NATURAL EXTRACTS AND APPLICATION IN BIOPRODUCT DEVELOPMENT

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ABSTRACT

The research and use of biofilms have been growing, especially in biodegradable packaging and other areas. Combining biodegradable films with plant extracts from the Asteraceae family is a form of sustainable development. This work aimed to quantify bioactive compounds in ethanolic extracts of macela, carqueja, calendula, chamomile, and guaco and incorporate them into chitosan-based biofilms. The antioxidant activity was realized using the DPPH method, which adopted Trolox as a standard. The quantification of phenolic compounds was carried out according to the Folin-Ciocalteu methodology, and the flavonoid content was quercetin as a standard. The biofilm was prepared through an adaptation of the casting method. As a result, higher antioxidant activity and total phenolic content were obtained with guaco, respectively 10758.33 µmol ET gL^{-1} and 4686.98 mg EAG L^{-1}, and a higher value for flavonoids observed with macela, with a value of 799.83 mg EQ L^{-1}. All biofilms with extracts demonstrated greater solubility, moisture content and thickness than the control biofilm, confirming the application potential.

Keywords: Asteraceae. Biofilm. Biodegradable packaging.

1 INTRODUCTION

The applicability of plants, whether for food or medicinal purposes, is intrinsically linked to the history of man[1]. Therefore, the constant research into plant extracts with biological activities, especially antioxidant and antimicrobial activities, is valid and necessary, as we currently know the significant impact caused to the body by the action of free radicals due to the instability of electrons, which cause damage to cells and various diseases such as cancer[2], as well as the constant and progressive resistance presented by many microorganisms to current antibiotics[3].

Containing the most significant number of species among Angiosperms, representatives of the Asteraceae family have significant economic importance given that such plants, in addition to being used in food and ornamentation[4], exhibit, among many other properties, therapeutic properties, thus allowing several species of this family to have medicinal use due to anti-inflammatory, antioxidant, astringent and antimicrobial characteristics[3].

Due to environmental concerns combined with the demand for sustainable packaging in the food industry and other areas, interest has emerged in the development of biofilms[5]. These biodegradable films are obtained from biological materials such as polysaccharides, lipids, and proteins[6]. They have protective functions and increase the useful life of the product[7]. Biofilms can assistant maintaining specific product characteristics such as moisture and structural integrity and improve sensory aspects, in addition to incorporating additives, such as antioxidant and antimicrobial agents, pigments, and aromas[5].

A polysaccharide with great potential for application in producing these biofilms is chitosan, a chitin derivative present in the shells of crustaceans that is low-cost, renewable, and widely used in biotechnology[8]. Also, it is widely used in the pharmaceutical and food areas due to its antimicrobial activity[9].

Therefore, the objectives of this study were to analyze the antioxidant activities, total phenolic and flavonoid content of five extracts from plants in the Asteraceae family, namely *Achyrocline satureioides* (macela), *Baccharis genistelloides* (carqueja), *Callendula officinalis* L. (calendula or marigold), *Matricaria recutita* L. (chamomile) and *Mikania glomerata* (guaco) and investigate their incorporation into chitosan biofilms.

2 MATERIAL & METHODS

Five samples of plants from the Asteraceae family were used for this study. Four samples were obtained *in natural*, dehydrated form, including *Calendula officinalis* L., *Matricaria recutita* L., *Baccharis genistelloides* and *Achyrocline satureioides*, sourced from the Chamel company. The concentrated extract of *Mikania glomerata*, kindly provided by the company Sustentec, was stored in a refrigerator at a temperature of 5 °C. The fresh samples were ground using a knife mill and classified on 35 mesh sieves with a nominal aperture of 0.425 mm to prepare the ethanolic extracts. To obtain the ethanolic extracts, 4 g of the fresh sample was added to 100 mL of 70 % ethanol. They were then placed in an ultrasonic tip sonicator with an acoustic cabinet (Eco-Sonics) at a controlled temperature of 25 °C, a power of 90 % and a time of 10 minutes. The samples were filtered through 25 µm filter paper and stored in amber bottles under refrigeration.

The ethanolic extracts were characterized by the presence of bioactive compounds. Antioxidant activity was assessed by capturing 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, using Trolox 200 µmol L^{-1} as a standard. The absorbance was read in a UV-VIS spectrophotometer (Thermo Fisher Scientific) at 517 nm, and the result expressed in µmol ET L^{-1} of extract[10].

The phenolic compounds were quantified using the Folin-Ciocalteu method, with a calibration curve using gallic acid 200 mg L^{-1}; the samples read at 725 nm, and the content displayed in mg EAG L^{-1} of the extract[10]. To determine the flavonoid content, the standard used was quercetin and the readings were taken at 425 nm with quantities mentioned in mg EQ L^{-1} of extracts[10].

The biodegradable film was prepared using the casting method with adaptations. The film-forming solution was prepared using acetic acid at a concentration of 0.5 mol L^{-1}, 2 % chitosan, 1 % sorbitol 70 % solution in distilled water for more remarkable plasticity of the film, and 1 % of the extract. The film-forming solution was subjected to magnetic stirring with heating at 50 °C until the chitosan was completely solubilized. Subsequently, 30 g of the solution was dispersed into 8 cm diameter Petri dishes (90 x 15 mm) and dried in a circulating air oven (SOLAB, model SL.102/125) at 40 °C for 16 hours.

The biofilms were evaluated for visual aspects based on homogeneity and handling, and the thickness was measured with a digital caliper at five different points. To calculate the moisture content, square cuts of 4 cm² with the biodegradable film were weighed before and after 24 hours in an oven at 105 °C with air circulation. For the solubility analysis, the films dried at 105 °C were placed separately in erlenmeyer flasks with 50 mL of distilled water and transferred to an incubator with orbital agitation at 68 rpm and 25 ± 2 °C for 24 hours. Subsequently, the films were dried again in an oven at 105 °C for 24 hours, and a third weighing was performed to determine the solubility.

3 RESULTS & DISCUSSION

After obtaining the extracts, the antioxidant activity and the content of phenolic compounds and flavonoids were determined using standards to construct the calibration curves and get the straight-line equations. These results can be seen in Table 1.

Table 1 Results of antioxidant activity using the DPPH method and quantification of the content of phenolic compounds and flavonoids in extracts of plants from the Asteraceae family obtained using ultrasound.

Popular name	Scientific name	DPPH (µmol ET L^{-1})	Phenolic (mg EAG L^{-1})	Flavonoids (mg EQ L^{-1})
Calendula	*Calendula officinalis* L.	975.33	309.79	98.58
Chamomile	*Matricaria recutita* L.	2871.67	673.20	322.28
Carqueja	*Baccharis genistelloides*	4042.50	980.11	122.28
Macela	*Achyrocline satureioides*	6630.00	1271.68	799.83
Guaco	*Mikania glomerata*	10758.33	4686.98	377.02

The results indicate that guaco has a high antioxidant activity, followed by macela and carqueja, similarly, for the content of phenolic compounds. These results indicate that phenolic compounds play a significant role in the antioxidant activity observed in the extracts since the same plants showed the best results in both characterizations. As for flavonoid content, macela stood out with 799.83 mg EQ L^{-1}, and guaco with 377.02 mg EQ L^{-1}. These values indicate that flavonoids, a class of phenolic compounds, are the majority in these extracts. Thus, it contributes to the antioxidant activity.

Therefore, the high antioxidant activity and phenolic contents of guaco, macela and carqueja are expected, given that previous studies have already proven the presence of bioactive compounds in these plants[3, 11,12]. As for flavonoid content, macela stands out, consistent with studies identifying active flavonoids in the plant[13]. Also, the guaco has a high flavonoid content, contributing to its overall antioxidant activity.

These results have important implications for the development of sustainable food films. Plants with high levels of phenolic compounds and flavonoids, such as those included in this study, can effectively protect food against oxidation, prolong its shelf life and improve quality. Previous studies indicate that films containing plant extracts such as guaco, macela and carqueja can significantly delay lipid oxidation in food, which is the leading cause of food degradation[14].

Regarding the characterization of the films, all showed a brown-greenish color, homogeneous characteristics, absence of bubbles and insoluble particles, and easy handling due to good flexibility. The average thickness values of each film, as well as solubility and moisture content, are shown in Table 2.

Table 2 Characterization analysis of chitosan biofilms incorporated with extracts from plants of the Asteraceae family.

Popular name	Scientific name	Thickness (mm)	Moisture content (%)	Solubility (%)
Control	-	0.156 ± 0.04	19.48 ± 1.31	24.89 ± 5.447
Calendula	*Calendula officinalis* L.	0.152 ± 0.08	21.29 ± 0.48	30.88 ± 0.003
Chamomile	*Matricaria recutita* L.	0.144 ± 0.01	20.90 ± 1.55	43.84 ± 0.782
Carqueja	*Baccharis genistelloides*	0.180 ± 0.06	20.53 ± 0.89	27.09 ± 4.095
Macela	*Achyrocline satureioides*	0.172 ± 0.06	21.64 ± 0.53	26.1 ± ND*
Guaco	*Mikania glomerata*	0.179 ± 0.04	20.66 ± 2.45	28.39 ± 1.444

* ND: Not done.

Considering that these biodegradable films can be used as biodegradable packaging, the analysis of the film's thickness is essential as it is directly related to oxygen penetration, which can accelerate the food deterioration process[15]. It was observed that two films (calendula, chamomile) decreased their thickness compared to the control, and three increased (carqueja, guaco, macela), probably due to the interaction of the extracts with the chitosan, which can alter its structure.

Water solubility and moisture content are essential analyses since packaging with high solubility is unsuitable for food due to its low water resistance,[16] and moisture content indicates the film's ability to absorb moisture from the environment. Regarding moisture content, all films had similar increases compared to the control, resulting in the extracts not significantly affecting the film's hygroscopicity. Concerning solubility, all biodegradable films showed an increase, with chamomile presenting the most change. Nevertheless, these increases were expected as this behavior occurs in many biofilms after adding extracts[17].

4 CONCLUSION

In conclusion, the extracts used in this study contain bioactive compounds; therefore, flavonoids that belong to the group of phenolic compounds, when incorporated into biofilms for packaging or other products, can act as antioxidants and improve the conservation of products. Moreover, the results obtained in this study demonstrated the great potential for the application of plants from the Asteraceae family not only in the field of biodegradable packaging but also in other areas that aim for a more sustainable economy, requiring even more research into the properties of such plants and other possible applications.

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