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Potential Use of Saga Leaf Extract (*Abrus precatorius*) as Anti Bacteria *Aeromonas hydrophila by* in Vitro

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Abstract: Many diseases are caused by bacterial pathogens, for example, *Aeromonas hydrophila*. One alternative that can be done to overcome this problem is to use saga leaves (*Abrus precatorius*) which have bioactive ingredients as antibacterial ingredients. The aims of this study were to determine the antibacterial activity of saga (*A. precatorius*) leaf extract, to study the character and structure of bacteria inhibited by saga (*A. precatorius*) leaf extract and to study the bioactive components contained in saga (*A. precatorius*) leaf extract. The results of the disc test showed that the average inhibition zone was 14,29 mm while the MIC test results showed that at the dose 1 mg/L capable of inhibiting bacterial growth (bacteriostatic) and dose 10 mg/L It has been able to kill bacteria (bactericidal). Crude extract of saga leaf extract (*A. precatorius*) affects the character and structure of *A. hydrophila* bacteria by damaging the cell walls and cell membranes of bacteria. The results of Infrared Spectrophotometry analysis of saga (*A. precatorius*) leaf extract and Ultra Violet Spectrophotometry analysis with 96% absorption ethanol were suspected to contain flavonoid compounds.

Keywords: Antibacterial activity; *Abrus precatorius*; *Aeromonas hydrophila*

Introduction

Indonesia is a maritime country because most of it consists of waters. The vastness of Indonesian waters has great potential in the development of aquaculture both sea water and fresh water. One of the main obstacles in the sustainability of aquaculture production is death caused by infection with pathogenic microorganisms, one of which is the bacterium *A. hydrophila*. Excessive use of chemical drugs or antibiotics can lead to disease resistance. Herbal medicine is an alternative that can be used and does not cause negative effects for cultivators. Therefore, an alternative disease control method is needed that is effective and does not cause negative effects for cultivators and consumers, and is environmentally friendly, one of which is saga (*A. precatorius*) leaves.

Saga (*A. precatorius* Linn.) including medicinal plants from the Leguminosae family. These vines require other stems as a host for vines. Reaching 6-9 m in length with a trunk diameter of up to 1.5 cm twisting to the left. Compound leaves with 8-15 pairs of ovoid leaves, rounded base, flat edge, glabrous upper surface and hairy lower surface. Inflorescences appear in the axils or at the tips of the leaves 5-7 flowers per bunch, light purple in color with a butterfly shape. Pods, oval, slightly flattened, 2.5 x 1.2 cm. The oval seeds are slightly flattened, shiny red with black at the base of the seeds, 6-8 mm long (Wahyuni et al., 2013).

The saga plant (*A. precatorius*) contains a number of important ingredients such as alkaloids, flavonoids, tannins, triterpenoids, proteins, etc., among which are toxalbumin, abrin, which are thought to play an important role against poisons (Majumdar et al., 2014). It is known that the leaves, stems and roots of *A. precatorius* have been used by humans and animals as antimicrobials (including *Mycobacteria tuberculosis*), antiprotozoal drugs, insecticides and to treat poisonous snake venom. Several groups of secondary metabolites such as alkaloids, triterpenoids, isofluranoquinones,

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anthocyanins, starch, tannins, flavonoids, orientin are proven to also be contained in this plant. Some of these compounds have potential as medicinal properties derived from plants.

Method

Location and Time of Research

The research was conducted at the Disease and Fish Health Laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, in November 2022.

Extraction

The process of making the crude extract of saga leaves (*A. precatorius*) is done by taking 1 kg of saga leaves, then drying them for 2 weeks with just letting them air out. After that, the dried saga leaves were crushed with a blender to become powder. Saga leaf extraction procedure (*A. precatorius*) according to Pramiastuti et al. (2020), namely using a ratio of 1:10 by weighing 200 grams of finely ground sample dissolved in 2 L of ethanol for 3 days (Nisak, 2021). According to Tabasum et al. (2018), the filtrate obtained from the two solvents was evaporated inrotary evaporator under reduced pressure, vacuum dried and packaged in airtight containers, labeled and stored in the refrigerator (2-4°C) until required for experimental purposes.

Disc Test

The disc test was carried out with the aim of testing antimicrobials by measuring the diameter of the inhibition area that occurs around the disc paper which already contains antimicrobial material and compared with the antibiotic chloramphenicol. The crude concentration of saga leaf extract was prepared for the disc test with several treatments used namely 50, 100, 150, 200 and 250 mg/L. For the positive control treatment the disc paper was soaked in antibiotics, while the negative treatment was disc paper soaked without saga leaf extract. Bacteria *A. hydrophila* was taken 100 microlit and put in a petri dish containing TSA media and then flattened with a triangle. Sterile paper discs were soaked in the crude extract of sage leaves for 15 minutes based on a predetermined treatment dose. Disc paper that has been soaked in crude saga extract is drained and placed on the surface of the agar plate. The results were read after incubation at room temperature 300°C for 18-48 hours by measuring the diameter of the inhibition zone formed around the disc paper using a vernier caliper.

Test Minimum Inhibitiry Concentration (MIC)

Bacterial inhibition test was carried out by determining MIC (Minimum Inhibition Concentration) and Minimum Bactericidal Concentration. Add 1 µl of A. *hydrophila* bacterial suspension based on optical density into the treatment and control test tubes, then vortex until homogeneous. All tubes were incubated at 31°C for 24 hours. After 24 hours, the turbidity was observed and recorded in all tubes with a spectrophotometer. The first time the MBC test stage was carried out by inoculating the bacteria in all the liquid seed used for MIC. Dilution with 0.9% NaFis was performed to facilitate colony counting. The last 3 dilutions made are the concentrations to be planted for the MBC test. Furthermore, the cultivation of bacteria was carried out using the pour plate method on PCA media with 0.1 ml of bacterial suspension from each dilution tube obtained. Then incubated for 24 hours at 31°C. The media that has been incubated is then counted for the number of colonies in the colony counter. The MBC value was determined from the lowest concentration of the extract which showed no growth of bacterial colonies in the petri dish.

Observation with Electron Microscope (SEM)

Damage analysis of *A. hydrophila* bacteria was carried out by comparing SEM (Scanning Electron Microscope) observation photos between normal conditions and bacteria that were given sage leaf extract (A. *precatorius*) and looking at the damage to the bacterial cell wall. Based on the results of the damage analysis will be continued in the next stage.

Result and Discussion

Disc Test

The inhibition or clear zone was indicated by the absence of *A. hydrophila* bacteria around the paper discs that had been soaked with saga leaf extract at different doses. This clear zone appears because the extract has antibacterial compounds that can inhibit bacterial growth. In the results of preliminary research at a dose of 50 ppm, sage leaf extract has been able to demonstrate its ability as an antibacterial by showing clear zones that are starting to appear.

The concentrations used in the disc test were 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L and 250 mg/L. While the extract used is extract *A. precatorius*. Measurements of the clear zone around the disc paper were observed at 24 and 48 hours after incubation. The results of the clear zone measurements around the disc paper are as presented in Table 1.

Saga leaves (A. *precatorius*) contain alkaloids, flavonoids, saponins and steroids (Pramiastuti et al., 2020). This antibacterial compound must be effective in controlling the growth of bacteria and the problem of resistance to the materials used, especially bacteria that are harmful to organisms. Therefore, the characteristics

of the effectiveness of an antibacterial substance are the formation of broad inhibition zones and the absence of bacterial growth (Singkoh, 2011).

The active component compounds contained in plant extracts can cause an inhibitory effect on the growth of microorganisms. According to Sunday et al. (2016), stated that the leaves, stems and roots of *A. precatorius* have been used by humans and animals as antimicrobials (including *Mycobacteria tuberculosis*), insecticides, antiprotozoa drugs and treating poisonous snake venom. Several groups of secondary metabolites such as alkaloids, triterpenoids, isofluranoquinones, anthocyanins, starch, tannins, flavonoids, orientin are proven to also be contained in this plant. Some of these compounds have potential as medicinal properties derived from plants.

Table 1. The Results of the Disc Extract Test A. *precatorius*

Concentration (mg/L)	Average Inhibition Zone Diameter (mm)		Classification of Response Zones of Inhibition
	24 jam	48 jam	
Control -	0.00±0.00^{a}	0.00±0.00^{a}	Weak
100 mg/L	13.66±0.08^{b}	13.79±0.08^{b}	Strong
200 mg/L	13.83±0.11^{b}	13.93±0.12^{b}	Strong
300 mg/L	14.06±0.08^{c}	14.08±0.08^{b}	Strong
400 mg/L	14.33±0.17^{d}	14.44±0.19^{b}	Strong
500 mg/L	14.18±0.07^{cd}	14.29±0.05^{b}	Strong
Control +	23.03±0.14^{e}	23.76±0.90^{c}	Very Strong

Saga leaves contain flavonoids, alkaloids and polyphenols. Flavonoids are present in all vascular plants but some classes are more widespread than others. The *A. precatorius* plant has different levels of phytochemicals in each part of the plant, the amounts are presented in Table 1. Flavonoids contain conjugated aromatic systems and therefore show strong absorption bands in the UV and visible spectra. Flavonoids are generally found in plants, bound to sugar as glycosides and flavonoid aglycones contained in one plant in several forms of glycoside combinations. Flavonoid compounds are the main ones that dissolve in water (Putri et al., 2015). The content of flavonoids in *A. precatorius* leaves is influenced by the region and the time of harvesting. The area where the *A. precatorius* plant habitat is the main factor influencing the decrease in the flavonoid content in *A. precatorius* leaves. In addition, the active components in *A. precatorius* leaves that grow for more than one year are not positively related to the year of plant growth, which may be related to the catalysis and decomposition of photosynthetic-related enzymes (He et al., 2022).

The MIC Test Results

The MIC test results were measured from the absorbance value with a spectrophotometer. The absorbance value indicates the ability of saga leaf extract (*A. precatorius*) in inhibiting the growth of bacteria *A. hydrophila.* The results of measuring the absorbance values are presented in Table 2.

Based on the data in table 2, it shows that saga leaf extract (A. *precatorius*) with ethanol solvent at a concentration of 100 mg/L has the lowest absorbance value with a value of 0.704 which is close to the positive control. Data at all concentrations showed that the level of absorbance decreased with increasing concentration of the extract given at each dose given.

Table 2. MIC Test Results

Concentration (mg/L)	Absorbance Value
Control (-)	0.986
1 mg/L	0.959
10 mg/L	0.911
100 mg/L	0.704
500 mg/L	0.764
1000 mg/L	0.856
Control (+)	0.054

Note: Negative Control (C+) using chloramphenicol 5 mg/L, Negative Control (C-) without treatment, only bacteria

OD measurement (Optical Density) using a spectrophotometer by looking at the absorbance value. The spectrophotometer works by passing light with a certain wavelength according to the type of atom on a glass object called a cuvette. Some of the light will be absorbed and the rest will be missed. The absorbance value of the light that is passed is proportional to the concentration of the OD solution in the cuvette. This absorbance application is used to analyze the content of certain materials. The main advantage of the spectrophotometric method is that it provides a simple way of determining very small quantities of a substance (Seniati et al., 2019).

FTIR Test

Crude extract of saga leaves (*A. precatorius*) analyzed using FTIR intends to determine the content of compounds contained in the extract. In addition, FTIR is used to see the absorption values obtained as well as the functional groups displayed in the Figure 1.

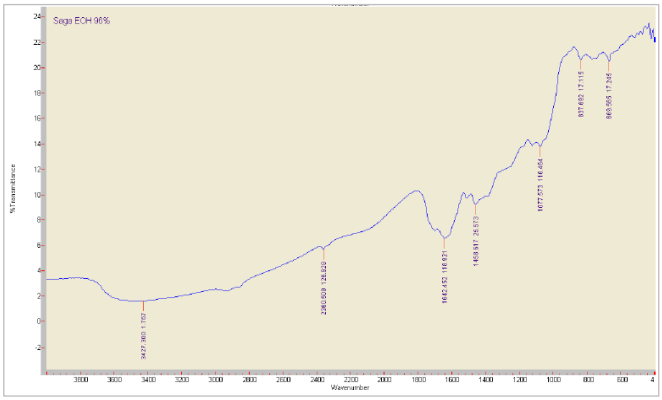

Figure 1. The results of FTIR of *Abrus precatorius*

The results of FTIR spectrophotometer observations found that there was an alcohol -OH group at wave number 3427. The absorption that appeared at wave number 1642 was a C=C alkene bond. Whereas the number 1458 is an aromatic C=C group. The absorption at number 1077 is the C-O alcohol group. At wave numbers 837 and 669 it is suspected to be C-H aromatic. Therefore, the results of the IR spectrum of saga leaf extract (*A. precatorius*) indicates the presence of flavonoid compounds.

The results of phytochemical screening of saga leaf extract (*A.* precatorius) which showed positive results for phenols and their derivatives (such as flavonoids and tannins), proved that saga leaves (*A.* precatorius) contain phenolic compounds. Phenolic compounds contained in woody plants such as saga can inhibit bacterial growth by breaking down cell membranes and peptidoglycan content in bacteria (Suhartini, 2017). The antibacterial activity of phenolic compounds is the cause of cytoplasmic leakage. The hydroxyl group in phenolic compounds interferes with the active sites of bacterial enzymes (Janakat et al., 2015).

Flavonoid compounds as derivatives of phenolic compounds contained in the roots and leaves of saga (*A.* precatorius) also have antibacterial activity, the first is associated with the partition of more non-polar compounds in the interior hydrophobic membrane, while the second includes the formation of bonds between polar lipid groups and more hydrophilic flavonoids at the interfacial membrane (Górniak et al., 2019).

UV-Vis Test

Based on data from the UV-Vis spectrophotometer test results of extract *A. precatorius*. The dominant compound comes from the flavonoid group. Determination of flavonoid levels in a plant sample using a UV-Vis spectrophotometer is one method by utilizing the interaction of light with atoms and molecules. The incident light that hits the surface of the substance and the light after passing through the substance cannot be measured, what can be measured is the ratio of the intensity of the incident light to the intensity of the light after passing through the sample (Purnamasari et al., 2022).

This flavonoid isolate showed the presence of functional groups O-H, C=O, C=C, C-H, C-OH, and C-O indicating that this isolate positively contained flavonoid compounds. Flavonoids are a group of natural substances with the main constituent structure being phenolic. These components have beneficial effects on health, which are related to anti-oxidative, anti-inflammatory, antimutagenic and anti-carcinogenic properties so that they have the potential to increase the immune system in the body (Yulia et al., 2022).

However, if the solution has reached an equilibrium point, there will be a decrease in the absorbance value. In addition, the longer the maceration time after reaching equilibrium, the more water is absorbed so that the number of water molecules as a solvent decreases, resulting in less extract being produced (Wiraningtyas et al., 2019).

Scanning Electron Microscope Test

SEM test results showed that there was an effect of treatment with saga leaf extract (*A. precatorius*) on the bacterial cell structure presented in Figure 2.

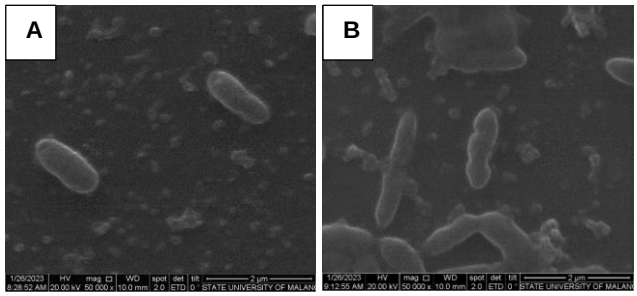

Figure 2. Morphology of *A. hydrophila* bacteria after SEM test; (A): without treatment, (B) administration of saga leaf extract (*A. precatorius*)

Figure 2 (A) shows that the morphology of bacteria *A. hydrophila* in good condition without damage to the cell wall, while image 2 (B) shows that the morphological picture of bacteria *A. hydrophila* which experienced lysis due to cell wall instability due to the influence of *A. precatorius* there by disrupting bacterial metabolism. The content of saga leaf extract (*A. precatorius*) in the form of active antibacterial compounds such as flavonoids, alkaloids, tannins and terpenoids are thought to be factors in the morphological damage of bacteria *A. hydrophila*.

In cell of *A. hydrophila* exposed to the extract, there was an elongation of cell size when compared to normal cells. The presence of cell size elongation indicates the damage caused by exposure to the extract. In addition to

cell enlargement, Figure 2 (B) also shows swelling or swelling of *A. hydrophila* cells exposed to extracts. Cell swelling was also found by Ultee et al. (2002) on the exposure of carvacrol and cymene compounds to *Bacillus cereus*, which occurs due to the accumulation of antibacterial compounds in the cell, causing the cell to enlarge, followed by leakage and cell death. In hypertonic conditions, cell dining damage causes the formation of spheroplasts in gram-negative bacterial cells which cause cell swelling, so that the cell membrane will be pushed towards the cell wall causing the cell to rupture (Gangga et al., 2007).

In hypertonic conditions, damage to the cell wall causes the formation of spheroplasts in gram-negative bacterial cells which cause cell swelling, so that the cell membrane will be pushed towards the cell wall which results in cell rupture (Asriani et al., 2007; Jawetz et al., 2001). Figure 2 (B) shows the formation of holes on the surface of the cell wall indicating damage.

Some antibacterial compounds are able to damage cell walls, one of which is by making holes (Nursidika et al., 2014). The same thing happened in Asriani et al. (2007), a perforated cell surface causes the permeability of the cell membrane to change so that cytoplasmic fluid can seep out which causes the cell wall to weaken. The release of fluid in the cells in large quantities will result in shrinkage and cell death.

According to Gilman et al. (1991), the process of damaging cell membranes is caused by the -OH hydroxy groups of phenolic compounds and their derivatives (flavonoids), so that phospholipid molecules break down into glycerol, carboxylic acids and phosphoric acids. This causes the phospholipids to be unable to maintain the shape of the cell membrane, and causes the membrane to leak and bacteria to experience growth inhibition and even death.

Based on the results of phytochemical tests, saga leaf extract (*A. precatorius*) contains flavonoids. Flavonoid compounds can damage bacterial cell membranes by forming complex compounds with extracellular and dissolved proteins, so that intracellular compounds in bacteria will come out. Flavonoids also disrupt the bacterial cytoplasmic membrane, inhibit nucleic acid synthesis and inhibit energy metabolism (Teng et al., 2023).

Conclusion

Based on the results of research on the effect of giving saga leaf extract (A. *precatorius*) against the inhibition of bacteria A. *hydrophila* regularly in vitro, it can be concluded that saga leaf extract (A. *precatorius*) effect on the inhibition of the growth of bacteria A. *hydrophila* and is bactericidal with the highest dose of inhibition, namely in treatment D (400 mg/L) which produced an inhibition zone of 14.44 mm and the lowest yield in treatment A (100 mg/L) which was 13.79 mm.

Author Contributions

Conceptualization, Kenitasari, R. E., Prajitno, A., Fadjar, M. and Gerrine, G.; methodology, Kenitasari, R. E and Gerrine, G..; validation, Prajitno, A. and Wilujeng, A.; formal analysis, Kenitasari, R. E.; investigation, Kenitasari, R.E. and Gerrine, G.; resources, Kenitasari, R. E., Prajitno, A., and Gerrine, G; data curation, Kenitasari, R. E.; writing—original draft preparation, Kenitasari, R. E.; writing—review and editing, Kenitasari, R. E.; visualization, Gerrine, G.; supervision, Prajitno, A. and Wilujeng, A.; project administration, Kenitasari, R. E., Prajitno, A., Wilujeng, A. and Gerrine, G.; funding acquisition, Kenitasari, R. E. and Prajitno, A.

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Conflicts of Interest

The authors declare no conflict of interest.

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