Antibacteria Activity of *Shewanella* and *Pseudomonas* as Endophytic Bacteria from the Root of *Ageratum conyzoides* L.

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ABSTRACT— ***Most of plants have endophytic bacteria that are able to synthesize some compounds that have biological activities as antibacteria. Four isolates of endophytic bacteria from root of Ageratum conyzoides was isolated and identified. The result of the identification of their morphology and biochemical activity shows that those bacteria are from Shewanella and Pseudomonas. The purpose of the research is to obtain crude extract of supernatant of endophytic bacteria in root A. conyzoides that performs antibacteria activity and to discover the identity of potential antibacteria compounds from the crude extract. The extraction of supernatant was done by using ethyl acetate. Then, antibacteria of each crude extract was tested on three pathogenic bacteria; i.e. Eschericia coli, Pseudomonas aeruginosa, and Staphylococus aureus. The result of the test indicates that those crude extracts perform antibacteria activity. The crude extract of supernatant of Shewanella presents the highest level with zone of inhibition of 11.92 ± 1.24 mm (E. coli), 12.85 ± 0.32 mm (P. aeruginosa), and 15.13 ± 0.88 mm (S. aureus). The result of the test on crude-extract compound by using GC-MS discovers the existence of two active compounds; those are 2-amino-3-quinoline carbonitrile and boric acid. The result of the research asserts that endophytic bacteria from root A. conyzoides demonstrate the potential to be a promising source of antibacteria compound.***

Keywords--- *Ageratum conyzoides*, Antibacteria, Endophytic bacteria

1. INTRODUCTION

Endophytic microorganisms are bacteria and fungi living in plant tissues, but they do not harm the host due to their symbiosis of mutualism. The host provides space and nutrition from exudates and the endophytic microorganism protects the host from pest and pathogen [1,2]. Some endophytic microorganisms have been isolated from various plant tissues and they are considered as a prospective source of bioactive compounds[3, 4].

Ageratum conyzoides is one type of plants that holds symbiotic endophytic bacteria. This is a medicinal plant that people have used extensively as a resource of traditional medicine for healing and preventing particular diseases, such as influenza, fever, rheumatism, inflammation, malaria, and bacterial or fungal infection, since its compounds have antimicrobial activity [5, 6, 7].

It has been informed that endophytic bacteria in medicinal plants are able to synthesize a particular compound with some biological activities, such as antimicrobia or antibacteria activities [3, 8]. Sun *et al*. [9] conveys that 29 isolates of potential endophytic bacteria isolated from *Polygonum cuspidatum* perform antibacteria and antifungal activities toward the development of *Gibberella fujikuroi*, *Aspergillus niger*, *Aspergillus fumigatus*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Eschericia coli*, and *Bacillus subtilis*. Some bacillus endophytic bacteria, such as *Paenibacillus sp* [10], *Bacillus subtilis* BSn5 [11], *Bacillus amyloliquefaciens* [12], and *Bacillus cereus* [13] isolated from different plants, also show antibacteria on pathogenic bacteria.

Based on that information, this research dealt with the extraction of supernatant of endophytic bacteria from *A. conyzoides* root and the identification of active compounds to obtain crude extract of supernatant of entophytic bacteria

from *A. conyzoides* root that perform antibacteria activity and to identify potential antibacteria compounds from the crude extract.

2. MATERIALS AND METHODS

2.1 Bacterial samples

Samples of bacteria used in this research are two isolates of endophytic bacteria from *A. conyzoides* root taken from earlier research.

2.2 Identification of endophytic isolates

Endophytic isolates were identified by observing their morphological characteristics, including cell shape, Gram staining, and the existence of endosperm, and their biochemical activity, including hydrolysis test on the extract, lipid, gelatine, nitrate reduction, IMViC, carbohydrate fermentation, catalyse, and urease [14]. Furthermore, the result achieved was conformed to the keys of determination referring to *Bergey`s manual of determinative bacteriology 9th* [15].

2.3 The extraction of secondary metabolites

Those two endophytic isolates were cultured in Luria *broth* (LB) medium for 24 hours in a temperature of 37^{o}C at 121 rpm. Afterwards, the culture was processed in a centrifuge at 10.000 rpm to obtain the supernatant. Supernatant was then moved into a split tube by adding ethyl acetate (1:1 v/v), mixed for 15 minutes, and held it down for 15 minutes until it forms several layers [16]. Next, those ethyl acetate layers were evaporated in a temperature of 40^{o}C to obtain dried extract. After that, it was dissolved in DMSO 1% with concentration 40 mg/ml and put into dark bottle in a temperature of 4^{o}C [2].

2.4 Antibacteria activity test

The test was taken employing of disk diffusion method. Bacteria used are pathogenic bacteria in human; specifically *Eschericia coli*, *Pseudomonas aeruginosa*, and *Staphylococus aureus*. Every 1 ml of the bacteria was tested with 1.5 x 10^{8} cfu/ml concentration (McFarland 0,5) and suspended in Luria Agar (LA) medium [17]. Subsequently, each 20 µl of the extract was tested using disk paper (Macherey-Nagel6mm MN 827ATD) and incubated in a temperature of 37^{o}C for 24 hours. Then, it went through the measurement of inhibition zone [14]. Ethyl acetate and DMSO 1% were used as negative control and ampicillin was used as positive control.

2.5 Identification of compounds in crude extract

The crude extract of supernatant was tested using GC-MS (Shimadzu QP 2010 ULTRA, column BD5). The temperature of the column was programmed to be at 60^{o}C – 280^{o}C with rate increase of 8^{o}C/minute. 'He' gas was used as carrier with flow rate of 1.32 ml/minute. The temperature of injector was set at 27^{o}C with injection volume of 0.2 µl, and split ratio 200.

3. RESULTS AND DISCUSSION

The result of identification of morphology and biochemical activity of two endophytic bacteria of *A. conyzoides* suggests that two isolates are bacteria from the genus of *Shewanella* and *Pseudomonas*.The crude extract of supernatant of those bacteria demonstrates inhibition activity on pathogenic bacteria tested (Table 1). GC-MS analysis of secondary metabolites presents the existence of five active compounds (Table 2).

Based on the result, the crude extract of their supernatant shows antibacteria activity on pathogenic bacteria tested (Table 1). The crude extract of *shewanella* has the strongest antibacteria among other extracts. It offers the greatest resistibility against all pathogenic bacteria tested and has high sensitivity with the diameter of inhibition zone of more than 12 mm [18]. On the other hand, the crude extract of *Pseudomonas* shows the lowest level of antibacterial activity and low sensitivity. It only inhibits the growth of *E. coli* (7.95 ± 0.51) mm and *S. aureus* (6.42 ± 0.05) mm, but not that of *P. aeruginosa*. For that reason, it can be assumed that the dose given (40 mg/ml) is not enough to inhibit the growth of pathogenic bacteria, *P. aeruginosa*. It is considered that the content of pathogen antibacterial compound of *P. aeruginosa* in 40 mg/ml concentration of the extract is so insufficient that it cannot exert a stress to pathogenic bacteria, *P. aeruginosa*. However, further study is still necessary to explore this consideration.

Table 1. Antibacteria Activity of Crude Extract of Endorhizosphere Bacteria *Ageratum Conyzoides*

Sample	Zone of Inhibition (mm)		
	S. aureus	*P. aeruginosa*	*E. coli*
Shewanella	15,13 ± 0,08	12,85 ± 0,32	11,92 ± 1,24
Pseudomonas	6,42 ± 0,05	0	7,95 ± 0,51
DMSO 1%	0	0	0
Ethyl Acetate	0	0	0
Ampicillin	28,84 ± 0,49	20,47 ± 0,52	21,54 ± 1,29

The result of analysis on the identity of compounds, they exist four active compounds (Table 2). In general, compounds identified from the crude extract can potentially become antibacteria agents. 2-amino-3-quinoline carbonitrile, one of alkaloid compounds, is successfully identified from the crude extract of *Shewanella*. This compound is the derivative of quinoline. Some researchers have reported that quinoline and its derivatives have many biological activities [19], for instance anti-malaria [20, 21], anticancer [20], antifungal, and antibacterial agents [22]. A research conducted by Kitagawa and Tamura [23] informs the existence of compounds with antibiotic activities isolated from *Rhodococcus erythroplois* bacteria, i.e. 1-hydroxy-2-methyl-3-(3,7,11-trimethyldodeca-9-hydroxy-2,6,10-trienyl)-quinoline-4-one that are the derivatives of quinoline compound.

Basically, quinoline compound and its derivatives are contained in plants useful for anti-malaria or anti-plasmodium. Some researchers have proven that *A. conyzoides* present anti-malaria or anti-plasmodium activity [5, 6, 7] so that it is considered to be able to synthesize quinoline compound or its derivatives.

Table 2. The Result of Identification the Contents of Compound by Using GC-MS

Crude Extract	Compound
Shewanella	2-amino-3quinolinecarbonitrile Boric Acid Dimethyl Sulfoxide (Solvent)
Pseudomonas	Ethyl Alcohol (Solvent) 9-octadecenoic acid (Oleic Acid) 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester

Based on the result, it can be identified that the existence of active compounds in endophytic bacteria and host plants comes from the interaction between them. It is corresponding to the notion from [1,3] that the characteristics of endophytic microorganisms are correlated or similar to those of the host. They say that both organisms are able to synthesize some compounds with similar characteristics or functions and possibly in the same line of compound derivation.

Some other compounds identified in this research are 9-octadecenoic acid and 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester from the crude extract of *Pseudomonas*. 9-octadecenoic acid is another term for oleic acid compound classified as fatty acid compound. It is notified that some fatty acid compounds are produced by microorganism in stationer phase and some can perform biological activities [27]. 9-octadecenoic acid compound or oleic acid and its derivatives are recognized to present antifungal [28, 29], antibacterial [30], anti-cancer [31] and antitumor [32] activities. Moreover, Zheng *et al*.[33] conveys that oleic acid compound is able to inhibit the development of *S. aureus* and *S. pyogenes*.

The second compound is from phenol compound, which is 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester. It is identified that phenol compound group present a lot of biological activities, one of which is antibacteria activity [34]. 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester compound is also called as phthalic acid. Generally, phthalic acid compound and its derivatives are considered as plasticizer compound, which is usually used in textile industry, glass industry, and plastic industry because it can improve product's flexibility, transparency, and endurance [35]. In addition to that benefit, some researchers have proven that most derivatives of phthalic acid present biological activities, such as antifungal [36, 37], antibacterial [38, 39], anticancer, melanogenesis inhibition [40], and some more of health benefits. In the same line, [41] informs that 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester compound derived from the extract

of ethyl acetate of *Burkholderia cepacia* shows antibacterial activity on *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Vibrio ordalli*.

The last compound is boric acid identified from the crude extract of *Shewanella*. Boric acid is a compound with rather high level of toxicity for living things and it also has some biological activities, including antibacteria activity [42]. One of its derivates is tartrolon compound, which is antibiotic compound derived from boron contained in the extract of bacteria metabolite.

Elshahawi *et al*. [43] is successful to identify the presence of tartrolon antibiotic compound in the crude extract of *Teredinibacter turnerae*, symbiotic bacteria on seashells. Two tartrolon compounds successfully identified present antibacteria activity on some pathogens, such as *P. aeruginosa*, *S. aureus*, *E. coli*, and *B. Subtilis*, and antifungal activity on *Candida albicans*. A research delivers similar report about antibacteria activity from tartrolon compound on the crude extract of *Sorangium cellulosum*. Other than tartrolon, there is another derivative of boron presenting antibacteria activity, i.e. boromycin that is reported to be identified in the crude extract of *Streptomyces* [44, 45].

In conclusion, it can be considered that endophytic bacteria from the root of *A. conyzoides* have potentials to be a promising source of antibacterial compound. Even if there are only five potential compounds identified in this research, it is possible that there are more bioactive compounds that have not been identified from the crude extract and can be useful for human life.

4. ACKNOWLEDGEMENT

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