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Phytochemical Screening and Antibacterial Activity of Flower, Stem, and Tuber of *Polianthes tuberosa* L. Against Acne-Inducing Bacteria

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***Abstract*—Objectives: The aim of this study was to determine the antibacterial activity of flower, stem, and tuber of Polianthes tuberosa L against *Staphylococcus epidermidis* and *Propionibacterium acnes*. Method: The extraction was conducted by a maceration method using different polarity of solvent (n-hexane, ethyl acetate, and methanol). The antibacterial activity and the minimum inhibitory concentration (MIC) were determined using the agar diffusion method. The analysis identified by thin layer chromatography (TLC), bioauthography assay, and scanning electron microscopy (SEM). Results and Discussion: The antibacterial activity showed the most active extract was a methanol extract of the flower against *Staphylococcus epidermidis* and *Propionibacterium acnes* with MIC value 30 mg/ml. Observations using SEM showed that the methanol extract of flower caused to morphological change on a cellular wall of *Propionibacterium acnes*, and therefore the antibacterial activity was related to damaged the bacterial cellular wall. The result of bioautography assay showed the methanol extract of flower have an antibacterial activity against *Staphylococcus epidermidis* and *Propionibacterium acnes* with Rf value at 0.87.. Conclusion:. The methanol extract of the *Polianthes tuberosa* L. flower has antibacterial activity against acne-causing bacteria by causing damage to cell walls**

Keywords:* Polianthes tuberosa *L., antibacterial, thin layer chromatography (TLC), bioautography, SEM

I. INTRODUCTION

Acne is a skin disease that occurs due to increased sebum production, keratinocyte decay, bacterial growth and inflammation [1]. *Staphylococcus epidermidis* and *Propionibacterium acnes* play a role in the pathogenesis of acne by producing metabolites that can react with sebum thereby increasing the inflammatory response. Treatment using antibiotics generally causes skin irritation so natural ingredients with antibacterial activity can be an alternative [1,2].

Tuberose (*Polianthes tuberosa* L.) comes from the Agavaceae family whose flowers are widely used as a complement to flower arrangements, because of their fragrance and beauty. Tuberose (*Polianthes tuberosa* L.) is also a plant that has potential as a medicinal plant, but has not been widely used [3].

Previous studies showed that the dried methanol extract of flower extracted in a dry state had antibacterial activity on *Proteus mirabilis* at a dose of 60 μg / ml (16 ± 1 mm) and *Eshcerichia coli* at a dose of 40 μg / ml (12.33 ± 1, 52 mm)[4]. The antibacterial potential was also demonstrated by the tuber of *Polianthes tuberosa* L. Methanol extract and fraction of n-hexane, carbon tetrachloride, chloroform, and water from the methanol extract concentrate at a dose of 400 μg / disc showed activity on gram positive (*Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Sarcina lutea*) and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi Salmonella typhi, Shigella boydii, Shigella dysenteriae, Vibrio mimicus Vibrio parahemolyticus*) with large resistance ranging from 9-11 mm [5].

This research will reported antibacterial activity based on the minimum inhibitory content (MIC) of flower, stem and tuber of *Polianthes tuberosa* L.) against *Staphylococcus epidermidis* and *Propionibacterium acnes* using different solvent variations in polarity.

II. MATERIAL AND METHOD

A. Materials

Plants materials

The *Polianthes tuberosa* L. (flower, stem and tuber) were collected from Sukabumi, West Java, Indonesia during September 2015. Taxonomic determination was conducted at Herbarium Bandungense, ITB, Bandung, West Java. The samples were dried in dying cabinet (40-50 ^{O}C), and cut into fine pieces.

Chemicals

Clindamycin hydrochloride was purchased from *Zhejiang Hisoar Pharmaceutical* Ltd. All chemicals were of analytical-grade purity.

Microorganisms

Staphylococcus epidermidis (S.e) and *Propionibacterium acne (P.a)* were obtained from Microbiology Laboratory, Faculty of Pharmacy, Padjadjaran University.

B. Determination of Antibacterial activity

Phytochemical Analysis

To knowing the secondary metabolites of flower, stem and tuber of *Polianthes tuberosa* L., phytochemical screening was

determine the content of alkaloid, flavanoid, polyphenol, tannin, monoterpene and sesquiterpene, steroid, kuinon and saponin [6].

Extractions

For each powder samples (150 g) was macerated successively at room temperature using n-hexane, ethyl acetate and methanol (1.5 L) for 3 x 24 hours respectively. Each extract was evaporated using rotary evaporator (IKA®) at 50 °C, dried completely and stored in tight container.

Culture media Bacterial inoculum

All microorganisms were maintained on Nutrient agar (NA) Petri dish sterile for 24 hours at 37ºC ± 1ºC. Nutrient agar was purchased from Difco. The turbidity of the resulting suspensions was diluted with sodium chloride 0.9 % w/v to obtain a transmittance of 25.0 % at 580 nm. The percentage was compared to McFarland turbidity standard using spectrophotometry ultraviolet (Shimadzu® UV 180). The level of turbidity is equivalent to approximately 3.0 x 10^8 CFU/mL.

Paper disc diffusion method

Tests conducted on the antibacterial activity of the extract n-hexane, ethyl acetate and methanol flower, stem and tuber tuberose and as a standard used clindamycin hydrochloride (P-003-WS15021001). Antibacterial activity test performed against *Staphylococcus epidermidis* and *Propionibacterium acnes* using paper disc diffusion method. A total of 15 ml of Nutrient Agar (NA) medium is poured into a petri dish and allowed to solidify, and then 0.1 ml of bacterial suspension was spread on an agar medium has solidified using a Drygalski and adapted for 15 minutes. Next, put the paper discs with a diameter of 6 mm which had been previously soaked in the extract at a concentration of 100; 50; 25; 12.5%, and tween-80 5% w/v as a negative control and clindamycin hydrochloride 50 mg/ml as a positive control. The Nutrient Agar (NA) medium was incubated for 24 hours in an incubator at a temperature of 37°C. Diameter of clear zone was measured using calipers to determine the width of inhibition zone (the test performed in triplicate).

Minimum inhibitory concentration (MIC) evaluation

The MIC was evaluated on plant extract that showed the highest antimicrobial activity. This test was using the same paper disc diffusion assay. The MIC was performed concentrations 15; 14; 13; 12; 11; 10; 9; 8; 7; 6; 5; 4; 3; 2; 1 %, and tween-80 5% b/v mg/mL for *Staphylococcus epidermidis* and *Propionibacterium acnes*.

Cell Morphological Observation

Clear zone derived from the treatment in the antibacterial test soaked with a solution of 2% glutaraldehyde overnight. Test solution is centrifuged and the supernatant discarded. The residue was added to a solution of 2% tannin acid, then soaked a few hours. Test solution was centrifuged back and disposed of fixative solution was then added chocodylate buffer, and soaked for 20 minutes. Test solution centrifuged and the supernatant is separated, then added 1% osmium tetraoksida and soaked for 1 hour. the test solution was centrifuged and the supernatant discarded and soak together 50% alcohol for 20 minutes. the residue is dried in a row with alcohol 70%, 80%, 95% and absolute alcohol for 20 minutes each. Samples were suspended with the addition of butanol and soaked 20 minutes, then the suspension placed on the cover slip, dried with a freeze dryer and then coated with gold and observed using *Scanning Electron Microscopy* (SEM) (JEOL JSM-5310LV®)

Bioautography assay

The contact bioautography was used for detection of antibacterial compounds in methanol extract of flower with modification. TLC plat e (Merck®, Silica Gel GF_{254}). The solvent system used was ethyl acetate : ethanol (7:3). The chromatogram was kept for evaporation of the solvent. The chromatogram is placed face down onto the inoculated 15 mL nutrient agar layer and left for thirty minutes to enable diffusion. Then the chromatogram is removed and the agar layer is incubated for 24 hours at 37ºC ± 1ºC. TLC plate without samples was added as negative control. The areas of inhibition were marked and relevant Rf values were recorded [7].

III. RESULTS

Table 1. The Phytochemical Screening of Flower, Stem, and Tuber on different solvent variations

Phytochemical screening		Alkaloid	Phenolic	Quinone	Saponin	Tannin	Flavanoid	Monoterpene dan Sesquiterpene	Steroid	Triterpenoid
Flower	Simplisia	+	+	+	-	-	+	+	-	-
	n-Hexane	+	-	+	-	-	-	+	-	-
	Ethyl acetate	+	+	+	-	-	+	+	-	-
	Methanol	+	+	+	-	-	+	-	-	-
Stem	Simplisia	+	+	+	+	+	+	-	-	-
	n-Hexane	-	-	+	-	-	-	-	-	-
	Ethyl acetate	+	+	-	+	-	+	-	-	-
	Methanol	+	+	+	+	+	+	-	-	-
Tuber	Simplisia	+	+	+	+	+	+	+	-	-
	n-Hexane	+	+	-	-	-	-	+	-	-
	Ethyl acetate	+	+	-	-	-	+	-	-	-
	Methanol	+	+	+	+	+	+	-	-	-

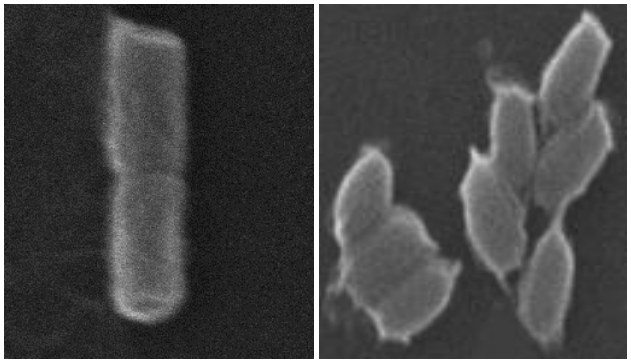
Note: + = detected, - = not detected

Table 2. Antibacterial activity of flower, stem and tuber extracts against *Staphylococcus epidermidis* and *Propionibacterium acnes*

Plant Section	Traetment group	Inhibition diameter (mm)							
		Staphylococcus epidermidis				*Propionibacterium acnes*			
		1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml
Flower	Control	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
	N-Hexane	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
	Ethyl Acetate	9,50±0,17	8,10±0,08	7,54±0,28	6,00	7,98±0,03	7,03±0,01	6,83±0,04	6,00
	Methanol	13,95±0,20	10,19±0,42	10,06±0,03	9,91±0,07	12,84±0,25	10,06±0,03	10,03±0,04	9,29± 0,10
Stem	Control	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
	N-Hexane	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
	Ethyl Acetate	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
	Methanol	10,61±0,38	7,10±0,03	6,00	6,00	8,49±0,41	7,11±0,04	6,00	6,00
Tuber	Control	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
	N-Hexane	6,00	6,00	6,00	6,00	6,00	6,00	6,00	6,00
	Ethyl Acetate	10,94±0,06	9,95±0,21	7,65±0,27	6,00	9,19± 0,13	8,18± 0,45	6,00	6,00
	Methanol	11,52±0,22	10,54±0,06	8,70±0,08	6,00	9,75± 0,07	8,31± 0,27	6,00	6,00

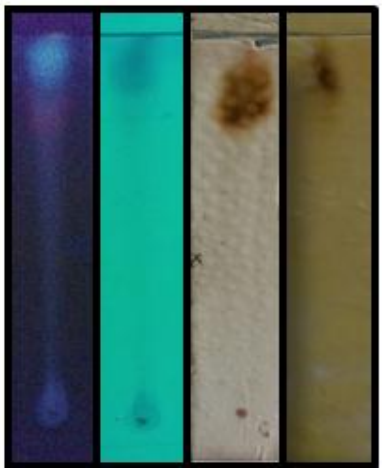
Table 3. MIC of flower methanol extract of *Polianthes tuberose* L.

Extract concentration (mg/ml)	Inhibition diameter (mm)	
	Staphylococcus epidermidis	*Propionibacterium acnes*
150	11,83±0,07	10,96±0,34
140	11,09±0,01	9,92±0,23
130	10,06±0,06	9,61±0,01
120	9,16±0,20	9,11±0,04
110	8,97±0,07	9,01±0,13
100	8,71±0,01	8,91±0,16
90	8,62±0,06	8,71±0,13
80	8,52±0,03	8,33±0,18
70	8,36±0,06	8,18±0,03
60	8,23±0,07	8,05±0,04
50	7,92±0,03	7,92±0,03
40	7,77±0,01	7,25±0,21
30	7,05±0,35	6,96±0,23
20	6,00	6,00
10	6,00	6,00



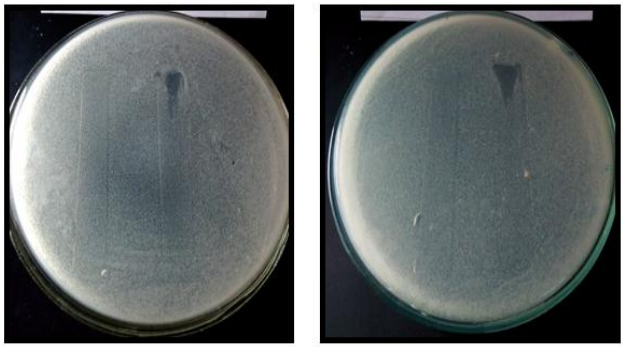
(A) (B)

Figure 1.Propinobacterium acnes morphological cellular in scale 10.000x (A) untreated, (B) bacteria treated with flower methanol exctract



(A) (B) (C) (D)

Figure 2. Thin Layer Chromatography (TLC) of flower methanol extract in silica gel GF_{254} and ethyl acetate: ethanol (3: 7) on UV light 366 (A), UV light 254 (B), spotting viewers H2SO4 10% (C), spotting $FeCl_3$ (D).



(A) (B)

Figure 3 Thin Layer Chromatography (TLC) -Bioautography of flower methanol extract in silica gel GF254 using ethyl acetate: ethanol (3: 7) on *Staphylococcus epidermidis* (A) and *Propionibacterium acnes* (B)

IV. DISCUSSION

Phytochemical screening is preliminary information in knowing chemical compounds that have biological activities of a plant. The result of phytochemical analysis showed that flower, stem, and tuber have a different secondary metabolite content. The difference in solvent also affects the type of secondary metabolites that are attracted based on their polarity.

The antibacterial activity of flower, stem and tuber extract of *Polianthes tuberose* L. against *Staphylococcus epidermidis* and *Propionibacterium acnes* showed that ethyl acetate and methanol extracts from all parts of the plant had antibacterial activity, whereas n-hexane extract had inhibitory zones of control, which meant extracts of n-hexane does not have antibacterial activity and from the whole treatment group it can be seen that the flower methanol extract has the best activity. The results of statistical analysis using ANOVA with a significance value $\alpha < 0.05$ showed that there was a difference in inhibitory strength between the flower extracts and control group, then Tukey test was carried out as a follow-up test which showed that only the methanol extract of flower had a different inhibitory effect on the control with a significance value $\alpha < 0.05$.

Minimum Inhibitory Concentration (MIC) aims to determine the minimum concentration required that are still able to inhibit bacterial growth. The MIC determination was carried out on methanol extract of flower, the most active antibacterial activity. The MIC value for *Staphylococcus epidermidis* and *Propionibacterium acnes* was 30 mg / ml.

Observation of cell morphology was carried out on Propionibacterium acnes using Scanning Electron Microscopy (SEM). The results showed the morphology of Propionibacterium acnes cells changed after administration of flower methanol extract when compared to normal cells. It is suspected that the flower methanol extract can damage the walls due to the presence of antibacterial active compounds in the flower methanol extract.

Thin layer chromatography test was carried out on the ethanol extract of the petty flower by using ethyl acetate: ethanol (3: 7) developer. Identification was carried out on UV light 254, UV light 366, 10% H_2SO_4 spotting view, and $FeCl_3$ spotting view. Detection results under UV light 366 showed that there were 2 spots with Rf 0.79 with red fluorescence and Rf 0.87 with blue fluorescence. When sprayed with 10% H_2SO_4 spots, blackish-brown spots appear on the spot with Rf 0.87. After spraying with $FeCl_3$ patches appear gray spots on the spot with Rf 0.87 indicating the presence of phenolic compounds [8]. Bioautographic results showed that the flower methanol extract contained antibacterial compounds at Rf 0.87 which were thought to be phenolics.

Hydroxyl groups in phenolic compounds cause damage to membrane integrity [9]. Hydrophobic phenolic groups that accumulate in lipid bilayers can increase membrane permeability which causes changes in membrane structure thereby facilitating the entry of more antibacterial agents [10].

V. CONCLUSION

The methanol extract of the *Polianthes tuberosa* L. flower has antibacterial activity against acne-causing bacteria by causing damage to cell walls.

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