Fibre based bioplastic film from *Morus sp.* (mulberry) leaves for medical purpose

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Abstract. New trend was shown the application of biodegradable plastic was used in the medicinal field especially as a template for the 3rd and 4th degree of burning is extensively studied. Currently, a bioplastic in medicinal applications for burning treatment comes from various materials based on fossil oil and synthetic chemical. *Morus sp.* among abundant plant and normally used for skin and inflammation treatment in traditional practices. The aim of this research is to synthesis bioplastic by using Polyvinyl alcohol (PVA) incorporates with *Morus sp.* leaves as the alternative of the skin template for a burning patient. With the phytochemical in the leaves such as phenolic, flavonoid and high content of antioxidant, hopefully, it will able to enhance the cell growth on the burning site without any contamination from the bacteria. 2 g *Morus sp.* leaves was blend with different concentration of the PVA (20%, 40%, 60%, 80%, and 100%) to produce plastic films. This mixture was stirred under constant magnetic stirring at 95°C for 1 hour. The mixtures are then pouring into a glass petri dish and dried in an oven at 50°C for 24 hours. The characterization of bioplastic is based on an antimicrobial test, tensile test, microstructure analysis using SEM and chemical composition analysed using FTIR. According to the results, a bioplastic made up from mixes *Morus sp.* leaves with 80% PVA has the best mechanical and antimicrobial properties. Therefore, the result showed a possible application of the film as an alternative for the medical purpose.

1. Introduction

Nowadays, antimicrobial and biodegradable films have been used in many fields due to their function and safety aspect. The antimicrobial film is a type of films or coatings with antimicrobial properties that can prevent the growth of microorganisms. Some studies have shown that the purpose of antimicrobial film produced was to ensure the safety, quality and shelf life of a product by preventing microbial growth [1]. Antimicrobial film inhibits the growth of microorganisms and pathogenic and increase the safety and quality of a product [2] compared to other films that can only increase in term of the safety aspect. By improving the concept of artificial skin or xenograft tissue which is a transplanted tissue from donor to a recipient of a different species, the films may able to reduce the rejection rate of the implants because most of the tissue implants will activate the host immune response which results in rejection of the implants [3]. But the films only cannot enhance cell growth and reduce the infection of the bacteria.
Morus sp. contains high antioxidant properties [4], and the plastic films able to reduce and prevent the inflammation and growth of microorganisms such as Escherichia coli and Staphylococcus aureus, and degraded harmlessly after completing their function which made it as a user-friendly template. Morus sp. is a tropical tree with fast-growing ability that possesses great potential in the medical field [4]. Morus sp. tree has been used for various health benefits and proved to be rich in mineral, dietary fiber, vitamins as well as it is considered as a good source of nutrients. The Morus sp. leaves are been choose because it possesses the highest active component such as antioxidants [4] that can lower the risk of infection. The phytochemical compounds in the Morus sp. leave which is flavonoids and phenolic compound. The Morus sp. leaves extract such phenolic compound will act as an active antimicrobial agent that can help to prevent the growth of bacteria and chronic inflammation [5]. By the incorporation between Morus sp. and poly-vinyl alcohol (PVA), the biodegradable plastic that contains antimicrobial properties and able to degrade harmlessly can to be produced.

PVA is a type of synthetic polymer which is soluble in water and crystalline in structure. This type of polymer has been used in industrial, commercial and one of the studies stated that PVA also has been used in pharmaceutical and biomedical areas due to its excellent film-forming [6]. PVA has a great potential to be used as a material for biofilm due to its good film-forming capability, odorless, non-toxic, high elasticity and tensile strength, transparency and resistance to the chemical [7]. By incorporation of Morus sp. in the production of antimicrobial and biodegradable plastic which partially replacing the plastic’s polymer, it can reduce the production cost and increase the degradation process. Therefore, this project was carried out to develop a film based on blends of Morus sp. with PVA and to evaluate the characterization and the effectiveness of the film against the microbial growth

2. Materials and Methods

2.1. Chemical and Reagents
Fresh Morus sp. leaves were collected in Perlis, Malaysia. The Morus sp leaves were collected 2 hours earlier before the experiment is started to maintain the freshness of the leaves. Poly-vinyl alcohol (PVA) 96% hydrolysis, glycerol, Nutrient Agar (NA) and Tryptic soy broth (TSB) were purchased from Merck-Millipore (Selangor, Malaysia).

2.2. Preparation of samples
After collection, Morus sp leaves were washed with running tap water, followed by rinsing with distilled water [4] and left in room temperature for 5 minutes to dried water. After that, the Morus sp leaves were cut into small pieces [4] by using scissors and then placed in an incubator at 50°C for 24 hours [8] until it was completely dry. The dried leaves were grounded by using the electrical blender and then sieved through a plastic filter to get a fine powder. Finally, the Morus sp. leave powders were kept in a sealed container with silica to prevent the contamination of fungus.

2.3. Blend PVA/Morus sp.
The Morus sp leaves powders were blended with different ratios of PVA (20%/40%/60%/80%) and were processed into films by a casting method.

2.3.1. Preparation of Morus sp. solution.
2g of Morus sp leaves powders were mixed with 100ml of distilled water. The mixture was heated and stirred at 75°C for at least 1 hour on a magnetic stirrer until completely dissolved. This mixture was continue stirred at 85°C and 15ml of glycerol was added into the solution as the plasticizer which can improve the permeability of the films [9]. Heat and stir the solution for 1 hour until completely mixed.

2.3.2. Preparation of PVA solution.
20%, 40%, 60% and 80% of PVA were mixed with 100ml distilled water respectively. The mixture was heated and stirred at 85°C for 1 h until completely dissolved.
2.3.3. Preparation of bioplastic film.
A *Morus sp.* solution was added into the PVA solution. Then, the mixture was heated and slowly stirred at 85°C for 1 hour until completely mixed to avoid the formation of bubbles. The aliquot was spread over a circular glass petri dish with a diameter 10cm and placed into an incubator at 50°C for 24 hours. After 24 hours, bioplastic films peeled off from glass petri dish, labeled and placed into the desiccator for future analysis.

2.4. Antimicrobial test

2.4.1. Bacterial preparation.
*Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*Staphy*) were cultured on nutrient agar (NA) for 24 hours at 37°C to activated a strain and to get a single colony. Then, from the single colony, the bacteria were prepared for analysis by adjusted an inoculum to 0.5 McFarland standards (5 × 10⁵ CFU/ml).

2.4.2. Broth dilution method.
1g of each bioplastic film (20%/40%/60%/80%) was dissolved into 10ml of Tryptic Soy Broth (TSB) using a double-boiling method for the plastic film solution preparation. Then, 0.1g/ml of plastic film solution was added into 1ml of inoculum then tested in triplicate, respectively. The preparation of positive control using Doxycycline 100mg was dilute in 10ml of distilled water. Then, 10mg/ml of Doxycycline were added into 1ml of inoculum. For the negative control, only added 1ml of TSB into 1ml of inoculum. All test tubes then incubated at 37°C for 12 h to 24 h. After that, the determination of turbidity and growth of bacteria using OD₆₀₀ was observed.

2.5. Characterization

2.5.1. Scanning electron microscope (SEM).
The surface and cross-section of synthesis bioplastic films were studied using the TM3000 Tabletop Microscope. The film samples were cut with a dimension of 10mm x 10 mm. The samples were coated using platinum at the lower surface and the side surface to make the films conductive. The magnification used to observe the surface morphology was 100X and 300X while for cross-section was 300X. SEM is used to identify the polymerization structure of the sample.

2.5.2. Fourier transform infrared spectroscopy (FTIR).
The FTIR spectra of synthesis bioplastic films were studied using a Perkin-Elmer Spectrum One Spectrometer, United States. The film samples were cut into (10mm x 10 mm) and the spectra were collected from 4000 cm⁻¹ to 600cm⁻¹ frequency range. All data analyzed using FTIR Spectrum Software with a 4 cm⁻¹ spectral resolution.

2.5.3. Mechanical properties testing.
The tensile properties were tested using an Instron 5848 Micro Tester testing machine. The initial grip was set to 6cm and the load used was 1000N. The tensile strength (TS) and the elongation at break (EB) of the film samples (50mm x 10mm) were evaluated from the force-displacement data.

3. Results and discussions

3.1. Film production.
The structure and the ability to peel off of the films produced is increased as the ratio of PVA increase while the color of the films becomes lighter from dark green to light yellowish. Results indicated that the PVA was able to increase the structure of the films to become stronger due to the strong interaction between *Morus sp.* and PVA.
Figure 1. Morphology of (A) 20% PVA bioplastic film, (B) 40% PVA bioplastic film, (C) 60% PVA bioplastic film, and (D) 80% PVA bioplastic film

3.2. Antimicrobial test.
Figure 2 (A-B) shows the result of the antibacterial test of 20%, 40%, 60% and 80% synthesis plastic film respectively. Antimicrobial property is the important criteria for biodegradable films to have. It shows the ability of the films to inhibit bacteria and can safely be used for medical purposes. Based on the results, the ability to inhibit bacteria is increased as the PVA ratio is increased. 80% PVA film shows the lowest absorbance density when tested with *E. coli* and *Staph. aureus* indicating that the bacteria were inhibited and lysis in the medium.

![Graph](image)

Figure 2. Graph show the (A) *E. coli* and (B) *Staph. aureus* of antimicrobial test with different concentration of PVA.

3.3. The microstructure of film.
Fig 2 (A-D) shows the result of microstructural analysis of the cross-section of 20%, 40%, 60% and 80% of PVA, respectively. The purpose of the test was to observe the microstructure of the synthesis plastic film based on cross-section using 300X of magnification. Figure 2 (D) show very good compatible morphologies with a smooth surface without a cavity. Thus, a smooth surface of the films resulted that the film becomes stronger and the bonding and arrangement of the particles are strong and close to each other. It shows by an increasing of PVA, the structure of the films becomes relatively smooth and homogeneous surfaces, without pores and crack. This condition was shown the compatibility of the PVA and *Morus sp.* as a biofilm [12]. SEM analysis on 20%, 40%, 60% PVA bioplastic film (Fig. 2A-2C) shown a roughness surface with undissolved particle and more voids. Presence of voids and undissolved particle may be due to the degree of the plasticizer in the synthesis plastic film [13].
Figure 3. SEM show the (A) 20% PVA bioplastic film, (B) 40% PVA bioplastic film, (C) 60% PVA bioplastic film, and (D) 80% PVA bioplastic film.

3.4. FTIR.
The purpose of the test is to observe the interaction and chemical bonding between PVA and Morus sp. which is an important parameter to evaluate the strength and structure of the films. Fig. 4 shows that the –OH groups on PVA and starch formed hydrogen bonding interaction, which could improve the compatibility of Morus sp. and PVA. So, as the PVA ratio is increased, the interaction and the bonding between Morus sp. and PVA becomes strong. Strong interaction could also indicate that the blend films are strong enough to use as the skin template.

![FTIR spectra](image)

Figure 4. FTIR spectra are given for Morus sp. Leaves and bioplastic made with different percentage of PVA.

3.5. Mechanical properties.
One of the most important criteria required for biodegradable plastic is the mechanical stability of films during handling. Tensile strength (TS) and elongation at break (EB) are the most important parameters used to evaluate the mechanical properties of biodegradable films. Based on the results, the TS is increased as the PVA ratio increased while the EB is decreased [10]. The TS and EB indicate that the films become stronger and less elastic as the PVA ratio increased. This is because, intermolecular interaction and hydrogen bonds between polymer chains, make a rigid and brittle matrix with low elongation [11].
Figure 5. Tensile strength (A) and elongation (B) of Moris sp. Leaves and bioplastic film.

4. Conclusion
The incorporation of PVA with Morus sp. formed a smooth and strong structure of films due to the high compatibility of both materials. Increasing the ratio of PVA improved the structure and the physical properties of the resulted blend films, while it increases the color and transparency of the films. Also, results from the antimicrobial test demonstrated the ability of Morus sp. with the increasing ratio of PVA able to inhibit the growth of bacteria, E. coli and Staphy. According to the result, blending 80% PVA and Morus sp. leaves synthesis plastic film had the best structure, antimicrobial properties, mechanical and physical properties. These results suggest a high potential of this blend film be used as the template for skin burning.

From the SEM cross-section, it has shown this synthesis plastic film has good compatible morphologies between PVA and Morus sp. leaves. The FTIR spectra indicate that the –OH groups on PVA and starch formed hydrogen bonding interaction, which could improve the compatibility of Morus sp. and PVA. Strong interaction could also indicate that the blend films are strong enough to use as the skin template. A clear surface of the blend films exhibited a smooth surface. With the increasing of PVA, the structure of the films become smoother and less fracture can be found which was resulted that the film becomes stronger. The tensile strength (TS) increased with the increase of the PVA. However, the elongation at break (EB) decreased with the increase of PVA. Results show that the film becomes stronger and less elasticity as the PVA increased. Therefore, considering the possible application of biodegradable plastic for the medical purpose especially to the 3rd and 4th degree of skin burning.

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