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Tridimensional Alginate Films with Cat's Claw (*Uncaria tomentosa*) Extract or Aloe Vera (*Aloe barbadensis*) Gel for Potential Use as Wound Dressings †

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Abstract: Cat's claw and aloe vera gel contain active compounds and could be used to enhance the properties of wound dressings. Cat's claw is known for its anti-inflammatory, anti-arthritic and anti-asthmatic properties; and aloe vera is commonly used for wound healing and skin hydration. In this study, we elaborated microparticles from an emulsion made of alginate solutions with aloe vera gel or cat's claw extract with ultrasound and tri-dimensional membranes were obtained by solvent evaporation. The 27 to 33 μm-thick membranes showed a porous surface on SEM; the contact angle of water on the membranes increased in hydrophilicity due to the use of aloe vera gel. Furthermore, the presence of aloe vera also improved water absorption in an acetate buffer (pH 5.5) at 37.5 °C. Finally, the presence of cat's claw extract in the microparticles significantly enhanced radical scavenging in ABTS decoloration assay, in comparison to tri-dimensional alginate membranes with no active compounds. Alginate films with cat's claw extract or aloe vera gel could be used as wound dressings materials.

Keywords: alginate; *Uncaria tomentosa*; *Aloe barbadensis*; antioxidant activity; biodegradable

1. Introduction

The use of wound dressings is common practice when there is a skin injury. Wound dressings help in the healing process and their role is to provide an adequate environment as they are in direct contact with the wound, this includes the need of reducing the risk of infection while minimizing the loss of function of the skin [1]. Wound dressings can be classified into traditional and polymeric dressings. Traditional materials, such as gauze or bandages, present certain risks since they do not provide a suitable humid environment and are prone to adhere to the wound, which can cause a prolongation of the healing process, an aggravation of the wound condition, and, in severe cases, even hospitalization [1,2]. On the other hand, polymeric dressings (natural or synthetic), although they present various improvements compared to traditional materials like they could be used in different types of wounds as acute and chronic and are good to permeability to water vapor; they also have disadvantages, such as being opaque and poor absorbents of wound fluid [1,2].

Among the polymeric materials, the use of biopolymers—such as alginate—is of great interest. Alginate is a linear polysaccharide that is biocompatible, biodegradable and known for its ability to form films. Previous studies [3] consider that this polymer can be used together with bioactive substances, such as aloe vera or a plant extract, to optimize its therapeutic properties.

Aloe vera (*Aloe barbadensis*) has been used in traditional medicine and therefore it is a highly studied plant due to its biological activities. Aloe contains a gelatinous matrix that contains between 98.5% to 99.5% of water and a solid phase with more than 75 active components like polysaccharides, enzymes, amino acids and vitamins [4]. Previous studies [5,6] consider that acemannan, a polysaccharide, is the major solid component of the plant. Aloe vera gel is anti-inflammatory, antibacterial and accelerates the healing of small wounds and burns [3,7].

Cat's claw (*Uncaria tomentosa*, UT) is a popular medicinal plant used as an antiinflammatory, immune system stimulator, antiantioxidant, antiviral, as well as a treatment for cardiovascular diseases [8], tumors [9], etc. Most of the studies on cat's claw are centered on its alkaloids [8,10] (tetracyclic and pentacyclic oxindole, indole, and triterpenoid) specially because it's abundancy. Also, cat's claw has a great number of polyphenolic compounds that can be classified in the following groups for a better comprehension: hydroxybenzoic acids (including gallic, salicylic and vanillic acids), hydroxycinamic acids (caffeic, p-coumaric, ferulic and isoferulic acids), flavonoids ((+)-catechin and (−)-epicatechin monomer), condensed tannins (several procyanidins and propelargonidin) and flavalignans (four cinchonain) [11].

The main contribution of the polyphenolic compounds to the properties of cat's claw is their antioxidant activity. Structurally, polyphenols possess numerous conjugated systems and hydroxyls that can react with radical species and deactivate them—process called radical scavenging—. However, polyphenolic compounds also play a role on the modulation of pathways involved in chronic inflammation [12]. Some studies also show positive results when evaluating the antimicrobial activity of rich polyphenolic extracts over *Staphyloccus aureus* and the in vitro cytotoxic activity of such extracts over colon adenocarcinoma cell lines [13,14]

2. Experimental Part

2.1. Materials

Alginate (AL) and the non-ionic surfactant poloxamer 407 were purchased from Sigma-Aldrich. Cat's claw bark Naturandes® and aloe vera leaves were obtained from a local market.

2.2. Plant Extraction Process

The cat's claw extract (UT) was obtained by ultrasonic bath for 20 min using ethanol 60% (*v*/*v*) as solvent (three times). The liquid phase was centrifuged to separate the fine particles, then the ethanol was removed at low pressure and finally the extract was lyophilized to obtained a powder.

Aloe vera leaves were washed and the gel was separated from the skin and washed with plenty of water. After that, the gel was cut into small pieces, blended and filtered to separate the fibers. The liquid was centrifuged and stabilized at 65 °C for 15 min. The gel was stored at 4 °C.

2.3. Microparticles and Film Formation

A 1% (*w*/*v*) aqueous alginate solution was mixed with cat's claw extract to a final concentration of 0.1% (*w*/*v*) (AL-UT). Surfactant (poloxamer 407) was added (0.1% or 0.5%, *w*/*v*) and also the organic solvent (hexane) in a 1:9 (*v*/*v*) ratio. The mixture was cooled for 15 min in an iced bath and then sonicated (Sonics, 20 kHz, 750 W, 40% amplitude) for 3 min. The emulsion obtained was poured on a Petri dish and left to dry at 50 °C for 24 h.

Aloe vera gel (AVG) with alginate films were prepared at the same manner as AL-UT films in different concentrations: AVG 10% (*v*/*v*) or AVG 25% (*v*/*v*).

Aditionally a control film—with no UT or AVG—was obtained with the same procedure with poloxamer 0.5% (*w*/*v*). Finally, all the films obtained were crosslinked with 1% calcium chloride solution for 5 min, rinsed with water and left to dry at room temperature.

2.4. Characterization of Films

2.4.1. Thickness and Morphological Analysis

Thickness of films was measured with a micrometer (Mitutoyo). Six thickness measurements were taken along the film, including the center.

Scanning electron microscopy (LVEM) was used to study the surface of films. SEM images were taken at an accelerating voltage of 5 kV.

2.4.2. Swelling Behavior

This experiment was performed using a 2 × 2 cm slice of the films. Pre-weighted films were immersed in an acetate buffer (pH 5.5) at 37.5 °C. After a period of time, the hydrated films were collected, the excess of buffer was removed with a filter paper and weighted. Weights were measured after 20, 40, 60, 180, 300 and 1440 min. The swelling was determing according to Equation (1):

$$Swelling\ (\%) = \frac{m_{swell} - m_{dry}}{m_{dry}} \times 100, \quad (1)$$

where m_{swell} represents the mass of the swollen film and m_{dry} corresponds to the mass of the dry piece of film. Three samples were evaluated for each type of film.

2.4.3. Wettability

Contact angle of films was measured using a goniometer (Ramé-Hart, model 250) and distilled water as solvent (5 μL drop). The measurements were made at six different points on the surface of films; fifty measurements were collected every 0.005 s at each point. Drop Image software was used to collect the data.

2.4.4. Antioxidant Activity

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ABTS radical cation decoloration assay was used to evaluate antioxidant activity. ABTS was dissolved in ethanol and a slice of film was used as sample. The absorbance was measured at 734 nm after 1, 5, 15, 30, 45 min. The inhibition was calculated with Equation (2):

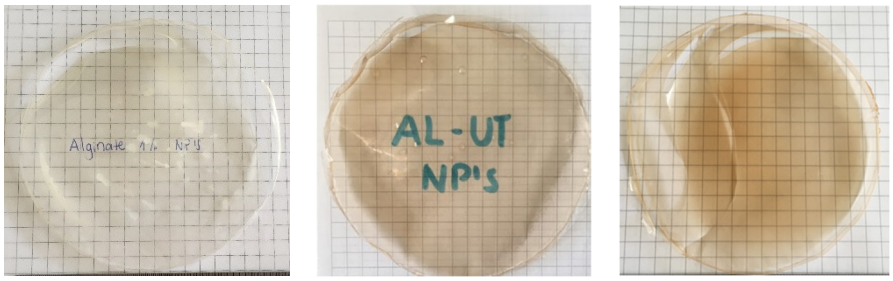
$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100, \quad (2)$$

where $A_{control}$ is the absorbance of just ABTS solution and A_{sample} represents the absorbance of ABTS solution with the sample after a certain time.

3. Results

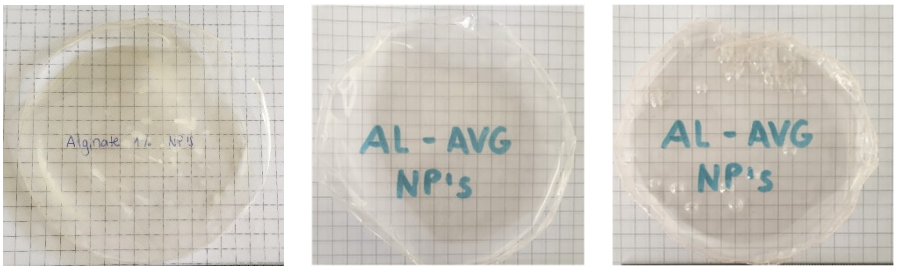
3.1. Physical Appearance, Thickness and Morphological Analysis

Figures 1 and 2 show the physical appereance of films of alginate with cat's claw extract (UT) and alginate with aloe vera gel (AVG), respectively, a control film (alginate and poloxamer only) is also shown. All membranes are transparent and, in case of cat's claw, the films have a brown color. On the other hand, AL-AVG films are similar in comparison to the control film.



(**a**) (**b**) (**c**)

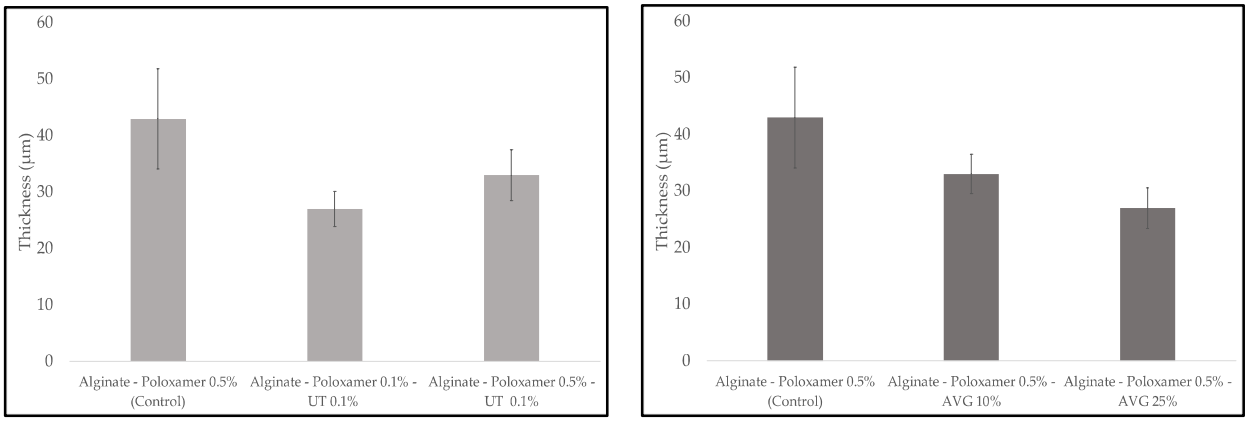
Figure 1. Physical appearance of alginate with UT extract powder: (**a**) Alginate-Poloxamer 0.5% (control); (**b**) Alginate-Poloxamer 0.1%-UT0.1%; (**c**) Alginate-Poloxamer 0.5%-UT0.1%.



(**a**) (**b**) (**c**)

Figure 2. Physical appearance of alginate with aloe vera gel: (**a**) Alginate-Poloxamer 0.5% (control); (**b**) Alginate-Poloxamer 0.5%-AVG10%; (**c**) Alginate-Poloxamer 0.5%-AVG25%.

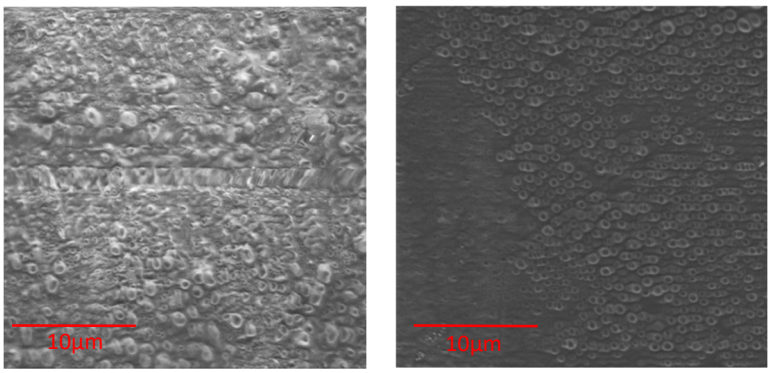
Figure 3 shows the thicknesses of alginate membranes, UT and AVG, which are between 27 to 33 μm. The control film's thickness was 43 μm. The addition of cat's claw or aloe vera gel decreased the films thicknesses. Moreover, the increase of aloe vera gel content reduced even more the thickness.



(**a**) (**b**)

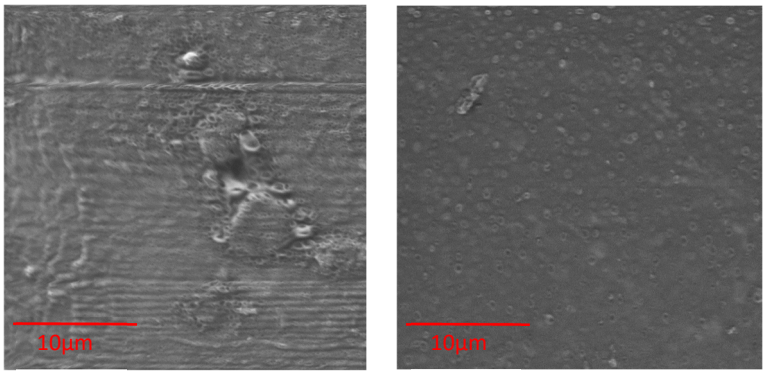
Figure 3. Average thickness of alginate films with: (**a**) cat's claw extract; (**b**) aloe vera.

The surface topography of alginate membranes with UT and with AVG was analyzed with SEM (Figures 4 and 5). A membrane prepared with alginate solution–no emulsion–showed a smooth surface with no cracks (data not shown), while films made with microparticles presented a porous surface. Films of alginate with cat's claw produced rough surfaces, this was also observed in control films of alginate and poloxamer (Figure 4a).



(**a**) (**b**)

Figure 4. SEM images of alginate with UT extract powder: (**a**) Alginate-Poloxamer 0.5% (control); (**b**) Alginate-Poloxamer 0.5%-UT0.1%.

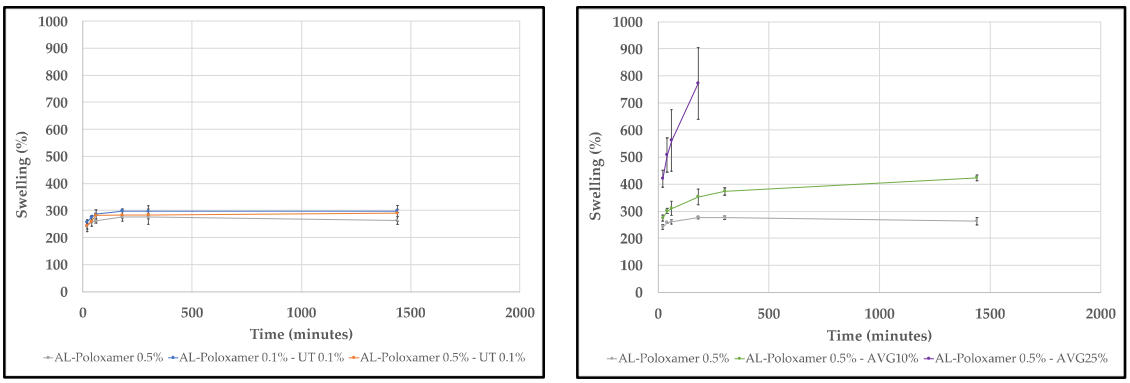


(**a**) (**b**)

Figure 5. SEM images of alginate with AVG: (**a**) Alginate-Poloxamer 0.5%-AVG10%; (**b**) Alginate-Poloxamer 0.5%-AVG25%.

3.2. Swelling Behavior

Figure 6 shows the swelling behavior of alginate with UT and AVG membranes in acetate buffer at 37.5 °C and pH 5.5 simulating skin. In both cases a control film (alginate-poloxamer 0.5%) was also studied. The average swelling behavior of alginate with cat's claw films increased slightly compared the control film; in the case of alginate with aloe vera films, the swelling was greater than in the control and it increased with the concentration of aloe vera gel. However, the alginate-AVG25% started to dissolve in the buffer after 3 h.

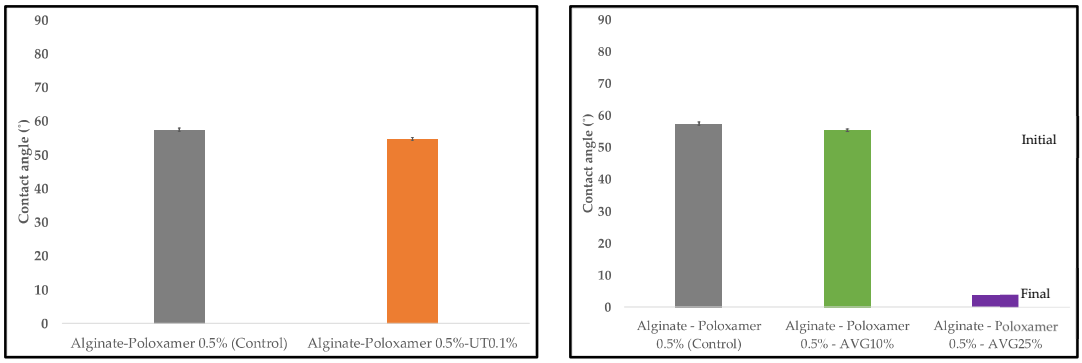


(**a**) (**b**)

Figure 6. Swelling behavior of: (**a**) alginate with UT extract powder films; (**b**) alginate with AVG films.

3.3. Wettability

Wettability was evaluated by the contact angle of a water drop which showed the hydrophilic/hydrophobic character of the films. Figure 7 shows the contact angles on AL-UT and AL-AVG films. The values on the AL-UT and control films are similar. In the case of the AVG, the film with 10% (*v*/*v*) of the gel has a similar contact angle to the control, but when AVG was increased to 25%, the hydrophilicity of the film increased sharply and the water drop was absorbed changing its contact angle from 53° to 4°, within seconds.

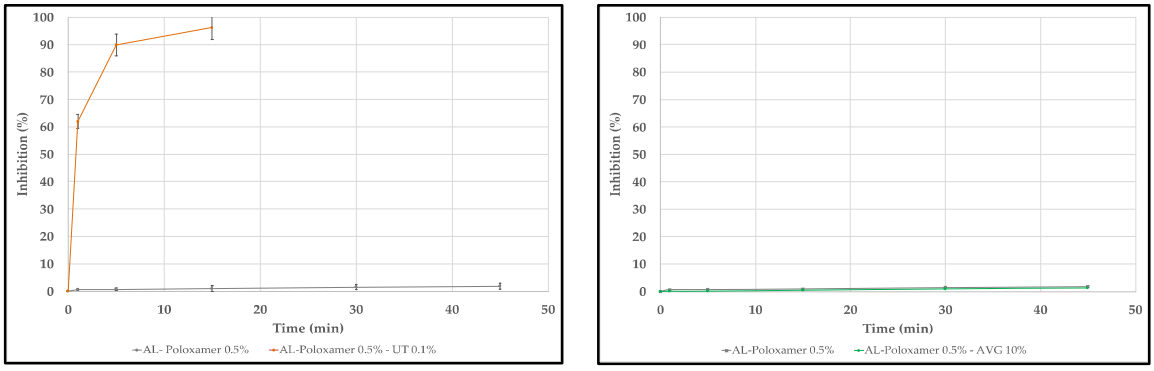


(**a**) (**b**)

Figure 7. Wettability of: (**a**) alginate with UT extract powder films; (**b**) Alginate with AVG films.

3.4. Antioxidant Activity

Figure 8 shows the antioxidant activity of the alginate films with the natural compounds. The film with UT shows an excellent antioxidant activity with almost 100% of inhibition after 15 min compared with the control film–only alginate and poloxamer film–which has less than 5% of inhibition even after 45 min. On the other hand, the film with AVG 10% has a similar activitiy to the control film. The AL-AVG25% film could not be evaluated because it dissolved in the solvent.



(**a**) (**b**)

Figure 8. Antioxidant activity of: (**a**) alginate with UT extract powder films; (**b**) Alginate with aloe vera gel films.

4. Discussion

4.1. Physical Appearance, Thickness and Morphological Analysis

All films were transparent and this is an important characteristic for wound dressings for to be monitored injuries without removing the film [3]. The brown color of AL-UT films is due to the components in cat's claw extract. The increase of AVG in films lead to a decrease of the film thickness, which is due to more water content from the gel during preparation, this was observed also by Pereira et al. [3].

SEM analysis shows a porous surface of the films that contained the active compounds due to the formation of dispersed microparticles previous to the formation of the film. Similar results have been reported by Shankar et al. [15].

4.2. Swelling Behavior

The high values of water absorption of AL-AVG films could be explained by the hydrophilic character of the components of the gel, such as acemannan and others polysaccharides. Fluid

absorption is important for wound treatment in order to maintained an adequate moisture environment on the injury [3].

4.3. Wettability

The contact angle analysis of the films showed an slight increase in the hydrophilicity of AL-UT films (3°), which could be due to the incorporation of phenolic compounds (rich on hydroxyl groups) in the cat's claw extract. On the other hand, the noticeable increase of hydrophilicity of the AL-AVG films was due to the nature of the compounds present in AVG as was described previously for the swelling behavior.

4.4. Antioxidant Activity

The high antioxidant activity of the AL-UT film can be explained by the presence of phenolic compounds. As explained above, plyphenols can easily deactivate free radical species. In this case, when the ABTS radical cation comes near any of the polyphenolic compounds from the UT extract, it gains readily either a hydrogen atom or an electron from the hydroxyl groups in the antioxidants, which causes the solution to lose it's characteristic blue color.

5. Conclusions

Tridimensional membranes made of alginate and cat's claw extract (AL-UT), and alginate and aloe vera gel (AL-AVG) were produced. The thickness of the membranes varied between 27 and 33 μm, all of them were uniform, transparent and, only the AL-UT films had a brownish color. Surface analysis through SEM shows that films have porous surfaces.

The swelling study showed that AL-UT films remained intact in buffer acetate (pH 5.5) at 37.5°C after 24 h, while AL-AVG25% film started to dissolve after 3 h. Surfaces of membranes of alginate with active compounds showed a hydrophilic character and a higher content of AVG decreased the contact angle. The AL-UT film showed a high antioxidant activity with almost 98% of inhibition after 15 min, while AVG does not show activity when compared to the control film. AL films functionalized with cat's claw extract or aloe vera gel show important properties as potential use as wound dressing materials.

Author Contributions: S.K., A.D., M.E. and J.N. conceived and design the experiments; B.G. designed and performed the SEM analysis; M.E. and A.D. performed the experiments; all authors analyzed the data; M.E. and A.D. wrote the paper draft; J.N. and S.K. reviewed and edited the paper.

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, nor in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

UT:	Cat's claw (*Uncaria tomentosa*)
AVG:	Aloe vera gel
AL:	Alginate

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