The role of betel (Piper betle) leaf extract and glycerol on physical properties of bioplastic based on sago starch

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Abstract. Starch-based bioplastic from sago is one of the potential sources in Indonesia. Since it is made from natural ingredients, it is easier to absorb water and susceptible to the activity of microorganisms. Betel leaf extract is one of natural antimicrobial agent that rich of tannin and flavonoid. This study aims to investigate physical properties and antimicrobial activity of sago (Metroxylon sp.) starch-based bioplastic. The betel leaf was extracted by using the ultrasonication method with ethanol as a solvent. Betel leaf concentrated extract is diluted using aqua dest with a concentration of 2 g/100 mL (2%) to be applied as an antimicrobial agent in bioplastics. The compositions of glycerol were varied from 10-30% whereas betel leaf extract from 3-9%(v) in the manufacture of bioplastics. The results show that the highest density of 1.5 g/cm³ was achieved at the composition of glycerol and betel leaf extract, respectively, 30%(v) and 9%(v). The highest water absorption percentage was shown by sample with 30%(v) glycerol without addition of betel leaf extract, that was 83.31%. The antibacterial activity showed that the addition of betel leaf extract to bioplastics was able to inhibit the activity of bacteria (Bacillus cereus). The best inhibition were shown in bioplastics with a variation of 20%(v) glycerol and 9%(v) betel leaf extract addition. However, all variation did not show their inhibition with fungal activity (Aspergillus niger).

1. Introduction

Due to the negative environmental impacts of synthetic plastics, the development of bioplastics for commercial applications is essential today. Biodegradable plastics are made from starch, cellulose, chitosan, and protein extracted from renewable biomass. Some related studies have discussed the manufacture of bioplastics from several starch sources such as rice [1], corn [1], potato [2], banana peel [2], and sago [3]. Sago is potential starch source to be used in bioplastic because it is widely available in South East Asia [4]. The sago starch is composed of 70-80% amylopectin and 20-30% amylose. It makes sago starch is easy to gelatinize and to dissolve in cold water. It also makes sago has a high viscosity [5]. Nevertheless, starch in bioplastics have several weaknesses since it is neither resistant to oxidation nor microbiological activity (no antibacterial and antifungal) [6].

Betel leaves is widely used as a post-meal mouth freshener and the crop is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan, and other Southeast Asian countries [7]. Betel leaves are rich in polyphenols compounds such as tannins and flavonoids. Phenolics compounds are a large diverse group of secondary plant metabolites including phenolic acids, tannins, and flavonoids. Tannins are phenolic compounds that shown potential antiviral, antibacterial and anticancer effects.
Flavonoids are polyphenolic compounds that are the most abundant of phenolics plants [8]. The previous study had reported 0.5%-3% betel leaves extract showed the antibacterial activity against bacteria *Staphylococcus aureus* by clear zones around the well [9].

One method that can be used for the betel leaf extraction is ultrasonic method. The ultrasonic method can minimalize the extraction time and maximalize the amount of yield. However, this method is not suitable to extract heat-resistant natural sources that contains bioactive compounds [10]. The ultrasonic bath is the most popular type of ultrasonic device. It has one or more ultrasonic transducers with stainless steel tank. It also has temperature control with frequency of around 40 kHz [11].

In the process of preparing bioplastics, plasticizers are usually used as additives. The plasticizer is a non-volatile compound with low molecular weight. It makes the polymer chain become more flexible, more resistant to fracture, and the dielectric constant becomes higher. Besides, it makes the tension of deformation, viscosity, density, hardness, and an electrostatic charge of a polymer become lower [12]. Glycerol is one type of plasticizer that the most widely used mainly in polymer industry [13].

This research aims to find out the effect of various betel leaf extract and glycerol concentrations on the physical properties and antimicrobial properties of sago starch bioplastic. The physical properties such as density and water absorption were investigated in this study. The antimicrobial was done for bioplastic films by investigating its inhibition on bacteria *Bacillus cereus* and fungi *Aspergillus niger*.

2. Methods
The materials needed in this study are betel leaves, ethanol, sago starch, aquadest, glycerol, and the starter of *Bacillus cereus* and *Aspergillus niger*. Pieces of equipment that needed are blender, oven, ultrasonic bath, 50 mesh sieve, filter paper, measuring cylinder, erlenmeyer flask, beaker glass, rotary evaporator, analytical balance, hotplate, magnetic stirrer, stirring rod, calipers, petri dish, and acrylic plates.

2.1. Preparation of Betel Leaf Extract
Betel leaves were cut and cleaned by washing. Dried betel leaves were obtained after the drying process in an oven at 50 ºC for 24 hours. Dried betel leaves were mashed with a blender until became powder. Betel leaf powder was sieved by using a 50 mesh sieve. The ultrasonic extraction was done by using an ultrasonic bath and ethanol 96% as a solvent with the betel leaf powder to the solvent ratio 1: 5 (w/v). The extraction process was extracted for 30 minutes at 40 ºC with filter paper. The filtrate was concentrated with rotary evaporator. Betel leaf concentrated extract was dissolved with water until its concentration become 2% (2 g/ 100 mL) for bioplastic antimicrobial application.

2.2. Preparation of bioplastic
Sago starch with 10 grams weight was dissolved with 100 mL aquadest. This mixture was added with variation of 0, 10, 20, 30% (v) glycerol. The starch solution is heated by using an hotplate at 70 ºC for 25 minutes and stirred by a magnetic stirrer agitation. After it was gelatinized, poured betel leaf extract without and with 3%, 6%, 9% (v) extract. The bioplastics were then casted on acrylic plates.

2.3. Density of Bioplastics
The samples of bioplastic film from each variation were cut become 2 x 2 cm in size. The film sample’s thickness is measured with a digital thickness gauge. The sample’s volume is calculated. The samples were weighed. Then, the density of sample can be determined with equation (1) [14].

\[
\rho = \frac{m}{v}
\]

where \( \rho \) is density of sample
\( m \) is mass of sample
\( v \) is volume of sample
2.4. Water Absorption of Bioplastics
The samples of bioplastic film from each variation were cut become 2 x 2 cm in size and were immersed in water. The samples were weighted once for each 30 minutes of immersion. Then weighing will be stopped when the constant weight got. The percentage of water absorption (\%WA) value can be determined with equation (2) [15].

\[
\%WA = \left( \frac{M_t - M_0}{M_0} \right) \times 100\%
\]  

where \( M_t \) is mass of sample after immersed in water
\( M_0 \) is mass of sample before immersed in water

2.5. Antimicrobial Activity Analysis
The disk diffusion method was used in this study for finding out the inhibition of samples on bacteria Bacillus cereus and fungi Aspergillus niger. The bacteria and fungi starter are inoculated on agar media on two separate petri dish and incubated. Samples of bioplastics film are cut become a circle with 6 mm diameter size and put on the dish surface. The petri dish is incubated at 37 °C for 24 hours. The diameter of the inhibitory zone was shown by the clear color surrounding the sample. It is measured by using calipers [6].

3. Result and discussion

3.1. Density of Bioplastic
Figure 1 shows the betel leaf extract and glycerol impact on bioplastic film’s density. The highest density value is 1.515 g/cm\(^3\) from the variation of the bioplastic sample with 9% betel leaf extract and 30% glycerol while the lowest density is 1.164 g/cm\(^3\) was obtained in the variation without the addition of betel leaf extract (0%) and without the addition of glycerol plasticizer (0%).

![Figure 1](image.png)

**Figure 1.** The effect of betel leaf extract and glycerol addition on density

Figure shows that the glycerol and betel leaf extract addition in bioplastic film has increased the density. It is due to the betel leaf extract has a higher density value than water whose composition dominates bioplastic. During the gelatinization process, the glycerol plasticizer and betel leaf extract
are absorbed into the starch granules. This led the bioplastic structure to become denser with the increasing composition of glycerol and betel leaf extract added to bioplastics [16].

3.2. Water Absorption of Bioplastic

Figure 2 shows the betel leaf extract and glycerol impact on bioplastic film’s water absorption. The highest water absorption value i.e 80.2% was obtained by bioplastics without the addition of betel leaf extract and the addition of 30% (v) glycerol. While, the lowest water absorption value viz. 36.1% (v) was obtained with the addition of 30% (v) betel leaf extract and without the addition of glycerol.

Figure 2 shows that betel leaf extract addition decreases bioplastic film absorption in water. This is due to the strong bond between polyphenolic compounds (tannins and flavonoids) in betel leaf extract and sago starch molecules, causing hydrophobic interactions in bioplastics.

Tannins and polyphenolic compounds interact with amylose and amylpectin molecules in gelatinized starch. Increased swelling of amylose and amylpectin chains allows polyphenol compounds to bind specifically to starch molecules through hydrogen bonds and cause hydrophobic interactions [17].

Meanwhile, glycerol addition represents the increasing of water absorption. This is due to the glycerol’s hygroscopicity. It makes bioplastic film tends to absorb water. This hygroscopic nature is due to the presence of three hydroxyl groups in the glycerol molecule [18].

3.3. Antimicrobial activity

Figure 3 shows that all variations of betel leaf extract and glycerol in bioplastic have an antimicrobial activity for bacteria Bacillus cereus growth. It is showed with the inhibitory zone that is a clear area around the samples. The higher betel leaf extract composition, the higher inhibitory zone diameter will be. It is because more concentration of betel leaf extract in bioplastic makes more tannin and flavonoids. Tannins are compounds that can deactivate microbial adhesion and obstruct hydrolytic enzymes such as carbohydrates, proteases, and cell wall transport proteins [19]. While, flavonoids are compounds that can kill bacteria, activate antibiotics, and weaken the pathogenicity of bacteria [20].

Figure 2. The Effect of glycerol and betel leaf extract addition on water absorption.
Glycerol makes an antibacterial impact to bioplastic since it is polyol compound that has three free hydroxyl groups [21]. More composition of glycerol makes the inhibitory zone larger. However, bioplastics at 30% glycerol variation have a difference that diameter inhibitory zone becomes smaller. It is due to glycerol is hygroscopic. Glycerol will increase the moisture and absorption of water in bioplastics. Bacteria loves moisture to grow and reproduce.

Antimicrobial agents can kill microbes, for example for disinfection and protection of materials against microbes. Antimicrobial agent organic compounds derivation with three until six hydroxyl groups and consist of: ethers, esters, carbonates, or carbonate derivatives of polyols. While glycerol is a polyol compound with free hydroxyl groups [21].

![Figure 3. Antimicrobial activity analysis of bioplastic for different glycerol concentration](image)

(a) without Betel Leaf Extract (b) 3% Betel Leaf Extract (c) 6% Betel Leaf Extract (d) 9% Betel Leaf Extract

Figure 4 shows the result of antifungal activity analysis for all variation of bioplastic films. All variations of bioplastics do not show any inhibition for *Aspergillus niger* growth. It indicates that all variations of bioplastic at these concentration have no ability to inhibit fungi growth. Neither betel leaf extract nor glycerol can inhibit the *Aspergillus niger* growth. This proves that at all these concentrations it is still not sufficient to inhibit or kill fungal in bioplastics. Further studies are needed to find out the minimum concentration of betel leaf extract to against the *Aspergillus niger*. 
Figure 4. Antifungal activity analysis of bioplastic for different glycerol concentration (a) without Betel Leaf Extract (b) 3%(v) Betel Leaf Extract (c) 6%(v) Betel Leaf Extract (d) 9%(v) Betel Leaf Extract

4. Conclusion
The highest density was obtained by sample with 30%(v) glycerol and 9%(v) betel leaf extract. Sample with 30%(v) glycerol and without betel leaf extract shows the highest water absorption percentage which was 83.3%. All samples of bioplastics have ability to inhibit the *Bacillus cereus* growth. All variations of bioplastics do not show any inhibition for *Aspergillus niger* growth. Further studies are needed to find out the minimum concentration of betel leaf extract to against the *Aspergillus niger*.

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