Characteristics of bioplastics based on sago starch contain betel (piper betle) leaf extract: Effect of glycerol as plasticizer

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Abstract. The presence of glycerol as plasticizer can improve elasticity of bioplastic. Betel (Piper betle) leaf extract contains tannin and flavonoid which both of them take a role in antioxidant, antibacterial, and antifungal. This study aims to investigate the effect of various glycerol as addition in bioplastic based on sago starch (Metroxylon sp.) which includes density, water absorption, and antimicrobial activity. The betel leaf extract is also added in the bioplastic to help the antimicrobial activity. The betel leaf extraction process used the ultrasonication method for 30 minutes using ethanol as a solvent. The betel leaf concentrated extract then is diluted using aquadest with a concentration of 2 g/100 mL (2%) to be applied as an antimicrobial agent in bioplastics. The results show the highest density was 1.515 g/cm$^3$ indicated by the variation of 30% glycerol. The highest water absorption percentage was shown by samples with 30% glycerol addition which was 75.62%. The antibacterial activity showed that the addition of glycerol to bioplastics was able to inhibit the activity of bacteria (Bacillus cereus) with the best inhibition results were shown in variation with 20% glycerol. However, all variation did not show their inhibition with fungal activity (Aspergillus niger).

1. Introduction
Biodegradable plastics or commonly known as bioplastics are plastics that can be used like conventional plastics, but after they are used, bioplastics can be easily discarded because they will break down decomposed by a microorganism [1]. Thermoplastic Starch (TPS) is relatively new material as biodegradable plastic. Starch is an inexpensive natural biopolymer [2]. One of the plants that have the potential to be a source of starch is sago (Metroxylon sp).

However, prior to production of such material, the structure of native starch should be suitably modified. This is necessary because starch is a multi hydroxyl polymer. There were vast intermolecular and intramolecular hydrogen bonds in starch that native starch is considered not a true thermoplastic. But in the presence of plasticizer at high temperature and under shear it readily melted and flowed [2]. The addition of plasticizers to edible films is important to overcome the brittle nature of the film due to extensive intermolecular forces [3].

Plasticizers are an important class of low molecular weight non-volatile compound that are widely used in polymer industries as additive. These substances reduce the tension of deformation, hardness, density, viscosity and electrostatic charge of a polymer, at the same time as increasing the polymer chain flexibility, resistance to fracture and dielectric constant. Other properties are also affected is resistance to biological degradation [4]. The most widely used type of plasticizer is glycerol [3].

This research aims to investigate the effect glycerol as plasticizer on the characteristic of bioplastic based on sago starch. Besides the glycerol addition, betel leaf extract is also added in the bioplastic to
improve the biological resistance properties of starch against bacteria and fungi [5]. Both betel leaf extract and bioplastic are analysed in this study. The analysis of betel leaf extract are yield, tannin content, and flavonoid content. The analysis of bioplastic are density, water absorption, and antimicrobial activity.

2. Research methods
Material used in this study are sago starch, aquadest, glycerol, betel leaves (from the market in Medan), ethanol, folin-ciocalteu, gallic acid, quercetin, and isolate of bacteria (Bacillus cereus) and fungi (Aspergillus niger). The equipment that used in this research are analytical balance, beaker glass, hotplate, magnetic stirrer, acrylic plates, thickness gauge digital, petri dish, oven, ultrasonic bath, blender, 50 mesh sieve, Whatman no.41 filter paper, rotary evaporator, and UV-Vis Spectrophotometric.

2.1. Preparation of betel leaf extract
1-2 cm betel leaves were dried using the oven for 24 hours at a temperature of 50 °C. The betel leaves were then blended and sieved to get 50 mesh size. 50 g of powder is weighed and mixed with 96% alcohol at a ratio of 1: 5 (w / v). The mixture was processed using an ultrasonic bath at 40 °C for 30 minutes. The mixture is then filtered using filter paper. The filtrate is then evaporated so that it becomes a thick liquid using a rotary evaporator. In application for bioplastic, betel leaf extract was diluted with distilled water at a concentration of 2% (2g / 100 mL).

2.2. Preparation of bioplastic
100 mL of aquadest was added to 100 g of sago starch in a Beaker glass. The sago starch solution was then added glycerol with variations of 0%, 10%, 20%, and 30% (v/v). Nine percent (9%) of betel leaf extract was added to the solution and heated at 70 °C for 25 minutes while stirring using a magnetic stirrer. The mixture was then moulded on acrylic plate by casting method.

2.3. Yield determination
Yield determination was done by weighing the betel leaf extract concentrated. Then the mass of betel leaf extract concentrated was compared with the mass of betel leaves powder. Yield percentage was calculated by using equation (1) [6].

\[
\% \text{ Yield} = \left( \frac{\text{mass of betel leaf extract concentrated}}{\text{mass of betel leaf powder}} \right) \times 100\%
\]  

2.4. Determination of tannin and flavonoid content
Determination of tannin and flavonoid content is done by using UV-Vis Spectrophotometric. The determination of tannin content uses folin-ciocalteu as reagent and gallic acid as standard solution. The tannin was obtained at 765 mm wavelength. The determination of flavonoid content uses quercetin as standard solution. The flavonoid was obtained at 436 mm wavelength.

2.5. Density of bioplastics
Bioplastic density was obtained by cutting 2 x 2 cm of bioplastic and then weighing it (m). Volume (V) was determined by measuring the thickness of the bioplastic. Furthermore, the density is calculated using the equation (2) [7].

\[
\rho = \frac{m}{V}
\]
2.6. Water absorption of bioplastics

2 x 2 cm size of bioplastic was weighed \((M_0)\), and then immersed in water. Every 30 minutes the bioplastic was removed from the water and dried and weighed \((M_t)\), then soaked again until a constant weight is obtained. Water absorption \((%WA)\) is calculated using the equation (3) [8].

\[
%WA = \frac{(M_t - M_0)}{M_0} \times 100\%
\] (3)

2.7. Antimicrobial activity analysis

6 mm in diameter bioplastic was prepared and placed in a petri dish. In other petri dishes, Bacillus cereus and fungi Aspergillus niger were inoculated on agar medium. Furthermore, the bioplastics in petri dishes were incubated for 24 hours at 37 \(^\circ\)C. The inhibitory area was then measured which is indicated by the clear colored [9].

3. Result and discussions

3.1. Yield determination

Yield analysis is used to determine how success the extraction process is. Yield percentage is described the amount of compound active which can be extracted in the substance. The yield that was obtained in this study is 16.4\%. It shows that the ultrasonic extraction method is confirmed as an effective way to extract betel leaf.

3.2. Determination of tannin and flavonoid content

Determination of tannin and flavonoid content is done by using UV-Vis Spectrophotometric. The determination of tannin content uses folin-ciocalteu as reagent and gallic acid as standard solution. The tannin was obtained at 765 mm wavelength. The determination of flavonoid content uses quercetin as standard solution. The flavonoid was obtained at 436 mm wavelength. The tannin and flavonoid content of betel leaf extract were obtained in this study are 20.33\% and 5.99\% respectively.

3.3. Density of bioplastic

In order to evaluate the effect glycerol addition in bioplastic film, the density analysis were determined. The highest density value is 1.515 g/cm\(^3\) which is obtained in the variation of 30\% glycerol addition.

Figure 1. The effect of addition in bioplastic density

Figure 1 shows the effect glycerol addition in bioplastic film. The addition of glycerol increases the density value. This is because glycerol have a higher density value than water whose composition dominates bioplastics. High density values indicate that bioplastic has a dense structure and is difficult to penetrate fluids such as water and oxygen.
3.4. Water absorption of bioplastic

Water absorption in starch-based bioplastics significantly affects the properties and characteristics of the bioplastics. Therefore, it is necessary to investigate the ability of bioplastics to absorb water. The highest water absorption value was obtained by bioplastics with addition of 30% glycerol variation with 75.62% water absorption percentage.

Figure 2. The effect of glycerol addition in bioplastic water absorption

Figure 2 represents that glycerol addition increases the water absorption of the bioplastic film. This is because the glycerol plasticizer is hygroscopic so it tends to absorb water. The bioplastics water absorption rate has been influenced by the interaction between hydroxyl groups of starch and glycerol with molecules of water. Glycerol is hygroscopic and has -OH group to bind with water through hydrogen interaction [10].

3.5. Antimicrobial activity of bioplastics

The antibacterial and the antifungal activity analysis of bioplastics were done for all variation of bioplastic. Figure 3 shows the antibacterial activity of bioplastic films. The antibacterial activity show that all samples can inhibit Bacillus cereus growth. It was confirmed by the inhibition zone around the bioplastic film which was showed on Figure 3. The highest diameter of inhibitory zone is 25.8 mm which is obtained in 20% glycerol addition.

Figure 3. Antibacterial activity of bioplastic films
Figure 4. Effect of glycerol addition in antibacterial activity

Figure 4 shows the effect of glycerol addition in antibacterial activity of bioplastic. The increasing of glycerol is also increasing diameter of inhibitory zone. However, inhibitory zone diameter of bioplastic with 30% glycerol addition decreases. This is because glycerol is hygroscopic so the more glycerol, the higher water content and moisture will be. Bacteria need water and moisture to grow and reproduce.

An antimicrobial agent consists ether, ester, carbonate or carbamate derivatives of polyols. Antimicrobial agents are derived from organic compounds containing three until six hydroxyl groups. Antimicrobial agents are used to kill microbes on contact such as in disinfection applications as well as to preserve and protect materials against microbial attack [11]. Glycerol is a polyol compound which has several free hydroxyl groups [12].

Figure 5. Antifungal activity of bioplastic films

The antifungal activity analysis are done for all bioplastics. The results shows that no variation of bioplastics have inhibition for Aspergillus niger growth. Figure 5 shows that glycerol have no ability to inhibit the fungi growth. Fungi have a more complex structure than bacteria. Fungi are multicellular organisms and have cell walls while bacteria are unicellular and does not have a cell wall. This is the reason why the glycerol show inhibitory properties of bacteria but not fungi.
4. Conclusions
The ultrasonication extraction method is confirmed effective to extract betel leaf. The yield of extraction is 16.4%. The tannin and flavonoid content of betel leaf extract in this study is 20.33% and 5.99% respectively. The results show the best density was shown by bioplastic with the highest glycerol composition. The highest water absorption value was obtained by bioplastics with the addition of 30% glycerol. The antibacterial analysis shows that all samples of bioplastics were able to inhibit the Bacillus cereus growth. Bioplastic with 20% glycerol has the best antibacterial properties. The antifungal analysis shows that all variation of bioplastics do not have any inhibition for Aspergillus niger growth.

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