

Phytochemicals and The Ability of *Plantago major* Linn. Extract to Inhibit The growth of *Aeromonas hydrophila*

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Abstract

Aeromonas hydrophila is a type of gram-negative bacteria that can harm humans and major animals that have poikilotherm properties such as fish and shrimp. These bacteria can cause haemorrhages in fish until death and causing fish farming production to failure. The use of medicinal plants has been trusted by people all over the world to overcome various disease problems, one of which is a disease caused by bacteria. *Plantago major* L. is known that able to inhibit the growth of both gram-positive and gram-negative bacteria. The purposes of this study were to identify the extracted compounds contained in *Plantago major* L., using FTIR and UV-VIS spectrophotometer and to evaluate antibacterial activity against *A. hydrophila*. The results of this study showed that crude extracts of *Plantago major* L. contained polar compounds such as phenols, flavonoids, saponins, and tannins. The presence of phenols, tanin and flavonoids was confirmed by the results of FTIR and UV-VIS. Furthermore, the crude extract of *Plantago major* L. significantly inhibited the growth of *A. hydrophila* ($P < 0.05$).

Keywords: Antibacterial, Co-culture, Extraction, FTIR, UV-VIS.

INTRODUCTION*

Aeromonas hydrophila is a type of bacteria that was widespread in both fresh and brackish water environments. These bacteria can infect various animals especially animals that are poikilotherm, such as fish and shrimp. If these animals are infected by the bacteria, the quality of their meats will be reduced [1,2,3]. *A. hydrophila* is known as an agent of *Motile Aeromonas Disease* or MAS, present as secondary pathogens in fish that have been exposed to environmental stress. The symptoms of MAS were observed as haemorrhages and pathological damages in internal organs. Infected fish experienced mortality up to 80-100% in a short time [4,5,6].

The use of medicinal plants has been known and trusted by 80% of the World community. *Plantago major* L. is a well-distributed plant over the World and known as a 'weed' that live freely in nature [7]. In Indonesia, 28% of *Plantago major* L. is found growing wild on the roadside and used as a wound medicine [8]. It has an ability to cure white discharge problem [9], inflammation and coughing [10]. This weed was known to have several activities such as antibacterial, antifungal and antiviral agents as well as antioxidant and analgesic [11]. The previous study affirmed the benefits of *Plantago*

major L. in the field of health because of several compounds such as polyphenols, alkaloids, tannins, and steroids [12].

As an antibacterial agent, *Plantago major* L. extract was able to inhibit gram-positive and gram-negative bacteria such as *Lactobacillus* sp., *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, *Proteus* sp., *S. enteritidis* [13,14]. However, there is no report on the ability of *Plantago major* L. to inhibit the growth of *A. hydrophila*. Therefore the aim of this study was to determine the phytochemical content of *Plantago major* L. and its ability to inhibit the growth of *A. hydrophila*.

MATERIAL AND METHODS

Phytochemical Extraction and Analysis

The Phytochemical analysis was carried out by referring to the method of [15], to observe the content of phenols, flavonoids, alkaloids, tannins, saponin, terpenoids (steroids and triterpenoids). Plants of *Plantago major* L. aged 3 – 4 months were obtained from Materia Medica in Batu, dried for 5 days and made into powder using blender according to the method of [16], with modification. *Plantago major* L. powder (200 g) was macerated with 1L of ethanol PA (1:5) for 5 days. The results of maceration were filtered using Whatman paper No. 42 and evaporated using a rotary vacuum evaporator at 50°C, 65 rpm to obtain a thick green extract.

The extract was analysed using FTIR and UV-VIS spectrophotometry. FTIR is used to determine the functional group or type of the active compound based on the peak value of the

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wavelength. FTIR testing was conducted referring Jain et al. [17]. The obtained extract was mixed with KBr in a mortar and pressed with a pressure of 6 bars for 2 minutes. Samples were scanned on a spectrometer IR (FT 1000 Varian) 4000 - 400 cm^{-1} .

UV-VIS analysis was carried out by using UV-Visible spectrophotometry (Cary50 Conc Varian) [17]. Extract *Plantago major* L. was diluted using ethanol (1:10), then examined under UV wavelength 300 - 800 nm.

Antibacterial Test

This research was carried out at the Fish Health and Disease Division Aquaculture Library, Faculty of Fisheries and Marine Science, Brawijaya University from November – December 2018. A bacterial isolate of *A. hydrophila* was obtained from BBPBAT Jepara, Central Java. The bacteria was rejuvenated with TSA medium and re-cultured in TSB medium with a bacterial density of $2.35 \times 10^7 \text{CFU.mL}^{-1}$. TSA medium that used for antibacterial activity was 20 ml with spread method. Antibacterial activity tests were carried out using a disc diffusion method [18].

Blank disk with a size of 6 mm was dipped into the crude extract which has been diluted with 10% DMSO. The doses used were 100, 200, 300, 400, 500 mg.L^{-1} , with DMSO 10% as negative controls and Chloramphenicol as positive controls. All the treatments then incubated at the incubator 37°C for 24 hours. The diameter of the inhibition zone is measured using a caliper. Furthermore, co-culture tests were used to confirm the results of antibacterial activity by referring to the method [19] where bacterial culture and various concentrations of plant

extracts were mixed in tubes containing sterile TSB medium, then incubated for 24 hours. The cultures were then transferred in TSA plates and bacterial density was enumerated after 24 hours.

RESULT AND DISCUSSION

FTIR Analysis

The FTIR results of *Plantago major* L. and the absorption peaks are presented in Figure 1 and Table 1. From Figure 1, there are six types of absorption some of which are absorption at waves 3854.124 cm^{-1} indicating that there is an O–H group, the changes in wave 3740.940 cm^{-1} detected the presence of O–H groups, absorption at wave 3446.329 cm^{-1} shows the presence of O–H groups with stretching vibrations, at wave 1640.934 cm^{-1} detected a C=C group with stretch vibration, the strong absorption band at wave 1384.402 cm^{-1} was found to be a C–H group and the absorption of waves of 1050.935 cm^{-1} was found to have a C–O bond with strong intensity. Stretch bands of phenyl groups C=C, –OH, and –CH are characteristics of IR which indicate the presence of phenol and flavonoid and tannin compounds [20-23]. This absorption of the IR spectrum is similar to a previous study, which states that *Plantago major* L. contains a carboxyl, hydroxyl, and methyl group [24].

Tabel 1. FTIR Peak Result of *Plantago major* L.

No.	Peak Values (cm^{-1})	Functional Group
1.	3854.124	O – H carboxylic acid
2.	3740.940	O – H alcohol
3.	3446.329	O – H alcohol, fenol
4.	1640.934	C = C alkena
5.	1384.402	C – H alkana
6.	1050.935	C – O alcohol



Figure 1. FTIR Test Results of *Plantago major* L.

UV-VIS Analysis

UV-Vis results of *Plantago major* L. ethanol extract presented in (Fig. 2). The UV spectrum in the maximum wavelength at 227 nm, 411 nm, and 505 nm strongly suspected contained phenol compounds that are flavonoids. The main flavonoids presents are flavones, flavonol, and auron. This was confirmed that flavones were the main type of flavonoids in *Plantago major* L. [25]. There are several flavones in *Plantago major* L. but only one flavonol was detected in UV-spectrophotometry [26].

Phytochemical Extraction and Analysis

Result of the phytochemical analysis showed that crude extract of *Plantago major* L. contained phenols, flavonoids, saponins, and tannins compounds. On the other hand, there were no alkaloid and terpenoid compounds (Table 2).

However, another study stated that *Plantago major* L. extract contained a composition of alkaloids and terpenoids [25]. Phenols, flavonoids, saponins, and tannins are polar compounds which have various functions, one of which is an antibacterial activity [27].

Table 2. Phytochemical test result of *Plantago major* L.

No.	Compound	Result
1.	Fenol	+
2.	Flavonoid	+
3.	Alkaloid	-
	Mayer	-
	Dragendrof	-
	Bouchardat	-
4.	Saponin	+
5.	Tanin	+
6.	Terpenoid	-
	Steroid	-
	Triterpenoid	-

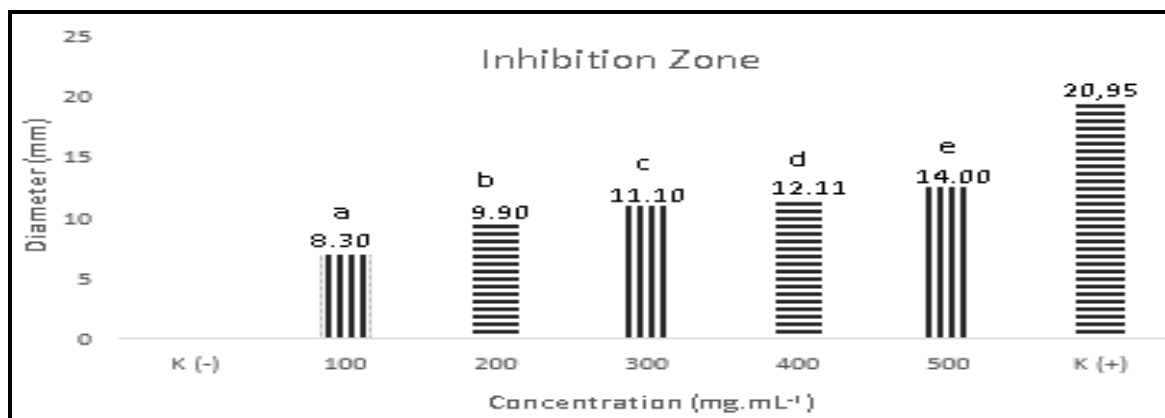


Figure 2. Antibacterial Test Results of *Plantago major* L.

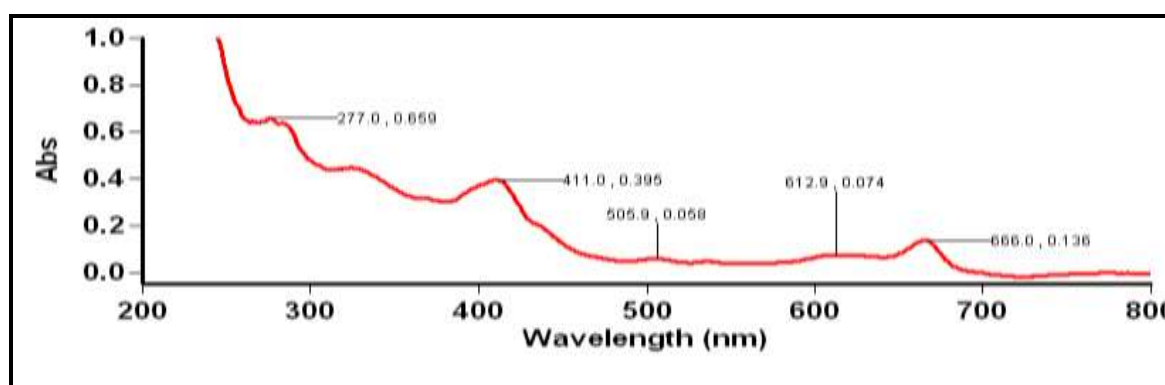


Figure 3. UV-VIS Test Result of *Plantago major* L.

Antibacterial Activity

The results of the antibacterial activity test showed that the crude extract of *Plantago major* L. had the ability to inhibit bacterial growth (Fig. 3). The inhibition of bacterial growth based on the extract dose. The higher dose, the greater

diameter of the inhibition zone were formed. At the dose of 100 and 200 mg.L⁻¹, an inhibition zone is formed with medium strength, doses of 300, 400 and 500 mg.L⁻¹ have a strong type of inhibition zone and the positive control using Chloramphenicol has a very strong inhibitory

zone characteristic while the negative control using DMSO 10% does not create an inhibition zone. The provisions of the inhibitory level refer to Eryln [28], the diameter of the inhibition zone ≥ 20 mm are included in the very strong category, 10 – 20 mm are included in the strong category, 10-5 mm has a medium category and ≤ 5 mm has a weak category.

The measurement results of the inhibition zone diameter were confirmed by the co-culture test presented in (Table 3). From the co-culture test explained that there was a decrease in the number of bacteria along with the larger dose given. This result strengthens the statement that *Plantago major* L. extract had the ability to inhibit the growth of *A. hydrophila*. Furthermore, *Plantago major* L. extract was able to significantly inhibit the growth of *A. hydrophila* ($P < 0.05$).

Table 3. Co-culture Test Result

No.	Dosage (mg.L ⁻¹)	Colony Forming Unit (CFU.ml ⁻¹)
1	C (+)	0
2	100	2.10 . 10 ¹³
3	200	1.81 . 10 ¹³
4	300	1.23 . 10 ¹¹
5	400	1.02 . 10 ¹⁰
6	500	8.6 . 10 ⁹
7	C (-)	3.65 . 10 ¹⁸

Based on phytochemical, FTIR and UV-VIS test results, *Plantago major* L. extract contain some compounds such as phenols, flavonoids, saponins and tannins which are capable to inhibit the growth of *A. hydrophila*. Phenol as antibacterial has a mechanism to inhibit bacterial growth by inactivating cell membrane proteins. Furthermore, phenol will bind to proteins in the structure of the bacterial cell wall to form hydrogen bonds [29]. Hydrogen bonds will make cell wall proteins and cytoplasmic membranes damaged so that there is an imbalance between the macromolecules and ions in the cell [30,31].

Flavonoid as antibacterial work in various ways, including inhibition of DNA gyrase, inhibition of the cytoplasmic membrane and energy metabolism [32]. Flavonoids can damage the permeability of bacterial cell walls by binding to cell wall proteins so that bacterial growth will be inhibited [33]. The other flavonoid activity as an antibacterial is by binding to proteins so that it affects permeability, then it will enter into bacterial cells resulting in coagulation of proteins and causing inactivation of bacterial enzymes [34].

Saponin works by influencing the bacterial

cell wall stress, this compound will bind to bacterial lipopolysaccharide which results in increased cell wall permeability and decreased surface tension of cell walls. Then saponin will enter the bacterial cell and disrupt the metabolism which causes the cell to lysis [36]. Tanin as an antibacterial will work by means of protein denaturation that prevents the metabolic process from being blocked. When the metabolic process is inhibited, growth and development of bacteria will be inhibited [36].

The antibacterial mechanism between one compound and another combined together will work synergistically and be more effective in fighting bacteria [37]. On the other hand, chloramphenicol was used as a positive control in this study because it is one of the antibiotics used to control the growth of *A. hydrophila* bacteria. Chloramphenicol is known to be able to inhibit the protein synthesis of *A. hydrophila* by binding to ribosome subunits so that peptide bond formation occurs [33,38].

CONCLUSION

Plantago major L. was known to have polar compounds such as phenol, flavonoid, saponin, and tannin. Phenol and flavonoid compounds contained in *Plantago major* L. are strengthened by wave detection from FTIR and UV-VIS results. *Plantago major* L. extract is known to have a significant effect on the inhibition of the growth of *A. hydrophila* bacteria significantly. In line with the result of this study, it can be suggested that further research on the antibacterial activity the plant extract with more solvent with a wider range of dosage.

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