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LEAF FRACTION ACTIVITY OF *AVICENNIA MARINA* AS ANTIFOULING AGAINST MACROFOULING AT SUNGSANG HARBOR SUMATERA SELATAN

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ABSTRACT

Mangroves are a type of high-level plant whose growth is influenced by the tides of seawater. *Antifouling* is a system used to prevent marine life from attaching to an object that is submerged in seawater. The use of natural *antifouling* is one of the efforts to reduce water and environmental pollution, such as natural *antifouling* from the mangrove plants *Avicennia marina*. The study was conducted in Sungsang, South Sumatra, Indonesia. *Avicennia marina* are mangrove plants that are suspected of containing *anti-fouling*, namely tannins, terpenoids and steroids. This study aims to determine the effect of the addition of *Avicennia marina* fraction on oil paint in the area of *Macrofouling* pasting on the test plate, how the effect of adding different *Avicennia marina* fractions on *Macrofouling* pasting on wood plates and steel plates and how the effect of the concentration level of fraction on *Macrofouling* pasting. The results showed that the fraction of *Avicennia marina* leaves was in the form of a methanol fraction which was obtained by steroids, flavonoids, and saponin compounds. The ethyl acetate fraction contains terpenoids, flavonoids, and saponins. Compounds that are active as antifouling present in the three fractions have a significant effect on plates that are not given treatment or control in reducing the sticking of fouling and there are different interactions between steel plates and wood plates.

KEYWORDS

Antifouling . *Avicennia marina*, Makrofouling



1. INTRODUCTION

Sungsang is a village located at the mouth of the Musi River with densely populated areas and transportation activities. The transportation that is widely used is sea transportation, where there is a pier as a place to stop ships or local community boats. Some piers are made of steel and wood as well as the ships used. The condition of the pier piers in Breech was found to have attached organisms that could be detrimental and also to transportation facilities such as ships (Afriyani *et al.*, 2017).

According to Julianti (2018) Attachment it doesn't only occur in natural substrates, but can also occur in various types of human interest facilities such as ships and docks. This attachment can cause biological contamination called *biofouling*.

Avicennia marina is a type of mangrove that has various bioactive compounds including flavonoids, tannins, steroids, alkaloids, glucosides which can be used as anticancer, antioxidant, antibacterial, antifungal, antiviral and anti-inflammatory. Environmental influences on mangrove vegetation habitat have influenced the presence of bioactive compounds. This is related to environmental limiting factors that trigger the rate of production of bioactive compounds (Nugroho *et al.* 2022). According to research by Abdelsalam *et al.* (2022) developed an effective *Avicennia marina* mangrove leaf extract as an antifouling agent in the form of a paint with a long duration. Ship hulls are painted to prevent pollution from unwanted marine organisms which can be controlled and toxic paints can affect unwanted living organisms, and plants such as *Avicennia marina* can be used as coatings or natural materials that do not dissolve harmful compounds such as TBT (in water and do not pollute the environment).

This study aims to determine the effect of adding *Avicennia marina* fraction to the paint oil to area sticking *macrofouling* on plate different test.

2. RESEARCH METHOD

2.1 Tools and Materials

The tools used in this study included a wooden plate with a length of 10 cm, a width of 7 cm and a thickness of 2 mm, a blender, a paint brush, a newspaper, a rotary evaporator, a dropper, a measuring cup, aluminum foil, cotton, a filter, an analytical balance, a jam jar, scissors, a large plastic scale, wire, scissors, a camera, a writing instrument. The materials used are *Avicennia marina* leaves, water, methanol, chloroform, 2 N sulfuric acid, ammonia, concentrated sulfuric acid, anhydrous sulfuric acid, FeCl₃, distilled water, 2 N HCl, ethanol, concentrated HCl, magnesium, n-hexane, ethyl acetate, and paint that does not contain TBT (Trybutyltin) *ingredients*.

2.2 How it Works

2.2.1 Extraction of *Avicennia marina* leaves

The extraction method used is the maceration method. *Avicennia* plant leaves *marina* taken in environment around Island Umbrella with take from a number of tree as much 2 kg (Shahara And Yenni, 2019). Leaf Which taken are mature leaves (dark green in color) then the leaves are cleaned with water flow. After that the leaves are dried by drying and baking. After drying the leaves cut small in blender until become powder (simplicia). Then simplicity taken each as much as 250 grams then placed in a different container and macerated using 750 ml methanol solvent for 72 hours at room temperature, Maceration was carried out 2 repetitions. Furthermore, the extract is filtered, then extract evaporated with use *Rotary evaporators* with temperature 50°C until obtained extract thick. After That extract thick entered in in bottle (Cahyaningtyas *et al.*, 2017). Extract thick *Rhizophora*

apiculata Then made 1 concentration with solvent paint that is control And 1250ppm (Andayani *et al.*, 2018).

2.1.2. Fractionation *Avicennia marina* and *Rhizophora apiculata*

Fractionation is separation compound based on polarity or polarity (Mukhriani, 2014). Solvent which used methanol, ethyl acetate, And n-hexane. Extract methanol is dissolved in methanol: water (1:1) until dissolved. The mixture is put in separator flask and fractionated with 250 mL n-hexane solvent (3 repetitions) until obtained fraction n-hexane And residue. Residue dissolved use ethyl acetate For fractionated again to get the ethyl acetate fraction again and residue. that fraction still liquid is condensed using a rotary evaporator to obtain a viscous fraction (Salni *et al.*, 2011). Fraction thick evaporated return use hair dryer until dry. Furthermore, activity test was carried out *antifouling*.

2.1.3. Screening Phytochemicals

A.marina fraction and the crude fraction of *R.apiculata* were then identified compound metabolites secondary. Identification done by screening phytochemicals:

a. Test Alkaloids

A total of 0.1 gram of extract and each fraction was mixed with 5 ml of chloroform and 5 ml of ammonia is then heated, shaken and filtered. Added 5 drops of sulfuric acid 2N in each filtrate, then shaken and allowed to stand. The top of each Each filtrate was taken and tested with Dragendroft reagent. Formed a yellow precipitate show exists alkaloids (Nur and Rahmawati, 2019).

b. Test Steroids

A total of 0.1 gram of extract and each fraction was dissolved in 20 ml of ether for 2 O'clock, Then filtered. Furthermore 5 ml filtrate Which obtained evaporated in Cupvaporizer until dry. Then added 2 drops sour sulfate concentrated And 2 drops sour acetic anhydrous. A change in color from purple to blue or green indicates the presence of steroids (Nur and Rahmawati, 2019).

c. Test terpenoids

A total of 0.1 gram of sample extract and each fraction was put in a tube reaction, then added with 2 mL of ethyl acetate and shaken. The ethyl acetate layer was taken then dripped on plate drops left until dry. After dry, added 2 drops sour acetate anhydrous and 1 drop of concentrated sulfuric acid. If a red or yellow color is formed, terpenoid positive (Muthmainnah, 2017).

d. Test tannins

As much 0.1 extract grams And fraction each added as much 1 ml solution $FeCl_3$ 1%, then observe the changes. When a blue or green-black color is formed indicate exists compound tannins (Kasitowati *et al.*, 2017).

e. Test Saponins

As much 0.1 grams extract And fraction each added 5 ml aquades, furthermore shaken until formed foam stable, then added 1 drops HCl 2N. When foam formation Which still stable indicates there is saponins (Acacia *et al.*, 2021).

f. Test Flavonoids

A total of 0.1 gram of extract and each fraction was added to 5 ml of ethanol, then added a few drops of concentrated HCl and 1.5 grams of magnesium. When a red color is formed it indicates

the presence of flavonoid compounds in *Avicennia marina* (Akasia *et al.*, 2021). when formed red indicate exists flavonoids (Acacia *et al.*, 2021).

2.2.1. Test Antifouling Fraction Leaf *A. marina* and Extract Leaf *R. apiculata*

2.2.1.1. Preparation Plate

The leaf fractions of *A.marina* and *R.apiculata* were used as test plates is a steel plate with a length of 10 cm, a width of 7 cm and a thickness of 2 mm and a wooden plate with a size of 10 cm, a width of 7 cm and a thickness of 2 cm. Then perforated on the right and left sides as a place to tie the wire. Then plate on clean, this is done so that the test plate is free of dirt that will interfere with the process sticking paint on test plate (Amen, 2017).

2.2.2.2. Process Mixing paint And Painting

Paint Which used to mix extract is paint Which No contain material- antifouling agents such as copper oxide (CuO) and zinc oxide (ZnO) boosters as well as titanium oxide (TiO) (Sonjaya, 2016). Stage painting done with use paintbrush paint. Furthermore prepared fraction leaf *A. marina* (fraction methanol, n-hexane, And ethyl acetate) concentration 1250 ppm And control (Andayani *et al.*, 2018).

Fraction leaf *A. marina* each mixed with paint until with 3 time repetition. Then paint applied on plate steel And plate wood. After That plate dried, furthermore plate steel and plate wood the will tied with wire And installed on pole buffer dock in breech.

2.2.2.3. Process Installation Plate Test

Plate test that has been painted is prepared, then the test plate is installed 50 cm below water level at the lowest ebb and tied with wire to the port of the Sungsang pier so that No drift away. Plate test left submerged during 4 Sunday (30 day). On moment installation of plates and monitoring carried out by measuring environmental parameters. Parameter environment which observed among them temperature, pH, And salinity (Marhaeni, 2012).

2.1.2. Identification of attachment area measurements and biofouling biomass

Observations were made every 7 once a day to identify the type of organism *macrofouling* And measure wide the pasting. Identification *macrofouling* done based on morphological similarities by using the *macrofouling* identification book " Archipelago Sea (Nontji, 1993). After being submerged for 4 weeks, the test plates were taken for the attachment area and *biofouling biomass* were calculated (Idora *et al.*, 2015).

Measurement area sticking *biofouling* done with method measure long And Also wide sticking *macrofouling* on plate surface test with use a ruler.

Before soaking each test plate will be weighed to determine the initial biomass and weighed again after soaking for 4 weeks to determine the final biomass. According to Vedhaprakas *et al* (2013) the *biofouling biomass* accumulated in the test plate can is known with method reduce heavy end plate test with heavy beginning test plate.

Parameter Observation

Parameters observed in this study is the concentration of extracts that are good in reduce sticking *biofouling* , wide sticking, biomass *biofouling* as well as type *macrofouling* Which stick on plate steel and wooden plate.

Analysis Data

Draft studies This use method design random group. For comparison area And biomass *biofouling* analyzed with ANOVA with level 95% confidence and further testing using Duncan's test with a 95% confidence level (Amen, 2017). Research result This will served in form picture, chart And table. The percentage of attachment area can be calculated using the following formula:

$$\% \text{ area of attachment} = \frac{\text{area of attachment}}{\text{Test panel area}} \times 100\%$$

Table. 3.1. Category percentage of *biofouling attachment area*

| No. | Area Percentage | Category |
|-----|-----------------|------------|
| 1. | less than 10 % | Low |
| 2. | 10% - 30% | Rather low |
| 3. | 30% - 50% | Currently |
| 4. | 50% - 80% | Tall |
| 5. | 80% - 100% | Very high |

Source: Xiangrong and Guiqiao (2005)

3. RESULTS AND DISCUSSION

Based on Parameter measurements that have been carried out include temperature, salinity, and pH. Measurement of environmental factors is measured directly at the location of the plate immersion test, results from measurement environmental parameters can seen on Table 4. 1.

| Parameter | Quality standards | Average |
|-----------------|-------------------|---------|
| pH | 7- 8.5 | 6,62 |
| Salinity (‰) | 33-34 | 10,2 |
| Temperature(°C) | 28-32 | 24,4 |

Note: Quality standard according to Ministerial Decree State of the Environment No.51. 2004 Appendix III concerning Seawater Quality Standards for Marine Biota

The salinity at the research location, precisely at the Breech Pier, is around 10.2 ‰ , this salinity is still low for the seawater group and is still below the quality standards for water for the growth of organisms in the sea. As stated in the Decree of the State Minister for the Environment No. 51 (2004) the magnitude of the salinity optimal for the life of marine organisms by 33 to 34% . Low salinity measurements at the time of the research and caused by seawater in low tide. According to Patty and Nebuchadnezzar (2018) , low salinity is influenced by several factors, including the mixing of river water and fresh water which causes the salinity in the area to be abnormal.

The research location has a normal temperature of 29-30 °C , this temperature is the optimal temperature for the growth of marine organisms. The temperature that is measured once a week has a different temperature, but the temperature difference is not too far but almost the same

every week. Sea growth is strongly influenced by several environmental factors, one of which is temperature. According to Murniati *et al.* (2015), *biofouling* growth influenced by environmental factors such as salinity, pH, and temperature.

Measurement of the pH parameters that have been carried out at Breech Pier, the pH is known, namely 6.01. pH is the optimal pH for the growth and life of marine organisms. According to Romimohtarto (1991), marine organisms live optimally in the sea with a pH of 6-9, because marine organisms grow at a normal pH. Getting lower pH in an area of waters, the less likely it is that only a few marine organisms exist in a sea area. According to Persulesy and Ine (2018) water areas that have mark pH low can decrease the amount oxygen dissolved existing in the waters.

3.1. Analysis of *Avicennia marina* Secondary Metabolite Compounds

Leaf fraction *A marina* used for research is the methanol fraction, the n-hexane fraction and the ethyl acetate fraction. The results of the phytochemical screening analysis that has been carried out show that there is class compound metabolites secondary that is steroids, tannins, terpenoids, saponins, flavonoids and alkaloids. The results of the identification of each fraction can be seen in Table 4.1 shows that the n-hexane fraction contains the most metabolites due to the nature of the n-hexane compounds.

| Compound | Fraction | | |
|------------|----------|----------|---------------|
| | methanol | n-Hexane | Ethyl Acetate |
| Steroids | + | - | - |
| tannins | - | + | - |
| terpenoids | - | + | + |
| Alkaloids | - | + | - |
| Flavonoids | + | + | + |
| Saponins | + | + | + |

Information (+) indicates the presence of compounds, (-) indicates the absence of compounds.

which can attract polar and nonpolar compounds. Study related Which do screening phytochemicals leaf *A. Marina* got the results compound metabolites secondary Which different research Alhaddad (2019), results screening leaf *A. marina* show exists compound tannins, steroids, flavonoids in solvents methanol, on solvent Ethyl acetate is present compound alkaloids, Flavonoids, triterpenoids, And solvent n-hexane contain compound flavonoids and triterpenoids.

Compound metabolites Secondary plants produced by plants have a role in self-defense from the environment and from attack by other organisms including adhering organisms or epiphytes, and preventing infection from pathogens (Marhaeni *et al.*, 2010). Pakidi and Suwoyo (2017), secondary metabolites are compounds that can generated creature life, as well as compound *antifouling* Can obtained from natural ingredients with the presence of antibacterial and antimicrobial properties. According to Trepos *et al.* (2013), the difference in the results of the attachment area *for macrofouling activity* can be expected Because compound bioactive generated on every faction different.

3.1.2 Attachment and measurement of *biofouling biomass*

Measurement of *biofouling biomass* found on plates according to Vedhaprakas *et al.* (2013), by subtracting the final weight from the initial weight to find out the biomass. In this study, the test plates

were weighed to determine the initial weight and then after immersing the plates for 30 days, the plates were removed and weighed again.

4.3.1. Macrofouling Attachment Area

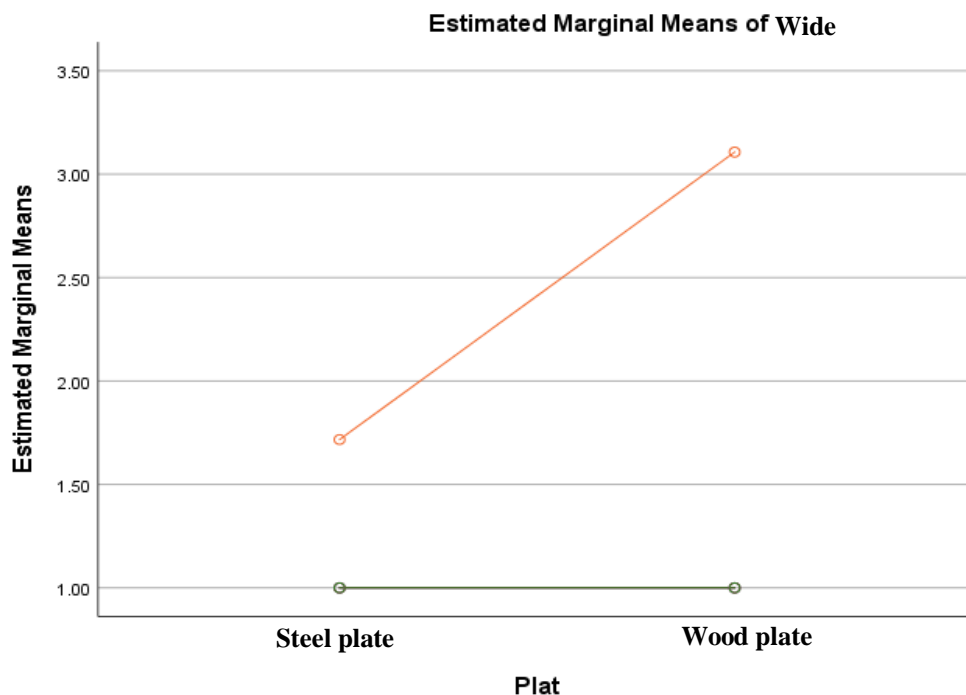


Figure 3.1. Comparison of wood plate and steel plate

Steel plate and wood plate have significant differences seen that *macrofouling* is found namely *Ancylus recurvus* more in wood plates than steel plates. The steel plate has a smooth surface with the interaction between the fractions containing saponins which minimizes corrosion *macrofouling* sticks and minimizes the toxicity of alkaloid compounds. Concentration used 1250 ppm with different plate and fraction treatments, so that the compounds present in the fractions play a role in the attachment of *fouling organisms* which produce biomass. *The macrofouling* did not stick because the test plate was not attached to the substrate at the breech dock. According to Marhaeni (2012) The denser the substrate, the more effective it is in preventing biofouling from sticking. The smoother the surface of the substrate, the more effective it is in preventing *biofouling from sticking*. The lowest biomass value is found in the n-hexane fraction and shows that the fraction has a significant effect by showing the value of the *Two Way Anova* test greater than 0.05.

4. CONCLUSION

Based on the research results that have been obtained, several conclusions are obtained as follows:

1. *Avicennia marina* leaf fraction in the form of the methanol fraction obtained steroid, flavonoid and saponin compounds. The ethyl acetate fraction contains terpenoids, flavonoids, and saponins.
2. Compounds that are active as antifouling present in the three fractions have a significant effect on plates that are not given treatment or control in reducing the sticking of fouling and there are different interactions between steel plates and wood plates.
3. The macrofouling attached to the test plate was identified as a Gastropod in the form of *Ancylus recurvus*.

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