
Analysis of Metabolit And Antibacterial Control of Mengkudu Leaf Extract (*Morinda citrifolia*) on the Bacteria of *Aeromonas hydrophila*

Yora Utami Putri Pertiwi, Arief Prajitno, Mohamad Fadjar

Faculty of Fisheries and Marine Sciences, University of Brawijaya

Email Address : yorabp2011@gmail.com

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Abstract *A. hydrophila* is an anaerobic facultative bacterium, which is a bacterium that can develop in conditions with or without oxygen. Therefore, it is necessary to use alternative materials that can be used to eliminate *A. hydrophila*, one of which is to use herbal medicines. The purpose of this study was to determine the content of active compounds in mengkudu leaf extract (*M. citrifolia*) using the disc test method. To test the content of secondary metabolites, phytochemical testing and FTIR (Fourier Transform Infrared Spectroscopy) were carried out. The results obtained are 100 ppm which is the minimum concentration that can inhibit the growth of *A. hydrophila*. Phytochemical test results obtained crude extract of positive mengkudu leaves alkaloids, saponins, triterpemoid and flavonoids. And in the FTIR test the most dominant content of phenol compounds contained in Mengkudu leaf extract which has a very important function that can be used as an antibacterial in this study. Further research is expected to carry out more in-depth characterization testing of active compounds through several tests including using LCMS or GCMS to determine the active ingredient of mengkudu leaves.

Introduction

Constraints that affect cultivation systems include maintenance systems, nutrition, seed quality, pest and disease attacks. One of the obstacles in fish farming is disease attack. Among these diseases are infectious diseases caused by parasites, viruses, bacteria and fungi. One of the bacteria that often attacks freshwater fish is *A. hydrophila*. *A. hydrophila* is gram negative, positive oxidation and positive catalase (Isohood and Drake, 2002). One of the diseases feared by fish farmers is the red spot disease or Motile Aeromonas Septicemia (MAS) caused by *A. hydrophila* (Prajitno, 2005). Diseases that can be caused by *A. hydrophila* include: the appearance of red spots on the surface of the body, inflamed skin which eventually occurs ulcers such as boils, bleeding in the liver, bleeding fins, muscle bleeding, bleeding mucus in the rectum and the formation of bloody fluids (Prajitno, 2007).

This disease shows symptoms such as loss of appetite, injuries to the surface of the body, bleeding in the gills (pathological changes in the form of hemorrhages in the muscle tissue in the gill arc), enlarged fluid filled abdomen, loose scales, loose tail fins. If it is done surgically there will be swelling and damage to the liver, kidney and lymph tissue. This disease is caused by *A. hydrophila* (Post, 1987). Red spot disease or septicemia haemorrhagica is caused by *Aeromonas* sp. *aeromonas* bacteria including the Pseudomonadaceae family which consists of three main species, namely *A. punctata*, *A. hydrophila* and *A. liquiefacieus* which are pathogenic (Kordi and Ghufran, 2004). *A. hydrophila* is a pathogenic bacterium that causes disease in various types of freshwater fish. The characteristics of the fish affected by this disease are the color of the fish becomes darker or pale, the fish are alone, the movement of fish is not normal (swirling), there are

inflammatory patches on the skin, torn scales, bleeding inflammation in the mouth and internal organs (Prajitno, 2005). The attack can kill fish seeds with mortality rates reaching 80-100% within 1-2 weeks (Cipriano, 2001).

In controlling the disease can use chemicals, natural ingredients and antibiotics as an alternative to treatment. According to Satyantini *et al.* (2016) the continuity of the use of antibiotics/chemicals continuously with improper doses or concentrations raises new problems in the form of increasing resistance of microorganisms to these materials. Disease prevention in the cultivation system generally uses antibiotics. However, the use of antibiotics is now banned because it can cause a resistant effect on pathogenic bacteria and cause pollution to the environment. The use of antibiotics in consumption fish can leave a residue on the host's body, so it is not safe when consumed by humans, because it can cause an effect of resistance to bacteria that are infectious to humans. Therefore, another alternative treatment that is more environmentally friendly and does not have the effect of resistant to bacteria is needed. Another alternative that can be used is by utilizing herbal medicines (Kamaludin, 2011).

One of the natural ingredients that can be used in the treatment of fish attacked by bacteria is Mengkudu leaf (*M. citrifolia*). Mengkudu leaves have active ingredients antharakinone and proxeronin. Antharakinon functions as an anti-microbial and anti-fungal, while proxeronin is useful in cell rejuvenation. In addition, mengkudu leaves also contain catechin, crude fiber, protein, lime, carotene, whole amino acids and vitamin A. The active ingredients in mengkudu leaves can also function as anti-allergic as well as anti-bacterial (Nuraini, 2014). Mengkudu plants can be used as antibacterials starting from seeds, fruit and leaves. Some types of active compounds that can function as antibacterial agents are acubin,

alizarin, between quinone and flavonoids (Waha, 2001).

Mengkudu leaf (*M. citrifolia*) grows in the lowlands at altitudes below 1600 above sea level (asl), the height of the mengkudu tree ranges from 3-8 m (Pranata, 2013). Mengkudu plants can be used as antibacterials starting from seeds, fruit and leaves (Waha, 2001). Flavonoids prevent energy formation in the cytoplasmic membrane and inhibit bacterial motility, which also plays a role in antimicrobial actions (Widiastuti, 2008). In mengkudu leaves, there are active compounds that function as antibacterial substances (Sukandar *et al.*, 2008). Mengkudu plants contain antibacterial compounds, namely anthraquinone, alkaloids (Rukmana, 2002), flavonoids, acubin and alizarin (Bangun and Sarwono, 2002) that are able to fight pathogenic microorganisms. From some of the content of active compounds in mengkudu leaves, it can have the potential as an antibacterial.

Flavonoids prevent energy formation in the cytoplasmic membrane and inhibit bacterial motility which also plays a role in antimicrobial actions (Widiastuti, 2008). The mechanism of action of flavonoids as antibacterial is to form complex compounds with extracellular proteins and dissolve so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds (Bobbarala, 2012).

To overcome the disease caused by infection with *A. hydrophila*, the need to study the antibacterial produced by mengkudu leaves as an antibacterial agent is one of the test disk tests. To determine the content of active compounds in mengkudu leaves, phytochemical tests and FTIR tests were carried out. The purpose of this study was to determine the active compounds in mengkudu leaf extract (*M. citrifolia*) which are antibacterial. According to previous research, explaining the advantages of mengkudu leaves that can be used for antibacterial purposes, it is hoped that in the

future it can be used as an alternative in the treatment of *A. hydrophila* and contribute to aquaculture production in the world.

Material and Methods

Disc Test, Phytochemical Test and FTIR Test

The research was conducted at the Laboratory of Organic Chemistry at the Faculty of Science and Technology (Saintek) of Maulana Malik Ibrahim Malang State Islamic University, Laboratory of Fish Disease and Health and Environmental and Aquatic Biotech Laboratories at the Faculty of Fisheries and Marine Sciences (FPIK) from May to October 2018. One way to test antimicrobial material can be done with a disc test. Disc paper containing antimicrobial substances is placed on the agar plate which has been sown with testing microorganisms. The inhibition of the growth of microorganisms by antimicrobial substances is seen as a clear area around the growth of microorganisms (Lay, 1994).

In the Phytochemical Test Qualitative phytochemical tests were carried out by Harborne (1987) using plant coarse powder. Determination of the presence of alkaloids as many as 2 grams of sample powder were extracted with a small amount of chloroform, then added with 10 ml of chloroform-ammonia and filtered. The filtrate was added with a few drops of H²SO₄ 2M, then shaken to form two layers. The acid (colorless) layer is pipetted into another test tube, then the solution is divided into 3 then each solution is tested with a few drops of Dragendorf, Mayer, and Wagner reagents. This test is positive if the solutions produce orange (Dragendorf), yellowish white (Mayer), and brown (Wagner) deposits. Determination of the presence of flavonoids A total of 2 grams of sample was extracted with an absolute amount of methanol until the ingredients were all submerged. The mixture is boiled and then filtered. The filtrate is then added with 10% NaOH. The formation of red

color after the addition of 10% NaOH shows the presence of flavonoids. Determination of the presence of steroids / triterpenoids A total of 2 grams of sample powder is extracted with a number of absolute ethanol until it is completely submerged, then heated to boiling, and filtered. The filtrate is evaporated, then diethyl ether is added to the remaining deposits. In the diethyl ether fraction, Liebermann-Buchard was added (3 drops of anhydrous acetic acid + 1 drop of H₂SO₄). The steroid test is positive if a greenish color and a positive test for the triterpenoid are produced if a reddish or purple color is produced. Determination of the presence of phenol A total of 2 grams of sample powder was extracted with absolute methanol until completely submerged, then heated and filtered with filter paper. The filtrate was diluted with 10 ml of distilled water and then heated. After 5 ml of ether are cooled, leave it for a few minutes until the precipitate forms. The formed layer is taken and evaporated at 40°C. The part that does not evaporate is dissolved in 5 ml of ethyl acetate and then filtered. As much as 1 ml of the filtrate was evaporated to dryness, the remaining part was dissolved with 2 ml of 95% ethanol then added concentrated H₂SO₄ reagent. If a blackish green color is formed indicating the presence of phenol compounds.

FT-IR (Fourier Transform Infrared) spectrophotometer is a tool used for analysis based on the measurement of infrared intensity on the wave length and to detect the characteristics of the group vibration function of the compound in the sample. When infrared light interacts with a sample, molecules that are bound to each other in the sample will experience strain and experience bending (Kang *et al.*, 1998). Spectral results show the absorbance and transmission of molecules that described the molecular data recordings of these samples. There is no record of the same data for each different molecule so FT-IR spectroscopy can be used for various types of

analysis (Day and Underwood, 2001). Examples of FT-IR applications provide information such as determining the molecular structure of polymers, identifying covalent binding compounds, knowing the purity of materials, and functional groups of molecules (Suseno and Sofjan, 2008).

Results and Discussion

Disc Test

The results of the disc test study using various test concentrations obtained the results of the data in Table 1.

Table 1. Test results of mengkudu leaf extract discs on TSA media (Trypticase Soy Agar) during 24-hour observation. Positive Control (K +) Used Chloramphenicol 5 ppm antibiotics and Negative Control (K-) Without Treatment (Bacteria Only).

Concentration (ppm)	Methanol	Ethyl Acetate
Positive Control (K+)	32.58	32.58
Negative Control (K-)	0.00	0.00
1 ppm	0.00	0.00
10 ppm	0.00	0.00
100 ppm	8.08	6.42
500 ppm	10.41	8.95
1000 ppm	12.32	10.13

The graph of the diameter of the inhibition zone/clear zone obtained during the 24 hours observation can be seen in Figure 1 below:

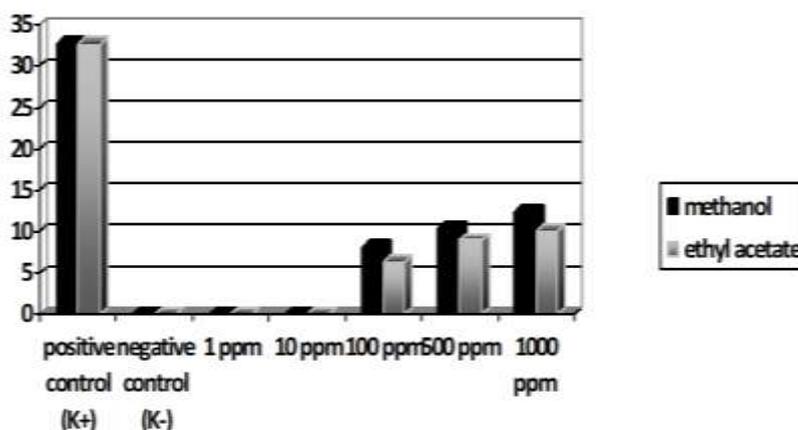


Figure 1. Graph of inhibition zone / clear zone in TSA (Trypticase Soy Agar) media tested for 24.

This research is an experimental test to determine the anti-bacterial effect of Mengkudu (*Morinda citrifolia*) leaf extract in inhibiting the growth of *A. hydrophila*. The results showed the inhibition of mengkudu leaf extract on the growth of *A. hydrophila* after an incubation process for 24 hours in an incubator at 37°C. This is stated by the appearance of bacterial inhibition zones around paper discs of mengkudu leaf extract.

This study used paper discs as an extract attachment medium. Based on this matter, this study took steps to minimize the bias by soaking five paper discs used in mengkudu leaf extract

simultaneously for thirty minutes then being removed simultaneously, then one paper disc soaked using Chlorampenicol antibiotics and one untreated paper disc (only given bacteria).

Disc test results showed that the concentration of 100 ppm can be said to inhibit bacterial growth, because there is a clear zone on TSA media. Then, when viewed from a concentration of 100 ppm to 1000 ppm, the clear zone obtained is also getting higher. According to Sommers (1994) the resistance zone formed as an area that does not show any bacterial growth around filter paper discs, or in other words is a clear zone around the paper disk. And at a concentration of 1000 ppm, the highest inhibition zone/clear zone is obtained. According to Haryani *et al.*, (2012), Flavonoids are the largest group of phenol compounds. Flavonoids and flavonols are synthesized by plants to respond to microbial infections. The mechanism of action of flavonoids in disrupting bacterial activity is by denaturing bacterial cell proteins and damaging the cytoplasmic membrane. This is supported by a statement (Doerge, 1972) that phenol can also function as a bacteriocidal substance.

Phytochemical Test

The results of mengkudu leaf extract (*M. citrifolia*) used methanol 96% as maceration solvent, were found to positively contain secondary metabolite content of flavonoids, saponins, alkaloids and triterpenoids through phytochemical test results. Data can be seen in Table 2.

The phytochemical test results showed that the active compounds contained in mengkudu leaves were flavonoids, saponins, alkaloids and triterpenoids (Table 2). This is in accordance with Diassanti 2011's statement that mengkudu leaves in mengkudu leaves have active compounds that function as antibacterial substances and contain saponins, flavonoids, polyphenols and triterpenes. Mengkudu plants contain various bioactive compounds including essential oils, saponins, triterpenoids, phenols, tannins, and glycosides (Rosman and Djauhariya, 2006).

From the phytochemical test that has been done, this shows that in Mengkudu leaf extract, among others, contains the active compounds of saponins, flavonoids, polyphenols, tannins, and triterpenes. Saponin comes from the Latin language Sapo which means soap, because it resembles soap. Saponins are triterpenoid and sterol glycosides (Robinson, 1995). The mechanism of action of saponins is included in the antibacterial group which disrupts the permeability of bacterial cell membranes which results in damage to cell membranes and causes the release of various important components from bacterial cells namely proteins, nucleic acids and nucleotides (Ganiswarna, 1995). Flavonoids are good reducing compounds, inhibiting many oxidation reactions, both enzymatic and non-enzyme. Flavonoids are the largest group of phenol compounds (Sjahid, 2008).

Table 2. Phytochemical Test of mengkudu Leaf Extract (*M. citrifolia*)

SECONDARY METABOLIT	OBSERVATION RESULT	RESULT (+/-)
ALKALOID	Orange Reaction (Dragendrof)	+
	The presence of yellowish deposits (Mayer)	+
FLAVONOID	Red Brick reaction	+
SAPONIN	There is a stable foam / foam	+
TANNIN	Green Color Reaction	-
TRITERPENOID	Brownish Color Reaction	++

The mechanism works by denaturing bacterial cell proteins and damaging cell membranes irreparably (Juliantina *et al.*, 2009). Polyphenols are compounds composed of many phenol compounds. Its mechanism of action is antibacterial by denaturing and coagulating proteins (Cowan, 1999). Tanin is one type of compound that belongs to the polyphenol group. This tannin compound is often found in plants. The mechanism of action of tannins is thought to be able to shrink the cell wall or cell membrane so that it disrupts the permeability of the cell itself. As a result of disruption of permeability, cells cannot carry out living activities so that their growth is hampered and dies (Ajizah, 2004). Triterpenoids are plant components that have an odor and can be isolated from vegetable materials by distillation as essential oils.

The triterpenoid mechanism as an antibacterial is reacting with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong polymeric bond that causes damage to the porin. Damage to the porin which is the entrance and exit of the compound will reduce the permeability of bacterial cell membranes which will cause bacterial cells to lack nutrients, so that bacterial growth is inhibited or dies (Rachmawati, 2009). Alkaloids are the largest group of secondary plant substances. The suspected mechanism is to disrupt the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death (Robinson, 1995).

Mengkudu plants can be used as antibacterials starting from seeds, fruit and leaves. Some types of active compounds that can function as antibacterial agents are acubin, alizarin, between quinone and flavonoids (Waha, 2001). The term Falvonoida is given for phenol compounds derived from the word flavone which is the name of one of the largest

types of flavonoida in plants. These flavone compounds have a 2-phenylchroman framework where the ortho position of the A ring and the karbon atom bound to ring B of 1,3-diarilpropane are connected by an oxygen bridge so as to form a new heterocyclic ring (Ring C). Flavonoid compounds consist of several types depending on the level of oxidation of the propane chain of the 1,3-diarilpropane system. Flavones, flavonols and anthocyanidins are types that are commonly found in nature, so they are often referred to as major flavonoids.

All flavonoid variants are interrelated because of the same biosynthetic pathway, which incorporates pre-substances from the cyclic grooves and acetate-malonate grooves. Flavonoids in plants are generally bound as glycosides, both O-glycosides and C-glycosides (Harborne, 1987). Flavonoids reported in *Passiflora edulis* Sims, are 6-C-C6-deo spiranosil J-3, 4, 5, 7-tetra hydroxiflavones or also called 6-chusnovosiluteolin. Phenol compounds and their derivatives (Flavonoids) are one of the antibacterial agents that work by disrupting the function of the cytoplasmic membrane. The presence of this phenol compound causes damage to the cytoplasmic membrane. H⁺ ions from phenol compounds and their derivatives (flavonoids) will attack polar groups (phosphate groups) so that the phospholipid molecules on the bacterial cell wall will break down into glycerol, carboxylic acid and phosphoric acid.

In such conditions, phospholipids are unable to maintain the shape of the cytoplasmic membrane as a result of which the cytoplasmic membrane will leak and the bacteria will experience barriers to growth and even death. Flavonoids prevent energy formation in the cytoplasmic membrane and inhibit bacterial motility which also plays a role in antimicrobial action (Widiastuti, 2008). Flavonoida compounds are the largest group of phenol compounds found in nature. These compounds

are red, purple and blue as well as yellow substances found in plants. Flavonoida has a carbon base framework consisting of 15 carbon atoms, where two benzene rings (C_6) are bound to a propane chain (C_3) to form a $C_6-C_3-C_6$ arrangement. The abundance of flavonoids is caused by various levels of hydroxylation, alkoxylation or glycosylation of these structures. Isoflavonoida and neoflavonoida compounds are only found in several plant species, especially the Leguminosae tribe.

Each type of flavonoida compound has a certain basic structure. Flavonoida has several characteristics, namely: Ring A of the structure of flavonoida has alternating oxygenation patterns which are at positions 2,4 and 6. Ring B flavonoida has one oxygen functional group in the position of the two or at the position of para and meta or three positions one in para and two in meta. Ring A always has a hydroxyl group that is located in such a way that it is possible to form a heterocyclic ring in a tricyclic compound. Some of the flavonoids are as follows:

Ring A – $COCH_2CH_2$ – Ring B	—————▶	Hidrokalon
Ring A – $COCH_2CHOH$ – Ring B	—————▶	Flavanon, kalon
Ring A – $COCH_2CO$ – Ring B	—————▶	Flavon
Ring A – CH_2COCO – Ring B	—————▶	Antosianin
Ring A – $COCOCH_2$ – Ring B	—————▶	Auron

From the observations in Table 2, it shows that in the noni leaf section, it contains active chemical compounds that can function as ingredients for treatment. The active chemical compounds contained in this noni leaf include Alkaloids, Steroids, Triterpenoids, Flavonoids, and Saponins. Existing results are obtained through observation of test solutions to changes that occur during the witnessing such as changes in color, the presence of deposits, or the emergence of foam.

The contents of these active chemical compounds have indeed shown that in the part of noni leaves can be used as medicinal ingredients for several diseases, but the presentation of the content of active chemical compounds is not known with certainty because the tests carried out are limited to qualitative testing through phytochemical testing. This observation is carried out with high accuracy on changes in changes that occur in the test solution, such as color changes, the presence of colored deposits and the emergence of foam.

Alkaloids

The presence of alkaloid compounds in this observation is characterized by the formation of white deposits in the test solution after reacting or added with reagents (Wagner reagents, Meyer reagents and Dragendof reagents). These compounds are a group of nitrogenous organic compounds derived from plants and have various pharmacological properties. Alkaloids include morphine, cocaine, atropine, kicine and caffeine. These compounds are mostly used in medicine as analgesics or anesthetics. According to Supriyadi (2001) cited by Budiwan (2003) that this compound also has pharmacological effects such as: (a) As a stimulant (stimulant) on the autonomic nervous system (b) As an analgesic material (c) As an insecticide material (d) As an ingredient anti cancer.

Steroids

The presence of steroids in this test is marked by the change of color to green in solution, steroids are a class of lipids derived from saturated compounds called cyclopentanaperhidrofenantrena, which has a core with 4 rings. Some important steroid derivatives are steroid / sterol alcohol. Other steroids include bile acids that help digest fat in the intestine, sex hormones (androgens and estrogens) and corticosteroid hormones produced by the adrenal cortex. Vitamin D also has a basic steroid structure (Dainith, 2000).

Steroids are not free but as derivatives of more complex compounds such as glycosides or esters with fatty acids / aromatic acids. Typical animal steroids in the form of cholesterol are found in surface lipids and plant organelles, but are often not found because only these exist as esters and glycosides which are insoluble in solvents commonly used for free sterols (Robinson, 1995).

Parenchymal resins in softwood and hardwood contain triterpenoids and steroids mainly present as fatty acid esters. Steroids and terpenoids are structurally related, but some special pathways in biosynthesis have produced certain structural characteristics and biological functions (Sjostrom, 1995).

Triterpenoid

The existence of this compound is marked by the occurrence of bluish discoloration in the test solution, this compound has a relatively complicated cyclic carotenoid pigment structure. Many triterpenoids are alcohol or colorless carboxylic acids, crystalline. Widely distributed triterpenoids are alpha amirin and beta amirin pentacyclic traits and their derivative acids, namely ursolic acid and oleanolic acid, which are found in waxy leaves and fruit such as apples and pears (Harborne, 1987).

Triterpenoids are widely distributed in amber, cork and plant warts. Tamarind is a triterpenoid acid which is often together with the polysaccharide gum in gum resin. Triterpenoids are free and also as glycosides, where glycosides are also present in more complicated forms as sulfates and aromatic acid esters. Important acyclic triterpenoids are only squalene hydrocarbons which are first isolated from shark liver oil but are also found in several epicyculas and vegetable oils (eg olive oil), because these compounds are considered as an intermediate compound in steroid biosynthesis.

Tricyclic triterpenoids are rare but for some tetracyclic triterpenoids are recognized. These

compounds attract attention because of their similarity and the possibility of linking biogenesis with steroids. Some interesting physiological activities are shown by several triterpenoids. This compound is an active component in medicinal plants that have been used for diabetes, menstrual disorders, snakebite wounds, skin disorders, liver damage and malaria. Some compounds may have ecological values for plants that contain them because these compounds work as antifungus, insecticides, or anti-predators, stimulate egg-laying insects, as well as anti-bacterial or anti-viral. Gossipol from cotton and malvaceae plants are grouped into triterpenoids because these compounds have 30 carbon atoms, but they may quickly be considered dimeric sesquiterpenes. Gossipol prevents certain hydrogen from binding to tubulin to interfere with oxidation phosphorylation, thus giving hope to male anti-fertility drugs (Robinson, 1995).

Flavonoids

The presence of Flavonoids in this test is indicated by the occurrence of changes in orange red color in the test solution. This compound is a group of natural material compounds from many phenolic compounds as plant pigments. Flavonoids include anthocyanins, flavonols and flavones. The distribution patterns of flavonoids are used in taxonomic studies of plant species (Dainith, 2000).

Flavonoids include many of the most common pigments and are found in all plants from fungus to angiosperms. High plants have flavonoids that are both in the vegetative and flowery parts. Flavonoids as flower pigments play an important role in attracting birds and flower pollinators. Another function of flavonoids is to absorb ultraviolet light to direct insects, regulate plants, regulate photosynthesis, work on anti-microbes and anti-viruses and work on insects.

The effects of flavonoids on various organisms are numerous and can explain why plants containing flavonoids are used in traditional medicine, including:

- Flavonoids can work as strong respiratory inhibitors
- Inhibits enzyme / non-enzyme oxidation reactions
- Acting as a hydroxyl radical reservoir and superoxidation thus protecting membrane lipids against incoming reactions
- Sugar bound to flavonoids tends to cause flavonoids to dissolve easily in water
- The antioxidant activity of flavonoids can explain the active components of plants used traditionally to treat liver function disorders (Robinson, 1995).

Fourier Transform Infrared Spectroscopy (FTIR) Test

FTIR analysis results of Mengkudu leaf extract with FTIR (Figure 2) showed that the value of wave number 3453 cm^{-1} is the -OH group with the type of phenol compound, then 2924 cm^{-1} is a CH functional group with the type of alkane compound, the next absorption value is 2361 cm^{-1} which is a $\text{C}\equiv\text{N}$ functional group

with the type of nitrile compound, the absorption value of 1638 cm^{-1} is a functional group of $\text{C}=\text{C}$ with the type of alkene compound, absorption value of 1424 cm^{-1} is a CH functional group with alkane type and absorption number 1384 cm^{-1} is a functional group of CH with the type of alkane compound (Skoog et al., 1998).

FTIR analysis of Mengkudu leaf extract obtained the wave value data of 3453 cm^{-1} with high uptake and widened -OH group which is a group of types of phenol compounds (Harborne, 2006). Flavonoids in plants are generally bound as glycosides, both O-glycosides and C-glycosides (Harborne, 1987) so that it can be assumed that phenol compounds are more dominant contained in mengkudu leaf extract.

Phenol compounds and their derivatives (Flavonoids) are one of the antibacterial agents that work by disrupting the function of the cytoplasmic membrane. Flavonoids in plants are generally bound as glycosides, both O-glycosides and C-glycosides (Harborne, 1987). Flavonoids prevent energy formation in the cytoplasmic membrane and inhibit bacterial motility, which also plays a role in antimicrobial actions (Widiastuti, 2008).

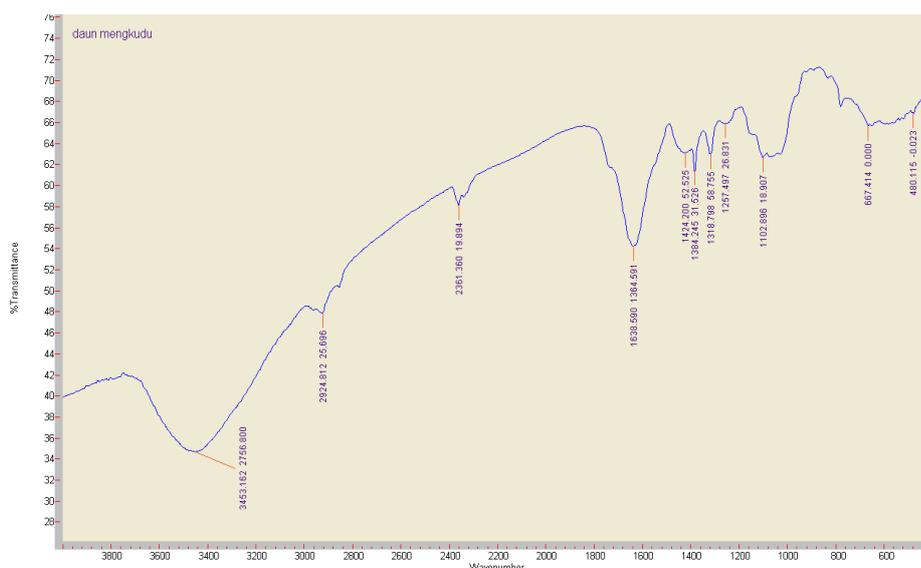


Figure 2. FTIR Test Result of Mengkudu Leaf Extract (*Morinda citrifolia*)

Conclusions and Suggestion

Based on several tests and the results of the research that has been done, it can be concluded that Mengkudu leaf extract is dominated by phenol compounds that have an antibacterial function. The disc test results obtained a concentration of 100 ppm is a minimal concentration that can inhibit the growth of *Aeromonas hydrophila* bacteria. Further research is expected to carry out more in-depth characterization testing of active compounds through several tests including using LCMS or GCMS to determine the content of the active compounds of mengkudu leaves and application directly to aquatic biota so that farmers can also use them.

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