An overview on antibiofouling agent from carica seeds waste as antifoulant coating

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Abstract. TBT (Tributyltin) is used to prevent biofouling, but it causes a dangerous effect on other marine biodiversity. In Wonosobo Regency, there are lots of Carica seeds (9 tons/month) which not utilized and considered as wastes. Whereas Carica seeds contain active compounds (saponins, alkaloids, phenols, flavonoids, and tannins) which are classified as antibacterial agent. Antibacterial compounds are used to prevent the formation of biofilms (which cause biofouling). This article discusses the antibacterial activity of Carica seeds and its prospects as an antifoulant coating. The general mechanism of the inhibition of the biofilm formation featuring bacteria were explained as sequences as follow (1) inhibiting cell division, (2) inhibiting DNA and RNA synthesis and (3) damaging cell membranes. By the number of antibacterial components contained in Carica seed, it has the potential to be used as an antifoulant coating because of its antibacterial and antibiofilm activity. However, further research needs to be done regarding the effective composition of the Carica seed waste and coating materials (binders, pigments, solvents, and additives) and their characteristics.

Keywords: Carica seeds, antibiofilm, antibiofouling, antifoulant coating

1. Introduction
Marine transportation in Indonesia is very important for economic development [1], there are several challenges in management or maintenance. One of the problems in marine transportation maintenance is the presence of biofouling. Biofouling is the attachment and growth of microorganisms or macroorganisms to each substrate that is submerged [2]. The occurrence of biofouling begins with the formation of a biofilm layer, which is a good medium for the attachment of microorganisms or macroorganisms [3]. Biofouling grows and develops rapidly in various submerged constructions. In the field of marine transportation, biofouling accumulation often occurs in ships, buoys, or constructions that are submerged in seawater [4].

The attachment of microorganisms or macroorganisms to the hull of a ship causes motion resistance, resulting in an increase in fuel consumption between 40-50% [2]. The increase in fuel consumption affects the level of carbon gas emissions which causes an increase in temperature on the earth's surface [4]. The overall cost associated with cleaning the biofouling pile is estimated at 56 million US dollars per year [3].
To prevent the occurrence of biofouling, antifoulant protective paints such as TBT (Tributyltin) have been used, but their use has been banned since 2008 by IMO because it is proven not only to be harmful to biofouling but also various organisms living in the sea [4]. Moreover, a new alternative is needed to overcome the problem of biofouling, which has criteria that are safe for the environment and cheap. It can reduce maintenance costs for marine vessels. As mentioned in Sustainable Development Goals (SDGs) no. 14 regarding Marine Ecosystems, one of which is by 2025 to prevent and significantly reduce all types of marine pollution and by 2020 sustainably manage and protect marine and coastal ecosystems. One of the ways that can be used to prevent biofouling, which is pollution in the sea, is to make coatings from natural materials that are environmentally friendly. Some of the compounds that can be used are saponins, alkaloids, and flavonoids because of their antibacterial properties. In nature, these compounds are generally found in plants, in the seeds, roots, stems, leaves, or fruit.

Wonosobo Regency, Central Java has many Carica home industries that produce seeds waste of 9 tons/month[6]. Generally, the waste is just thrown away. Even though the Carica seeds contain secondary metabolite compounds such as tannins, flavonoids, phenols, terpenoids, alkaloids, and saponins. All these compounds have antibacterial properties [7]. Antibacterial properties are needed to prevent the formation of biofilms (a good medium for microorganisms and attached microorganisms), which is the initial stage of biofouling.

This potential can be used as an anti-biofouling coating on marine vessels and port construction submerged in seawater. This narrative review aims to provide an overview of the activity of active compounds in Carica seeds waste and its mechanism of action in inhibiting the presence of bacteria that can cause biofouling. As well as the prospect of Carica seeds wastes as an antifoulant coating.

2. Biofouling and Antibiofouling
The formation of biofouling begins with the adhesion of microorganisms, where particles of organisms such as bacteria, spores, protozoa, and diatoms are adsorbed onto the surface of the material which causes sedimentation and causes primary adhesion of microorganisms which then forms a biofilm. A few days later formed several colonies of organisms such as primary colonies where microbes multispecies like larvae come and cause deposition on biofilm-forming a layer of the organisms most basic, then secondary colonies of organisms like barnacles that come as a result of the chemical activity of the resulting colonies of the main organisms and form a new layer and tertiary organisms colony where the substrate was previously colonized by invertebrate larvae and algae which grow and develop then become macroscopic individualist communities.

![Fig. 1 The Process of Biofouling](image-url)
Finally, within a few months, a macroscopic individualist community began to emerge which was the last layer consisting of complex organisms such as shells and ascidians, even containing salt deposits and corrosion [8][11]. Due to its non-cultivable, the exploration of marine bacteria has not been widely carried out [12]. Moreover, biofouling involves complex multi-species microbes.

3. Phytochemical content and antibiofouling activities of Carica seeds

Carica seeds component in the fruit itself reaches 14.3%. Carica seeds contain several secondary metabolite phytochemical compounds, namely phenols, alkaloids, flavonoids, tannins, and saponins [13]-[16]. Where these phytochemical compounds have been widely used in several studies, especially studies regarding antibacterial, anticancer, and cytotoxic effects [15]. Carica seeds extract (concentrations of 2.5%, 5%, 7.5%, and 10%) can inhibit the growth of bacteria (gram-positive and negative) [14]. Carica seeds have anticancer activity in female mice within 7 days of treatment. Apart from its activity as antibacterial and anticancer, Carica seeds also contain nutrients such as fatty acids, crude protein, potassium, calcium, magnesium, and myrosinase enzymes [16].

In the context of biofouling, the phenomenon of biofouling is initiated by the formation of a biofilm (bacterial layer) by marine bacteria [8]. The marine bacteria are non-cultivable, and their exploration is still rarely conducted [12]. Several studies have been conducted to determine the antibacterial activity in Carica seeds, such as antibacterial testing using gram-positive and gram-negative bacteria. The following is the quantitative data for the bacterial inhibition zone by Carica seeds.

Table 1. Antibacterial Activities of Carica Seeds

| Solvent           | Bacteria                          | Activities          |
|-------------------|-----------------------------------|---------------------|
| Ethanol 70% [18]  | *Salmonella typhi*                | Zone inhibition:10 mm |
| Ethanol 80% [19]  | *Staphylococcus aureus*           | Zone inhibition:11,5 mm |
|                   | *Escherichia coli*                | Zone inhibition:9,1 mm |
| Ethyl acetate     | *S. flexneri, E. Coli, S. aureus, dan B. cereus* | Significantly indicates antibacterial activity |
| Petroleum ether   | *Escherichia coli*                | Zone inhibition:3,6 mm |
|                   | *Staphylococcus aureus*           | Zone inhibition:2,9 mm |

The zone of inhibition is a clear area that will form around the bacterial area if there is an antibacterial substance in it. If a clear area is formed, it shows that these antibacterial substances can inhibit bacterial growth [22]. Based on the table, it is known that Carica seeds is the potential to inhibit bacteria based on the several sources above. Although these bacteria are not directly isolated from the sea, because of the nature of marine bacteria themselves, they are non-cultivable [12].

Antibiofouling agents can be obtained from antibacterial material. This is because the initial stages of biofouling are caused by a layer of bacteria that adheres to the surface of objects submerged in seawater [8]. On the other hand, the Carica seeds contain several secondary metabolite phytochemical compounds, namely phenols, alkaloids, flavonoids, tannins, and saponins [13][16]. The compounds have antibacterial activity. The following is the mechanism of active compounds in Carica seeds which have potential to become antibiofouling agent.
3.1 Damaging Cell Membranes/ Membrane Integrity

Figure above shows the conditions of bacteria exposed to the active compound. In general, phenols, alkaloids, and flavonoids inhibit biofilm-forming bacteria by means of bacteria under normal conditions (A), then exposed to active compounds that cause the condition of the cell membrane or the integrity of the membrane is damaged (penetrates the cell membrane) (B), therefore the active compound can enter into the internal bacteria and damage the internal parts, then the bacterial components will break down (C). Membrane integrity in bacteria plays an important role in maintaining internal conditions for the metabolism and transduction of bacterial energy. The relatively small damage to the integrity of the membrane results in inhibition of cell growth and even death from these bacteria [23]. Saponin compounds are also shown to work the same way, which causes lysis in bacteria. The following are observations by using Scanning Electron Microscopy (SEM) [24].

3.2 Acting as an Antibiofilm

Tannin compounds have anti-biofilm properties, which are the precursors to the formation of biofouling. In general, tannin compounds work by splitting the peptidoglycan layer, moreover, cause decrease in the thickness of the biofilm. Also, tannin compounds inhibit the primary adhesion of cells on the surface, and also reduce the production of adhesin between polysaccharide cells. The tannin compounds in Chinese Bayberry leaves can reduce biofilm formation by 84% by changing the membrane integration [25]. A compound derived alkaloids such as 2-aminoimidazole, berberine, and Pyrrole-imidazole alkaloids also can inhibit the formation of bacterial biofilms [26].

Fig. 2 Bacteria Exposed to Phenol, Alkaloids, and Flavonoids

Fig. 3 Bacterial Conditions after Exposure to Saponins

Fig. 4 Illustration of Thinning Biofilm Layer
3.3 Inhibits DNA and RNA Synthesis
Generally, alkaloids such as class pergularinine, tylophorinidine, and indolizidene work by inhibiting the synthesis of nucleic acids. This is caused by inhibition of dihydrofolate reductase, whose activity is very important in the production of pyrimidine and purine precursors for amino acids, RNA, and DNA biosynthesis [26]. Also, the flavonoid derivatives such as robinetin, myricetin, and epigallocatechin show their activity inhibiting DNA synthesis in bacteria. The ‘B’ ring in flavonoids plays a role in hydrogen binding, this binding has an impact on the inhibition of DNA and RNA synthesis [27].

Observing the antibacterial and antibiofilm activity by active compounds such as alkaloids, flavonoids, tannins, phenols, alkaloids, flavonoids, tannins, and saponins, it can be stated that as per literature Carica seeds has the potential as an antibacterial and antibiofilm and become an antifouling agent.

4. Extraction of antifouling agent from Carica seeds
Extraction methods are used to extract secondary metabolites, such as phenols, alkaloids, flavonoids, tannins, and saponins. Chloroform-methanol extract has active antioxidant activity (phenols and flavonoids). Where this antioxidant also has a role in antibacterial. The antioxidant activity (phenol and flavonoids) of Carica seeds was also found in the solvent extraction with ethanol, petroleum ether, ethyl acetate, n-butanol, and water. In ethyl acetate and n-butanol fractions, higher antioxidants (phenols and flavonoids) were found than other fractions [17].

Table 2. Extraction of Carica Seeds Content [13]

| Extracts and Fractions | Fenol (mg GAE/100 gDW) | Flavonoid (mg RE/gDW) |
|------------------------|------------------------|-----------------------|
| Petroleum ether        | 522.67±94.3            | -                     |
| Ethyl acetate          | 1945.48±45.55          | 117.48±15.54          |
| n-Butanol              | 832.25±125.91          | 32.04±2.45            |
| Water                  | 276.64±47.53           | 4.22±0.14             |
| Ethanol                | 1132.41±162.58         | 22.47±0.69            |

Based on the above, the content of phenols and flavonoids that have antioxidant activity is present in the seeds extract of Carica extracted with ethyl acetate [13][17]. On the other hand, using Carica seeds powder extracted with water has the highest content, namely tannins (0.7213±0.01 content/contents (%)), then phenol (0.6799±0.009), saponins (0.2468±0.007), and flavonoids (0.124±0.01) [14].

5. Synthesis of antifoulant coating from Carica seeds
Producing coating anti-biofouling (antifoulant coating) from Carica seeds waste extract is expected to reduce the waste caused by the activities of the home industry. The Carica Craftsmen Association said that 25 Small and Medium Units (UKM) that produced candies, needed 90 tons of Carica fruits. The waste of Carica seeds produced reaches 9 tons/month [6].

Table 3. Various Research Related to Antifoulant Coating

| Antifouling Coating Materials | Concentrations |
|------------------------------|----------------|
| Sea sponges: *Agelas tabulata*, Myrmekioderma gyroderma, Oceanapia peltata, Aplysina lacunosa, Neopetrosia sp. and Holoturia glaberrima* [28] | 1% w/w |
| Papaya fruit adsorbed on activated carbon [29] | 24 grams |
| Nerium oleander flowers [30] | 100% with adding an additive |
| Rhizophora apiculata bark with the addition of zinc tannate [31] | 35 mg/ml |
| Erica manipuliflora extract [32] | 100% |
Based on the data above, it is known that the concentrations used by several researchers in making antifoulant coatings are variative. The effective concentrations to see antibacterial activity are 75% and 100%. Extracting Papaya seeds with 96% ethanol produced a yield of 11.7 % (w/w) from 350 g of dry raw material \textsuperscript{[33]}. If in Wonosobo regency produces waste of Carica seeds as much as 9 tons/month, then dried so that it becomes ±4 tons, and then extracted, it will produce a yield of as much as ±468 kg yield per month. If the seeds of Carica waste can be processed in total, the agent anti-biofouling that can be produced per month is ±468 kg yield. Which will then be added with coating additives (resin, hardener, and thinner).

From the calculation above, the Carica seeds has good prospects to be used as a raw material for the manufacture of antifoulant coatings. Indeed, natural products can be used for antifouling applications by coating the appropriate paint. However, making the antifouling layer using microbes or natural materials is a major challenge because these compounds can be quickly damaged in the environment \textsuperscript{[34]}. Therefore, coating materials such as resin, hardener, and thinner are needed, then combined with the Carica seed extract waste.

6. Conclusions and future perspectives
Based on our research using literature review method, the Carica seed which consider as waste has an antibacterial active compound, which are needed to prevent biofouling, also used in the manufacture of antifoulant coatings. According to our rough calculation, each month produced a yield of Carica seeds waste extract ±468 kg. Therefore, it can be concluded, Carica seeds waste has the potential to be used as an anti-biofouling agent for antifoulant coating. However, corresponding to some literatures, natural-based antifoulants have a drawback, the compounds damaged easily after being immersed in the water. Thus, it needs coating materials such as resin, hardener, and thinner which are then combined with the Carica seeds waste extract. Further research is needed to find out its effective composition.

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