} CFU/mL because based on World Health Organization (WHO) and Food Agriculture Organization (FAO) for the number of bacterial colonies found in samples around 10^{6}- 10^{9} CFU/mL. Isolation of bandotan by maceration process in the medium many produce organic compound as a bioactive compounds like tannins, alkaloids and flavonoids. The calculation results of the number of colonies in samples are B1, B2, and B3 with dilutions of 266. 10^{5}, 9. 10^{7} and 104. 10^{9} CFU/mL.

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Figure 3. Multilevel dilutions from Bandotan

Purification of the bacteria was carried out on nutrient agar media using the zigzag streak method at 37 °C for 24 h with the aim of obtaining a pure culture of bacteria. From the results of bacteria purification.

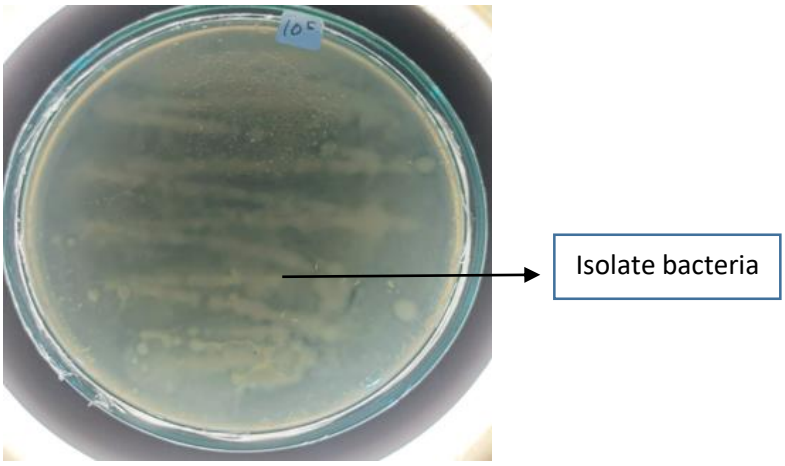

Figure 4. B1 Isolate Sample dilution of 10^5 CFU/mL

Morphological Identification

Identification of B1, B2 and B3 isolates with Gram staining is carried out to differentiate Gram positive or negative bacteria. The macroscopic and microscopic characteristics of sample are generally such as shape, size, elevation, edge shape, color and Gram test.

Table 1. Characteristics of Bandotan isolates

isolates	shape	size	elevation	edge shape	Color	Gram test
B1	circular	big and small	convex	circle	yellowish white	bacillus (positive Gram)
B2	circular	small	concave	Irregular round	White	bacillus (positive Gram)
B3	circular	small	convex	circle	yellowish white	coccus (positive Gram)

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This test is carried out through the Gram staining process and then observed under a microscope with 100X. The results of Gram staining identification showed that the B1, B2 and B3 isolates are bacillus, bacillus and coccus with Gram positive, indicated by the formation of a blue or purple color in the bacterial cells (**Figure 5**). Gram positive are bacteria that retain crystal violet dye during Gram staining process so that they appear blue or purple under microscope. Gram positive bacteria have been about 90 percent of cell walls are composed peptidoglycan while the rest is another teichoic acid molecule.

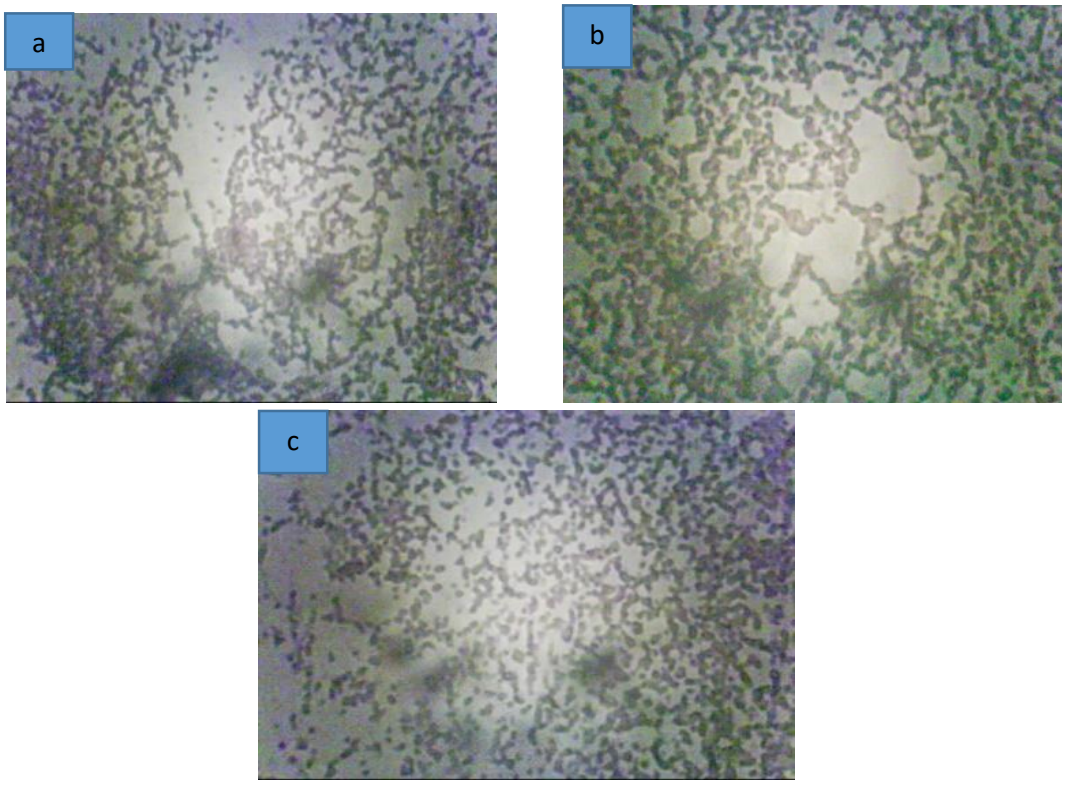

Figure 5. Gram staining from a) B1 Isolate; b) B2 Isolate ; c) B3 Isolate

pH Tolerance of Isolated Bacteria and Antimicrobial Activity

pH tolerance of isolated bacteria resistance and antimicrobial activity test using B3 sample which is considered superior isolate in test to inhibit or kill the growth of pathogenic bacteria in water. The condition of water having pH range like 4.0; 6.0; 7.0; 8.0 and 10. At this stage, the test treatment uses liquid media or nutrient broth whose pH is adjusted by adding concentrated HCl and NaOH as a acid and base in the water, then the pH value is measured using a calibrated pH meter universal. The results obtained can be seen from the level of turbidity of the test medium, if the medium becomes more turbid so more pathogenic bacteria will live in the water.

Table 2. pH tolerance of isolated bacteria

Isolates	-	Bacteria growth rate at pH variations				
		4.0	6.0	7.0	8.0	10
B3		+	+++	-	+++	+
control		-	-	-	-	-

note : + : little cloudy, ++ : cloudy , +++ : very cloudy, - : nothing

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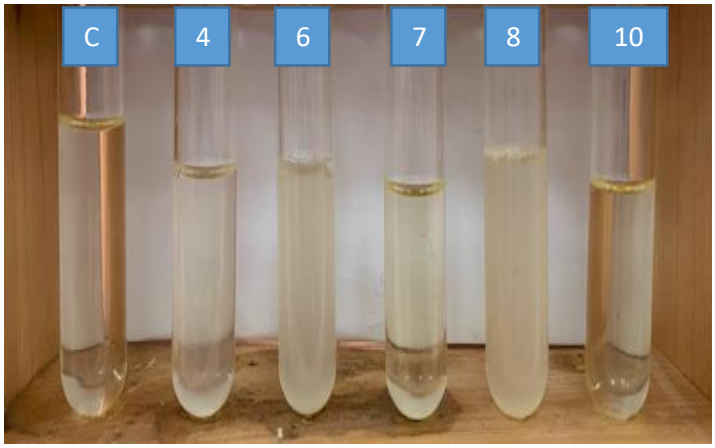

Figure 6. Water pH tolerance test from B3 Isolate

The pH value greatly influences the growth rate, survival and death of microorganisms. The importance of pH values for bacteria stems from the influence of pH on three key metabolic variables such as protein structure and function; kinetics and thermodynamics of chemical reactions involving protons and the synthesis of adenosine triphosphate. The bacteria thrive in different habitats, so bacteria from the sample have been very cloudy to pH of 6.0 and 8.0 because neutralophiles properties from the environmental affect the lifestyle based on classifying of bacteria acidophiles for pH 1.0 – 3.0, neutralophiles for pH 5.5-9.0 and alkalophiles for pH 10-13 [13].

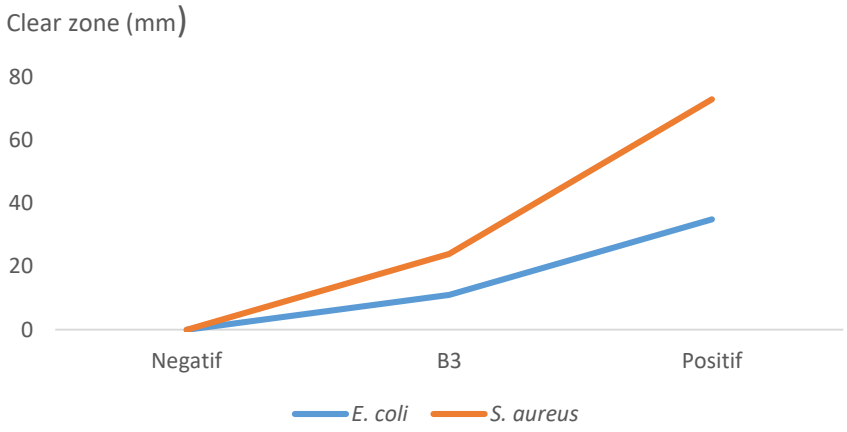

Figure 7. Antimicrobial activity for 24 h

The final stage is antimicrobial activity by looking clear zone of growth of certain bacteria. From the B3 isolate sample have high clear zone *S. aureus* of 13 mm more than *E. coli* of 11 mm because differences from the structure and peptidoglycan component bacteria cell wall. The peptidoglycan layer in the cell wall of Gram negative bacteria is thinner , while in Gram positive bacteria and component Gram negative bacteria are more complex because its have an additional outer membrane layer so that it's easier to penetrate Gram positive cell walls than Gram negative ones, the antimicrobial activity caused by the extract is due to the content of secondary metabolites such as flavonoids, phenolic and terpenoids. Flavonoids can inhibit bacteria growth through damaging DNA gyrase, thereby inhibiting the function of cytoplasmic membrane. Phenolic compounds also have the potential as antimicrobials which cause lysis of cellular components and damage the enzymatic mechanisms of bacteria cells. Terpenoids are also known to play an antimicrobial

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role by involving the breakdown of membranes by lipophilic components like *E. coli* and *S. aureus* bacteria. So, the bandotan is able to inhibit the growth of pathogenic bacteria in the water.

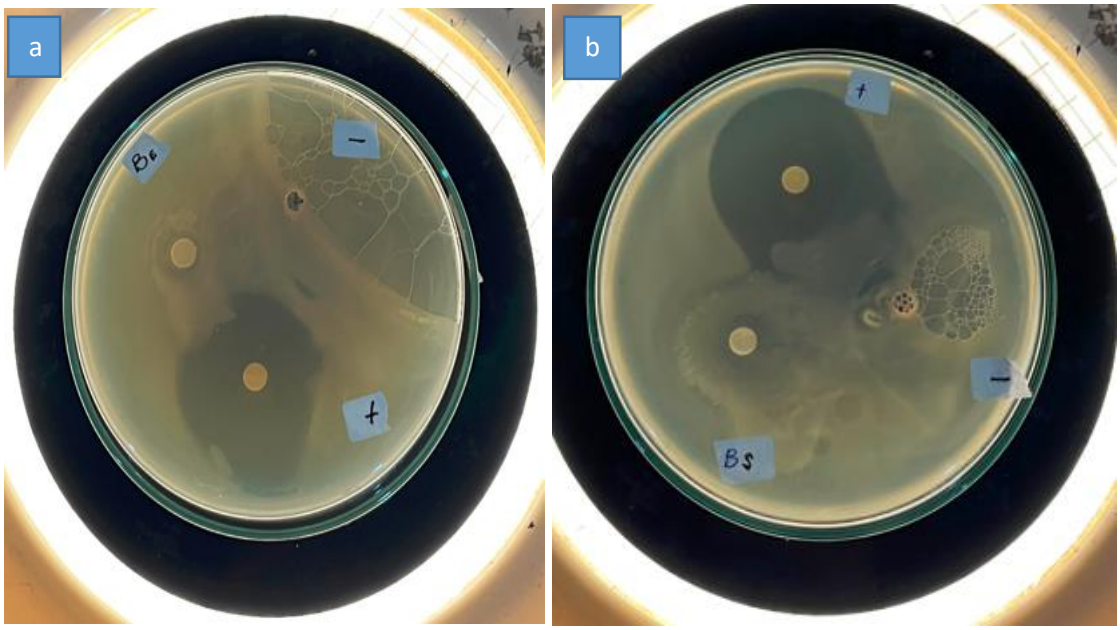

Figure 8. Clear zone of antimicrobial activity from the B3 Isolate; a) *E. coli*; b) *S. aureus*

Conclusion

The maceration process from the morphological identification and antimicrobial activity from bandotan the number of colonies were obtained B1, B2, and B3 with dilutions of 266. 10^5, 9. 10^7 and 104. 10^9 CFU/mL. The results of Gram staining identification showed that the B1, B2 and B3 isolates had the shape are bacillus, bacillus and coccus (round) with Gram positive. The bacteria thrive in different habitats, so bacteria from the sample have been very cloudy to pH of 6.0 and 8.0 because neutralophiles properties. From the B3 isolate sample have high clear zone for Gram positive *(S. aureus)* of 13 mm more than Gram negative (*E. coli*) of 11 mm.

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