natural ingredients extracted from potentially pathogenic plants are needed to manage A. *hydrophila* infection. Within vitro studies looking at the zone of inhibition, this research aims to determine which solvent is most useful for use as an antibacterial and determine the antibacterial activity of black fruit seed extract against A. *hydrophila*.

Indonesia has rich plant biodiversity that can be used to treat a range of ailments. Traditional medicinal plants are unique to each area in Indonesia and are a national cultural heritage that must be protected. Traditional medicine plays a significant role in Indonesian public health services; as a result, it can be created [7]. West Papua is a province rich in indigenous knowledge and plants, including black fruit (Haplolobus*monticola*). Because of its economic value, this black fruit has the potential to be grown. Black fruit has been used as a natural preservative in food for decades. The flesh portion of the black fruit is commonly used by the locals because it is mixed with sago and then burned over a pit, increasing the shelf life[8].

Research on black fruit has been conducted by several researchers [9] who have stated that the results of research on black fruit raw leaf extract contain an alkaloid, flavonoid tannins compounds. The tannin content obtained in black fruit leaves ranged from 8.34 percent to 37.26 percent by the Titrimetric Lowenthal-Procter method. [10], [11] suggested the antioxidant potential of black fruit skin and seed extracts. Black fruit skin with ethanol solvent obtained a total phenol content of 8.7102 mg/100 gr with 3.8202% renamed extract. At the same time, Andaman extract used 2.0748 percent hexane with a total phenol content of 6.108 gr/100 gr. Toja. et al. 2020 found that black fruit seed compounds predicted their interactions with proteins that play a role in peptidoglycan synthesis. Peptidoglycan was selected based on an analysis of the potential of black fruit seeds, which showed higher yields as an inhibitor of Peptidoglycan glycosyltransferase.

The FTIR test can be used to find substances that have the ability to be antibacterial. FTIR spectroscopy is a technique used to qualitatively and quantitatively classify compounds, specifically organic compounds. The shape of the spectrum was examined, and unique peaks suggesting functional groups possessed by a compound were identified [12]. The absorption bands developed during FTIR identification of S. Grandiflora revealed that the n-hexane fraction contained flavonoids and terpenoids [13]. Investigating in vitro antioxidants and FTIR analysis of Mannar bay seaweed. It was found that the highest total phenolic content was observed in S. wightii (0.65 ± 0.02 mg GAE / g) when compared to U. Lactuca. The FITR spectrum of standard gallic acid was compared with seaweed, and the number of similar peaks located between 449.32 and 3 495.89 cm-1 and 462.89 and 3 407.05 cm-1 was recorded [14].

Various antibiotics, such as ampicillin, tetracyclines, and disinfectants, are often used by fish farmers to avoid disease. Antibiotics are commonly used to treat fish disease. Their use in aquaculture is costly, induces bioaccumulation, and has negative health effects for consumers, such as immunosuppression, residue accumulation in tissues, and the development of drug-resistant pathogens. It also kills fish and pollutes the water. Prevention and treatment, while ensuring biological protection, are the best ways to cope with disease problems. The ethyl acetate extract of black fruit seeds can effectively prevent Aeromonas *Hydrophila*.

Alkaloids, flavonoids, tannins, polyphenols, triterpenoids, and steroids present in black fruit seeds have been shown to inhibit bacterial growth in phytochemical studies. The local Papuan black fruit seeds are thought to have antioxidant and antibacterial properties, according to these phytochemical studies. There hasn't been any study on black fruit seed extract as a fish medicine until now. As a result, further research is needed to understand the effect of black fruit seed extract on tilapia infected with A. *Hydrophila* and how it can be used in the fishing industry, especially as an antibacterial agent for tilapia disease. With in vitro studies looking at the zone of inhibition and FTIR, this research aims to determine which solvent is most efficient for use as an antibacterial, as well as the antibacterial activity of black fruit seed extract against A. *Hydrophila*.

II MATERIAL AND METHOD

Preparation of Black Fruit Seeds (Haplolobus monticola)

Black fruit seeds were used as a source of material. The sample was prepared before extraction in order to achieve a Simplicia sample. The black fruit is removed from the pulp, cleaned of the skin, and thinly sliced to make smoothing with a blender easier. After cutting, it is dried in a 40oC oven until the moisture content of the dried black fruit seeds is approximately 12 percent bb, then mashed in a blender to make a powder, which is then sieved using a 40 mesh sieve to obtain black fruit seed powder.

Extraction

The maceration method was used to remove the samples, with ethyl acetate (3: 1 w/v) as the solvent. The pulp extract was separated after immersing 50 grams of black fruit seed powder in 200 mL of ethyl acetate in an Erlenmeyer tube with a screw cap and extracting the pulp extract. Following the completion of the maceration process, the filtrate is collected and concentrated using a rotary vacuum evaporator at a temperature of 40 ° C, yielding a thick extract of the black fruit seeds. This procedure takes 48 hours to complete. The more time spent extracting, the more parts are collected. [15].

Fractionation

Ethyl acetate extract in the form of a paste obtained from maceration, dissolved 5 grams in water solvent, then placed into a separating funnel and partitioned with n-hexane, ethyl acetate with a volume of 75 ml each, partitioned three times (Mogi et al. ., 2016). The funnel is then sealed and vigorously shook to combine the two phases of the solution. After that, the horn is turned over and the tap is opened to let go of the excess vapour pressure. The funnel is then set aside to allow the separation of the two phases to occur. The plug and funnel taps are then opened, and the separating funnel tap is used to isolate the two phases of the solution [16]. The antibacterial activity of the water fraction, n-hexane fraction, and ethyl acetate fraction was then measured at doses of 1000 ppm, 750 ppm, and 500, 250 ppm.

Antibacterial activity test

The disc approach was used to determine the antibacterial activity of Aeromonas *Hydrophila*. Prepare a clean petri dish first. Fill the petri dish halfway with ready-made media and set aside to cool. Then, using a triangle, uniformly spread two drops of bacteria from the liquid press on the surface of the Petri dish. The disc paper that has been treated with black fruit seed extract (H.monticola) is then placed on the media and gently pressed so that the portion is absorbed after 15-30 minutes. So, there you have it. The press was then incubated for 18-24 hours at 35 0C. Following inclusion, the presence or absence of a particular site that is formed and measured can be used to observe the inhibition region. To assess the type of H.momticola extract, if it is bacteriostatic or bactericidal, two 24-hour incubations can be performed. If the barrier area remains clear for two 24-hour incubations, it is bacteriocidal; if it is overgrown with bacteria within two 24-hour incubations, it is bacteriostatic.

FTIR Procedure: The FTS 1000 variant spectrophotometer system is used to observe and display specific and functional groups during the analysis process. The Fourier transform infrared spectroscopy (FT-IR) is a technique for identifying the functional groups in a compound. The chemical bonds visible in the annotated spectrum are differentiated by the wavelengths of the absorbed light. Using the chemical bonds in an undetermined molecule, the infrared absorption spectrum can be estimated. For FT-IR analysis, we used dried black fruit seed fraction. For a transparent preparation, 100 mg of dry extract is compressed into KBr pellets. A specimen of loaded black fruit seed samples was scanned with a scanning range of 400-4000 cm-1 and a resolution of 4 cm-1 using FT-IR spectroscopy.

III RESULTS AND DISCUSSION

Disc Method: The use of The wondama local black fruit seed extract here is thought to be novel since they have not been use previously fo used as a natural antibacterial in the prevention of Aeromonas *Hydrophila* disease.This section illustrates the results of our procedure. The antibacterial activity of the active compounds of black fruit seeds was tested in vitro using disk paper. The inhibitory test uses several solvents as a comparison to find the best solution for the most significant inhibition, including the water fraction, the n-hexane fraction, and the ethyl acetate fraction. Besides, the inhibitory test was performed using the best fraction previously tested with different concentrations, including 15.63 ppm, 31.25 ppm, 62.5 ppm, 125 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm, so that the best concentration value can be found to be applied to the in vivo test at a later stage. The results of the two types of tests are presented in Table 1. and Table 2.

The best fraction that produces a larger inhibition zone is ethyl acetate with an average value of 13.65 +0.06, based on Table 1. On the other hand, the water fraction gives an inhibition zone with an average value of 11.21 + 0.09. The n-hexane fraction is the weakest, with an average inhibition zone of 5.31 + 0.04. Based on these results, ethyl acetate was chosen as the fraction to be used in the next in a vitro test phase.

Table 2 shows the results of the best concentration test used in this study, where the inhibitory power activity began to appear at a concentration of 125 ppm with an average value of 5.16 + 0.15 and continued to show an increase in the inhibition zone along with an increase in the concentration tested, with an average inhibition zone of 9.25 +0.25 ppm at a concentration of 250 ppm. The highest concentration used in this study is 1000 ppm, which is shown to be inhibitory with an average area of 11.43 + 0.02.

The results obtained are then concluded with a comparison of the inhibitory capacity of the active compound against the bacterial activity. The inhibition zone formed after the incubation period is measured and recorded[17]. [18]classified the criteria for bacterial inhibition by suggesting that the inhibition zone diameter of 0 mm does not inhibit, 5-10 mm is weak, 11-20 mm is strong, > 22 mm is very strong.

The above classification shows that the inhibitory ability of black fruit seed extract against A. *hydrophila* bacteria at the highest concentration, i.e. 1000 ppm, shows intense activity. This is also supported by several opinions of [19], which state that the bacterial inhibitory response ≤ 5mm 6-10mm is weak, 11-20 mm is strong ≥ 21 mm is very strong. [20] classified the four reactions as <5 mm weak, 5-10 mm moderate, 10-19 mm strong, >20 mm very strong. With an inhibitory ability that continues to increase with increasing concentrations used, the raw extract of black fruit seeds may be a candidate for use at the next stage of the research, namely in vivo.

Minimum Inhibitory Concentration (MIC) Test Results: The Minimum Inhibitory Concentration (MIC) test was used to assess the lowest dose of ethyl acetate extract of black fruit seeds that were effective in destroying A. *hydrophila* bacteria. Using a spectrophotometer, the MIC test was observed by looking at the Optical Density, which is determined by the turbidity level of the liquid media. This test employs a variety of doses, ranging from 15.63 ppm to 1000 ppm, as well as positive (+) and negative (-) control tests. The following are the results of the MIC exam, as shown in Table 3.

According to the table above, the black fruit seed extract will inhibit the growth of A. *hydrophila* bacteria, with a decrease in the OD value with each increase in the number of doses measured, indicating a decrease in bacterial growth. The OD value is decreasing, indicating that the test media is becoming clearer and the spectrophotometer is absorbing fewer particles. Bacterial cells that grow on the test media make up the particles. However, at a dose of 500 ppm, the OD value started to drop dramatically, to 0.061 and continued to fall at a dose of 750 ppm, to 0.059, and a dose of 1000 ppm, to 0.035. This indicates that the antibacterial activity of black fruit seed extract will destroy A. *hydrophila* bacteria at a MIC dose of 500 ppm, while a dose less than 500 ppm can only inhibit A. *hydrophila* bacteria development.

The flavonoid compounds found in black fruit can inhibit the growth of bacteria by interfering with the permeability of the bacteria's cell walls. Flavonoids have antibacterial properties because flavonoids as derivatives of phenols can cause damage to the structure and change the bacterial cell wall's permeability mechanism. Besides, the flavonoid is a flavonol group, so that it has chemical properties of phenol compounds. The mechanism of action of flavonoids as antimicrobials can be divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism (Hendra et al., 2011). Damage to bacterial cell walls, changes in cell permeability, damage to bacterial nucleic acids, changes in protein molecules, and inhibition of nucleic acid synthesis processes can all prevent bacteria from reproducing. As A. *hydrophila* infects the host by releasing an enzyme that can break down the host's blood cells, the function of enzymes released by bacterial cells is inhibited [21], [22].

Bacterial cells are encased in a peptidoglycan cell wall, which is made up of a long sugar polymer. The peptidoglycan uses trans glycosidase to crosslink the glycan chains, and the peptide chain extends from the polymer's sugar and forms cross-links between peptides. Bacteria are classified into two groups based on their chemical composition: gram-positive bacteria and gram-negative bacteria [23]–[25]. cytoplasmic membrane is bound by a rigid and durable tissue called the cell wall in Gram-positive bacteria. Gram-negative bacteria, on the other hand, have a thin cell wall surrounded by a second lipid membrane known as the outer membrane (OM). OM is an extra layer of protection on gram-negative bacteria that prevents various substances from entering the cells. This membrane, however, contains porins, which enable molecules from outside the cell, such as drugs, to reach the cell [26].

In general, antibacterials work by damaging bacterial cell walls, which is the first step to inhibiting bacterial growth. The cell wall itself is the earliest defense possessed by bacteria; if the cell wall is damaged, the antibacterial compounds will more easily enter the cell and interfere with the cell organelles' function in it. The active compounds contained in black fruit seed extracts such as flavonoids, polyphenols, alkaloids, and terpenoids can damage the bacterial cell membrane by changing the phospholipids that make up the cell membrane into other forms such as phosphoric acid, carboxylic acid, and glycerol which cause changes in the shape of the cell membrane or the cell is unable to maintain its shape and maintain the organelle that will work in the process of bacterial cell growth so that in the end the bacterial cell becomes lysis and experiences death [27], [28]. One of the processes of the growth inhibition of A. *hydrophila* bacteria is by inhibiting its enzyme activity because these bacteria work to infect targets by transferring enzymes that can enter the blood cells, causing infection in the host.

The active compounds in Black Fruit Seed Extract were identified and characterized using Fourier Transform Infrared (FTIR): Fourier Transform Infrared (FTIR) Spectrophotometry can be used to determine the functional groups of a chemical compound. This test is needed to ensure that the chemical compounds in the black fruit seed extract (H. *monticola*) can be identified using the absorption expression of a specific wavelength number. The following are the FTIR test results of black fruit seed extract (H. *monticola*) with ethyl acetate solution, as shown in Figure 2.

The graph of the absorbance value of black fruit seed extract with ethyl acetate solvent at a wavelength of 3430 cm-1 shows the absorption band with the most detailed shape, which shows the strongest vibration of the hydroxylate (-OH) functional group. At absorbance wavelengths of 1707-1619 cm-1, the vibrations of the carboxylic acid functional group (C = C) are noticeable, while 1384 cm-1 indicates the presence of a methyl functional group (-CH3) and 1344 cm-1 indicates the functional group of the compound. Aromatic nitro (-C-NO2) indicates the presence of an alcohol functional group (CO) at a wavelength of 1213-1037 cm-1, an aromatic alkene functional group (CH) at a wavelength of 764-689 cm-1, and monobranched alkane functional group vibrations at a wavelength of 604-518 cm-1 (-CH3). The results of FTIR identification of the chemical compounds of black fruit seed extract contained in it are hydroxylates, carboxylic acids, methyls, aromatic nitrogen, alcohols, aromatic alkenes, monobranch alkanes.

IV Conclusion

These findings support the conclusion that the black fruit seed herbal extract tested was proven to inhibit the growth of A. *Hydrophila* in vitro. Of the three solvent fractions used, ethyl acetate produced a more significant inhibition zone with an average inhibition zone value of 13.65 +0.06. Test the concentration of 125 ppm, 250 ppm, 500 ppm, and 1000 ppm obtained a large inhibition zone at a concentration of 1000 ppm with an inhibition zone area of 11.43 + 0.02. The results of FTIR identification of the chemical compounds of black fruit seed extract contained in it are hydroxylates, carboxylic acids, methyls, aromatic nitrogen, alcohols, aromatic alkenes, monobranch alkanes.

AUTHOR’S CONTRIBUTION

YTT designed the research and wrote the manuscript., ES AA and UY reviewed the manuscript.

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Table 1. Inhibitory Test Results of Various Black Fruit Seed (H. *monticola*) Fractions

Fraction	**Inhibition Zone (mm)**			
	Repetition			Mean ± SD
	1	2	3	
Water	11.29	11.12	11.22	11.21±0.09
N-hexane	5.36	5.29	5.29	5.31±0.04
Ethyl Acetate	13.40	13.51	13.40	13.65± 0.06

Table 2. The Result of Inhibitory Test of Ethyl Acetate Fraction of Black Fruit Seeds (H. *monticola*)

Concentration (ppm)	**Inhibition Zone (mm)**			
	Repetition			Mean ± SD
	1	2	3	
1000	11.45	11.42	11.41	11.43± 0.02
750	11.16	11.29	11.22	11.22± 0.06
500	10.31	10.22	10.4	10.31± 0.09
250	9.19	9.31	9.24	9.25± 0.06
125	5.17	5.31	5.02	5.16± 0.15
62.5	-	-	-	-
31.25	-	-	-	-
15.63	-	-	-	-

Table 3. Results of the Minimmune Inhibitory Concentration (MIC) test of ethyl acetate extract of black fruit seeds (H. *monticola*) against A. *hydrophila*

Concentration (ppm)	**Od value**
K+	0.001
K-	0.275
1000	0.035
750	0.059
500	0.061
250	0.104
125	0.120
62.5	0.148
31.25	0.167
15.63	0.181

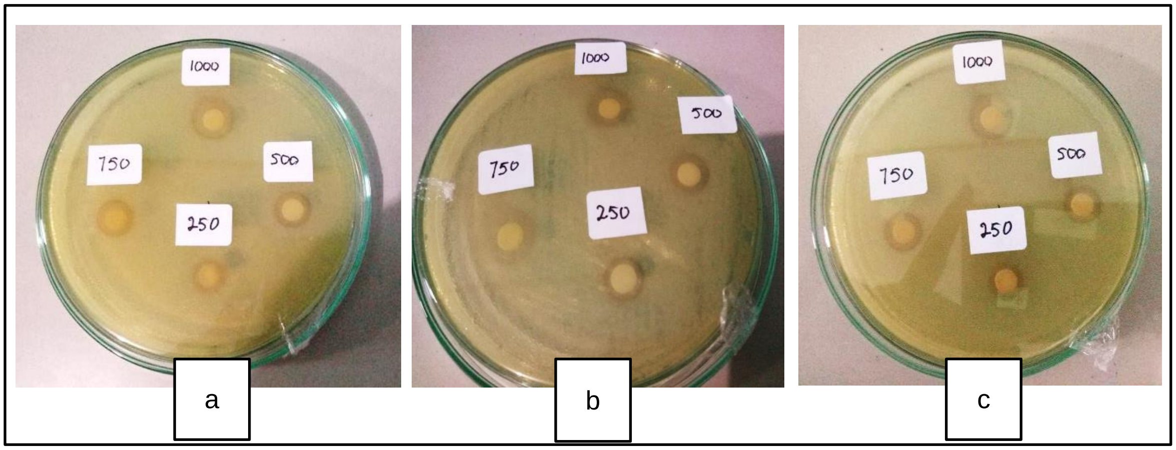

Figure 1. Inhibitory test of black fruit seed (H. *monticola*) extracts at doses of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. a) The inhibitory test of repetition 1, b) The inhibitory test of repetition 2, c) The inhibitory test of repetition 3

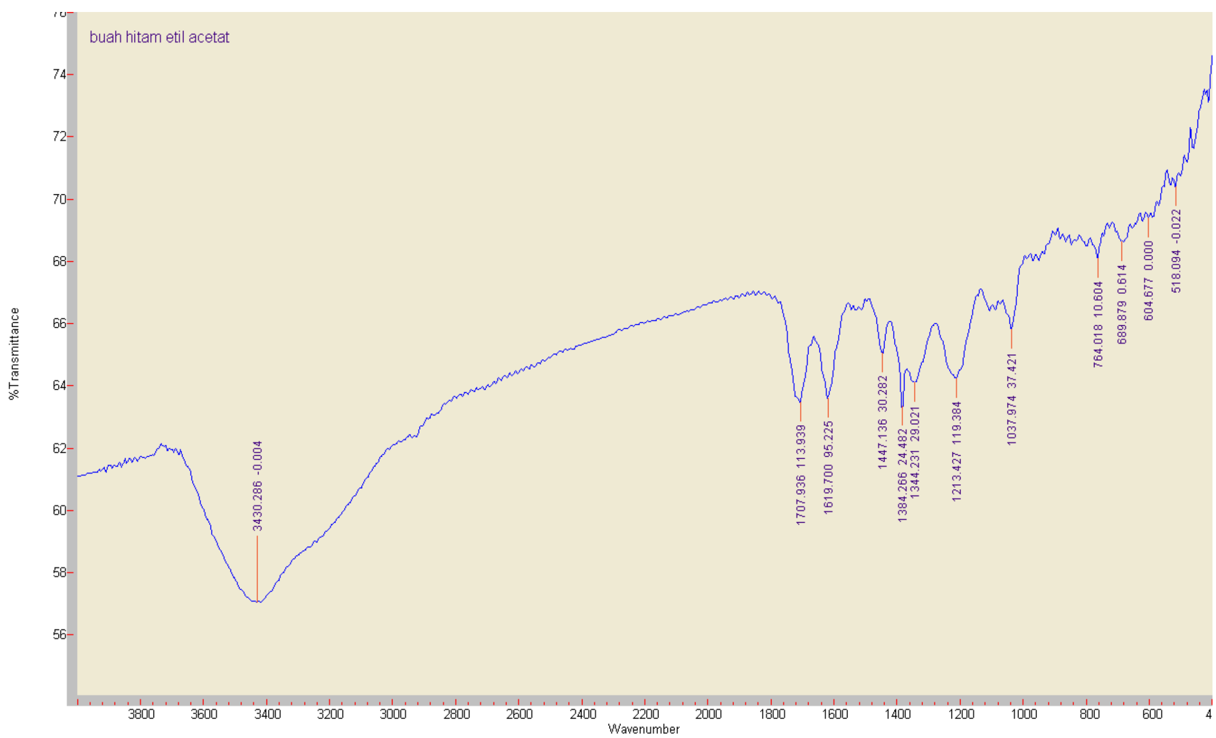

Figure 2. Infrared Wavelength Spectrum of Ethyl Acetate Extract of Black Fruit Seeds (H. *monticola*) 4000-500 cm-1