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Antibacterial Activity from Ethanol Extracts and Fractions of Family *Asteraceae* Leaf Against *Bacillus cereus* and *Vibrio cholera*

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***Abstract*—The use of plants as traditional medication has been widely used in the community. *Asteraceae* is a family plant with activity against many diseases. The bioactivity dues to bioactive compounds contained in the plants as flavonoids, alkaloids, terpenoids and saponins. This study aims to determine the antibacterial activity in *Asteraceae* family plants using the paper disc diffusion method. Antibacterial activity was evaluated using Activity Index (AI) and Proportion Index (PI) calculations. The activity test shows the leaves of *Asteraceae* family plant is potential as antibacterial agents. According to AI and PI calculation, the most effective antibacterial against *B. cereus* and *V. cholera* is the ethyl acetate fraction of Insulin (*Tithonia diversifolia*) leaves. Bacterial cell morphology was observed using Scanning Electron Microscope (SEM), showing a change in bacterial morphology which are pores formation and cell shrinkage.**

Keywords:* Asteraceae, *Scanning Electron Microscope, Tithonia diversifolia

I. INTRODUCTION

Indonesia has a high prevalence of infectious diseases, especially the ones caused by bacteria such as diarrhea. According to WHO, there are 13 million deaths around the world each year are caused by infectious diseases. While the Indonesia Ministry of Health (2018) stated, there were 7,077,299 cases of diarrhea in health facilities in Indonesia[1]. Bacillus cereus is a gram-positive bacterium which causes food poisoning with certain symptoms as vomiting and diarrhea. These pathogenic bacteria are often found in foods which contain carbohydrate-based ingredients, such as rice, flour, noodles and other foods [2]. Vibrio cholera is a gram-negative bacterium that causes diarrhea by producing cholera toxin (enterotoxin), mucinase, and endotoxic which is able to stimulate water and chloride hypersecretion and inhibits sodium absorption[3].

The main therapy bacterial infection is by the use of antibiotics[4]. However, the increasement of bacterial resistance to antibiotics causing the necessity to discover new drugs with better activity than former medicines, economically affordable, with less of adverse effects. One of them is by utilizing Indonesian plants that have efficacy[5].

Efficacious plants as traditional medicine to treat a disease has been widely used by the people of Indonesia. Asteraceae family plant is one of the largest plant kingdoms in the world that has many activities to treat a disease. It dominates the vegetation of plants on earth with a number of species of 24,000-30,000 species and is spread almost all over the world and is found in almost all environments[6]. The activity produced by plants is caused because it contains active secondary metabolites, including lactone sesquiterpenes, pentacyclic triterpenes, alcohols, alkaloids, tannins, polyphenols, saponins, and sterols[7].

II. MATERIAL AND METHOD

A. Material

The tools used in this study were autoclave, rotaryvaporator, incubator, Scanning Electrom Microscopy (SEM) instrument of JSM-6360 LA series, paper disc, test tube, ose needle, petri dishes, pipette, micropipette, vortex and Laminar Air Flow (LAF) cabinet.

The materials used in this study were leaves of selected Asteraceae plants consisted of leaves (*Tithonia diversifolia*) , Bandotan leaves (*Ageratum conytoides*), African tree leaves (*Vernonia amygdalina*), Sembung leaves (*Blumea balsamifera*), *Bacillus cereus* and *Vibrio cholera.*

B. Preparation of Asteraceae Plants Leaves Extracts

After characterization and phytochemical screening, the each simplicia was extracted using cold method by maceration in 96% ethanol. Maceration was carried out for 3x24 hours by immersing an amount of simplicia in 96% ethanol. After 24 hours, it filtered and the solvent frequently changed by a new one. Collect all the liquid extract to be evaporated by the rotaryvaporator to obtain a viscous extract.

C. Preparation of Asteraceae Plants Leaves Fractions

The viscous extracts were later fractionated by Liquid-Liquid Extraction (LLE) using separating funnel. The solvents used were n-hexane as nonpolar agent, ethyl acetate as semi polar agent and methanol-water (1:5) as polar agent.

LLE was carried out using 4x250mL for each solvent. Each liquid fraction was collected to be evaporated in rotaryvaporator to obtain viscous fraction.

D. Antimicrobial Assay

Antibacterial activity test was performed using paper disc diffusion method to obtain the MIC of the extracts and fractions by observing the inhibition zone appeared on the petri dishes. The principle of the method is to solidify the agar media after the bacteria suspension poured into the dish and placing a paper disc infused by the extracts and fraction above the agar. It incubated at temperature of 37°C for 18-24 hours. The observed parameter was the inhibition zone appeared surrounding the paper disc and measured using calipers.

E. Observation of Bacterial Cell Morphology by Scanning Electron Microscope

The solutions were incubated at 37°C, and centrifuged at 3500 rpm for 20 minutes. Cell deposit was separated with the supernatant and soaked overnight by 2% glutaraldehyde. Chocodylate buffer was added and soaked for an hour. Dried by alcohol 70%, 80%, 90% and absolute alcohol for each 20 minutes in a row. The cell deposit was then precipitated using butanol and placed above dried slip cover. The cover was coated with gold using vacuum for 20 minutes and observed with a JEOL JSM-6360 LA11 Scanning Electron Microscope (SEM)[8].

III. RESULTS

TABLE I. SIMPLICIA CHARACTERIZATION OF ASTERACEAE PLANTS LEAVES

Plants	Total Ash Content (%)	Water Content (%)	Content of Water Soluble Extract (%)	Content of Ethanol Soluble Extract (%)
Afrika	0,83	24,20	1,75	11,32
Bandotan	0,95	11,26	2,09	9,96
Insulin	0,61	6,90	1,93	9,33
Sembung	0,83	4,68	2,39	11,07

BA: Bandotan Leaves, SE: Sembung Leaves, IN: Insulin Leaves, AF: Afrika Leaves

The four plants have different characteristics. The water content has fulfilled the requirements <10%, the highest ash content was found in Africa leaves (21.20%), the highest water soluble extract content was found in Sembung leaves (2.39%), and the highest ethanol soluble extract was found in Africa leaves (11, 32 %).

TABLE II. SIMPLICIA PHYTOCHEMICAL SCREENING OF ASTERACEAE FAMILY PLANTS LEAVES

Test of		Bandotan	Sembung	Insulin	Afrika
Alkaloid	S	+	+	+	+
	E	+	+	+	+
Tannin	S	+	+	+	+
	E	+	+	+	+
Flavonoid	S	+	+	+	+
	E	+	+	+	+
Saponin	S	-	+	+	-
	E	-	+	+	-
Kuinon	S	-	-	-	-
	E	-	-	-	-
Steroid/ Terpenoid	S	+ (s)	+ (s)	+ (s)	+ (t)
	E	+ (s)	+ (s)	+ (s)	+ (t)

Information : S = Simplicia; E = Extract ; (+) = Found; (-) = Not Found; (s) = Steroid; (t) = Triterpenoid

Phytochemical screening results shows that all plants tested contain alkaloid, flavonoid, tannin, steroid/ triterpenoid, especially Insulin and Sembung leaves contain saponin as well.

TABLE III. ANTIMICROBIAL ACTIVITY OF ASTERACEAE FAMILY PLANTS LEAVES

Plants		Diameter of the Inhibition Zone (mm)					
		K.10%		K.5%		K.1%	
		BC	VC	BC	VC	BC	VC
Extract	BA	8,68	0	8,30	0	8,16	0
	SE	8,65	0	8,30	0	0	0
	IN	14,46	16,13	9,10	13,30	0	9,07
	AF	11,78	11,80	8,30	9,55	7,56	8,03
N-Hexane Fraction	BA	10,21	0	7,56	0	0	0
	SE	8,65	0	8,30	0	0	0
	IN	16,46	15,50	10,81	11,20	9,05	6,20
	AF	12,10	10,77	9,46	8,87	0	0
Ethyl Acetate Fraction	BA	8,15	8,17	7,63	6,15	0	0
	SE	9,23	10,27	8,43	7,97	7,65	7,00
	IN	18,36	18,80	14,36	15,00	7,93	9,47
	AF	15,40	16,42	12,50	13,78	8,28	8,00
MeOH Fraction	BA	8,80	0	0	0	0	0
	SE	0	0	0	0	0	0
	IN	8,81	0	8,03	0	0	0
	AF	9,60	0	8,43	0	0	0
Ciprofloxacin (Standard)		12,90					
DMSO 8% (Control +)		0					

BA: Bandotan Leaves, SE: Sembung Leaves, IN: Insulin Leaves, AF: Afrika Leaves

The comparative test was carried out using ciprofloxacin in concentration of 50ppm as standard amount, and 8% DMSO as control positive. The test shows 10% ethyl acetate fraction of Insulin leaves has the biggest yield.

TABLE IV. ANTIMICROBIAL ACTIVITY OF ASTERACEAE FAMILY PLANTS LEAVES

Plants		Diameter of the Inhibition Zone (mm)					
		4%	3%	2%	0.75 %	0,5 %	0,25 %
Extract	BA				7,9	7,6	0
	SE				0	0	0
	IN	8,4	7,55	0			
	AF				0	0	0
N-Hexane Fraction	BA				0	0	0
	SE	7,65	0	0			
	IN				8,35	0	0
	AF				0	0	0
Ethyl Acetate Fraction	BA	0	0	0			
	SE				0	0	0
	IN				7,75	0	0
	AF				7,65	7,3	0
MeOH Fraction	BA	0	0	0			
	SE						
	IN				0	0	0
	AF				0	0	0
Ciprofloxacin (Standard)		11,89					
DMSO 8% (Control +)		0					

BA: Bandotan Leaves, SE: Sembung Leaves, IN: Insulin Leaves, AF: Afrika Leaves

TABLE V. ANTIMICROBIAL ACTIVITY OF ASTERACEAE FAMILY PLANTS LEAVES

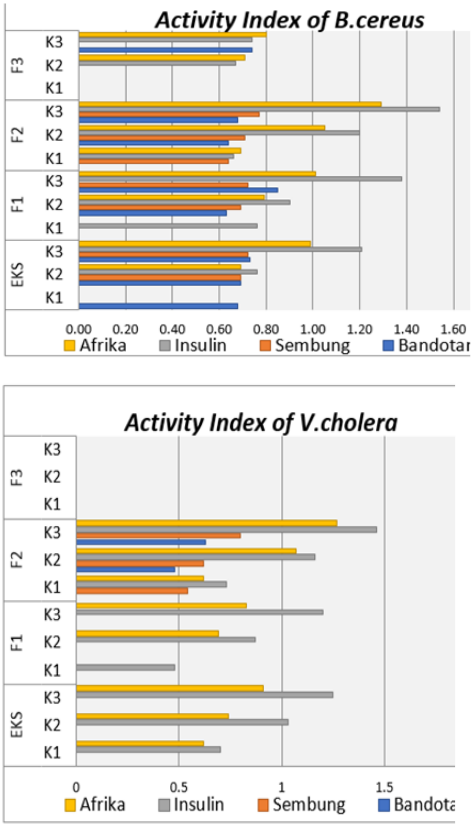
Plants		Diameter of the Inhibition Zone (mm)					
		4%	3%	2%	0.75 %	0,5 %	0,25 %
Extract	BA				7,9	7,6	0
	SE				0	0	0
	IN	8,4	7,55	0			
	AF				0	0	0
N-Hexane Fraction	BA				0	0	0
	SE	7,65	0	0			
	IN				8,35	0	0
	AF				0	0	0
Ethyl Acetate Fraction	BA	0	0	0			
	SE				0	0	0
	IN				7,75	0	0
	AF				7,65	7,3	0
MeOH Fraction	BA	0	0	0			
	SE						
	IN				0	0	0
	AF				0	0	0
Ciprofloxacin (Standard)		11,89					
DMSO 8% (Control +)		0					

BA: Bandotan Leaves, SE: Sembung Leaves, IN: Insulin Leaves, AF: Afrika Leaves

TABLE VI. MINIMUM INHIBITORY CONCENTRATION OF ASTERACEAE FAMILY PLANTS LEAVES AGAINS V. CHOLERABACTERIUM

		Diameter of the Inhibition Zone (mm)					
		4%	3%	2%	0,75%	0,5%	0,25%
Extract	BA						
	SE						
	IN				8,73	7,3	0
	AF				0	0	0
N-Hexane Fraction	BA						
	SE						
	IN				6,00	0	0
	AF	8,50	8,00	7,35			
Ethyl Acetate Fraction	BA	0	0	0			
	SE				0	0	0
	IN				8,15	0	0
	AF				7,95	7,60	0
MeOH Fraction	BA						
	SE						
	IN						
	AF						
Ciprofloxacin (Standard)		12,9					
DMSO 8% (Control +)		0					

BA: Bandotan Leaves, SE: Sembung Leaves, IN: Insulin Leaves, AF: Afrika Leaves



EKS = Extract, F1 = N-Hexane Fraction, F2 = Ethyl Acetate Fraction, F3 = MeOH Fraction, K1 = Concentration 1%, K2 = Concentration 5%, K3 = Concentration 10%

Fig. 1. Data Analysis Graph Using Activity Index(AI)

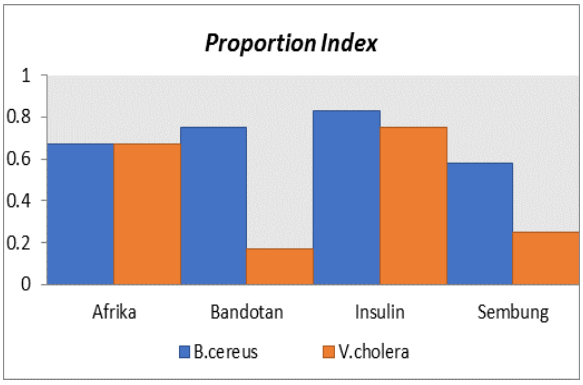


Fig. 2. Data Analysis Graph Using Proportion Index (PI)

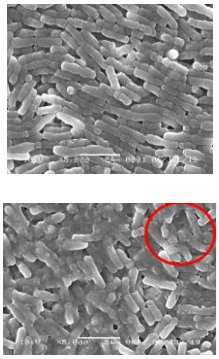


Fig. 3. SEM observations (5000x magnification), left - right: normal *V.cholera*; *V. cholera* exposed to ethyl acetate fraction of Insulin leaves (*Tithonia diversifolia*) with concentration 10%

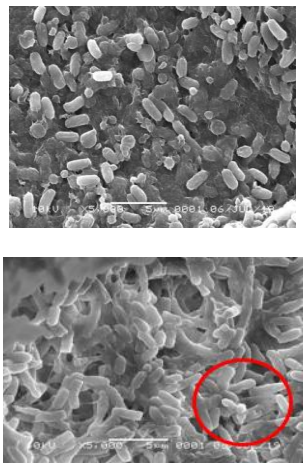


Fig. 4. SEM observations (5000x magnification), left - right: normal *B.cereus*; *B.cereus* exposed to ethyl acetate fraction of Insulin leaves (*Tithonia diversifolia*) with concentration 10%

IV. DISCUSSION

A. *Characteristics of Asteraceae Family Plants Leaves*

Characterization tests are carried out to ensure that simplicia has required characteristics and is suitable for use as traditional medicine. Water content test is performed to find out the water content of simplicia, this will affect the simplicial condition,if it exceeds the percentage of 10%, the bacteria and fungus will be susceptible to grow. Ash content test is conducted to determine the amount of residual mineral and organic compounds after combustion. Water-soluble extract determination is conducted to determine the percentage of water soluble compounds in simplicia. The ethanol soluble extract determination test is performed to determine the ethanol soluble percentage in.

B. *Phytochemical Screening of Simplicia and Extract of Asteraceae Family Plants Leaf*

Alkaloids are considered to have antibacterial properties by its ability as DNA interchelator to inhibit bacterial cell topoisomerase[1]. Tannins are known to have antibacterial activity by its activity to denaturate bacterial cell proteins[9]. Flavonoids are considered to have antibacterial activity by its ability to inhibit bacterial cell respiration systems[10]. Saponin has antibacterial activity because it may lower the surface tension in bacterial cell and causing cell leakage[11, 12]. Steroids and triterpenoids can interfere the bacterial cell membrane function, to become brittle and undergo a lysis [12, 13].

C. *Antimicrobial Activity of Asteraceae Family Plants Leaves*

Antibacterial activity of the four Asteraceae family plants shows that the four plants have antibacterial activity. The activity was resulted from diffusion process of extracts and fractions in agar as growth media grown by the bacteria. The antibacterial activity was affected by the concentration of extracts and fractions. The higher concentration of extract and fraction, the higher antibacterial activity against *B. cereus* and *V. cholera*. It means the concentration affects the antibacterial sensitivity. The further antibacterial activity mechanism analyzed by Scanning Microscope Electron (SEM). The inhibition zone obtained are calculated to be analyzed by the Activity Index (AI) and Proportion Index (PI). And continued by the reduction of the concentration of each plant to find the Minimum Inhibitory Concentration (MIC)

The results of the MIC test show that ethyl acetate fraction of Insulin leaves has MIC of 0,75% against *B. cereus* and *V. cholera* with inhibition zone diameters of 7.75 mm for *B. cereus* and 8.15 mm for *V. cholera*.

D. *Activity Index and Proportion Index Calculation Analysis*

The antibacterial activity result analyzed by AI calculation. Figure 1 shows ethyl acetate fraction of Insulin leaves in concentration of 10% has the most inhibition against *B. cereus* and *V. cholera*. The results indicate that the fraction is effective in inhibiting the growth of gram-positive and gram-negative bacteria.

Whereas based on PI calculation, Insulin leaves has better activity against *B. cereus* than *V. cholera*. This is caused by the antibacterial activity of Insulin leaves has more positive results against *B. cereus* instead of *V. cholera* yet the inhibition zone of the fraction is appeared larger on the plate of *V. cholera* 18,80 mm and 18,36 mm against *B. cereus*.

This indicates that Insulin leaves have a better antibacterial sensitivity to gram-negative bacteria compared to gram-positive bacteria. It happens because of the differences of both bacteria. Gram-positive bacteria has thicker cell wall structure of peptidoglycan than gram-negative bacteria which cause the antibacterial substance has more adversity to penetrate the bacteria cells[8].

E. Observation of Bacterial Cell Morphology with Scanning Electrone Microscope Electron (SEM)

Based on analysis using AI and PI, morphological observations of bacterial cells were carried out using a Scanning Electron Microscope (SEM) on *B.cereus* and *V.cholera* bacteria after exposure to the ethyl acetate fraction of Insulin leaves of concentration of 10%

Observation of bacterial cell morphology using SEM in figures 3 and 4, shows change in cells between normal and 10% ethyl acetate fraction of insulin leaves exposed bacteria. These changes are the formation of pores and the shrinkage of bacterial cell walls. It is suspected that these changes occur due to interactions between compounds in the ethyl acetate fraction of insulin leaves and bacterial cell peptidoglycan. Peptidoglycan of the bacteria is composed of hydrophilic polypeptides, when hydrophilic antibacterial compounds will be easily pass through the bacteria cell and causing cell membrane permeability disruption and the bacterial cell wall formation is inhibited[14].

V. CONCLUSION

In this study, the leaves of Asteraceae family plants are found to have antibacterial activity after the test against *B. cereus* and *V. cholera*. The strongest antibacterial activity is shown in ethyl acetate fraction of Insulin Leaves by the change of cell morphology with the shrinkage and pore formation of the cell.

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