Antibacterial Activity of Biodegradable Plastic from *Chromolaena odorata* (Pokok Kapal Terbang) Leaves

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**Abstract.** The aim of this research project was to develop antimicrobial films from blends of *C. odorata* and PVA and test the films for microbial activity using broth dilution methods for Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. The result shows that CO/PVA₈₀ successfully inhibit the growth of target bacteria. In antibacterial activity analysis, CO/PVA₈₀ showed 50% higher compare with pure PVA film, PVA₁₀₀. Other than that, the high percentage of PVA in the blend films, the greater the thickness, Tensile Strength (TS) and Young’s Modulus (YM), while the Elongation Break (EB) of the prepared films decreased. The 0.5 mm CO/PVA₈₀ film shows a good result in mechanical properties which is TS 6.55 MPa, YM 182 MPa and EB is 7.47%. A CO/PVA₈₀ were show a smooth texture, lacked of macropore and good characteristic with a SEM analysis. These results suggest that CO/PVA₈₀ films have good compatibility to form an antimicrobial film as a new material for medical application especially for wound healing.

1. Introduction

Nowadays, natural antimicrobials have become very important for global health due to their potential as antibiotics for many diseases [1]. Since the last half of the last century, many types of antimicrobials have been developed to destroy and inhibit the growth of bacteria. But now it is becoming more complicated as bacteria are increasingly developing resistance to certain vaccines or antibiotics. A study has shown that biofilms are a target for new compositions to inhibit bacterial growth in the medical field [2]. The addition of an active ingredient can improve the quality of a film. Some studies have shown that plants rich in phenolic compounds can be used as active agents due to their high antioxidant properties [3]. *Chromolaena odorata* (*C. odorata*) can be used as an active ingredient as it contains phytochemicals such as phenol which can act as an antioxidant, it also has good film forming properties. Previous study has proved the medicinal potential of *C. odorata* leaves for many skin diseases [4]. Other than that, many studies have found that *C. odorata* leaves contain many phytochemicals such as phenols, tannins, flavonoids, saponins and alkaloids which act as anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial and antioxidant on human skin [1,3,5–7] and also been used as a traditional medicine to treat skin infections and wounds [8,9].

To increase the hardness or mechanical stress of bioplastic, synthetic polymers such as polyvinyl alcohol (PVA) are sometimes used as one of the components of biodegradable plastic. PVA is also water soluble and a semi-crystalline polymer with technological potential as a biodegradable polymer [10]. Moreover, PVA are nontoxic, colourless, and odourless. This type of synthetic polymers has been used...
for many fields such as medicine, commerce, and industry to produce many products such as surgical threads, paints, and resins [11]. Antimicrobial film is a type of film that has antimicrobial properties following the concept of artificial skin to inhibit the growth of bacteria in open wounds. In the medical field, biodegradable polymers such as PVA, polylactic acid and polyamine acid can be used to develop the antimicrobial film because they degrade harmlessly over time after their function is completed. Many studies have shown that antimicrobial films are very effective in inhibiting the growth of bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Salmonella*, *Pseudomonas aeruginosa* and *Escherichia coli* [12,13].

In this research work, development of antimicrobial films from different percentage of PVA blends (20%, 40%, 60% and 80%) with *C. odorata* was fabricated. With the presence of antibacterial properties in *C. odorata* such as flavonoid will consider as a best source of raw materials that can be used to develop biodegradable films and polymer like PVA is a safer, inexpensive, non-toxic and efficient to use in medical sectors. Currently, only a synthetic polymer such as PVA was used for medical application and lacked of antibacterial property. Hence, the development of new bioplastic with an antibacterial property is one of the alternatives in medical applications especially in wound healing.

2. Methodology

2.1 Bioplastic fabrication

The leaves of *C. odorata* were collected from Kedah, Malaysia. The leaves were washed and dried at a temperature of 40°C for 24 hours. After drying, the leaves were ground into powder using a mechanical blender. After that, 2 g of *C. odorata* powder are mixed with 50 ml of distilled water and heated with magnetic stirrer at a temperature of 75°C for at least half an hour. Then 7.5 ml of glycerol is added and stirred further with constant magnetic stirring for at least half an hour. Then 20 g of PVA is mixed with 50 ml of distilled and heated and stirred at 90°C with magnetic stirring until the PVA is completely dissolved and a good film is formed. Then the films are prepared with different PVA concentrations from 20% to 100% (w/v) shows in Table 1. A *C. odorata* and PVA solution was blend together and heated at 85°C for 1 hour with a magnetic stirrer until homogenize before a casting process. For casting process5-10ml of CO/PVA was pipette into 5mm glass petri dish. The air bubble was removed by flaming, and dried that solution at 30°C for 24 hours until future used.

| Table 1. Bioplastic film ratio |
|-----------------------------|
| PVA (v/v) | Plant fibre (w/v) | Total volume (ml) |
| CO/PVA<sub>20</sub> | 20 | 2 | 100 |
| CO/PVA<sub>40</sub> | 40 | 2 | 100 |
| CO/PVA<sub>60</sub> | 60 | 2 | 100 |
| CO/PVA<sub>80</sub> | 80 | 2 | 100 |
| PVA<sub>100</sub> | 100 | 0 | 100 |

2.2 Antibacterial activity

Typical Soy Broth (TSB) is used as a medium in broth dilution methods. 9 g of TSB dissolved in 300 ml of distilled water before autoclave this TSB at 121°C, 1 atm for 15 min. A bacterial strain was getting from Biomaterial Laboratory, Faculty of Electronic Engineering Technology. This bacterium was culture onto Nutrient Agar (NA) for 16-24 hours. After 24 hours, a working culture was prepared by subculturing an overnight bacterium onto new NA plate. The inoculum of bacteria was prepared after the confluency of bacteria more than 70%. One millimetre of sterile distilled water was added into subculturing plate and ‘L-shape’ rod was used to harvest a bacterial inoculum. A number of bacterial cells was determined by use 0.5 McFarland, approximately 1.5 x10<sup>8</sup> CFU/ml bacteria. After that, bacterial inoculum was diluted in TSB to bring the concentration to 1.5x10<sup>4</sup> CFU/ml. Approximately 200 mg samples of CO/PVA were immersed in 12 ml of distilled water, sonicated at 50°C for 30
minutes. After 30 minutes, the sample were filtered by used Whatman no.4 and 500 µl of supernatant was added into 1500 µl TSB containing target bacteria. By use a 2-fold dilution, 1 ml of inoculum with an additive of CO/PVA film were transfer to the second tube until finish. All test tubes were incubated at 37°C for 16 to 24 hours. A Visible Spectrophotometer was used to determine a turbidity and density of all sample at OD600 after an incubation period.

2.3 Mechanical and Morphology analysis
Four hundred milligrams of CO/PVA bioplastic are immersed in beakers containing 50 ml of distilled water and incubate at 37°C for 24 hours. After that, films were removed from the water and dried in the desiccator for 24-48 hours until it reaches a constant weight before the measurement of final film dry weight. Solubility in water was performed for each film in three replicates and the solubility in water (%) is calculated using the following equation:

\[ \text{Solubility in water (\%) } = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100 \quad (1) \]

For the morphological studies, film was cut into a square shape (10 mm x 10 mm) and test by use Scanning Electron Microscope (SEM) to determine a surface roughness. The bioplastic CO/PVA was cut at 5 cm x 1 cm and by use a calliper to measure the film thickness. However, a Tensile Strength (TS), Elongation at Break (EB) and Young’s Modulus (YM) of elasticity were use the Instron 5848 Micro Tester testing machine. Lastly, the samples were analysed by a FTIR spectroscopy with a scan range of 400-4000 cm⁻¹ to evaluate their chemical composition.

3. Result and Discussion

3.1 Broth dilution test
The results of broth dilution methods for *E. coli* (Gram negative) were presented in Figure 1 and for *S. aureus* (Gram positive) in Figure 2. Three concentrations of broth dilution were tested, which are 200 mg/ml, 100 mg/ml and 50 mg/ml [14]. It is clearly seen that the absorbance decreases with increasing PVA concentration except for pure PVA or PVA100.

![Figure 1. Absorbance value for *E. coli*.](image-url)
PVA100 has the highest absorbance value for all concentrations, indicating that the number of bacteria in the solution was very high. The CO/PVA80 has the lowest absorbance value for both bacteria showed a good antibacterial activity of that film. Figure 1 and Figure 2 show decreasing of antibacterial activity from CO/PVA20 > CO/PVA40 > CO/PVA60 > CO/PVA80 compared without additivity of C. odorata in PVA100, it shown a high number of bacterial cells. This antimicrobial activity of CO/PVA have an excellent ability to inhibit growth of E. coli and S. aureus. This result supported by [11,15], an active phytochemical compound in plant will revealed its minimum bactericidal concentration against E. coli and S. aureus.

3.2 Solubility
Among all the films, CO/PVA20 had the lowest percentage of solubility, 38%, and PVA100 had the highest percentage of solubility, which is 70%. In this study, a film solubility increased with the higher amount of PVA in CO/PVA film which is CO/PVA80 (64%) > CO/PVA60 (58%) > CO/PVA40 (49%) > CO/PVA20 (40%). The main factor for a high solubility of the blend films is probably due to the percentage of hydrophilic compounds of PVA.

3.3 Thickness and tensile test
The thickness for CO/PVA80 was the thickest compared to the other blend films as it contains a high proportion of PVA. This study showed increase a PVA concentration in film blend cause increase in tensile strength of film. The greater addition of PVA matrix increases the strength and YM on the CO/PVA film and its shows that PVA is a polymer that has a high elasticity. In comparison, CO/PVA80 has the highest TS and YM, 6.55 MPa and 182 MPa respectively, compared with CO/PVA20 has the lowest TS (1.87 MPa) and YM (42.17). However, result from this study showed an EB decreased with the additional amount of PVA in film because of stress-strain changes from CO/PVA20 (19.62%) > CO/PVA40 (13.54%) > CO/PVA60 (8.55%) > CO/PVA80 (7.47%) show in Figure 3. In addition of amount of PVA decrease the value of EB and contradict with YM and TS value. Table 2 shows the result for thickness and tensile test for all blend films.
Figure 3. Tensile Strength and Elongation of Break

Table 2. Result for thickness and tensile test

| Sample    | Thickness (mm) | Maximum load (N) | Tensile Strength, TS (MPa) | Elongation at Break, EB (%) | Young Modulus, YM (MPa) |
|-----------|----------------|------------------|----------------------------|-----------------------------|-------------------------|
| CO/PVA20  | 0.38           | 6.7158           | 1.8655                     | 19.62                       | 42.1724                 |
| CO/PVA40  | 0.39           | 10.0275          | 2.5712                     | 13.54                       | 113.6401                |
| CO/PVA60  | 0.41           | 20.5222          | 5.0054                     | 8.55                        | 172.7964                |
| CO/PVA80  | 0.50           | 32.7466          | 6.5493                     | 7.47                        | 182.0021                |

3.4 Cross-section morphology
Figure 4 showed the SEM image for cross-section in 100x magnification for CO/PVA20, CO/PVA40, CO/PVA60 and CO/PVA80. The cross-section for CO/PVA80 shows the smooth morphology because the film contains 80% of PVA. The binding between C. odorata and PVA in this CO/PVA80 film was compact, smooth and strongest structure compare to CO/PVA60, CO/PVA40 and CO/PVA20. The characterization of CO/PVA80 easier to handle, lacked of macropore and fracture and this result was similar like Silva et al. (2021) [16], which is the accorporate a high PVA will increase a good characteristic of film. However, a morphology of CO/PVA20 cross-section showed a brittle characterization and has a visible macropores because of low concentration of PVA.

Figure 4. SEM for cross-section in 100x magnification of films, a) CO/PVA20; b) CO/PVA40; c) CO/PVA60; and d) CO/PVA80

4. Conclusion
In conclusion, the objective of development a bioplastic with antibacterial properties achieved. The bioplastic CO/PVA80 exhibit the highest antibacterial activity showing the best film sample for further use. In antibacterial activity analysis, PVA80 shows the least number of E. coli and S. aureus after 18-
20 hours of incubation compared to negative control, PVA_{100}. Therefore, CO/PVA_{40} has the best antimicrobial ability for both Gram-positive (S. aureus) and Gram-negative (E. coli). In the degradation test, a PVA_{100} film was highest solubility in water as the PVA is one of degradable polymer. The 80% CO/PVA blend films showed the second highest solubility compared to the other CO/PVA bioplastic. In term of mechanical properties, the maximum load that can be applied to CO/PVA_{40} was 32.75 N and TS was 6.5493 MPa, but the elongation was only 7.47%. The tensile strength for CO/PVA_{40}, CO/PVA_{40} and CO/PVA_{20} were 5.0054 MPa, 2.5712 MPa and 1.8655 MPa, respectively. For the future work, to increase the antibacterial properties of C. odorata film, maybe some of raw material treatment such as an acid-base treatment or different type of polymer needs to be used.

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