Sodium Alginate/Ageratum Conyzoides Extract Film for Wound Dressing Materials

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Abstract. This study focuses on the effect of Ageratum conyzoides extract (ACE) in Sodium alginate (SA) films to their mechanical, physical, and thermal properties. The tensile strength results show that the addition of ACE into the SA film improved the stress of the SA-ACE6 film to 5.1 ± 0.8 MPa compared to 4.0 ± 1.0 MPa for blank SA film. Water Vapor Transmission Rates (WVTRs) of SA incorporated Ageratum conyzoides extract (SA-ACE) were decreased, while swelling rate values of all films were increased upon addition of ACE. It was found that the thermal stability of the SA film also improved after the addition of ACE. Overall, the addition of ACE enhances the properties of SA film. This makes the SA-ACE film is suitable to be applied as wound dressing materials.

1. Introduction
Biopolymers are receiving greater attention than synthetic petrochemical-based polymers due to the environmental concerns. A variety of renewable biopolymer such as sodium alginate (SA) derived from brown algae, Laminaria hyperborean, has been studied in the development of wound dressings material.

Wound dressing based on alginic material is well known in literature as well as from commercial point of view, in wound management [1]. SA is approved by the United States Food and Drug Administrator (US FDA) and European Union (EU) for use in the food industry and has been in biomedical applications, for examples wound dressing, [2] scaffolds, [3] and dental or surgical impression materials [4].

Ageratum Conyzoides is among such medicinal plants that are effective against diseases and may contain bioactive compounds, which are effective against ill health [5]. It has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments, such as burns and wounds, headaches pneumonia, analgesic, inflammation, asthma, spasmodic and haemostatic effects, stomach ailments, gynaecological diseases, leprosy and other skin diseases [6]. Ageratum Conyzoides can be used as an anti-microbial agent due to presence of bioactive compound, phenol. This is because phenol and phenolic compounds have been extensively used in disinfection and remain the standard with which other bactericides are compared [7]. Hence, it can be used to treat skin disease and wound [5].

Up to our knowledge, no single study has been conducted to produce sodium alginate films incorporated with Ageratum conyzoides. This study fabricated and characterized the sodium alginate incorporating Ageratum conyzoides. The mechanical characteristic, water vapor transmission rates, swelling properties, and thermal study of the film were examined.
2. Materials and method

2.1. Materials
Sodium alginate (product number- W201502 lot number-9005383), glycerine (product number-G2289, lot number SHBC2650V), and anhydrous calcium chloride, CaCl₂ (product number-C5670, lot number SHBC2650V) were obtained from Sigma Aldrich, St Louis, MO, USA. Ageratum conyzoides was obtained from Kg. Tok Jembal, Kuala Terengganu, Terengganu. All materials were used as received without any purification while Ageratum conyzoides were extracted using ethanol 95%.

2.2. Preparation of sodium alginate incorporated Ageratum conyzoides (SA-ACE)
The sodium alginate (SA) films were prepared via casting method. The SA solution was prepared by dissolving 2% (w/v) of SA in 100 mL distilled water, followed by glycerine at 50% w/w with continuous stirring for 1 hours 30 minutes at 70°C. After SA was fully dissolved, 10mM CaCl₂ was added into the mixture and stirred for another 30 minutes. The Ageratum conyzoides extract (ACE) was added and the solution was stirred for 15 minutes. These SA solutions containing 0.02%, 0.04% and 0.06% (w/v) ACE were known as SA-ACE2, SA-ACE4, and SA-ACE6 respectively. The SA-ACE solutions were deposited onto petri dishes (90 mm x 15 mm) and dried in the oven at 50°C for 24 hours.

2.3. Characterization of SA-ACE films
2.3.1. FTIR Characterization. ATR-FTIR spectra were determined by using Perkin Elmer Spectrum 100 FTIR spectrophotometer with MIKE Miracle ATR accessory with single-bounce beam path, 45° incident angle, and 4 cm⁻¹ resolution. Then all spectra were corrected by using Perkin Elmer Spectrum 100 software. The wavenumber region studied was between 4500-400 cm⁻¹. The resulting spectra and peak of functional groups were recorded.

2.3.2. Swelling Percentage. The swelling percentage was carried out by immersed dried samples of dimensions 20 mm × 20 mm in 20 mL of phosphate buffer solution at pH 7.2 in water bath (37 ± 0.5°C). The samples were removed after 24 hours and lightly wipe with tissue to expel surface solution and weighed. Swelling degree was determined by the equation below:

\[
\text{Swelling percentage (\%) = } \frac{(W_f - W_i)}{W_i} \times 100
\]

Where, \(W_i\) = initial weigh of sample and \(W_f\) = final weigh of sample

2.3.3. Water Vapour Transmission Rates (WVTR). The WVTR was carried out by put the films (30 mm × 30 mm) as a cap on a glass vial with diameter 16 mm that contain 10 mL of distilled water. Then, it was weigh (\(W_i\)) and left in the desiccator containing silica gel for 24 hours. After 24 hours, it was weigh again (\(W_f\)). The value of WVTR (g m⁻² day⁻¹) was calculated as bellow:

\[
\text{WVTR} = \frac{(W_i - W_f)}{(A \times \text{day})}
\]

Where, \(W_i\) = initial weigh of sample, \(W_f\) = final weigh of sample, \(A\) = area of vial opening.

2.3.4. Tensile Strength. The tensile strength were performed by using Instron tensile machine, model 3366 with cross-speed at 10 mm/min. All films were cut into 60 mm × 20 mm for characterization. The tests were repeated triplicates per sample for defined ratio.

2.3.5. Thermogravimetric Analysis. Thermogravimetric analysis were performed using Thermogravimetric Analyzer model Mettler Toledo. The films were cut into small pieces to maximized the surface area of the samples. The rate flow of nitrogen gas are 30 mL/min at the heating rate of 10 °C/min while the range of temperature used are 25 °C to 700 °C. Sample used was approximately 10 mg.

3. Results and discussion
3.1. ATR-FTIR Spectrum
Figure 1 shows the FTIR spectra of SA films incorporated Ageratum conyzoides extract (ACE).
There are shifting and disappearance of few functional peaks in spectrum of SA film compared to a representative of SA-ACE2 film.

All films show a broad peak at 3200 cm\(^{-1}\) for SA and SA-ACE2 while 3380 cm\(^{-1}\) for ACE due to the stretching vibration belong to O-H group [8]. At 2838 cm\(^{-1}\), 2939 cm\(^{-1}\) and 2850 cm\(^{-1}\) the spectra show the presence of C-H group at SA film, ACE and SA-ACE2 respectively. A peak was shown at wavenumber around 1620 cm\(^{-1}\) and 1420 cm\(^{-1}\) for SA film and SA-ACE2 film corresponding to symmetric and asymmetric stretching vibrations for COO group, respectively [9]. At 1634 cm\(^{-1}\) for ACE spectrum, it is possible to observe a weak absorption band probably related to C=O linkage of ketone or carboxylic acid groups. Furthermore, the weak peak at 1445 cm\(^{-1}\) and 1250 cm\(^{-1}\) indicate the presences of CH\(_3\) and COC groups respectively. Overall, it seems that there is no significant difference between SA film and SA-ACE film except for the shifting of some functional groups such as C-H and COO groups and disappearance of COC group around 1200 cm\(^{-1}\).

3.2. Swelling Percentage
Swelling properties of a film is important characteristics to determine the ability of the sample to absorb exudates from any wound. The swelling percentages of all SA-ACE films were summarized in Table 1. The swelling degree of film keep increasing upon addition of ACE. For instance, the SA film absorbed 134 ± 59 % and increased to 177 ± 36% for SA-ACE2 film and further increased to 254 ± 94% for SA-ACE6 film. The increased values of swelling of SA-ACE films could be due to the presence of hydroxyl functional group (OH) of SA-ACE available to bind with the hydroxyl group (OH) from water. In fact, an ideal wound dressing normally present water absorption in the range of 100% to 900%[10]. Overall, all the swelling behaviour of the prepared SA-ACE film were in the range of ideal wound dressing criteria.

3.3. Water Vapor Transmission Rates (WVTRs)
WVTR can determined the ability of a wound dressing to control the water loss from a wound at an optimal rate, in which it could prevent extreme dehydration of wound and accumulation of exudates [11]. The WVTRs values of all films were summarized in Table 1. The increasing of ACE concentration in SA film resulted in decreasing the WVTR value to 1214 ± 35 g m\(^{-2}\) d\(^{-1}\) for SA-ACE6 compared to 1689 ± 8 g m\(^{-2}\) d\(^{-1}\) of free standing SA film. The lowest WVTR values of SA-ACE6 is due to inclusion of ACE into SA film and disturbed the diffusion of water through the film. Besides, SA-ACE2 film with the lower amount of ACE possess higher WVTR value at 1688 ± 164 g m\(^{-2}\) d\(^{-1}\).
Nevertheless, all samples show the WVTR values of commercial wound dressing product in the range of 90 – 2893 g m⁻² d⁻¹[12].

| Sample     | Swelling Percentage (%) | WVTR (g m⁻² d⁻¹) |
|------------|-------------------------|------------------|
| SA         | 134 ± 59                | 1689 ± 80        |
| SA-ACE2    | 177 ± 36                | 1688 ± 163       |
| SA-ACE4    | 241 ± 81                | 1481 ± 84        |
| SA-ACE6    | 254 ± 94                | 1214 ± 35        |

3.4. Tensile Test

Tensile tests were examined to determine the stress and strain of the fabricated film. Figure 2 shows the stress-strain curves of SA-ACE film at different concentrations of ACE and Table 2 summarized the tensile stress (σ), tensile strain (ε) and Young’s Modulus (YM) of the films. The inclusion of ACE into the SA film enhances the stress of the film from 4.0 ± 1.0 MPa (SA) to 5.1 ± 0.8 MPa (SA-ACE6). In contrast, the strain of SA-ACE film was decreased to 20.0 ± 1.3% after the addition of ACE and maintain the value even after addition of ACE at high concentration (20.2 ± 1.9%). There is only a slightest difference of Young’s modulus after the addition of ACE which in the range of 28.9 ± 5.6 MPa to 35.6 ± 2.9 MPa. The increasment of stress values of the films is a result of an increased in hydrogen bonding interaction between SA-ACE[13, 14]. Overall, the addition of ACE was enhanced the strength of SA films.

![Figure 2. Tensile stress-strain curve of sodium alginate (SA) and SA films at different concentrations of ACE (SA-ACE).](image)

3.5. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis are performed to determine the amount of weight change as a function of time and temperature. Figure 3 shows the thermogravimetric thermograms and derivative thermograms of SA-ACE films while Table 3 summarized the temperature onset (T₀), temperature completion (Tₚ) and weight loss (%). The degradation of SA film started approximately at temperature onset, T₀ = 34°C and the temperature completion, Tₚ at 500 °C. The inclusion of ACE does not significantly affect the temperature onset (T₀ in range 34°C to 36°C), but increased the temperature of completion, Tₚ from 500°C (SA) to 556°C for SA-ACE6. The weight loss of all films does not have significant difference after the addition of ACE.
Figure 3. (a) Thermogravimetric thermograms and (b) derivative thermograms sodium alginate (SA) and SA films at different concentrations of ACE (SA-ACE).

Table 2. Tensile stress ($\sigma$), the tensile strain ($\varepsilon$), and Young’s Modulus (YM) of sodium alginate (SA) and SA films at different concentrations of ACE (SA-ACE).

| Sample     | Tensile Stress, ($\sigma$) (MPa) | Tensile Strain, ($\varepsilon$) (%) | Modulus (YM) (MPa) |
|------------|---------------------------------|-------------------------------------|-------------------|
| SA         | 4.0 ± 1.0                        | 23.4 ± 3.7                          | 30.9 ± 5.8        |
| SA-ACE2    | 4.0 ± 0.4                        | 20.0 ± 1.3                          | 29.8 ± 5.3        |
| SA-ACE4    | 4.5 ± 0.8                        | 20.0 ± 1.8                          | 35.6 ± 2.9        |
| SA-ACE6    | 5.1 ± 0.8                        | 20.2 ± 1.9                          | 28.9 ± 5.6        |

Table 3. Thermogravimetry properties of sodium alginate (SA) and SA films at different concentrations of ACE (SA-ACE).

| Sample     | Temperature Onset, $T_o$ (°C) | Temperature Completion, $T_c$ (°C) | Weight Loss (%) |
|------------|-------------------------------|-----------------------------------|-----------------|
| SA         | 34                            | 500                               | 76              |
| SA-ACE2    | 34                            | 520                               | 76              |
| SA-ACE4    | 36                            | 560                               | 71              |
| SA-ACE6    | 35                            | 556                               | 77              |
4. Conclusions
Sodium alginate films incorporated with Ageratum conyzoides extract (SA-ACE) were successfully prepared. The addition of ACE into the SA film increased the swelling degree of film and lowered the values of WVTRs. The tensile performance shows the enhancement of stress of the films and increased the temperature completion of the film after have been tested using thermogravimetric analysis. The film has shown promising properties to be an ideal wound dressing material.

5. References
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