Field Optimization of *Durio zibethinus* as Macro-Antifouling at PT Dok and Shipping, Surabaya, Indonesia

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Abstract. The use of TBT (Tributyltin) as a mixture of antifouling compounds in ship paint has been banned since 2001, and the use of Natural Product Antifoulant (NPA) as a substitute continues to be made by utilizing many resources. *Durio zibethinus* is one of the plants suspected of having antifouling compounds, so it needs to be explored its potential as an NPA with field test mechanism on ship plates for 28 days. The result of phytochemical screening test showed that extract of the skin of *D. zibethinus* positive contains secondary metabolite compound Alkaloid, Terpenoid, Tannin, Flavonoid, and Sapnin. Field test results show that *D. zibethinus* extract has an effect on the area of adhesion and biomass biofouling. In the fourth week of the experiment, the biofouling attachment was recorded at concentrations of 0, 25, 50, 75, and 100 ppm respectively 66.1%, 62.1%, 60.9%, 54.2%, and 47.8 %. As for biomass biofouling recorded 36.6 g, 30.3 g, 20.4 g, 9.6 g and 8.9 g. The identified species of biofouling are only one species of *Balanus amphitrite*.

Keywords: *Durio zibethinus*, area of adhesion and biomass biofouling

1. Introduction

Biofouling is a mechanism of accumulation of microorganisms, plants, and animals attached temporarily or permanently to the surface of objects submerged in seawater [1]. On the hull of the ship, biofouling can increase the surface roughness of the ship's body as well as the added weight of the vessel significantly which increases the drag force so that fuel consumption rises 40%, the speed decreases 50% and the shipping time can be delayed 10-15% [1].

Many research continues to be developed as an effort to prevent and eliminate biofouling. According to [2], until this time antifouling paint uses chemo-biocidal principles, which are chemically based such as heavy metal compounds and organometallic. Such compounds can accumulate even persistent and are toxic in nature, one of them is TBT (Tributyltin) [2]. TBT may also cause abnormalities of the imposex in Gastropoda [3]. Therefore, non-toxic antifouling paint alternatives become an urgent need. One of the current research techniques is to look for environmentally friendly antifouling compounds by examining the natural mechanisms of marine organisms in dealing with biofouling problems, resulting in the idea of developing Natural Product
Antifoulants (NPA) products. According to [4], Terpenoid, Alkaloids, and Steroids can be used as NPA feedstock. These three compounds are suspected to be found on the skin of *Durio zibethinus*. The potency of *D. zibethinus* as the antifouling product is very big because in Indonesia its production rate reaches 1.818.949 ton/year with skin waste reach 60-75% / fruit [5]. Therefore, this study needs to be done to explore the content of compounds and the potential of *D. zibethinus* skin as an active ingredient NPA.

2. Materials and methods

The research was conducted in Chemical Laboratory of Microorganism Department of Chemistry and Ecology Laboratory of Department of Biology ITS, while field test was conducted in PT Dok and Perkapalan Surabaya (figure 1). The material used is the skin of *D. zibethinus* for the manufacture of extracts and reagent compounds for phytochemical screening tests. Equipment required for example extraction of materials, painting, and analysis of the area of adhesion and biomass biofouling.

![Image](image.png)

**Figure 1.** Location of soaking (immersion) activity at PT Dok dan Perkapalan Surabaya in coordinates 7012'07.38 "S 112043'55.27" E

2.1. Extraction

*Dario* parts used in this study are white skin, cut, and dried without having to be exposed to direct sunlight, and then blended until it becomes a powder. The resultant powder was processed by maceration with an addition of methanol using 1: 10 ratios for 3 x 24 hours and stirring four times a day. After the powder was filtered, part of the solution was taken to evaporate using a rotary evaporator to obtain the extract in gel form. The next step was phytochemical and GC-MS screening test to determine the components of the active compound contained. Phytochemical screening test using *H*₂SO₄, alkaloid test with Mayer, Wagner, and Dragendorff reagents, steroid and terpenoid test
with Lieberman Burchard, saponin test with HCl, and tannin test with FeCl₂. After that, *D. zibethinus* skin extracts were made in concentrations of 25, 50, 75, and 100 ppm.

2.2. Preparation of reagents

The preparation of reagents in the phytochemical screening process refers to [6] and [7]:

2.2.1. Mayer’s reagent. Dissolved 1.36 g HgCl₂ in 60 ml water. On the other side, 5 g of KI was dissolved in 10 ml of water. Both of these solutions are then mixed and diluted with water up to 100 ml. The reagent is stored in a dark bottle to avoid light.

2.2.2. Wagner’s reagent. A total of 1.27 g of Iodine and 2 g of KI were dissolved in 5 ml of water. Then this solution was diluted to 100 ml with water. The precipitate formed was filtered and stored in a dark colored bottle.

2.2.3. Dragendroff’s reagent. A total of 8 g of KI was dissolved in 20 ml of water, while on the other 0.85 g bismuth sub nitrate was dissolved in 10 ml glacial acetic acid and 40 ml of water. Both solutions are mixed and the result was stored in a dark colored bottle. In use, one solution was diluted with 2/3 of a 20 ml solution of glacial acetic acid in 100 ml of water.

2.3. Phytochemical screening

Phytochemical screening tests refer to [6] and [7], namely:

2.3.1. Flavonoid test with \( \text{H}_2\text{SO}_4 \). The extract was put into the test tube, then \( \text{H}_2\text{SO}_4 \) 2N was added and shaken until homogeneously mixed. Positive samples contain flavonoids when the solution undergoes a very striking discoloration into yellow, red, or brown.

2.3.2. Alkaloid test. One mL sample extract was added 10 drops of \( \text{H}_2\text{SO}_4 \) 2 N and shaken in one direction, allowed for several minutes to form 2 layers. The top layer is transferred into three test tubes each of 1 mL. Tube 1 added 3 drops of reagents Mayer, Wagner and Dragendorff. Positive samples contain alkaloids when Mayer's reagents give white deposits, Wagner's reagents give brown deposits and Dragendorff’s reagent gives an orange precipitate.

2.3.3. Steroid and Terpenoid tests. One drop of sample extract was placed on the drip plate at 3 points (the first point for standard and the other for terpenoid and steroid testing) and left to dry. After dry, Lieberman Burchard added (1 drop of concentrated sulfuric acid and 3 drops of anhydrous acetic acid) then observed the color change. Positive samples when subjected to discoloration to red or brown to terpenoids (triterpenoids) and blue, violet, or green changes to steroids.

2.3.4. Saponin test. One mL of sample extract was inserted into the test tube, then added 5 mL of hot aquades and added 2 drops 2 HCl 2 N and shaken. After that, see if the foam formed after silence for 10 minutes. Positive samples contain saponins when there is foam with a lot of intensity and consistent for 10 minutes.

2.3.5. Tannin test. One mL of the extract was inserted into the test tube, then 1% FeCl₃ added about 2-3 drops. Positive samples contain tannin when subjected to discolouration to a blackish green.

2.4. Gas Chromatography – Mass Spectre (GC - MS)

GC-MS is useful for determining the mass or molecular weight of a compound with its fragmentation as either structural analysis or structural elucidation. Identification of the main components of *D. zibethinus* skin extract using GC-MS and its interpretation was done by grouping the peak chromatogram that changed in process variation. The compounds are grouped according to the amount
of C in the compound and the pattern of concentration changes on the temperature change; In which case the compound undergoes a breakdown of the carbon chain at a rise in temperature or a compound having an increase in percentage.

2.5. Painting and treatment in the field
Test material used in the form of steel plate with size 5 x 7 cm and a thickness of 0.8 cm (figure 2). Then formed a hole diameter of 2 cm in the middle of the plate to place the rope. Steel plate blasted to remove dust, dirt, grease, and small animals attached, then painted with a gray color with a thickness of 100 µm. Paint is treated by the addition of a mixture of gel extract at concentrations of 0, 25, 50, 75, and 100 ppm. After painting, the plate soaked in the location of PT Dok and Perkapalan using a strap that hung up the plate submerged at least 20 cm above the water level at the lowest tide.

![Figure 2. Soaking model of steel plate at the study site](image)

Note:
A: pole line to the waters.
B: pathway
C: sea level height at high tide
D: sea level height at low tide
E: rope along 3 meters (from top surface of concrete to sea level)
F: boards or wooden/beams along the 25 cm
G: Straps to the vessel plate along the 20 cm
H: Steel plate size 5 x 7 cm and 0.8 cm thickness
I: distance between plate; 5 cm
J: distance between boards/beams; 5 cm

2.6. Data retrieval.
Every seven days, the steel plate is taken for each concentration to obtain the attachment and biomass data without the need to be returned again. Field physics-chemical data are checked, including brightness using Secchi disk, water temperature using a thermometer, and salinity using salinometer.

Biomass biofouling data; before being soaked, the steel plate is weighed first so that the initial biomass is obtained, and then weighed after immersion for each treatment time (every 7 days, for 28 days) as the final biomass. Biomass biofouling data is obtained by subtracting the final biomass and the initial biomass. Data obtained in grams.
The biofouling attachment data was calculated using a transparent mica that had been given a grid of 1 x 1 cm for the entire surface area of the steel plate. On each grid, the percentage area of adhesive by biofouling is calculated by the formula:

\[ C = \frac{\sum (M_i \times f_i)}{\sum f_i} \]

Note:
- \( C \) = percentage area of adhesive by biofouling
- \( M_i \) = percentage of the midpoint of the presence of biofouling class type
- \( f_i \) = many sub plots with class attendance the same type of biofouling

Note: The criteria for cover area category to attachment

| Class | Broad area cover | % Cover area | % Midpoint (M) |
|-------|-----------------|--------------|---------------|
| 5     | 1/2 - full      | 50 – 100     | 75            |
| 4     | 1/4 - 1/2       | 25 – 50      | 37.5          |
| 3     | 1/8 – 1/4       | 12.5 – 25    | 18.75         |
| 2     | 1/16 – 1/8      | 6.25 – 12.5  | 9.38          |
| 1     | < 1/16          | < 6.25       | 3.13          |
| 0     | Not available   | 0            | 0             |

Figure 3. Left (a) a steel plate profile with the size specification used, right (b) a technical calculation of percentage of cover area by using a transparent mica that has been grided with a size of 1 x 1 cm

2.7. Data analysis.
This research uses the statistical tools for the calculation. The independent variable is the concentration of \( D. zibethinus \) extract, the dependent variable is the area of adhesive and biomass biofouling. These data are expected to illustrate the effect of \( D. zibethinus \) skin extract on the area of adhesion and biomass biofouling. Statistical analysis used is ANOVA one way and MANOVA. One-way ANOVA is used to see the effect of extract concentration on the area of adhesion and biomass biofouling. MANOVA is used to see the effect of concentration simultaneously in the area of adhesion and biomass biofouling. In anticipation, a simple linear regression test was conducted to see if the attachment area variable changed when the biomass variable also changed.

3. Results
The \( D. zibethinus \) skin used in this study was white, weighing about 1000 g. The skin is processed and extracted to produce about 127 g thick red-colored gel. Components of the compounds contained in the gel extract were analyzed secondary metabolite compounds and produced as follows (table 1):
Table 1. Results of skin phytochemical screening test of *Durio zibethinus*

| Phytochemically test | Reagent          | Result                       | Conclusion |
|----------------------|------------------|------------------------------|------------|
| Alkaloid             | Mayer            | No white precipitated formed | Negative   |
|                      | Wagner           | Reddish color                | Positive   |
|                      | Dragendorff      | Orange color                 | Positive   |
| Flavonoid            | $\text{H}_2\text{SO}_4$ | Orange color               | Positive   |
| Saponin              | Air + HCl        | Stable foam formed           | Positive   |
| Steroid              | Liebermann Burchard | Green color not formed      | Negative   |
| Terpenoid            | Liebermann Burchard | Reddish brown color      | Positive   |
| Tannin               | $\text{FeCl}_3$  | Blackish green-colored      | Positive   |

The attachment of *Balanus Amphitrite* (the only one macrofouling that detected) has begun since the first week. Figure 4 shows the observed extent of biofouling attachment applied to all steel plates immersed for 28 days. The result of biomass biofouling after 28-day project is showed in figure 5.

![Percentage of biofouling areas of adhesion](image)

**Figure 4.** Percentage of areas of adhesion of biofouling each week sampling until 28-day project

Note: categorization of percentage of covering area

| Percentage of areas of adhesion | Categorized areas of adhesion |
|---------------------------------|-------------------------------|
| < 10%                           | low                           |
| 10-30%                          | rather low                    |
| 30-50%                          | middle                        |
| 50-80%                          | high                          |
| 80-100%                         | very high                     |
4. Discussion

4.1 Potential of Durio zibethinus skin extract.

The results in table 1 indicate that there is an undetectable or negative metabolite. Methanol solvent is thought to be the cause because it is polar, so it tends to form water. This is in accordance with the statement [8], which states that the increasingly polar solvent nature tends to form larger water than the intended secondary metabolite so that it becomes undetectable.

Alkaloids, terpenoids, tannins, saponins, and flavonoids have distinctive bacterial inhibitory mechanisms according to their individual characters [9]. The mechanism of action of these secondary metabolite compounds is to damage the permeability of bacterial cell walls so that bacteria become lysis. This condition shows that the methanol viscous extract of *D. zibethinus* acts as anti-biofouling, precisely micro-antifouling.

As an antibacterial, *D. zibethinus* skin is composed of 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl [5], which included in the type of flavonoid and benzoic acid compounds, parts of the phenol compound. Different results were observed in the study [6], with the same GC-MS analysis, they found that *D. zibethinus* skin contained methyl hexadecanoic which belonged to the essential oils and methyl octadecanoic groups included in the ester compound. Although the main components are different, they are included in secondary metabolite types that have anti-bacterial properties. And if flavonoids, phenols and essential oils have properties inhibiting the growth of bacteria, then they also have potential as anti-fouling because they inhibit biofilm formation by bacteria.

The results of different GC-MS analyze were also suspected to be due to differences in *D. zibethinus* varieties used as test materials. Researchers [6] used *D. zibethinus* varieties of “Petruk” as a test material, while in the study [5], including this study, used a whole variety of “durian” skins that were discarded by “durian” traders. Although none of the statement about 4H-pyran-4-one, 2,3-dihydro -3,5-dihydroxy-6-methyl compound was used in this study, another parallel study using different Sansevieria trifasciata varieties showed different saponin levels. Differences in levels of compounds between varieties are suspected because each variety has different genetic properties. This
will affect the ability of plants to cope with environmental conditions. This is consistent with the opinion of [10], which states that different varieties consist of a number of different genotypes, and each genotype has the ability to adapt typically to the environment in which it grows.

4.2 The composition of macro-fouling species.

The magnitude of colonies formed, species diversity, the development of the community of sticking organisms depends heavily on the material (chemical properties of the substrate, texture) used and the environmental conditions (tidal, brightness, light, current, wave, food supply, and space to grow) [11]. A 28-day observation of a steel plate as a test material found only one species of macro-fouling, *Balanus amphitrite*. The morphological identification results of this activity are in accordance with the description of [12], where *B. amphitrite* has a morphological characteristic of a round shell with a conical tip, finely textured and thin, having a blend of white and pink, or white and brown. The lines of color are lined with white tends to be in the middle.

*Balanus amphitrite* is a eurythermal and euryhaline organism, meaning it can live in a range of salinity and high temperatures. *B. amphitrite* can develop optimally in the salinity of estuary waters ranging from 5-30 ‰ to open water salinity reaching 41 ‰ and environmental temperature in the range of 25-30 °C [13]. The salinity and temperature at the soaking site indicate 30 ‰ and 31 °C, so that *B. amphitrite* is possible to grow and multiply, including on steel plate.

The dominance of *B. amphitrite* is thought to be caused by the Arthropodine compound it produces so that the same barnacles will congregate and grow until there is build-up [14]. In addition, the condition of the steel plate that is always submerged in sea water at a depth of ± 20 cm when the lowest tide allows for a decrease in the intensity of light from the sun so that *B. amphitrite* larvae can breed optimally. Larvae *B. amphitrite* has the behavior of avoiding light directly or called negative phototropic, so the low light that blends will make the larvae easily attached to the steel plate. In addition, the object surface also affects the ability of *B. amphitrite* in the pasting process. Research [15] and [16] using two types of carbon steel materials, one of which is coated with asphalt so that its surface becomes more-coarse, indicates that *B. amphitrite* tends to like the surface of rough objects rather than fine ones. However, apart from both, *B. amphitrite* means being able to stick to objects made of carbon steel plates, which are also used in this activity.

4.3 Area of adhesion and biomass biofouling

Figure 4 shows that the concentration of 0 - 75 ppm has a percentage of the covering area above 50% at 4 weeks, which according to [17] has entered into the high category (50-80%). While the concentration of 100 ppm was recorded only 47.8% and included in the middle category (range 30-50%). While at a concentration of 0-100 ppm for week 1 to week 3 none of the concentrations reached> 50%, except at 0 ppm concentration at week 3 which reached 49.9% or close to 50%.

Figure 4 also shows that there is generally a decrease in the percentage of biofouling attachment area as the concentration of *D. zibethinus* extract is mixed with the primary paint. Presumably, this is related to the higher concentration of the extract, higher the content of secondary metabolite compounds such as alkaloids, flavonoids, tannins, terpenoids, and saponins that provide anti-macrofouling effects.

However, after tested ANOVA one-way, p-value = 0.445 which means > 0.05, so it is concluded that there is no influence between each concentration in terms of biofouling attachment area. The concentration of 0 ppm with 100 ppm is not significantly different in terms of the biofouling attachment area, which means that the effect of *D. zibethinus* extract does not differ significantly for each concentration.

And the figure 5 shows that there is a general decrease in biomass biofouling weight as the concentration of *D. zibethinus* extract increases on each observation week. At week 4 the biomass decrease ranged from 7.29 - 52.94%. The highest biomass reduction occurred at concentrations of 50 to 75 ppm.
One-way ANOVA results showed that the concentration of *D. zibethinus* extract gave significant effect on the weight of biomass biofouling which was shown by p-value = 0.000 which means <0.05. This condition indicates that biomass biofouling is influenced by the concentration of *D. zibethinus* extract containing some secondary metabolite compounds that have the potential as anti-macrofouling.

The assumption that the extent of biofouling attachment and the weight of biofouling biomass is affected simultaneously by the concentration of the extract is necessary for further demonstration. Therefore, MANOVA test to know the two dependent variables are simultaneously influenced by the giving of different extract concentration. If p-value <0.05, it can be stated that the two dependent variables are indeed influenced by the gradation of *D. zibethinus* extract concentration. MANOVA calculation results are shown as follows (table 2):

**Table 2.** MANOVA test results related to extracting concentration of *Durio zibethinus* on the area of adhesion and biomass biofouling

| Criterion    | Test statistic | F  | Num | Denom | P   |
|--------------|----------------|----|-----|-------|-----|
| Wilk’s       | 0.15652        | 5,347 | 8   | 28    | 0.000 |
| Lawley-Hotelling | 5.34494 | 8,686 | 8   | 26    | 0.000 |
| Pillai’s     | 0.85034        | 2,774 | 8   | 30    | 0.020 |
| Roy’s        | 5,33672        |      |     |       |      |

Referring to table 2, actually, extract of *D. zibethinus* has significant effect simultaneously in the area of adhesion and biomass biofouling with p-value <0,05. From these results will arise a new question, is it true that the percentage of adhesion area and biomass biofouling influence each other? This question arises as a result of environmental conditions or material conditions of the test, both of which affect the percentage of plant attachment and biomass biofouling. In addition, the area of adhesion can also affect biomass biofouling because the surface area is limited by the initial adhesion over time. That's why Simple Linear Regression Test was done to prove both mutually influential, with the free variable is the percentage of the adhesion area while the dependent variable is biomass biofouling. The result of simple linear regression test stated that: Biomass = 6.5 + 0.343 Adhesion. The result of the equation is stated that if another variable is constant, then the biomass value will change by itself at a constant value of 6.5; and if another variable is constant, then the biomass value will change by 0.343 per one unit of adhesion area.

In principle, the variable of adhesion of area and biomass biofouling are indeed affecting each other, but the data model is classified as less good. This is seen in the value of P on partial T and R-Sq values. The value of R-Sq is only 11.5%, which means that the biomass variable is influenced by the attachment or adhesion attribute variable is only 11.5%, and the remaining 88.5% (100 - 11.5% = 88.5%) is influenced by other variables (alleged: The number and size of individual biofouling). Thus, the effect of extensive attachment to biomass biofouling can be neglected. The low percentage of the attachment area to biomass biofouling is also indicated by the P value for partial T = 0.526 which means> 0.05, which means that the variable attachment area does not affect the biomass biofouling variable. These conditions give the result of simple linear regression test for the attachment area and biomass biofouling with the value of P 0.144> 0.05, and it is concluded that the adhesion area does not have significant effect to biomass biofouling, so the result of ANOVA one way for both variables is considered appropriate.

Other assumptions that could be proposed include *B. amphitrite* having a chemical compound called arthropodine [14]. This compound is thought to have a function as attractant compounds for similar larvae to come. Although it remains unclear and surely the function of the compound when it is an attractant, the existence of an individual *B. amphitrite* on a steel plate affects the attachment of another individual *B. amphitrite* on the same steel plate. Researchers [13] also convey the same thing that *Balanus* sp. adults will emit a signal of a chemical compound called glycyl-glycyl l-arginine (GGR) that calls the larvae to attach to the substrate and grow into adulthood. This will cause the
surface area to be attached by the genus *Balanus* to be widened around it. One of the images allegedly associated with this assumption is shown in figure 6 below:

![Figure 6](image)

**Figure 6.** Several examples of the extent of the closure area by *B. amphitrite* in some concentrations of *D. zibethinus* extract and different sampling weeks through the appearance of grid counter.

The condition of competition for the seizure of place remains between individuals of *B. amphitrite*. Individuals who are unable to compete with other individuals, or fail to adhere to the substrate are evident in the following sample images (figure 7):

![Figure 7](image)

**Figure 7.** (left) the 2nd-week treatment plate at 25 ppm, (right) the 4th-week treatment plate at concentration 50 ppm. The white color indicating the dark circles indicates a diseased *B. amphitrite* attachment, presumably due to intraspecific competition or the inability to attach permanently.

### 5 Conclusion

Skin extract of *Durio zibethinus* positive contains secondary metabolite compounds Alkaloids, Terpenoids, Flavonoids, Tannins, and Saponins. The macrofouling organisms are identified by only one species: *Balanus amphitrite*. Skin extract of *D. zibethinus* able to decrease macro fouling attachment area along with increasing concentration during four weeks of treatment that is equal to 66.1%; 62.1%; 60.9%; 54.2% and 47.8% at concentrations of 0, 25, 50, 75, and 100 ppm. In addition,
macro fouling biomass also experienced a downward trend of 36.6; 30.3; 20.4; 9.6 and 8.9 grams at concentrations of 0, 25, 50, 75, and 100 ppm for four weeks of treatment.

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