The Effect of Betel (piper betle) leaf extract as antimicrobial agent on characteristics of bioplastic based on sago starch

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Abstract. Betel leaf extract contains many phenolic compound such as tannin and flavonoid which have antibacterial and antifungal properties. This study aims to investigate the effect of betel leaf extract addition in bioplastic based on sago starch (Metroxylon sp.) which includes density, water absorption, functional group characterization and antimicrobial activity. The betel leaf was extracted by using the ultrasonication method with ethanol as a solvent. Then betel leaf concentrated extract is diluted using aquaest with a concentration of 2 g/100 mL (2%) to be applied as an antimicrobial agent in bioplastics. The compositions of betel leaf extract as antimicrobial were varied in bioplastics making. The results show the highest density was 1.448 g/cm³ indicated by the variation of 9% betel leaf extract. The highest water absorption percentage was shown by sample without betel leaf extract addition (0% betel leaf extract), which was 75.56%. 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 with 9% betel leaf extract addition. However, all variation did not show their inhibition with fungal activity (Aspergillus niger).

1. Introduction

Nowadays starch-based bioplastics have become the most studied because they are renewable, cheap and abundant in nature. There are several sources of starch that have been applied in bioplastic making such as potato starch [1], banana peel starch [1], corn starch [2], rice starch [2], and sago starch [3]. Sago is a plant that has the potential to be used in South East Asia because of its high amount [4]. The sago starch is consist of 20-30% amylose and 70-80% amyllopectin which make sago starch difficult to dissolve in cold water, easy to gelatinize, high viscosity, hard and not expands in baking process [5]. However, starch-based bioplastics have several disadvantages such as having poor anti-oxidation and bad biological activity (no antibacterial and antifungal) [6].

Betel (piper betle) leaf is known as a traditional medicinal plant which contains polyphenol compounds such as tannins and flavonoids [7]. Tannins and flavonoids are phenolic compounds that have antifungal and antibacterial inhibitory properties.

The ultrasonic extraction method is non-destructive and non-invasive, so it can be easily adapted to various applications [8]. The advantages of this method are speeding up the extraction process, being safer and increasing the amount of yield. Ultrasonics can also reduce the operating temperature of extracts that are not heat-resistant, making it suitable for application in the extraction of heat-resistant bioactive compounds [9].

This research aims to investigate the effect of betel leaf extract as antimicrobial agent on the characteristic of bioplastic based on sago starch. Both extract and bioplastic are analysed in this study. The analysis of betel leaf extract are functional groups and antimicrobial activity. The analysis of bioplastic are functional groups analysis by FTIR, density, water absorption, and antimicrobial activity.

2. Methods

Material used in this study are betel leaves (from the market in Medan), ethanol, sago starch, glycerol, aquaest, folin-ciocalteu, gallic acid, quercetin, the starter of Bacillus cereus and Aspergillus niger. The
equipment that used are oven, ultrasonic bath, blender, 50 mesh sieve, Whatman no.41 filter paper, analytical balance, beaker glass, petri dish, rotary evaporator, hotplate, magnetic stirrer, acrylic plates, and Fourier Transform Infrared (FTIR).

2.1. Preparation of piper betle leaf extract
Betel leaves are washed with water and cut until 1-2 cm size. The leaves are dried in oven at 50 °C for 24 hours then pulverised by using blender and sieved by using 50 mesh. The betel leaves powder is weighed as much as 50 gram and added ethanol 96% with ratio 1: 5 (w/v). The betel leaf powder be extracted using ultrasonic bath for 30 minutes at 40 °C and filtered by using filter paper. The filtrate is thickened by using rotary evaporator. The functional groups of the betel leaf extract are investigated by using FTIR. Betel leaf concentrated extract was dilute with water with 2% concentration (2 g/ 100 mL) for bioplastic antimicrobial application.

2.2. Preparation of bioplastic
10 grams of sago starch is weighed and put in beaker glass then added with 100 mL aquadest and 20% glycerol (v/v). The starch solution is heated for 25 minutes at 70 °C with magnetic stirrer agitation and added 9% betel leaf extract with variation 0%, 3%, 6%, 9% (v/v). The bioplastics are casted on acrylic plates.

2.3. FTIR analysis
The FTIR is used to investigate functional groups of betel leaf extract and bioplastic film. The sample is scanned by using infrared spectrum with wavenumber 4000-400 cm⁻¹. The transmittance and wave number are plotted. The FTIR peaks indicate a specific functional groups.

2.4. Density of bioplastics
Bioplastic film is cut with 2 x 2 cm size and the thickness is measured. The volume is calculated. The sample is weighed. The density was calculated by using equation (1) [10].

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

where m and v are mass and volume of samples, respectively.

2.5. Water absorption of bioplastics
Bioplastic film is cut with 2 x 2 cm size and soaked in water. Weighing the sample is carried out every 30 minutes of immersion until a constant weight was obtained. The percentage of water absorption (%WA) value was calculated by using equation (2) [11].

\[
\%WA = \left(1 - \frac{M_{t}}{M_{o}}\right) \times 100\%
\]

where \(M_{o}\) and \(M_{t}\) are weights of sample before and after soaking, respectively.

2.6. Antimicrobial activity analysis
Microbes or microorganism are very small organism which can only be observed with a microscope. Bacteria and fungi belong to microbes. The bacteria Bacillus cereus and fungi Aspergillus niger are inoculated on agar media on different petri dish. Sample betel leaf extract and bioplastic film are cut into 6 mm disk and put on the dish surface. The petri dish is incubated for 24 hour at 37 °C. The diameter of inhibitory zone which has the clear colored is measured by using calipers [6].
3. Result and discussions

3.1. FTIR analysis

The FTIR is used to investigate functional groups of betel leaf extract and bioplastic with betel leaf extract and glycerol addition. Then, both of the analysis results be compared. Figure 1 shows that both samples have similar graph. The data of FTIR spectrum range has been known by spectroscopy handbook [12].

Table 1 shows that both betel leaf extract and bioplastic with betel leaf extract and glycerol addition has O-H groups, C-H stretching groups, C-N groups, and C-O groups. Besides, C-O-H groups, C=O groups, and C=C aromatic groups are only found in betel leaf extract and not found in bioplastics with betel leaf extract. Meanwhile, the alkene group (C = C) is also only found in betel leaf extract bioplastics. The functional groups that have been identified through FTIR analysis are in accordance with previous research [13-14]. Ethanol extract of betel leaf was proven containing amine groups (NH), phenol alcohol (OH), ether/ester (CO) and alkenes (CH) [13]. Bioplastic with glycerol addition is showed alkyne groups, alkane functional groups (C-H), and in all spectra according to the groups (C=C) [14].

![Figure 1. FTIR characterization of betel leaf extract and bioplastic](image)

| Functional Group          | Wave number (cm⁻¹) | Betel leaf extract (cm⁻¹) | Bioplastic with betel leaf extract and glycerol (cm⁻¹) |
|---------------------------|--------------------|--------------------------|-------------------------------------------------------|
| Alcohol, Phenol (O-H)     | 3400-3200          | 3372                     | 3277                                                  |
| C-H stretching            | 3000-2850          | 2925                     | 2926                                                  |
| Aldehyde (C-O-H)          | 2900-2800          | 2855                     | -                                                     |
| Ketone (C=O)              | 1820-1660          | 1715                     | -                                                     |
| Alkene (C=C)              | 1680-1600          | -                        | 1647                                                  |
| Aromatic (C=C)            | 1600-1475          | 1510                     | -                                                     |
| Amina (C-N)               | 1350-1000          | 1268                     | 1336                                                  |
| Ether, ester (C-O)        | 1300-1000          | 1268                     | 1150                                                  |
3.2. Density of bioplastic

In order to evaluate the effect of betel leaf extract addition in bioplastic film, the density analysis were determined. The highest density value is 1.448 g/cm³ which is obtained in the variation with 9% betel leaf extract.

![Figure 2. The effect of betel leaf extract addition in bioplastic density](image)

Figure 2 shows the effect of betel leaf extract addition in bioplastic film. The addition of betel leaf extract increases the density value. This is because the betel leaf extract have a higher density value than water whose its composition dominates bioplastics. High density values indicate that bioplastic has a dense structure and is difficult to penetrate fluids such as water and oxygen.

3.3. Water absorption of bioplastic

The effect of betel leaf extract addition in water absorption of bioplastics are shown in Figure 3. The water absorption value decreases with the betel leaf extract addition. The highest water absorption value was obtained by bioplastics without betel leaf extract addition (0% betel leaf extract) with 75.56 % water absorption percentage. Meanwhile, the lowest water absorption value was obtained by bioplastics with the addition of 9% betel leaf extract with 68.35% water absorption percentage.

![Figure 3. The effect of betel leaf extract addition in bioplastic water absorption](image)

This result is accordance with previous research that reported that natural extract addition in bioplastic also decreases the water absorption value. The interactions between the functional groups of betel leaf extract and bio-polymers decrease polysaccharide-water interactions [6].
3.4. Antimicrobial activity of betel leaf extract

Antibacterial activity of betel leaf extract is investigated for betel leaf extract concentrated and betel leaf extract with 2% (2 g/100 mL) dilution. The antibacterial activity analysis is shown that both of the sample can inhibit the growth of *Bacillus cereus*. The inhibitory zone of extract is exhibited in Figure 4 by the formation of a clear zone around bacterial growth.

![Figure 4](image)

**Figure 4.** Antimicrobial activity analysis of betel leaf extract (a) Antibacterial activity of betel leaf extract concentrated (b) Antibacterial activity of betel leaf extract with 2% dilution (c) Antifungal activity of betel leaf extract concentrated (d) Antifungal activity of betel leaf extract with 2% dilution

The diameter inhibitory zone of antibacterial activity of betel leaf extract concentrated and betel leaf with 2% dilution is 44 mm and 14.4 mm respectively. It means that betel leaf extract concentrated has more inhibition ability better than betel leaf extract with 2% dilution. Tannins is related to their ability to inactivate microbial adhesions and inhibit hydrolytic enzymes such as proteases and carbohydase and cell envelope transport proteins [15]. Meanwhile, the flavonoid have three ways to inhibit the bacteria growth (directly kill the bacteria, synergistically activate the antibiotics, and attenuate the bacterial pathogenicity) [16].

The antifungal activity is exhibited in Figure 4. The antifungal analysis of the betel leaf extract concentrated is shown a large inhibition area which means it has potential to inhibit the fungal growth. However, betel leaf extract with 2% (2 g/100 mL) dilution has no inhibition for the *Aspergillus niger* growth. The diameter inhibitory zone of antifungal activity of betel leaf extract concentrated is 45.8 mm. It shows that actually betel leaf extract has potential to inhibit the fungal growth.

The betel leaf extract at 2% concentration is too dilute so that it is unable to inhibit the growth of *Aspergillus niger*. Fungi have a more complex structure than bacteria. Fungi are multicellular organisms and have cell walls. Meanwhile, bacteria have a simpler structure which is unicellular and does not have a cell wall. This is the reason why the betel leaf extract with a concentration of 2% show inhibitory properties of bacteria but not fungi. Therefore, it is necessary to investigate the minimum inhibitory concentration of betel leaf extract for *Aspergillus niger* species by doing the further research.
3.5. Antimicrobial activity of bioplastic

The antimicrobial activities of bioplastic are exhibited in Figure 5, 6 and 7. All of the bioplastic films can inhibit the growth of *Bacillus cereus*.

![Figure 5. Antibacterial activity of bioplastic films](image1)

![Figure 6. Antifungal activity of bioplastic films](image2)

Figure 7. Effect of betel leaf extract addition in antibacterial activity

Figure 6 shows the antifungal activity of bioplastic films. All variation of bioplastics do not have inhibition for *Aspergillus niger* growth. It shows that both betel leaf extract and 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. Meanwhile, bacteria have a simpler structure which is unicellular and does not have a cell wall. This is the reason why the betel leaf extract show inhibitory properties of bacteria but not fungi.

The effect of betel leaf extract addition is shown in Figure 7. The betel leaf extract addition is increased the diameter of inhibitory zone. An antimicrobial agent consist 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 [17].

4. Conclusions

The highest density was shown by bioplastic with the highest betel leaf extract composition. The highest water absorption value was obtained by bioplastic without the betel leaf extract addition (0% betel leaf extract). The antibacterial analysis shows that all samples of bioplastics can inhibit the *Bacillus cereus* growth. The antifungal analysis shows that all variation of bioplastics do not have any inhibition for *Aspergillus niger* growth. The betel leaf extract at 2% concentration is too dilute so that it is unable to
inhibit the growth of *Aspergillus niger*. Therefore, it is necessary to investigate the minimum inhibitory concentration of betel leaf extract for *Aspergillus niger* species by doing the further research.

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