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L. Ethanol Extract and *Ageratum Conyzoides* L Ethanol Extract as Antiacne.

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ABSTRACT

Acne is a skin disease that occurs due to inflammation caused by bacteria. *Cassia sp*. has been used by Indonesian people as antibacterial, antifungal and biofungicide. *Cassia siamea* L., was also known have antibacterial activity empirically, especially in the treatment of skin rashes, scabies and wounds. Bandotan leaves have been known as a medicinal plant, i.e. useful antiacne. The aim of this research was to determine *Cassia siamea L.* and *Ageratum conyzoides* L that have antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes*. The study started with reflux extraction using ethanol. The antibacterial activity and Minimum Inhibitory Concentration (MIC) were done by Agar Diffusion method. The result showed that determine *Cassia siamea* L. ethanol extract showed high antibacterial activity against *P. acnes* with MIC 5 mg/mL, but showed moderate antibacterial activity against *S. aureus* 25 mg/mL and *S. epidermidis* 35 mg/mL. Whereas, the activity of *Ageratum conyzoides* L ethanol extract showed that have antibacterial activity with MIC against *P. acnes* 5 mg /mL, 10 mg/mL against *S. aureus* and 7.5 mg/mL against *S. epidermidis*.

Keywords: *Cassia siamea L.*, *Ageratum conyzoides* L, antiacne, *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes*.

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INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous units and inflammatory papules caused by bacteria [1] [2]. The conditions usually start at the age of 14 to 19 years. A change in keratinisation pattern of hair follicle leads to blockage of sebum secretion. It is hypersensitivity to the stimulation of sebocytes and follicular keratinocytes by androgen leads to hyperplasia of the sebaceous glands and seborrhea which characterize acne [3].

In a few decades, many antibiotics were found to be resistant [4]. This has led to search for new, safe and effective antibacterial agents for natural plants [5] Indonesia have so many biodiversity, one of the plant that belong to family Fabaceae, *Cassia siamea L.* has been used as traditional medicine by Indonesian people for dermatologist which caused by bacterial and fungal, such as acne. Acne vulgaris is the most common skin disease. *Staphylococcus aureus, Staphylococcus epidermidis*, and *Propionibacterium acnes* were proliferate during puberty and can develop acne [4] [6].

Cassia siamea L. has been known contains phytochemical compounds like lupeol, chrysophanol, cassiamin A, cassiamin, siameadin, lupeone, rhein, chrysophanol- antrone, barakol, cassia chromone (5-acetonyl-7-hydroxy-2-methylchromone), p-coumaric acid, apigenin-7-o-galactoside, β-sitosterol, cassia chromonone and cassiadinine [7]. Finding the new source antibiotic from plants was needed fast screening methods for the detection of antibacterial activity. Antibacterial test should be simple, rapid, reproducible, inexpensive, could be done with extracts, fractions and its isolate [8].

A. conyzoides is an annual herb in the tropics and subtropics whose extracts are known to possess pharmacological and biocidal activity. It has a history of use in traditional medicine in various countries worldwide and is commonly used to treat wounds, burns and bacterial diseases. Various extracts of the plant, including water and methanol have been shown to inhibit the growth of *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *H. Pylori* [9][10][11].

The antibacterial activity can be determined by various methods like diffusion method (agar diffusion and MIC) and Bioautography methods. Bioautography was known as a sensitive method for detection of antibacterial compounds even in a small amounts [12][13]. This research purpose is to find the antibacterial activity by using agar diffusion method as qualitative method and quantitative method by determined the MIC value.

MATERIALS AND METHODS

Preparation of extracts from leaves of Cassia siamea L. and Ageratum conyzoides

The leaves of *Cassia siamea L.* were collected from Manoko, Lembang, West Java during February 2014 and authenticated in Herbarium Bandungense, Institute of Technology Bandung, West Java.e Whereas the leaves *Ageratum conyzoides L.* were collected from Cicanir, Kecamatan Puspahiang, Tasikmalaya and authenticated in Departement Biology Universitas Padjadjaran. Dried leaves (450 gram) of *Cassia siamea L.* leaves and *Ageratum conyzoides L.* leaves were powdered and extracted by reflux extraction using ethanol (5 Liter) as solvent. Each extract was then concentrated to dryness under vacuum at temperature 50^{o}C by using a rotary evaporator (IKA®), dried completely and stored in tight containers.

Determination of Antibacterial activity

Microorganisms used

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

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 [14].

Agar well diffusion assay

The antibacterial was using agar well diffusion assay with modification. The extracts were suspended in Dimethyl sulfoxide (DMSO-Merck®) and DMSO also used as negative control. Four serials of each extract (n-hexane, ethyl acetate, ethanol) yielded concentrations of 100; 50; 25; 12.5 mg/mL. For about 50 µL extracts were added to each of the 5 wells (6 mm diameter holes cut in the agar gel). The systems were incubated for 24 hours at 37ºC ± 1ºC. After incubation, clear zone around the holes were observed. Inhibition of the bacterial growth was measured in mm. Tests were performed in [12][14][15].

Minimum inhibitory concentration (MIC) evaluation

The MIC was evaluated on plant extract that showed the highest antimicrobial activity. This test was using the same modified agar well diffusion assay. The MIC was performed concentrations 25; 22.5; 20; 17.5; 15; 12.5 mg/mL for *Staphylococcus aureus,* then 50; 45; 40; 35; 30; 25 mg/mL for *Staphylococcus epidermidis* and 15; 12.5; 10; 7.5; 5.0; 2.5 mg/mL for *Propionibacterium acnes* [12][14][15].

Statistical analysis

The data were analyzed by one way ANOVA (analysis variance) and significant differences between the mean of the samples were determined by Tukey's test. The confidence limit was set at $P < 0.05$.

RESULT AND DISCUSSION

Phytochemical screening results from cassia ship ethanol extract and *Ageratum conyzoides* L ethanol extract can be seen in table 1

Table 1. The Result of Phytochemical screening

	Cassia siamea L.. ethanol extract	*Ageratum conyzoides* L ethanol extratct
Alkaloids	-	+
Flavonoids	+	+
Saponin	-	-
Polyphenol	+	+
Tannins	-	-
quinons	+	+
Monoterpenoid & Sesquiterpenoid	+	+
Steroid & Triterpenoid	+	+

The result of phytochemical screening showed that Seconder Metabolic of compounds that have the potential as an antibacterial such as flavonoids and polyphenols showed positive results. The flavonoids causing damage to the permeability of the bacterial cell wall, microsomes and lysosomes as a result of interaction between flavonoid with bacterial DNA. As according to Naim Osho [16], flavonoids have a lipophilic nature that is possible will damage the bacterial cell membrane.

Diffusion method was chosen because attractive of their simplicity, low cost and time intensive [8]. The diffusion method is not suitable for natural antimicrobial compounds, such as steroid, terpenoid, essential oil, that are insoluble in water, thus their hydrophobic nature prevents uniform diffusion through the agar media [17]. This study was using DMSO to suspense the extracts, so that the extracts could diffused in media, and inhibited the growth of bacterial tested [18].

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The antibacterial activity from *Cassia siamea L.* ethanol extract and *Ageratum conyzoides* L ethanol extract can be seen in table 2.

Table 2. The Antibacterial activity from *Cassia siamea L.* extracts

Plant material	Concentration (mg/mL)	Zone inhibition* (diameter in mm)		
		S.a	*S.e*	*P.a*
Ageratum conyzoides L ethanol extract	100	15.21±0.07	13.13±0.08	11.91±0.17
	50	12.0±0.1	10.21±0.12	9.39±0.2
	25	7.21 ±0.1	8.01±0.07	8.0 ±0.17
	12.5	7.11±0.17	7.33±0.1	6
Cassia siamea L.. ethanol extract	100	12.3±0.22	10.9±0.12	16.1±0.08
	50	9.3±0.16	9.1±0.1	13.5±0.07
	25	8.4±0.28	6	11.8±0.07
	12.5	6	6	10.7±0.07
DMSO absolute		6	6	6

S.a = Staphylococcus aureus, S.e = Staphylococcus epidermidis, and *P.a = Propionibacterium acnes*
* Value are the average of triplicate; includes the cup diameter = 6 mm

The MIC from *Cassia siamea L.*. ethanol extract can be seen in table 3.

Table 3. The MIC from Cassia siamea L. ethanol extract

Bacterial	Concentration (mg/mL)	Zone inhibition* (diameter in mm)	
		Ethanol extract	DMSO absolute
S.a	25	8.1	6
	22.5	6	6
	20	6	6
	17.5	6	6
	15	6	6
	12.5	6	6
S.e	50	8	6
	45	7.6	6
	40	7.2	6
	35	7	6
	30	6	6
	25	6	6
P.a	15	8.2	6
	12.5	8	6
	10	7.7	6
	7.5	7.4	6
	5	6.5	6
	2.5	6	6

S.a = Staphylococcus aureus, S.e = Staphylococcus epidermidis, and *P.a = Propionibacterium acnes*

* Value is the average of triplicate; includes the cup diameter = 6 mm

The MIC from *Ageratum conyzoides* L ethanol extract can be seen in table 4.

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Table 4. The MIC from *Ageratum conyzoides* L ethanol extract

Bacterial	Concentration (mg/mL)	Zone inhibition* (diameter in mm)	
		Ethanol extract	DMSO absolute
S.a	25	8.1	6
	12.5	7.21	6
	10	7.01	6
	7.5	6	6
S.e	25	8.11	6
	12.5	7.8	6
	10	7.7	6
	7.5	6.31	6
	5	6	6
P.a	25	8.19	6
	12.5	7.9	6
	10	7.91	6
	7.5	6.69	6
	5	6.5	6
	2.5	6	6

S.a = Staphylococcus aureus, S.e = Staphylococcus epidermidis, and *P.a = Propionibacterium acnes*

* Value is the average of triplicate; includes the cup diameter = 6 mm

The result of antibacterial activity showed that *Ageratum conyzoides* L ethanol extract have antibacterial activity. The Cassia sp ethanol extractshowed high antibacterial activity against *P. acnes* with MIC 5 mg/mL, but showed moderate antibacterial activity against *S. aureus* and *S.epidermidis*. Whereas, the activity of *Ageratum conyzoides* L ethanol extract showed that have antibacterial activity with MIC against *P. acnes* withMIC 5 mg /mL, 10 mg/mL against *S. aureus* and 7.5 mg/mL against *S. epidermidis*.Many pharmacological active compound have been found in*Cassia siamea L.* Ethanol extract and *Ageratum conyzoides* L ethanol extract which could be responsible for antibacterial effecting. They include flavanoids such as conyzoigun and dotriconthene, tannins and eugenol. Phenol is known as disinfectants as well other antimicrobial and insecticidal [11].

The statistic data were analyzed by one way ANOVA (variance analysis) with The confidence limit was set at $P < 0.05$, showed that a significant difference between the sample of extract and control. The Tukey test result showed that a significant difference between extract both *Cassia siamea L.* Ethanol extract or *Ageratum conyzoides* L ethanol extract with Dimethylsulfoxide (DMSO). This is indicated that DMSO didn't affect to the antibacterial activity of *Cassia siamea L.* Ethanol extract or *Ageratum conyzoides* L ethanol extract.

CONCLUSION

Cassia siamea L. ethanol extract showed high antibacterial activity against *P. acnes* with MIC 5 mg/mL, but showed moderate antibacterial activity against *S. aureus* 25 mg/mL and *S. epidermidis* 35 mg/mL. Whereas, the activity of *Ageratum conyzoides* L ethanol extract showed that have antibacterial activity with MIC against *P. acnes* 5 mg /mL, 10 mg/mL against *S. aureus* and 7.5 mg/mL against *S. epidermidis*.

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