Research Journal of Life Science
AUGUST 2021 Volume 8 No. 2 (103-111)
journal homepage: www.rjls.ub.ac.id

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

Annisa Farhana Dewi

Faculty of Fisheries and Marine Sciences, University of Brawijaya

Email Address : annisafarhana3@gmail.com

KEYWORDS

Antibacterial;
Extraction;
FTIR;
UV-VIS;
Co-culture.

Abstract *Aeromonas hydrophila* is gram-negative bacteria that can harm humans and major animals with poikilotherm properties such as fish and shrimp. These bacteria can cause hemorrhages in fish until death, causing fish farming production to fail. 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. This study aims to find out the compounds contained in *Plantago major* L., using phytochemicals screening, FTIR, UV-VIS and antibacterial activity against A. *hydrophila*. This study extracts *Plantago major* L. known to contain polar compounds such as phenols, flavonoids, saponins, and tannins. The results of FTIR and UV-VIS strengthen the presence of phenols and flavonoids. Furthermore, extract *Plantago major* L. is known to be able to significantly inhibit the growth of A. *hydrophila* ($P<0.05$).

Introduction

Aeromonas hydrophila is a widespread type of bacteria in both fresh and brackish water environments. These bacteria can infect various animals especially poikilotherm animals, such as fish and shrimp. It is the major causes of gastroenteritis and could cause skin infections and sepsis in the human body (Lowry *et al.*, 2014; Tahoun *et al.*, 2016; O'Ryan and Lucero, 2018). A. *hydrophila*, an agent of Motile Aeromonas Disease or MAS is present as secondary pathogens in fish 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 (Triyaningsih *et al.,* 2014; Peatman *et al.*, 2017; Shahi *et al.*, 2018).

The use of medicinal plants has been known and trusted by 80% of the community. *Plantago major* L. is a well-distributed plant over the World and known as a 'weed' that live freely in nature (Haddadian *et al.,* 2014). In Indonesia, 28% of *Plantago major* L. is found growing wild on the roadside and used as a wound medicine (Nahlunnisa *et al.,* 2015), have the ability to cure white discharge problems (Yamin *et al.*, 2018), inflammation and coughing (Kainde *et al.,* 2016). This weed was also reported to have several activities such as antibacterial, antifungal, antiviral, antioxidant and analgesic (Shirley *et al.*, 2015). Karima *et al.,* (2015) affirmed the benefits of *Plantago* major L. in the field of health because of several compounds such as polyphenols, alkaloids, tannins, and steroids.

As an antibacterial, *Plantago major* L. extract inhibited gram-positive and gram-negative bacteria such as *Lactobacillus* sp., *S. aureus, E. coli, P. aeruginosa, K. pneumonia, Proteus* sp., S. *enteriditis* (Metİner *et al.,* 2012; Razik *et al.,* 2012). However, there is no report on the ability of *Plantago major* L. to inhibit the growth of A. *hydrophila*. Therefore, this study

How to cite this article : Dewi, A. F. (2021). Phytochemicals and The Ability of *Plantago major* Linn. Extract to Inhibit The growth of *Aeromonas hydrophila* Research Journal of Life Science, 8(2), 103-111. https://doi.org/10.21776/ub.rjls.2021.008.02.5

aimed to determine the phytochemical content of *Plantago major* L. and its ability to inhibit the growth of A. *hydrophila*.

Materials and methods

Phytochemical Test and Extraction

Plants of *Plantago major* L. aged 3–4 months were obtained from Materia Medica Batu, dried for 5 days, and made into powder. The phytochemical test was carried out by referring to Adhayanti *et al*. (2018) method to observe the content of phenols, flavonoids, alkaloids, tannins, saponin terpenoids (steroids and triterpenoids). Extraction of *Plantago major* L. was carried out according to Putra *et al.* (2014), with modification. *Plantago majo*r L. powder 200 g macerated with 1L of ethanol PA (5:1) for 5 days. The results of maceration were filtered using Whatman No. paper. 42 and evaporated using a rotary vacuum evaporator at 50°C, 65 rpm to obtain a thick green extract.

FTIR (Fourier TransformInfra-Red) Analysis

FTIR is used to determine the functional group or type of the active compound based on the peak value of the wavelength. FTIR testing was conducted referring to Jain *et al.*, (2016). Extract was mixed with Kbr in a mortar and pressed with a pressure of 6 bars for 2 minutes. Samples were scanned on an spectrometer IR 4000 - 400 cm^{-1}.

UV-VIS Analysis

UV-VIS analysis was carried out with reference to Jain *et al.*, (2016), using UV-Visible spectrophotometry. 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, University of Brawijaya, from November to December 2018. Bacteria A. *hydrophila* was obtained from BBPBAT Jepara, Central Java. A. *hydrophila* was rejuvenated with TSA media and re-cultured on TSB media with a bacterial density of $10^7 CFU.mL^{-1}$. Antibacterial activity tests were carried out using a disc diffusion method (Sambuaga *et al.,* 2018). Blank disk with a size of 6 mm is inserted 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 control and Chloramphenicol as a positive control. All the treatments then incubated at the incubator 37°C for 1 x 24 hours. The diameter of the inhibition zone is measured using a caliper. Furthermore, co-culture tests were used to strengthen the results of antibacterial activity by referring to the method of Aamer *et al.,* (2015) where bacteria and extracts are poured in a tube containing sterile TSB media, then incubated for 24 hours. The incubation results were planted in petri dishes and bacterial density was calculated after 24 hours.

Result and Discussion

Phytochemical Test and Extraction

Phytochemical test results showed that *Plantago major* L. contained phenols, flavonoids, saponins, and tannins compounds. On the other hand, there were no alkaloid and terpenoid compounds (Table 1). Phenols, flavonoids, saponins, and tannins are included in polar compounds which have various functions, one of which is an antibacterial activity (Nugraha *et al.,* 2017).

E-ISSN 2355-9926

Table 1. Phytochemical test result of Plantago major L.

No.	Compound	Result
1.	*Phenol*	+
2.	*Flavonoid*	+
3.	*Alkaloid (Dagendroff, Mayer, Bouchardat)*	–
4.	*Saponin*	+
5.	*Tannin*	+
6.	*Terpenoid*	
	**Steroid*	–
	**Triterpenoid*	–

FTIR (Fourier Transform Infra Red) Analysis

The FTIR results of Plantago major L. and the absorption peaks are presented in (Figure 1, Table 2).

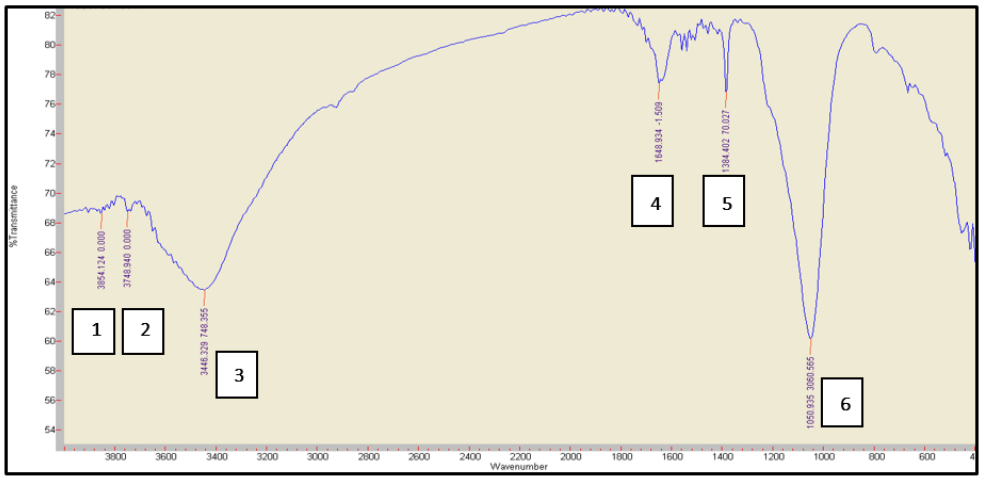

Figure 1. FTIR Test Results of *Plantago major* L.

Table 2. 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

E-ISSN 2355-9926

From the curve above, 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 flavonoids compounds (Kiswandono *et al.*, 2015; Zirconia *et al.,* 2015; Juliani *et al.*, 2016; Nugraha *et al.,* 2017). The presence of phenol and flavonoid compounds in *Plantago major* L. has been demonstrated (Samuelsen, 2000; Effat *et al.*, 2008; Haddadian *et al.,* 2014).

UV-VIS Analysis

UV-Vis results of *Plantago major* L. ethanol extract presented in (Figure 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 by Adom *et al.*, (2017), that flavones were the main type of flavonoids in *Plantago major* L. Kawashty *et al.*, (1994) states that there are several flavones in *Plantago major* L. but only one flavonol was detected in UV-spectophotometry.

Antibacterial Activity

The results of the antibacterial activity test showed that *Plantago major* L. had the ability to inhibit bacterial growth (Figure 3.). The inhibition of bacterial growth based on the extract dose. The higher dose, the greater diameter of the inhibition zone are formed. At the dose of 100 and 200 mg.L^{1-} an inhibition zone are 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 (Erlyn, 2016), 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.

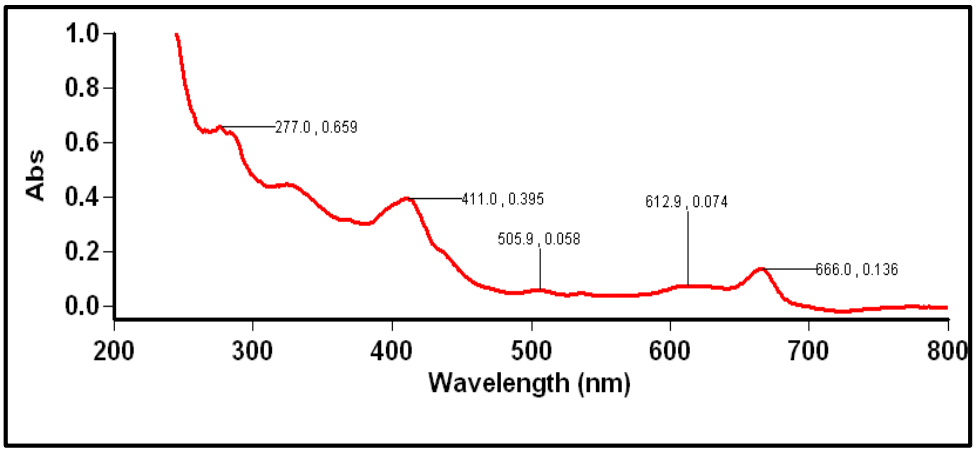

Figure 2. UV-VIS Test Result of Plantago major L.

E-ISSN 2355-9926

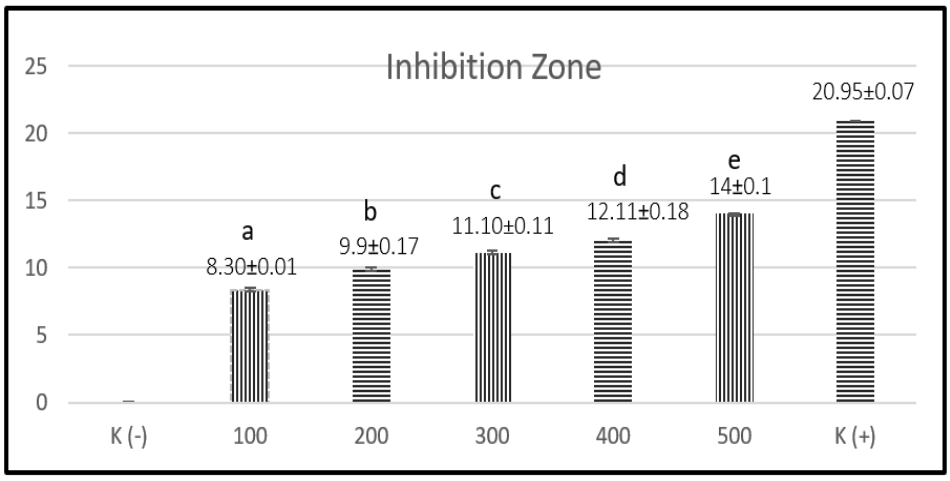

Figure 3. Antibacterial Test Results of Plantago major L. Extract

The inhibition zone diameter measurement results are proven by the co-culture test presented in (Table 3.). The co-culture test explained that there was a decrease in the number of bacteria and 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	500	$8.6 . 10^{9}$
3	400	$1.02 . 10^{10}$
4	300	$1.23 . 10^{11}$
5	200	$1.81 . 10^{13}$
6	100	$2.10 . 10^{13}$
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 capable of inhibiting 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 bacterial cell wall structure to form hydrogen bonds (Novita, 2016). Hydrogen bonds will damage cell wall proteins and cytoplasmic membranes, resulting in an imbalance between the macromolecules and ions in the cell (Bontjura *et al.,* 2015 ; Hariati *et al.*, 2018).

Flavonoid as antibacterial work in various ways, including inhibition of DNA gyrase, inhibition of the cytoplasmic membrane and energy metabolism (Suteja *et al.,* 2016). Flavonoids can damage the permeability of bacterial cell walls by binding to cell wall proteins so that bacterial growth will be inhibited (Putri *et al.,* 2016). 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 (Nirwana and Susilowati, 2017).

E-ISSN 2355-9926

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 (Dwicahyani *et al.,* 2018). 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 (Karmila *et al.,* 2017).

The antibacterial mechanism between one compound and another combined together will work synergistically and be more effective in fighting bacteria (Rempe *et al.*, 2017). 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 (Dian *et al.,* 2015; Putri *et al.,* 2016).

Conclusion and Suggestion

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 the further research on antibacterial activity Plantago major L. with more solvent with wide range of dosage.

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