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Phytochemical Screening and Quantitative Analysis of *Coleus arthropurpureus* Ethyl Acetate Fraction and Antibacterial Activity Against *Staphylococcus aureus*

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Abstract. *Staphylococcus aureus* (*S. aureus*) is a pathogenic microbe that is caused by various diseases in humans and animals. Infectious diseases caused by *S. aureus* in Asia reached 70% in 2007, while in Indonesia reached 23.5%. The plant provided several bioactive compounds that might function as an antibacterial which inhibits both bacterial growth and damaging the cell system and protein synthesis. *Coleus arthropurpureus* known contains alkaloids and tannins that supposed to be an antibacterial compound. Tannins have antibacterial activity, in general, the mechanism is to damage the bacterial cell membrane and induce the formation of complex compound bonds to enzymes or microbial substrates. This study aims to analyze the bioactive compounds contained in *C. arthropurpureus* in qualitative and quantitative which have an antimicrobial function using high-performance liquid chromatography (HPLC) in the reverse phase C-18 column and screening of antibacterial activity was carried out by disc diffusion method. The results of both qualitative and quantitative analysis by HPLC has obtained the presence of tannin bioactive compounds (1.48 ppm at a retention time of 2.806 minutes) and alkaloids (1.11 ppm at a retention time of 7.015). Moreover, we verified the diameter of inhibition of growth zone against S. aureus at a concentration of 15% extract was 12.80 mm. It was found that the highest percentage of the bioactive compound in *C. arthropurpureus* is tannin, and that is might an antibacterial agent.

Keywords : antibacterial activity, *Coleus arthropurpureus*, *Staphylococcus aureus*

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Introduction

An infectious disease caused by the *Staphylococcus aureus* (*S. aureus*) is quite high in Asia, up to 70% in 2007. While 2006 in Indonesia it reached 23.5%. S. aureus is a pathogen that is capable of causing various diseases in humans and animals. When *S. aureus* was attached to human tissue, it would be able to grow and survive in many ways. In vitro analysis showed that *S. aureus* can attack and survive in epithelial cells including endothelial cells, which theoretically this causes the microbes difficult to be recognized by the body's defense systems. *S. aureus* can form a small disparate colony or small-colony variant (SCVs), so the *S. aureus* infection often repeated and difficult to heal [1-5]. *S. aureus* had a significant role in nosocomial infections and other infectious diseases.

Gram positive bacterial infections are harder to treat with than those caused by gram negative bacteria [6]. During infection, *S. aures* produces various enzymes such as protease, elastase, lipase that have been used to invade and destroy human tissue even to move on to other locations. *S. aureus* can also raise septic shock. That's because there was an interaction between *S. aureus* and mediators inflammatory. Some strains of *S. aureus* produce superantigens such as food poisoning and toxic shock syndrome. The superantigens can induce sepsis due to cytokine activity such as selectivity, integration, PECAM, and ICAM. Efforts to overcome infections caused by S. aureus are to give antibiotics for gram-positive bacteria [7]. The antibiotic used work as an antibacterial which can impair bacterial growth by damaging its cell system and protein synthesis [8-9]. The advantage of using an antibacterial compound is the economic value that can be produced from plants (herbs).

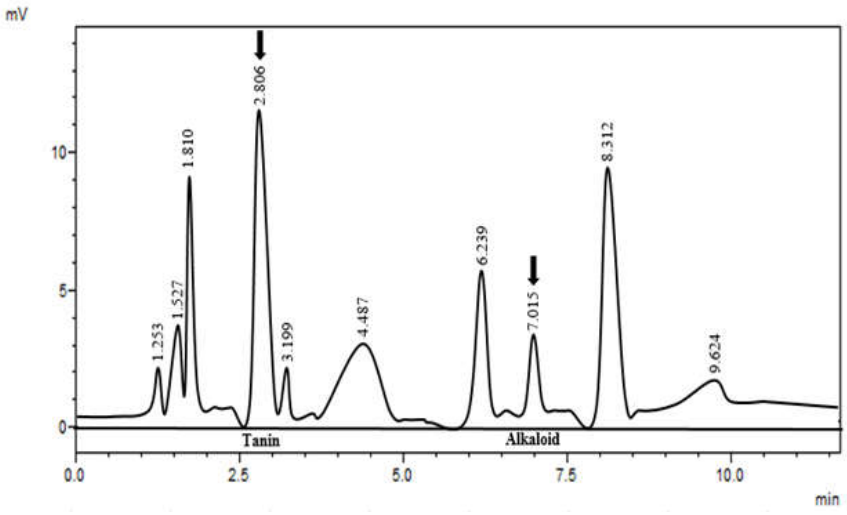
Until recently, the use of natural substances derived from plants (herbs) widely used to treat various diseases. Although modern medicine has been superseded by modernization in health care, in reality, herbal medicines are equally effective in treating disease. Even though herbal medicines also tend to be less expensive and have very small side effects. No wonder when the use of herbal medicines was once again common among Indonesian society [10-13]. Many plants have been examined for their chemical properties, which one of them is Myana plants. Myana (*Coleus arthropurpureus* L. Bent) is a decorative plant that has regularly been planted in the courtyard of the house, but beneath its function as a decorative plant, Myana has medicinal benefits because it has antibacterial properties [14]. Among these are flavonoids, tannins, and saponins that can dissolve in water and that can work to destroy cytoplasm membranes and regulate cell protein [16-19]. Flavonoids are proven empirically as anticancer, antivirus, anti-inflammatory, diuretic, and antihypertension. Saponin, however, is one of the substances that plants produce as antivirus, antibacterial, and immunodeficiency [20]. Tannins are a phenol substance with antiseptic properties. The antibacterial effect of tannins is through reactions to cell membranes, enzyme inactivation, and material function inactivation [21-22]. Antibacterial agents from the plant are agents that either inhibit or kill bacteria that cause wound infections. One of the antibacterial substances that can be used is the antibacterial from Myana plant extract.

Studies on Myana plant as antibacterial are rarely observed. However, earlier studies using ethanol solvents prove that Myana plants have antibacterial activity [4,5]. Myana leaves contain essential oil, phenol, tannin, fats, and phytosterols. Tannin has antibacterial activity in general; the mechanism is to damage the membrane of a bacterial cell, that astringent substance in tannin induces the building of complex bonds against enzymes or microbial substrates [18]. Flavonoids have antibacterial effects through their ability to form complex proteins with extraneous proteins and soluble proteins as well as walls of bacterial cells [23]. Usually, the portion of the Myana plant that used medicinally is on the leaf. Among various Myana, only Myana has the red-brownish one or blackish leaves with the edge of a jagged leaf that it can exploit as a medicinal plant. Myana leaves are usually boiled as cough drops, hemorrhoids, menstrual periods, and diabetes [24].

Based on these, in this research we want to analyze the quality and quantity of secondary metabolites of Myana leaf extract (*Coleus arthropurpureus* L. Bent) using ethyl acetate solvent and determine the antibacterial activity.

Materials and Methode

Materials used in this study are ethyl acetate 96%, nutrient media, NaCl, aquadest, Myana plant (*Coleus arthropurpureus* L Bent), *S. aureus*, Tetracycline antibiotic (Tetrasanbe), Wagner reactant, Mayer and Dragendorf, $FeCl_3$, HCl, and H_2SO_4. The tools



Peaks	Ret.Time	Area
1	1.253	181631
2	1.527	222446
3	1.810	246936
4	**2.806**	**950213**
5	3.199	83468
6	4.487	320404
7	6.239	297635
8	**7.015**	**163262**
9	8.312	663872
10	9.624	1210375
Total		4340242

Figure 1. Chromatogram of Secondary Metabolite of Myana leaf extract. Analysis condition: column C18; Volume of Injection = 2 µL; Phases of Movement = acetonitrile: Potassium dihydrogen phosphate on water pH 3 (10:90); rate of flow = 1 mL/second; l = 210 nm)

used in this study are a set of glasswears (Pyrex), pipet drops, blender, sifter, mixer, oven, petri dish, incubator, extraction pumpkin, a laminar airflow, thermometer, autoclave, scaling puzzle, photography tool, spatula, and analytic scale. Sorted Myana leaves are washed with water, drained and cut into small pieces around two inches. The sample is weighed as 4kg of wet samples and dried in the sun for two days. The final steps were weighed by simplity dry and smoothed out until a fine powder formed. Sifting it, and Simplicia ready to be extracted [25].

Extraction of Myana leaf. Myana leaf was obtain from Batu, Kediri Regency, West Java of Indonesia. Myana leaves are dried and mashed using a grinding machine to obtain a survivalist of the Myana leaves. 500 grams of delicate survivalist Myana leaves weighed and impregnated by ethyl acetate solvents in a sealed of macerate bottle and left for five days at room temperature, shielded from direct sunlight. After 5 days, then filtered to get the filtrate. The filtrate contained in a macerating bottle. The shags are stored in other containers and recalibrated with ethyl acetate, repeated three times. All the filtrate obtained is concentrated with the rotary evaporator at 50 ℃ until it receives a buxom extract [25].

Analysis of Qualitative and Quantitative Secondary metabolites Myana Leaves. The qualitative analysis was carried out by conducting of phytochemical screening of test reagents, including Mayer reagent, dragendorff, tannin, saponin, and others. While quantitative analysis is carried out using an HPLC, in the UIN Malang Chemical Laboratory with condition analysis: column: C18; Injection volume = 2 µL; mobile phase = acetonitrile: Potassium dihydrogen phosphate on water pH 3 (10:90); mobile phase = 1 mL/minute; with PDA detector l= 210 nm [26].

Determination of Antibacterial Activity. The antibacterial activity of the extract of Myana leaf was carried out using disc diffusion methods. We put up a media that has been inoculated with bacteria. The paper disks are prepared and sterilized with 6 mm in diameter. The various of Myana leaf extracts then added to a disk of 10 microliters. The sterile of disk paper that has been impregnated with Myana leaf extract is placed on the media surface by sterile tweezers and gently pressed down to ensure contact between disk paper and media surface. The

Table 1. Qualitative Analysis of the Secondary Metabolites of Ethyl Acetate Extract of Coleus arthropurpureus.

Groups	Result
Alkaloid	
Wagner Test	+
Dragendorf Test	+
Mayer Test	+
Flavonoid	-
Tanins	+
Saponins	-
Steroids	-
Terpenoids	-

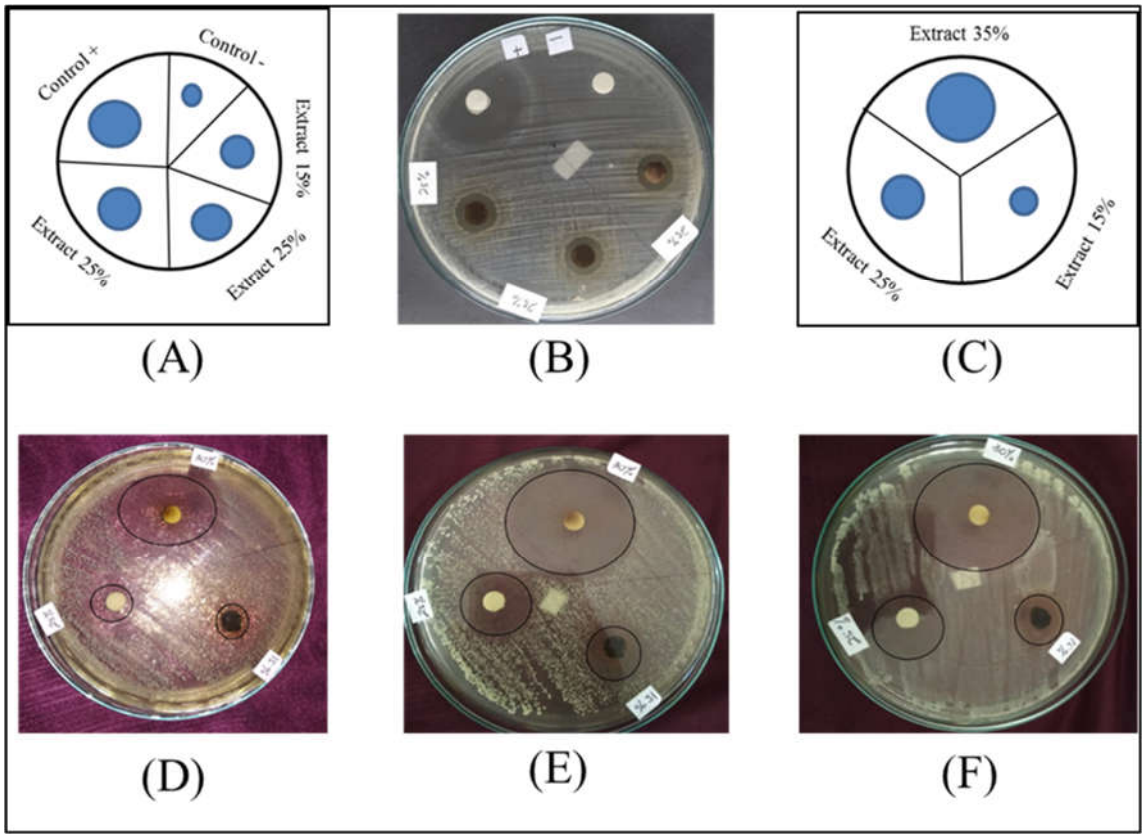


Figure 2. The diameter of inhibition of growth zone against S. aureus (A) Schematic of disc-diffusion of control (+), control (-), extract 15%, 25%, and 35% (B) The diameter of inhibition of growth zone for control (+), control (-), extract 15%, 25%, and 35% of Myana leaves against (C) Schematic of disc-diffusion of Replication extract 15%, 25%, and 35% of Myana leaves (D) Replication 1 (E) Replication 2 (F) Replication 3 of inhibition of growth zone for extract 15%, 25%, and 35% of Myana leaves.

positive control is prepared by dipping the disk paper in tetracycline cream. The negative control is prepared by dipping the disk paper in ethyl acetate. Petri dishes were incubated at 37 ℃ temperatures for 24 hours. Determination of the antibacterial activity, we verified the diameter of inhibition of growth zone against *S. aureus* [27].

Result and Discussion

Secondary Metabolites. Secondary metabolic is an organic substance synthesized by plants and widely used as a pharmaceutical agent. A secondary analysis of metabolic compounds with ethyl acetic extract of Myana leaves was done with phytochemical tests and reinforced by using an HPLC instrument. Observations from phytochemical tests indicate the presence of alkaloids and tannins (Table 1).

Qualitative properties of secondary metabolic compounds in Myana extract are amplified with HPLC and show the same results as alkaloid and tannin compounds. The result can be known from the ratio of retention time between standard bioactive antibacterial compounds with the analyte in a Myana leaf sample. Tannin standard retention time is 2.772 minutes and the analyte in test samples is suspended at 2.806 minutes retention time. Then for an alkaloid standard retention time is 7.035 minutes and the analyte alkaloids in the alkaloid samples were attacked at 7.15 minutes retention (Figure 1) [28].

Table 2. The diameter of inhibition of growth zone extract against *S. aureus*

No.	Sample Test	The diameter of inhibition of growth zone
1	Extract 15%	12.80 mm
2	Extract 25%	17.00 mm
3	Extract 35%	39.60 mm
4	Control (+)	62.17 mm
5	Control (-)	00.00 mm

The results of the quantitative test, the concentration of tannins in the sample amounts to 1.48 and 1.11 ppm for alkaloid compounds, using a calculation of single calibration below:

$$[\text{Analyte}] = \frac{\text{Analyte area}}{\text{Standard Area}} x\ [\text{Standard}] \quad (1)$$

Known:

[analyte] : analyte concentrations of antibacterial bioactive compounds (tannins/ alkaloids) in the sample

[standard] : standard tannin concentration, alkaloids = 50 ppm

Tannin analytes area = 950213

Alkaloids analytes area = 163262

The tannin standard area is 50 ppm = 32002102

The standard alkaloid area is 50 ppm = 7346819

Asked: [tannins] and [alkaloids] in the sample?

[Tannin] = 1.48 ppm

[Alkaloids] = 1.11 ppm

Antibacterial Activity. Observation and measurement of the diameter of the clear zone formed around the paper disk indicate that the inhibition of ethyl extract of Myana leaves Measurement of inhibition zone of ethyl acetate extract of Myana leaves against bacteria *S. aureus* is shown in Table 2.

The results of the antibacterial activity of the extract that gave the optimum antibacterial activity to a concentration of 35% with a diameter of 39.6 mm (Table 2). The diameter response of the affected zone constitutes a strong bacterial growth response. It is supported by alkaloids and tannins compounds. The mechanism of Alkaloids as antibacterial is by interfering with the bacterial building blocks of peptidoglycan cells, thus preventing the cell walls from forming intact and causing death in the bacteria [25]. The mechanisms of tannins antibacterial have an antibacterial effect by applying proteins inhibition synthesis. The tannin-antibacterial effect through reactions to cell membranes, enzyme inactivation, and genetic material inactivation. The mechanisms of tannin as antibacterial are to halt reverse transcription enzyme and topoisomerase DNA so that the bacterial cell cannot form [28].

Conclusion

A secondary qualitative metabolic analysis with phytochemical tests indicates that Myana's iridescent leaves contain alkaloids and tannins. The analysis shows that alkaloid and tannins secondary metabolism using HPLC shows that an alkaloid content is 1.11 ppm and tannins 1.48 ppm. Moreover, the antibacterial activity indicates that the inhibition diameter zone (mm) on 15%; 12.80 mm, 25%; 17 mm, and 35%: 39.6 mm against *S. aureus*.

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