

Polymeric pullulan films incorporated with extract of *Cyclosporum leptophyllum* (pers.) Sprague for healing purposes

Filmes poliméricos de pululana incorporados com extrato de *Cyclosporum leptophyllum* (pers.)

Sprague para fins cicatrizantes

Películas poliméricas de pululano incorporadas con extracto de *Cyclosporum leptophyllum* (pers.)

Sprague con fines cicatrizantes

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Abstract

Healing films provide excellent results in the adjuvant treatment of injuries. Its association with plant extracts allows the achievement of therapeutic activities, favoring skin regeneration. This study aimed to develop pullulan films incorporated with an extract from the aerial parts of *Cyclosporum leptophyllum* to be used as healing dressings and to evaluate their morphological and physicochemical characteristics and their potential antioxidant activity. Polymeric films in the proportion of 4% PU (m/v) and 15% plant extract and control films composed only of the polymer matrix were produced using the casting method. The films were evaluated for thickness, visual aspects, solubility, water vapor permeability, swelling, degradation, and antioxidant activity. They showed good appearance and flexibility but heterogeneity due to the precipitation of components. A high degree of solubility and swelling was observed, and despite its rapid dissolution in contact with water, the formation of a hydrogel can enable greater adherence to wounds. The films have controlled water vapor transmission, that allows gas exchange. A DPPH radical inhibition rate of 17.23% was obtained, proving its antioxidant activity. Therefore, the biofilms produced have healing potential, which can be better studied in the future through tests demonstrating antimicrobial, and anti-inflammatory activities.

Keywords: *Apium leptophyllum*; Biofilms; Wild celery; Antioxidant; Dressing.

Resumo

Filmes curativos propiciam excelentes resultados no tratamento adjuvante de lesões. Sua associação à extratos vegetais permite a obtenção de atividades terapêuticas, favorecendo a regeneração cutânea. O objetivo do trabalho foi desenvolver filmes de pululana incorporados com extrato das partes aéreas de *Cyclosporum leptophyllum* para serem utilizados como curativos cicatrizantes, bem como avaliar suas características morfológicas e físico-químicas e sua potencial atividade antioxidante. Foram produzidos pelo método de casting filmes poliméricos na proporção de 4% PU (m/v) e 15% extrato vegetal e filmes controle compostos apenas pela matriz polimérica. Os filmes foram avaliados quanto a espessura, aspectos visuais, solubilidade, permeabilidade ao vapor d'água, intumescimento, degradação e atividade antioxidante. Eles apresentaram boa aparência e flexibilidade, mas heterogeneidade pela precipitação de componentes. Observou-se alto grau de solubilidade e intumescimento, e apesar de sua rápida dissolução em contato com a água, a formação de um hidrogel pode possibilitar melhor aderência à feridas. Apresentaram também controlada transmissão de vapor d'água, a qual permite a troca gasosa. Uma taxa de inibição do radical DPPH de 17,23% foi obtida, comprovando sua atividade antioxidante. Portanto, os biofilmes produzidos apresentam potencial atividade curativa, a qual pode ser melhor estudada em futuros estudos por meio da realização de testes que demonstrem suas exatas atividades antioxidante, antimicrobiana e antiinflamatória.

Palavras-chave: *Apium leptophyllum*; Biofilmes; Aipo silvestre; Antioxidant; Curativo.

Resumen

Las películas cicatrizantes proporcionan excelentes resultados en el tratamiento adjuvante de lesiones. Su asociación con extractos de plantas permite la consecución de actividades terapéuticas, favoreciendo la regeneración de la piel. El objetivo de este trabajo fue desarrollar películas de pululano incorporadas con extracto de partes aéreas de *Cyclosporum leptophyllum* para ser utilizadas como apósitos cicatrizantes, así como evaluar sus características morfológicas, fisicoquímicas y su potencial actividad antioxidante. Se produjeron películas poliméricas en la proporción de 4% PU (m/v) y 15% de extracto vegetal y películas de control compuestas únicamente por la matriz polimérica por el método de fundición. Se evaluó el espesor de las películas, aspectos visuales, solubilidad, permeabilidad al vapor de agua, hinchamiento, degradación y actividad antioxidante. Presentaron buena apariencia y flexibilidad, pero heterogeneidad debido a la precipitación de componentes. Se observó un alto grado de solubilidad e hinchamiento y, a pesar de su rápida disolución en contacto con el agua, la formación de un hidrogel puede permitir una mejor adherencia a las heridas. También mostraron transmisión controlada de vapor de agua, lo que permite el intercambio de gases. Se obtuvo una tasa de inhibición del radical DPPH del 17,23%, demostrando su actividad antioxidante. Por lo tanto, las biopelículas producidas tienen una actividad curativa potencial, que puede estudiarse mejor en futuros estudios a través de pruebas que demuestren sus actividades antioxidantes, antimicrobianas y antiinflamatorias exactas.

Palabras clave: *Apium leptophyllum*; Biopelículas; Apio silvestre; Antioxidante; Apósito adhesive.

1. Introduction

The skin acts as the first barrier against physical and chemical pathogens, shelters the internal organs preventing aggressions from the external environment, helps in the thermoregulation and hydration processes, and has numerous nerve endings that allow us to experience the tactile sensation. It comprises three main layers: epidermis, dermis, and hypodermis. Furthermore, the stratum corneum - the most superficial layer of the epidermis - is the initial obstacle to skin permeation by exogenous factors, including topical drugs (Lane, 2013; Santos & Costa, 2015; Souto et al., 2021).

Trauma to the skin, such as injuries, results in scarring. Healing stems from the need for tissue repair, in which the injured tissue is replaced by new tissue, restoring the skin barrier and making it able to prevent serious damage (Sorg et al., 2016). During the healing process, free radicals are produced in large quantities. These free radicals transpose the tissue's antioxidant capacity, generate oxidative stress, and react with other molecules, worsening the inflammatory process and delaying tissue repair. Pace (2002) and Pessoa (2014) demonstrated that antioxidant substances promote less formation of free radicals and reduce tissue damage, helping the skin repair process.

Encouraging research and the development of new technologies have given prominence to dressings. Adhesive gels, polymeric films, and hydrogels have generated excellent results in helping the healing process and improving existing pharmacological and non-pharmacological treatments. They are easy to use, and many are designed for topical drug administration (Bueno, 2019). Among the materials that can be used for this purpose, we have biopolymers, which are non-toxic, biocompatible and biodegradable (Hajji et al., 2021). The natural polymers used to manufacture polymeric films can be

of vegetable, animal, and even microbial origin, such as pullulan. PU is an extracellular polysaccharide produced by the fungus *Aureobasidium pullulans*, characterized as an α -glucan, consisting of repetitive maltotriose subunits joined by α -1,6- glycosidic bonds. Studies demonstrate its use as a prebiotic, a vehicle for prolonged release drugs, food coating, and food and curative films (Sueiro et al., 2016).

Substances such as plant extracts are incorporated for healing films based on polymers to have therapeutic properties. The species *Cyclospermum leptophyllum* (Pers.) Sprague, known as wild celery, belongs to the Apiaceae family. It is a herbaceous plant native to tropical America, found mainly in Brazil's South and Southeast regions. It is used in folk medicine to treat gastrointestinal and respiratory disorders, infections, and inflammations. It is often used in Ethiopia to treat a disease known locally as "Mitch," characterized by inflammation and loss of appetite (Helal et al., 2016).

As a dressing, an ideal polymeric film must guarantee effective protection and fixation, be biocompatible, non-toxic, flexible, comfortable, and permeable to gases and water vapor. PU decomposes without the emission of toxic gases, while its film is thermally stable, antistatic, elastic, compressible, and has excellent adhesive properties (Lima et al., 2017). The wide variety of secondary metabolites present in plants constitutes an inexhaustible source of substances of great therapeutic value. For example, *C. leptophyllum* (Pers.) Sprague, whose phytochemical composition, rich in terpenes, flavonoids, coumarins, and alkaloids, provides anti-inflammatory, antiseptic, antioxidant, and analgesic activities and antifungal potential (Helal et al., 2016).

The association of microbial biopolymer and plant extract for making healing films is of significant interest, both from the industrial and clinical aspects. These are easy-to-handle raw materials with ideal physical and biological properties, producing a highly acceptable and effective dressing. Thus, the study aims to develop PU-based biofilms and incorporate them with *C. leptophyllum* (Pers.) Sprague extract, to evaluate their antioxidant property to be used as healing dressings and to evaluate and compare the films produced according to their morphological and physicochemical characteristics.

2. Methodology

2.1 Collection of vegetable material

The collection of aerial parts of *C. leptophyllum* (Pers.) Sprague was carried out in the spring of 2021, at the Hospital Universitário do Oeste do Paraná - HUOP in the city of Cascavel, PR, Brazil (La -24.973201636946225, Lo - 53.493980273496724). Subsequently, an exsiccate of the plant was made and sent to the UNOP Herbarium of UNIOESTE/Cascavel for confirmation of the botanical species, which was identified as *Cyclospermum leptophyllum* (Pers.) Sprague ex Britton & P. Wilson, Registration Number UNOP10893.

2.2 Preparation of vegetable material

The plant material was selected and dried at the Laboratory of Pharmacognosy and Phytochemistry of UNIOESTE - Cascavel campus. The aerial parts were visually selected, discarding the unhealthy parts (dried or attacked by insects or fungi) for drying for twenty days, at ambient temperature, in the shade, and in low humidity. After drying, the plant material was ground in a knife mill (SL-31 Solab®) and weighed, resulting in 132 g of pulverized plant material. The powder was stored for further obtaining the plant extract.

2.3 Obtaining the vegetable extract and Phytochemical screening

The plant material obtained from the milling was subjected to extraction by maceration, using concentrated absolute ethyl alcohol as a solvent. Maceration was carried out by exhaustive extraction for 72 hours, in which 132 g of pulverized plant material were in contact with 528 mL of absolute ethyl alcohol, according to a 1:4 (w/v) ratio. After this period, the material

was vacuum filtered, and the liquid obtained was processed by rotary evaporation at 40 °C to concentrate the plant extract. The concentrated extract was stored in a refrigerator until conducting further tests. The extraction yield was calculated using Equation 1.

$$\text{Extraction yield (\%)} = (\text{mass of crude extract})/(\text{mass of dry plant}) \times 100 \text{ (Equation 1)}$$

The extract was submitted to standardized phytochemical screening tests adapted from the methodology of Simões et al. (2007), who employ colorimetric or precipitation reactions to identify classes of secondary metabolites. The tests used the plant extract diluted 1:10, with the investigation of steroids and terpenes (triterpenes) through the Liebermann-Burchard reaction, alkaloids with the Dragendorff reagent, flavonoids through the Shinoda reaction, tannins through the gelatin and ferric chloride reactions, coumarins by the reaction with potassium hydroxide and ultraviolet radiation, and amino acids by the ninhydrin reaction.

2.4 Preparation of pullulan films incorporating the extract of *C. leptophyllum* (pers.) Sprague

The polymeric films were produced through the casting method, associating the natural polymer PU, supplied by Attivos Magistrais, to the crude extract of *C. leptophyllum* (Pers.) Sprague in the proportion of 4% (m/v) PU and 15% extract (concerning the mass of PU). In parallel, films were produced only from the PU polymer matrix, which acted as control films. An aqueous dispersion of PU was prepared under stirring and heating to 50 °C. The crude extract was dissolved in a combination of ethanol, Tween 80, and methanol and slowly added to the PU polymer dispersion. After homogenization, the final dispersion was poured into Teflon[®] molds, 10 mL per sample, and left in an oven for 24 hours at 40 °C. After solvent evaporation, the films were removed from the molds and stored in a desiccator containing silica gel until their use (Lima et al., 2017).

2.5 Morphological evaluation of polymeric films

2.5.1 Thickness

The thickness of the films was determined using a Mitutoyo[®] 293-821-30 micrometer, with five random measurements per film and three samples of each formulation.

2.5.2 Color, size, texture and presence of bubbles

The morphological characteristics of color, size, texture, and presence of bubbles were visually evaluated. Cracked films, bubbles, or deformities were rejected.

2.6 Assessment of the physical and chemical properties of polymeric films

2.6.1 Solubility

Solubility was evaluated in distilled water by adapting the method by Silva et al. (2016). The films were cut into 2 cm samples and left in an oven at 50 °C for residual moisture evaporation. Subsequently, the samples were weighed and immersed in 50 mL of distilled water, remaining under immersion for 24 hours. The mixture obtained was filtered, and the material retained was dried in an oven at 40 °C until reaching constant mass. The amount of unsolubilized dry matter could then be calculated from Equation 2.

$$S\% = (M_s - M_f)/M_s \times 100 \text{ (Equation 2)}$$

Where M_s is the mass of dry starting material and M_f is the mass of unsolubilized material after drying.

2.6.2 Water vapor transmission

Water vapor permeability was evaluated using ASTM's "B" methodology, called E 96-66 (Standard Test Methods for Water Vapor Transmission of Materials, 2016). Permeability cups (Braive Instruments®) containing 10 mL of distilled water were used, where the 10 cm² films were fixed. The set was kept in a desiccator, and the system was weighed at certain times (0, 24, 48, 72, 96, and 120 hours) to obtain the lost mass value. The assay was performed in triplicate. The water vapor transmission rate (WVTR) was calculated using Equation 3.

$$\text{WVTR} = (g \times 24)/(t \times a) \text{ (Equation 3)}$$

Where g is the lost mass, t is the time in hours of monitoring the mass, and a corresponds to the area of the film used in m².

2.6.3 Degree of swelling and Degradation index

Based on the methodology of Lima (2017), aliquots of 1 cm² were removed from the films to calculate the degree of swelling. Their residual moisture was removed in an oven at 40 °C until constant mass. The samples were weighed and immersed in water at 37 °C. At each time interval (1, 3, 5, 7, 9, and 10 minutes), the films were removed from the immersion medium, removing excess liquid with filter paper to be weighed again. Concerning the degradation index, the films were filtered, oven dried for 24 hours at 60 °C, and weighed again after immersion. The assay was performed in triplicate.

The swelling and degradation indices were obtained from Equations 4 and 5, respectively.

$$\text{Is\%} = (M_i - M_s) / M_s \times 100 \text{ (Equation 4)}$$

$$\text{Id\%} = (M_s - M_{sf}) / M_s \times 100 \text{ (Equation 5)}$$

Where M_s represents the mass of the dry films, M_i, the mass of the films after immersion, and M_{sf}, the mass of the films after final drying in an oven.

2.7 Assessment of the antioxidant activity of polymeric films

The antioxidant activity of polymeric films incorporated with *C. leptophyllum* extract was determined, following the methodology of Zhai et al. (2018) using the DPPH method, which tests the rate of elimination of free radicals. Samples of 1 cm² of the films were solubilized in 10 mL of distilled water at room temperature. Then, 1 mL of this solution was added to 4 mL of DPPH solution in ethanol (150 μmol/L), and this mixture was left in an environment protected from light for 30 minutes. The control consisted of 4 mL DPPH solution and 1 mL distilled water. Absorbances were measured using a Biospectro® SP-220 spectrophotometer at 517 nm.

The level of capture of DPPH radicals was calculated according to Equation 6.

$$\text{Rate of elimination of DPPH radicals (\%)} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100 \text{ (Equation 6)}$$

Where A_{sample} is the absorbance of the sample and A_{control} is the absorbance of the control.

2.8 Statistical analysis

The results obtained were expressed as mean ± standard deviation. The analysis was performed with the GraphPad Prism application, using the paired t-test for the water vapor transmission, mass loss, and degradation index tests and the unpaired t-test for thickness and antioxidant activity, with a confidence level of 95%.

3. Results and Discussion

3.1 Obtaining the vegetable extract and Phytochemical screening

The ethanolic extraction yield was 9.88%, calculated using Equation 1. The phytochemical screening tests were positive for steroids, terpenes, and amino acids and negative for flavonoids, tannins, coumarins, and alkaloids. Steroids such as β -sitosterol and 7α -hydroxy-stigmasterol, and terpenes, such as γ -terpinene, carvacrol methyl ether, and thymol methyl ether, also found by Helal et al. (2016), have antioxidant and anti-inflammatory potential. Such potential is essential during the first phase of the healing process, the inflammatory phase, mediating inflammatory substances and eliminating free radicals (Almeida, 2015; Breda, 2010; Moreski et al., 2018).

The phytochemical analyses performed by Asamenew et al. (2008) and Pande et al. (2011) occurred using the methodology of gas chromatography coupled to mass spectrophotometry (GC/MS), demonstrating that the essential oil of the leaves of *C. leptophyllum* consists mainly of monoterpenes, such as thymohydroquinone dimethyl ether, isothymol methyl ether, thymol methyl ether, p-cymene, γ -terpinene, and carvacrol methyl ether. Sesquiterpenes and phenylpropanoids are also present, such as α -humulene and apiol, respectively. Helal et al. (2016) analyzed the composition of the methanolic extract of *C. leptophyllum* fruits using silica column chromatography (SCC), finding carbohydrates, flavonoids, terpenoids, phytosterols, glycosides, tannins, and proteins.

3.2 Morphological evaluation

3.2.1 Thickness

There was no statistically significant difference between the values of the control formulation and the formulation with 15% extract concerning the thickness measurement. As thickness values were 98.6 ± 6.5 and 93.7 ± 2.0 nm, the incorporation of the plant extract did not induce an increase in the biofilm thickening. Similar results were presented by Bilohan (2021) and Calceto and Romero (2017) when working, respectively, with PU films to develop food packaging and adhesive associated with *Calendula officinalis* extract for oral mucosa infections. In a study in which the PU films were incorporated with cinnamon essential oil and Tween 80 for use as food packaging, Chu et al. (2019) describe there is not always an increase in thickness due to the increase in total solids in the formulation, through the addition of essential oils for example. This is possible due to the hydrophobicity of these oils, whose lipophilic substances could weaken the hydrogen bonds between the PU molecules, leaving the structure of the films less loose.

3.2.2 Color, size, texture, and presence of bubbles

Films with a good appearance were obtained, without bubbles or cracks, with a smooth texture, great flexibility, and easy removal of the plates. The control films (Figure 1) showed translucency and homogeneity, as in the study by Bilohan (2021), while the films incorporated with extract (Figure 2) presented a greenish color due to the dark shade of the plant extract and the presence of accumulation points of the extract.

Figure 1 - Control film.



Source: Research data.

Figure 2 - Film incorporated with 15% extract.



Source: Research data.

The precipitation of solids in the films containing the extract may be related to the incomplete solubility of the extract in the polymeric solution since the PU solution is highly polar. In contrast, the extract may have hydrophobic metabolites such as steroids, terpenes, and coumarins, as stated in the article by Helal et al. (2016). Bilohan (2021) explains that natural compounds are commonly incorporated into biofilms, while for PU-based films, their hydrophilicity makes incorporating hydrophobic compounds more complex. Concomitantly, PU is insoluble in solvents such as ethanol, and the polymer itself may precipitate due to this incompatibility (Pradella, 2006; Takata, 2019).

3.3 Assessment of physical and chemical properties

3.3.1 Solubility

The water solubility of both the control films and the films incorporated with extract was 100% in 24h, so it was impossible to calculate the amount of non-solubilized dry matter. Oliveira (2014) states that the polymer structure influences its performance in an aqueous solution. The presence of polar, carboxyl, and hydroxyl functional groups increases the hydrophilicity of the polymer. Furthermore, the presence of α -1,6-glycosidic bonds in linear homopolysaccharides confers less structural rigidity. It increases solubility through the flexibility that the molecule has to rotate between its monomeric units, in the case of PU, the maltotrioses (Pradella, 2006; Silva et al., 2006; Chi et al., 2009; Oliveira, 2014).

3.3.2 Water vapor transmission

The barrier properties of biofilms are an indicator of their stability status. They are evaluated in terms of their water vapor transmissibility and directly depend on the mechanical characteristics of the polymers used. For example, film permeability increases by reducing its thickness or incorporating bioactive compounds (Bilohan, 2021; Luís, et al., 2021).

These properties can be used to evaluate, for example, the performance of polymeric films as food packaging or as a dressing for burns and wounds. As dressings, the films must act as a semi-permeable barrier, allowing proper gas exchange, covering the affected area, and ensuring its mechanical protection (Barros, 2022; Gomes, 2016; Luís, et al., 2021).

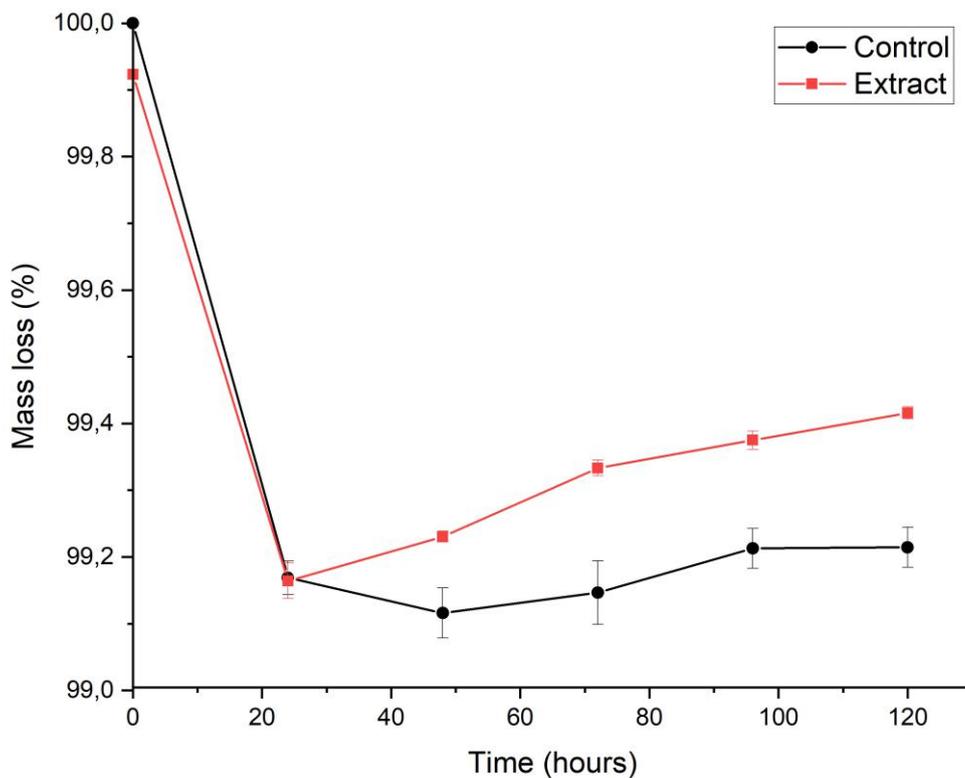
Table 1 - Table of water vapor transmission and weight loss values in 120 h.

Formulation	WVTR (g/m ² 24 h)	Weight loss values (g/120 h)
Control	1078.00 ± 11.37 ^a	5.39 ± 0.06 ^a
Extract	1081.33 ± 8.08 ^a	5.41 ± 0.04 ^a

^a Means in the same column with the same superscript do not differ significantly by paired *t*-test (*p*<0.05). Source: Research data.

When analyzing the test result (Table 1), no statistically significant differences in water vapor transmission were obtained between the films with and without extract. The control films obtained WVTR values of 1078.00 ± 11.37 g/m² in 24 hours, while the films incorporated with the extract obtained values of 1081.33 ± 8.08 g/m² in the same period, showing no increase or decrease in the permeability of the polymer due to the presence of the extract. Luís et al (2020) reported the same when performing the WVTR test and comparing the results between PU films and PU films incorporated with rock rose essential oil. Bilohan (2021) obtained similar results in pullulan and glycerol films incorporated with rock rose essential oil.

Figure 3 - Graph of mass loss of control films and films with 15% extract.



Source: Research data.

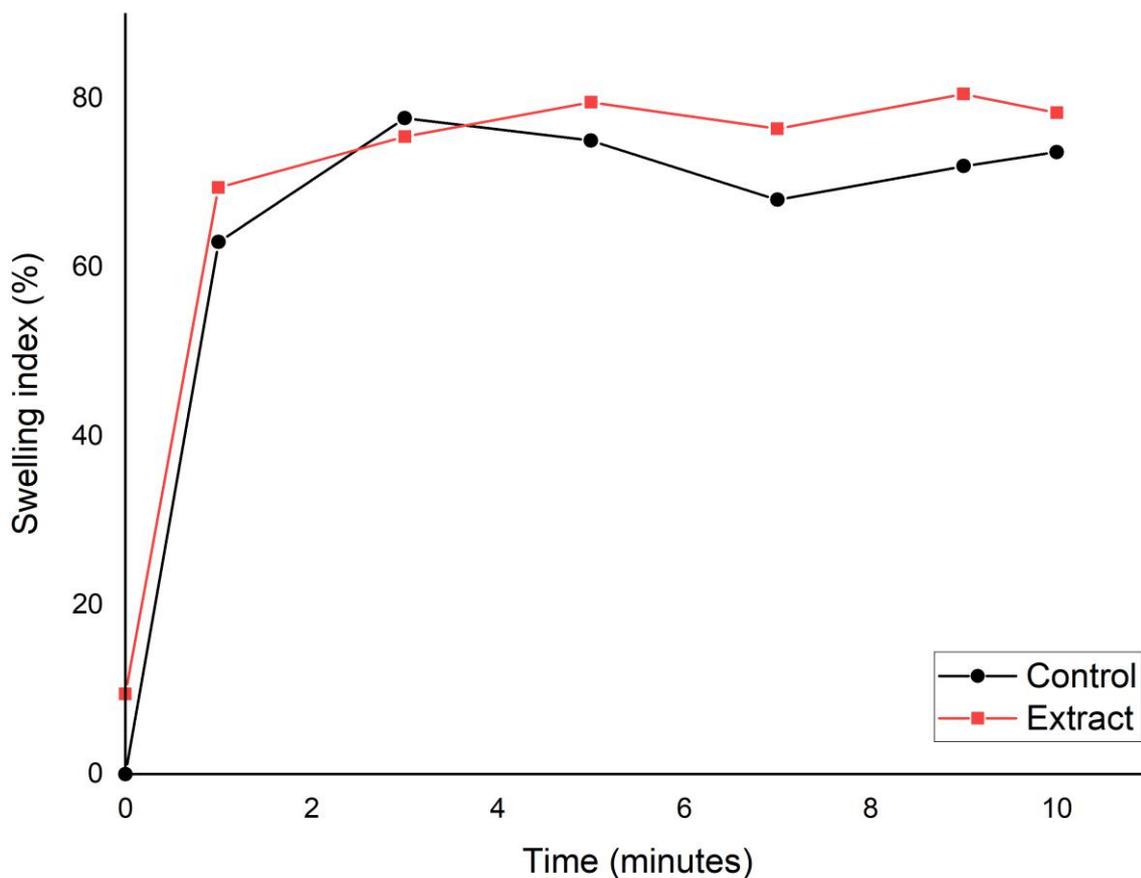
As seen in Figure 3, the mass loss was more significant in the first 24 hours, with subsequent stabilization. This is advantageous because it demonstrates that the biofilm allows gas exchange and remains structurally intact, maintaining its physical protection capacity.

3.3.3 Degree of swelling and Degradation index

Tests of swelling and degradation indices were performed to evaluate the films' degrees of hydration and decomposition. It was impossible to calculate the degree of swelling of the films produced because they had dissolved entirely during 7 minutes of immersion in water. It was also not possible to weigh the filtrates from 1 to 5 minutes because, in addition to part of the biofilms having already dissolved in the medium, the scale used for weighing was not sensitive enough for such small values. Although the films dissolved in the medium, a hydrogel was formed. Which can increase the mucoadhesiveness and adhesion of the films. Being able, therefore, still to protect and to act on the wounds.

Calceto and Romero (2017) argue in their thesis that the solubility of hydrophilic polymers significantly influences the degree of hydration, emphasizing that accelerated or excessive swelling results in the destruction of the films by rapid dissolution. It was also reported that the swelling was not significantly different with adding the plant extract compared to the PU-based polymeric system. Finally, they correlated that the polymer's solubility in the immersion medium and the variation of the pH of the medium mediated the water retention capacity of the films.

Figure 4 - Graph of the degradation index of control films and films with 15% extract.



Source: Research data.

Considering the PU polymeric system's high solubility and hydration capacity, it was possible to calculate its degradation index through Equation 5, as shown in Figure 4. There was more significant polymeric degradation in the periods of 1 to 5 minutes, while after 7 minutes of immersion, this index became less variable. When analyzing statistically, there was no significant difference between the results of the control films and the films with extract, demonstrating that the incorporation of the plant extract provided no greater resistance to the polymeric film.

3.4 Assessment of antioxidant activity

Asamenew et al. (2008) performed phytochemical analysis and DPPH antioxidant assay with the essential oil of *C. leptophyllum* leaves, pointing out the phenolic compounds (α -terpinene, γ -terpinene, and terpinen-4-ol) as the main responsible for the high antioxidant capacity obtained in the assay. It demonstrates that the higher the essential oil concentration, the greater the capture of free radicals. Sahoo et al. (2013) also evaluated the antioxidant capacity of *C. leptophyllum*, performing DPPH, hydroxyl, nitric oxide, and superoxide radical capture assays in aqueous, ethanolic, and methanolic extracts of the fruits of the plant species. The researcher reports that the fruits are sources of phenols and flavonoids and that the results of the tests showed intense antioxidant activity by inhibiting all the radicals tested. However, the methanolic extract showed more significant antioxidant activity and a greater amount of phenolic and flavonoid compounds compared to other extracts tested.

The results obtained in the present study demonstrate that there was no statistically significant difference in the antioxidant activity of the control films and the films incorporated with the extract of *C. leptophyllum*, corresponding respectively to 19.15 ± 13.38 and 17.23 ± 11.12 % of DPPH-free radical elimination rate. However, according to Oliveira (2015), the assessment of antioxidant activity should not be based on a single methodology. It requires the association of the DPPH assay with other tests, such as ABTS methods and lipid peroxidation, to fully determine a substance's antioxidant capacity. Furthermore, higher concentrations of the extract can be tested to assess whether there is an increase in DPPH inhibition as the plant concentration increases.

Probably the antioxidant activity of the produced films is related to the presence of pullulan. Li et al. (2022) observed a DPPH radical scavenging rate of 9.24% by pure pullulan. It also found that when adding pullulan to gelatin films, there was an increase in its antioxidant activity. This property is explained by the phenolic hydroxyl groups of pullulan. Therefore, even though in the present study there was no increase in the antioxidant property when incorporating the crude extract of the plant, the developed films present good inhibition of DDPH radicals, which could be beneficial in the healing process of wounds.

Moreover, the films incorporated with extract may have anti-inflammatory and antimicrobial activities that were not tested in this study. Helal et al. (2016) demonstrated the anti-inflammatory activity of the essential oil from the fruits of *C. leptophyllum*. It was greater than that of indomethacin - an effective non-steroidal anti-inflammatory. Concomitantly, research shows that the essential oil from the leaves of *C. leptophyllum* has antimicrobial activity against a broad spectrum of pathogens, including gram-positive and gram-negative bacteria, namely *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, and some fungal strains (Asamenew et al., 2008; Singh et al, 2013).

4. Conclusion

This study demonstrated that the PU film has potential as a dressing, given its great flexibility, mechanical protection, and performance as a semi-permeable barrier, allowing adequate gas exchange. These characteristics help to improve the tissue regeneration process and reduce the likelihood of complications. On the other hand, incorporating the extract brings some aspects that can limit the acceptability of the dressing by the patient, such as the staining and precipitation of compounds that restrict the visibility of the wound and the monitoring of healing, requiring removing the wound dressing. However, removing chlorophyll from the extract before its incorporation into the PU dispersion could be considered to avoid these drawbacks. In

addition, although the films have a high degree of water solubility, their addition to the aqueous medium allows the formation of a hydrogel, which can increase its adherence and mucoadhesiveness on wounds.

The developed films showed antioxidant activity, due to the presence of pullulan. The addition of the *C. leptophyllum* extract did not increase the inhibition of DPPH radicals, but verification is necessary from tests with other methodologies and extract concentrations. Other therapeutic activities, such as antimicrobial and anti-inflammatory ones, can be tested in future studies as they have been demonstrated in several international studies and are of interest to prove their effectiveness as an adjuvant in the healing process.

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