Advances in Health, Sports and Technology Sciences

DOI: 10.14744/ahsts.2025.57220
Adv Health Sports Technol Sci 2025;2(1):16–25

Original Article

Keratin-based Composite Films with *Hypericum triquetrifolium* and *Hypericum neurocalycinum* Extracts as a Base Material for Biomedical Applications

Ismail Sen, Behlul Koc Bilican, Tugce Karaduman Yesildal, Emel Cakmak

Department of Molecular Biology and Genetics, Aksaray University, Aksaray, Türkiye

ABSTRACT

Objective: Recycling of used materials and utilization of natural wastes have become even more important for the survival of future generations. For this reason, there has recently been an increasing number of experiments on upcycling methods using natural materials. Keratin, notable for its excellent biocompatibility, low immunogenicity, and abundance, presents itself as a strong candidate for biomedical applications. In here, we developed keratin-based composite films by incorporating of water and methanol extracts from two *Hypericum* species: *Hypericum neurocalycinumto* and *Hypericum triquetrifolium*.

Materials and Methods: The physicochemical properties of these films were characterized through thermogravimetric analysis, scanning electron microscopy and Fourier transform infrared spectrometry while their biological activities were evaluated using in vitro antioxidant and cytotoxicity tests on L929 fibroblast cells.

Results: The free radical removal rate increased with the addition of the *Hypericum* extracts (between 76.41% and 82.07%) compared to control (63.48%). The cell viabilities of control (without material), control film, *Hypericum neurocalycinumto* infusion (HNinfusion), *Hypericum neurocalycinumto methanol* (HNMeOH), *Hypericum triquetrifolium* infusion (HTinfusion) and *Hypericum triquetrifolium methanol* (HTMeOH) were 96.02±3.30, 79.33±4.11, 71.59±5.28, 79.37±7.49 and 71.25±3.33 %, respectively, at 24 h.

Conclusion: A new natural product that can be used for food and other industries has been produced with waste feathers as a source of keratin and *Hypericum* extracts for the first time.

Keywords: Antioxidant activity, characterization, cytotoxicity, feather, isolation, keratin

Cite this article as:
Sen I, Koc Bilican B, Karaduman Yesildal T, Cakmak E. Keratin-based Composite Films with *Hypericum triquetrifolium* and *Hypericum* neurocalycinum Extracts as a Base Material for Biomedical Applications. Adv Health Sports Technol Sci 2025;2(1):16–25.

Address for correspondence:
Emel Cakmak.
Department of Molecular Biology and Genetics, Aksaray University, Aksaray, Türkiye
E-mail: emelkunduz@gmail.com

Submitted: 02.03.2025
Revised: 18.03.2025
Accepted: 18.03.2025
Available Online: 27.03.2025

Advances in Health, Sports and Technology Sciences – Available online at www.advanceshsts.com

INTRODUCTION

Pollution is one of the leading environmental causes of disease worldwide today [1]. With increasing industrialization, the most prominent pollutant is plastic, whose production and release into the environment continues to increase. Since its popularization in the 1950s, the use of plastics has increased rapidly due to its benefits for public energy, safety and health [2,3]. Plastics have become indispensable today, not only because they are very practical and have many uses, but also because they are a polymer that is rich in diversity. The current consumption pattern of these polymer-based products, which are widely used worldwide, also results in piles of waste that pose major environmental, social and economic problems worldwide [4]. As much as 99% of the plastic products used worldwide are produced using petrochemical or fossil raw materials [5]. There is

an urgent need and growing interest worldwide to reduce the use of petroleum-based products and to develop biomaterials using sustainable and renewable resources. Future generations need to work on biomaterials to meet their basic needs for food, clothing, medicine, automobiles, cosmetics, packaging and other basic needs. This is where nature has become a focal point, providing many unique raw materials that are important sources of inspiration for pharmaceutical, food and biomaterials pioneers. These raw materials are cheap, environmentally sustainable and renewable resources for the development of biomaterials [6,7].

Natural polymers are widely used because they are environmentally friendly, emit low greenhouse gases, and originate from various renewable sources such as microorganisms, animals, plants, and their residues [8]. The main biopolymers utilized in this context include chitin, chitosan, collagen, silk, keratin, and elastin, all of which originate from animal sources. Additionally, there are plant-based natural polymers such as pectin, cellulose, and starch [9]. One of these biopolymers, the β-layered structure, which constitutes about a quarter of the keratin protein, predominantly forms the middle hydrophobic part of the structure. This high hydrophobicity is an indication that the fibers are soluble in organic solvents. Keratin is the main component of epithelial cells in horns, nails, feathers, hair and hooves of different organisms. The reason why keratin is an important, renewable and sustainable raw material for many applications is its high resistance to degradation compared to other animal tissues [10]. Polymers obtained from renewable resources and capable of biodegradation have garnered significant attention from both academia and industry on a global scale [11]. It is extremely important to ensure the durability, compatibility and stability of most polysaccharide-based films. At this point, it is possible to provide more flexibility to materials by incorporating polyols such as glycerol as plasticizers [12].

The economic viability, low toxicity and pharmacological properties of medicinal plants, including their analgesic, anti-inflammatory and antipyretic activities (due to their high phenolic content, antioxidant, antimicrobial and antiproliferative properties), have attracted great interest in the light of technological advances in the scientific field [13,14]. The addition of these herbal ingredients to edible films or food packaging can result in enhanced structural and functional properties compared to films made with single ingredients [15,16]. The genus *Hypericum*, rich in phytochemicals, is one of the 100 largest genera in the class of closed-seeded plants and encompasses more than 500 species that occur as trees, shrubs, annuals and perennials, which are highly variable in habitat. Globally, the medicinal properties of most *Hypericum* species are well known [17]. Moreover, plants belonging to the genus *Hypericum* and their compounds can exhibit anti-inflammatory, antifungal, cytotoxic, antioxidant, antihyperglycemic, antimicrobial and hepatoprotective activities *in-vitro/in-vivo*, as well as monoamine oxidase and acetylcholinesterase inhibitory activities [18]. For these reasons, research on the genus *Hypericum* has intensified in recent years.

In this study, keratin purified from waste goose feathers was mixed with methanol and infusion extracts prepared from the above-ground parts of *Hypericum triquetrifolium* and *Hypericum neurocalycinum* species in the presence of glycerol to produce antioxidant-enriched composite films. Structural and morphological characterizations (FT-IR, TGA, SEM and contact angle) enabled the films to be compared with each other as well as evaluating the efficacy of the plants. Soil and water degradation tests were conducted to assess the suitability of the films for sustainable applications. Antioxidant and cell viability tests were conducted to assess their potential applications in the food and other industries.

MATERIALS AND METHODS

Plant and Feather Samples Collection

Goose feathers were sourced from a slaughterhouse in Aksaray, Türkiye. Plant samples used for extraction were collected from different regions of Türkiye during the summer of 2019. *H. neurocalycinum* was gathered from Hadim village, Dedemli Valley (3,140 m, Konya), while *H. triquetrifolium* was obtained from Anamur village, near the ancient city of Anemurium (5 m, Mersin). Taxonomic identification was performed by Prof. Dr. Evren Yildiztugay, a botanist from the Department of Biotechnology at Selcuk University, Konya, Türkiye. The aerial parts of the plants (leaves, stems, and flowers) were separated from the roots. These plant materials were then dried in a shaded, well-ventilated environment. After approximately 10 days, they were ground into a fine powder using a laboratory mill and subsequently stored in a cool, dark place away from direct sunlight.

Plant Extraction

In the study, maceration was found to be a suitable method for obtaining the methanol extract. The procedure involved the following steps: five grams of powdered plant samples were stirred with 100 mL of methanol at room temperature for 24 h. The mixture was then filtered, and the solvent was evaporated using a rotary evaporator. The water extracts were subjected to infusion. Five grams of the material were boiled in 100 mL of water for 15 min, then filtered and lyophilized. The dry extracts were stored at 4°C.

Keratin Extraction

A quantity of 10 g of goose feathers was meticulously divided into small pieces and weighed. Subsequently, a solution was meticulously prepared by combining 0.2 N Na2S and 3 N NaOH in 200 mL of distilled water. This solution was then poured over the weighed goose feathers. The mixture was then placed in a hot water bath at 40°C for a duration of 2 h. Thereafter, a 2 N HCl solution was prepared with the objective of precipitating the extracted keratin. The HCl solution was added to the keratin solution while it was on a magnetic stirrer. The pH of the solution was then adjusted to 4.2, at which point the precipitation was completed. The precipitated keratins were washed with acetone and filtered using filter paper. Finally, the keratin was left to dry in an oven at 23°C for 24 h.

Preparation of Composites Films

5 g of keratin was dissolved in 200 mL of aqueous solution containing 5% H_2O_2 for 24 h on a magnetic stirrer. For each composite film sample, 20 mL of the dissolved keratin was taken from the stock solution and mixed with 250 mg of plant extract. Finally, 0.3 mL of glycerol was added and the mixture was stirred on a magnetic stirrer for 5 min. The extract was allowed to dissolve completely, then the samples were poured into Petri dishes and left to dry at room temperature.

Thickness of the Keratin-based Composite Films

The thickness was detected by using the digital micrometer Mitutoyo (Coolant Proof Micrometer-293) from 10 different sites of films, and the results were given by taking the average of the measurements.

Characterization of Composite Films

FT-IR

The ATR-FTIR spectra were measured with a Perkin Elmer FTIR spectrometer at a frequency range of 4000-400 cm^{-1} by weighing out 5 mg from Control, Hn-Infusion, Hn-MeOH, Ht-Infusion, Ht-MeOH films.

TGA

The thermal degradation of the Control, Hn-Infusion, Hn-MeOH, Ht-Infusion, Ht-MeOH films were obtained using the EXSTAR S11 730°C at a heating rate of 10°C/min to obtain TG and DTG curves.

SEM

The surface morphology of the Control, Hn-Infusion, Hn-MeOH, Ht-Infusion, Ht-MeOH films were analyzed using a FEI Quanta FEG 250 SEM. Prior to analysis, the mucilage-based keratin films were sputter coated with Au with a Gatan Precision Etching Coating System (PECS).

Contact Angle

The water contact angles of the Control, Hn-Infusion, Hn-MeOH, Ht-Infusion, and Ht-MeOH films were analyzed using the Data Physics OCA30 video-based contact angle measurement system. The hydrophobicity of the films was assessed through the Owens, Wendt, Rabel, and Kaelble (OWRK) method. To ensure accuracy, ten separate measurements were taken and averaged.

Free Radical Scavenging Activity

Antioxidant activities of Control, Hn-Infusion, Hn-MeOH, Ht-Infusion, Ht-MeOH films were determined by the following method [19]. The films were cut into small pieces and weighed at 10 mg before being placed in test tubes. Then, 6x10-5 M concentration of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared and 1.0 mL of this reagent solution was added to the sample tubes. The sample was subjected to incubation in conditions of darkness and ambient temperature for a period of 30 min. Subsequent to this, the volume of each solution was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Each sample was analyzed in triplicate. DPPH radical scavenging activities were calculated using the following equation:

$$\text{Inhibit (\%I)} = ((\text{Acontrol} - \text{Asample})/\text{Acontrol}) \times 100$$

Acontrol is the absorbance of the control, Asample absorbance is Control film, Hn-Infusion, Hn-MeOH, Ht-Infusion, Ht-MeOH films + DPPH.

Biodegradability

For film materials, beakers were filled with characterized soil. A certain number of samples was buried 3 cm below the surface in a metal net, the beakers were watered regularly and the soil was kept at 30% water-holding capacity. The samples were removed from the soil on days 10, 20 and 30 and carefully cleaned to avoid damage to the samples. The experiments were repeated three times and weight loss was calculated using the same equation.

$$\%\,WL = ((Wi\text{-}Wf)) / Wi \times 100$$

WL = weight loss, Wi = initial weight and Wf = final weight

Cell Culture Studies

L929 cell lines were obtained from the HUKUK Sap Institute in Ankara, and subsequently cultivated in DMEM medium with a high glucose concentration (4.5 g/L), which was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin. The cells were harvested using a trypsin-EDTA solution (0.25% trypsin and 0.05% EDTA) at 37°C for a duration of 5 min, once the cells had attained a confluence of 70%. Subsequent passages were performed at a

1:4 dilution interval, with a maximum interval of 2-3 days. Cell viability was assessed using trypan blue, and experimental continuation was contingent on viability exceeding 90%. It should be noted that all experiments were performed with cells at passages 18 and 19.

For the MTT assay, L929 cells were seeded in 96-well plates at a density of 1,104 cells per well. Following an overnight incubation, when the cells were approximately 70% confluent, fresh cell culture medium was added to the sterilized biomaterials for the treatment groups, and the media in the control wells were also refreshed. Following a 24-h incubation period, MTT solution (Serva, Germany) was added to each well at a final concentration of 0.5 mg/mL (10 µL). Three hours later, the formation of formazan crystals was checked, and the crystals were dissolved in 100 µL of DMSO. The resulting color was measured spectrophotometrically using a ChroMate® ELISA microplate reader set to a wavelength of 492 nm. Each experiment was performed in triplicate, and wells without biomaterial served as controls. The percentage of cell viability was calculated based on the control group.

$$\text{Cell Viability} = [(A\text{sample})/(A\text{control})]\ \chi 100 \quad (1)$$

A control is the absorbance of the control well and A indicates the absorbance of the wells containing sample biomaterial.

Statistical Analysis

Data analysis was performed using GraphPad Prism software (version 5, GraphPad Software®). Results are expressed as the mean ± standard error of the mean (SEM). Statistical differences were assessed using One-Way ANOVA, followed by Tukey's post-hoc test with a 95% confidence interval. A p-value below 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

SEM and Thickness of the Films

The appearance of the control keratin film and the *Hypericum neurocalycinumto methanol* (HNMeOH), *Hypericum neurocalycinumto* infusion (HNinfusion) and *Hypericum triquetrifolium methanol* (HTMeOH), *Hypericum triquetrifolium* infusion (HTinfusion) composite films produced by adding water and methanol extracts obtained from two different *Hypericum* species were shown in Figure 1. The homogeneous appearance of the films reveals that the extracts added into the films were successfully incorporated into the keratin membrane. The thicknesses of the control film and HNMeOH, HNinfusion, HTMeOH, HTinfusion composite films were recorded as 0.03±0.01, 0.15±0.02, 0.16±0.04, 0.16±0.04 and 0.15±0.03 µm, respectively. It was determined that the thicknesses increased at a similar rate with the addition of extracts to the keratin film compared to the control film.

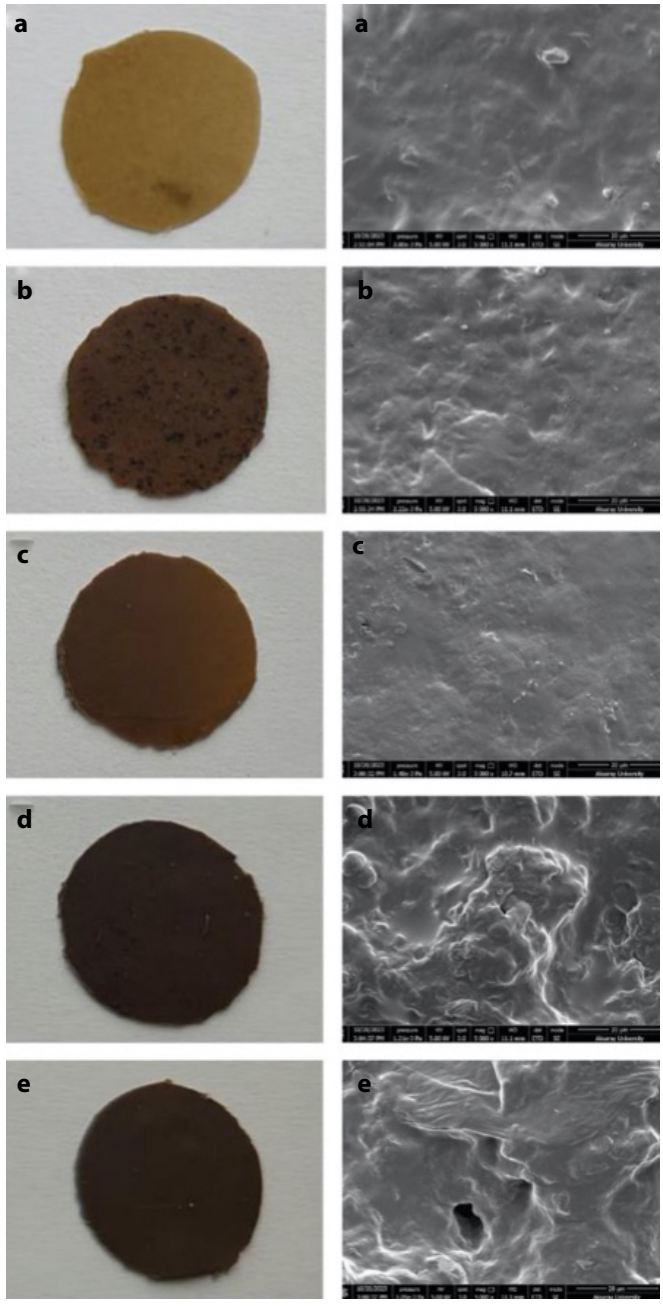

Figure 1. The appearance of the control keratin film (without plant extract) **(a)** and composite films; HNMeOH **(b)**, HNinfusion **(c)**, HTMeOH **(d)**, HTinfusion **(e)**.

One of the most important characterizations that determine the application areas of materials in film form is the determination of their surface morphology. It was observed that the control film produced with glycerol added to the keratin solution as a plasticizer had a very smooth, homogeneous morphology. The surface morphology of the

films produced by adding methanol and water extracts of *Hypericum neurocalycinum* the keratin solution has a rough, continuous morphology. The addition of methanol and water extracts of *Hypericum triquetrifolium* to the keratin solution was noted to have an irregular rough surface morphology. It is predicted that the extracts give the surface a rough, homogeneous morphology due to evaporation during the drying of the film. In previous studies, similar rough surfaces were recorded after extract added to natural films. The common point of all observed morphologies of the produced films with rough, protruding surfaces is that they are continuous and homogeneous. This supports that the added materials are successfully homogeneously incorporated throughout the film.

FT-IR

The present study employed FTIR analysis to evaluate the chemical changes that occurred in the matrices of HNMeOH, HNinfusion and HTMeOH, HTinfusion composite films are created by incorporating additives, water and methanol extracts obtained from the control keratin film and two different *Hypericum* species through the functional groups and their corresponding recorded spectra are presented below Figure 2.

The broad peaks recorded between 3200-3500 in all of the films produced are due to the H bonds formed between the molecules due to water and plasticizer in the structure [20]. At the same time, obvious peaks between 3271-3292 cm^{-1} were recorded in this range. These peaks can be attributed to the OH groups of the polysaccharides in the structure [21]. The peak recorded at 2924 cm^{-1} in all films produced is due to C-H stretching vibration in aliphatic CH_2 groups [22]. The peaks recorded around 1740 cm^{-1} for the composite films produced can correspond to the C-O stretching vibration of the ester functional groups in the extracts [23-25]. In addition, the peaks recorded between 1600-1400 cm^{-1} for the composite film can be attributed to benzene ring saturations in the phenolic content of the extracts [26]. Amide I and Amide II bands, which are the characteristic peaks for the keratin protein that forms the basis of the produced films, were recorded between 1620-1640 cm^{-1} and 1540-1570 cm^{-1} wavelengths, respectively [27,28]. In addition, the peaks recorded in the spectra between 1100-1200 and 1030-1060 cm^{-1} wavelengths can be attributed to sulfonic acid groups bound to keratin [29]. The recorded spectra support that water and methanol extracts obtained from two different *Hypericum* species successfully formed composites with keratin film.

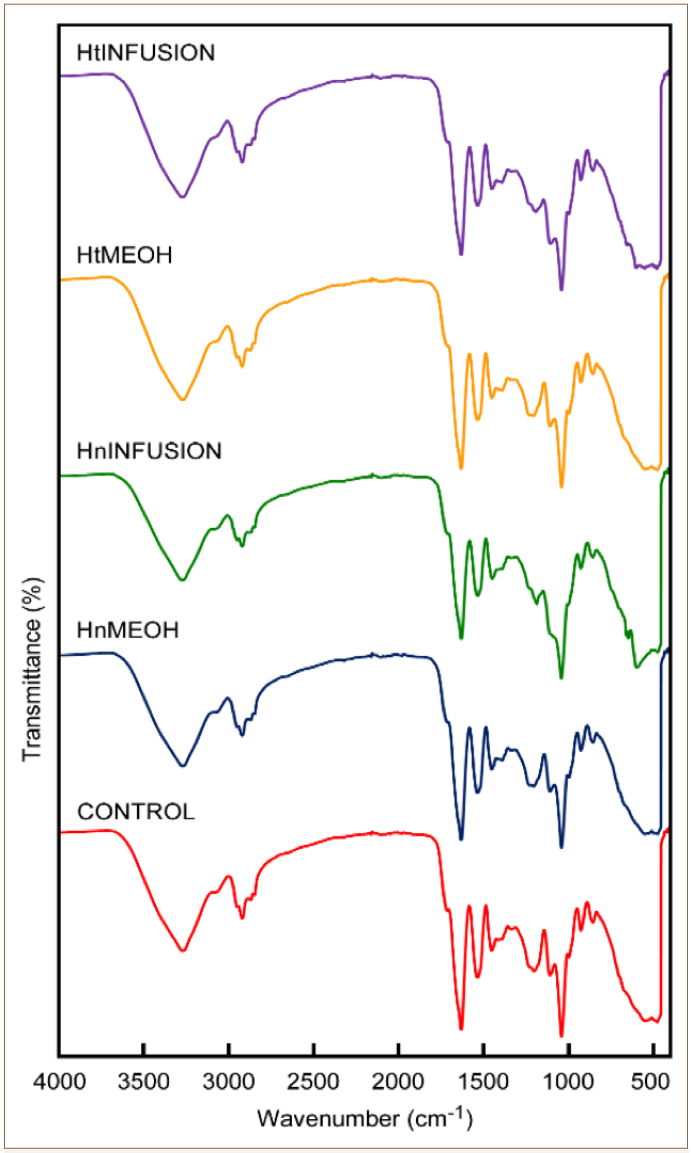

Figure 2. FT-IR analysis of control and composite films.

TGA

TGA analysis was performed to evaluate the thermal stability of HNMeOH, HNinfusion and HTMeOH, HTinfusion composite films produced by adding water and methanol extracts from two different *Hypericum* species to keratin solution and the thermograms recorded are shown in Figure 3.

In the literature, the peaks recorded at 0-100°C in the films are attributed to the desorption of intermolecular moisture in the polymeric structure of the films [30-32]. Composite films are generally brittle and plasticizers such as glycerol should be added to improve their flexibility and processability [33]. The degradation peak of glycerol can be observed approximately

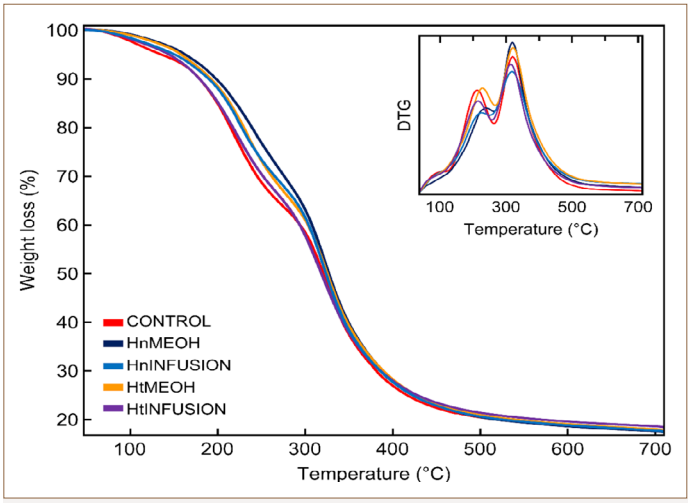

Figure 3. TGA of control and composite films.

between 150-280°C [34]. The degradation peaks of plant extracts are also recorded in similar ranges [35,36]. In the present study, the glycerol peak for the control film was recorded as 208.2°C. In composite films, glycerol and extract peaks screened each other and the second major peaks (DTGmax) for HNMeOH, HNinfusion, HTMeOH, HTinfusion films were recorded as 235.4, 223.5, 224.8 and 222.4°C, respectively. The other degradation peak recorded in the thermograms of the films belongs to keratin, which forms the basis of the films. The keratin peak recorded at 320.8°C for the control film was recorded at 319°C for HNMeOH film, 322.7°C for HNinfusion film, 321.1°C for HTMeOH film and 323.4°C for HTinfusion film. Similar degradation temperatures were recorded in the thermograms of hair, wool and chicken feather keratins in the literature [37-39]. This high thermal stability of the produced natural films may enable them to be used in different application areas in the future.

Contact Angle

The water contact angles of the keratin control film and HNMeOH, HNinfusion and HTMeOH, HTinfusion composite films produced in the study are shown in Figure 4.

Free Radical Scavenging Activity

The antioxidant activities of the control and keratin films containing *Hypericum* water and methanol extracts were evaluated by measuring the free radical scavenging activity. Accordingly, while the DPPH radical scavenging activity of the control was 63.48%, the antioxidant activities of the keratin-based composite films formed by the addition of extracts increased compared to the control. The free radical scavenging activities of the HNMeOH, HNinfusion, HTMeOH and HTinfusion were determined as 82.48%, 76.41%, 82.07%, and 81.54% respectively (Table 1). Compared to the chitosan-based film [10], and mucilage-based keratin film [40], current study showed a minimum of 3 times higher antioxidant activity. This is very important considering the potential for biomedical activities.

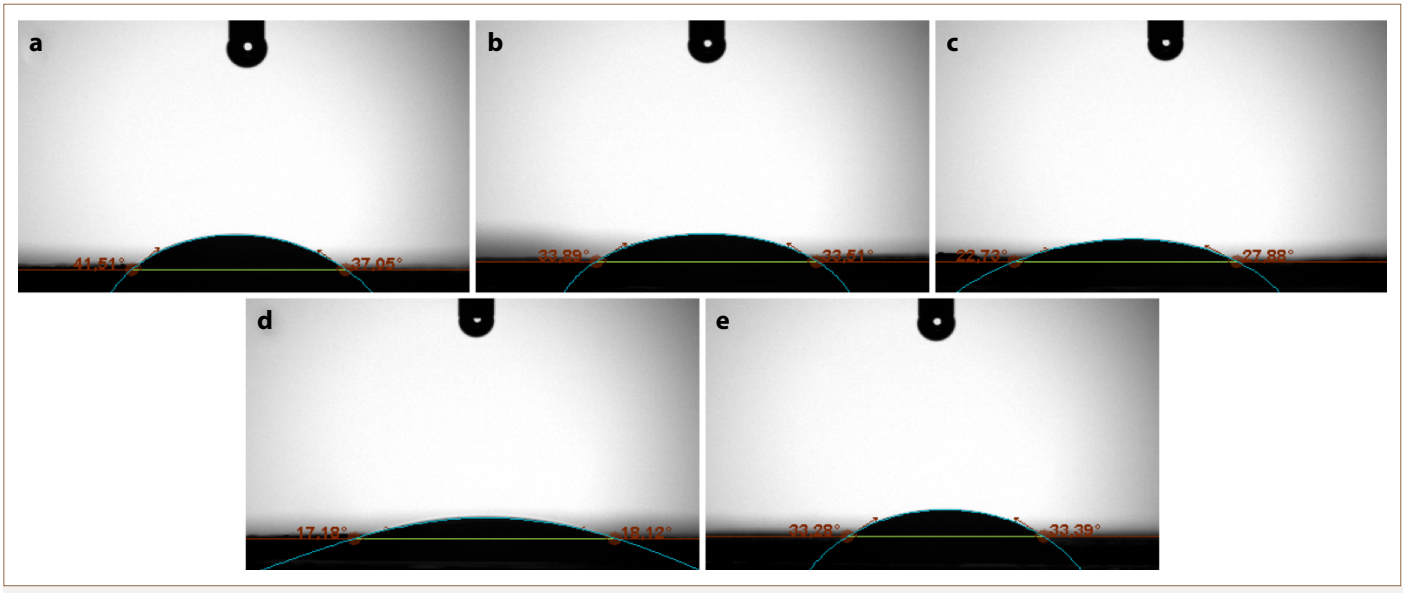

Figure 4. Water contact angle analysis results of control keratin film **(a)** and composite films; HNMeOH **(b)**, HNinfusion **(c)**, HTMeOH **(d)**, HTinfusion **(e)**.

Table 1. Free radical scavenging activities (%) of control and composite films; HNMeOH, HNinfusion, HTMeOH, HTinfusion

Films	Inhibition (%)
Control	63.48±2.57*
HNMeOH	82.48±0.74
HNinfusion	76.41±3.36
HTinfusion	81.54±0.39
HTMeOH	82.07±0.50

*Means of three parallel measurements±SD. HTMeOH: Hypericum triquetrifolium methanol; HTinfusion: Hypericum triquetrifolium infusion; HNMeOH: Hypericum neurocalycinumto methanol; HNinfusion: Hypericum neurocalycinumto infusion.

Biodegradation

The accumulation of plastics in the soil for many years without degradation causes major ecological problems. Therefore, the demand for natural films and alternative biomaterials to plastic is increasing day by day. The films produced in this study were incubated in soil for 30 days to determine their degradation rates in soil. The weight loss of the films is interpreted as an indicator of their degradation [41]. At the end of 15 days, 84±0.21% mass loss occurred in the control film and 98±0.8% mass loss occurred at the end of the experiment (Fig. 5). The mass losses of the produced HNMeOH, HNinfusion and HTMeOH, HTinfusion composite films were 79±0.31%, 77±0.42%, 80±0.01% and 87±0.10% at the end of the 15-day period, respectively, and 97±0.2%, 98±0.3%, 97±0.1% and 98±0.3% at the end of the experiment, respectively (Fig. 5).

The high degradability of the produced films in soil is a great advantage in terms of nature, but these results support the low strength of the films. The strength of the films is one of the most important properties in determining the usage areas [42-44]. It can be predicted that films with rapid degradability in soil can be an alternative to plastics that cause ecological destruction.

Cell Culture

The purpose of the MTT assay is to measure the viability of cells in proportion to the activity of the mitochondrial dehydrogenase enzyme. In living cells, ongoing mitochondrial activity results in the formation of formazan crystals. When these formazan crystals are dissolved in solvents such as DMSO, they form a purple color with an intensity proportional to cell viability. MTT assay results were acquired on a microplate reader (ChroMate®ELISA) at 492 nm.

MTT assay was performed at 24 h for Control film, HNinfusion, HNMeOH, HTinfusion, HTMeOH biomaterials. In this analysis, only cells without material containing DMEM medium were selected as the control group. Percent cell viability of the materials compared to the control group for 24 h is shown in Figure 6. The cell viabilities of control (without material), control film, HNinfusion, HNMeOH, HTinfusion and HTMeOH

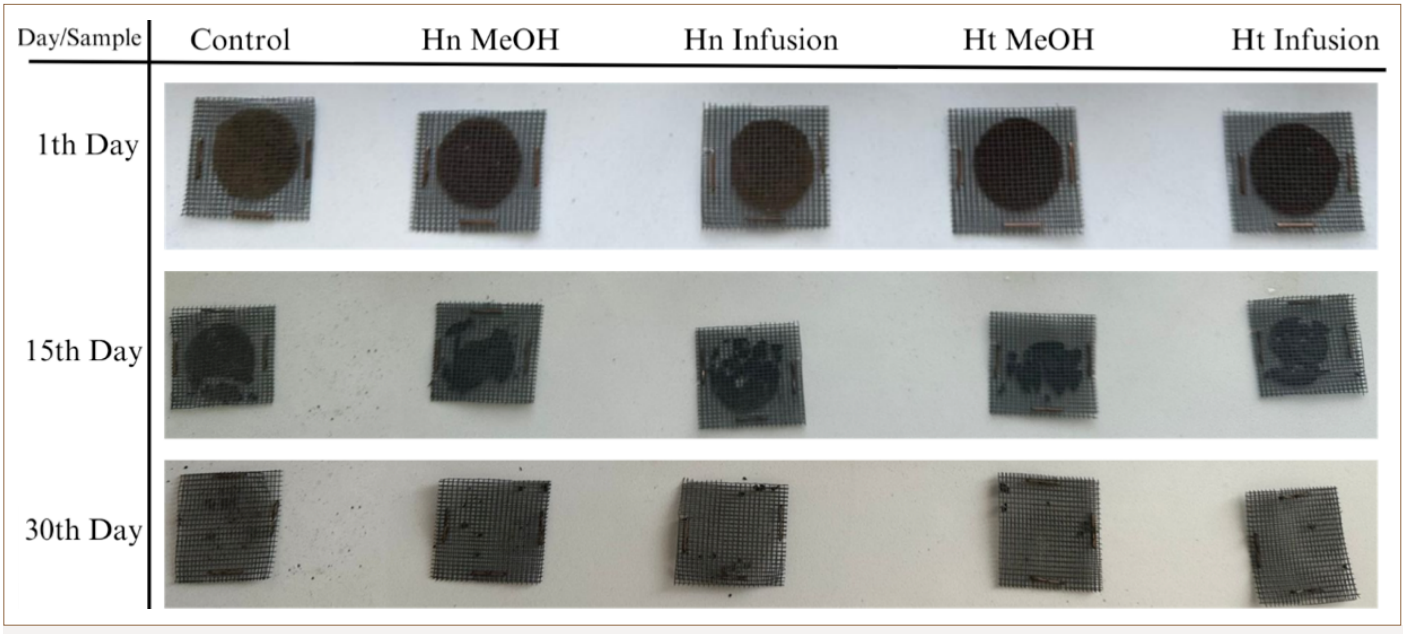

Figure 5. The weight loss of the control and composite films.

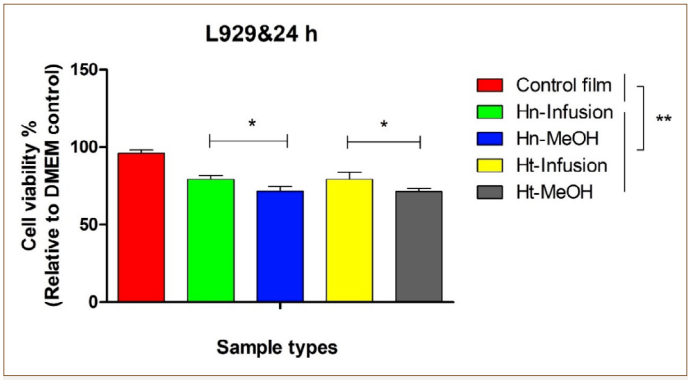

Figure 6. MTT assay results of the respective biomaterials.

were 96.02±3.30, 79.33±4.11, 71.59±5.28, 79.37±7.49 and 71.25±3.33 %, respectively, at 24 h. The results of cell viability (MTT results) showed that five different biomaterials with a diameter of 2x2 mm had no cytotoxic effect on L929 cells at 24 h (all values >=70%). Statistical significance levels between groups are indicated in the graphs (Fig. 6).

CONCLUSION

In this study, keratin-based composite films were successfully developed by incorporating methanol and infusion extracts from *Hypericum triquetrifolium* and *Hypericum neurocalycinum* into keratin extracted from waste goose feathers. The structural, morphological, and functional properties of the films were extensively characterized using SEM, FT-IR, TGA, and contact angle analysis. The results demonstrated that the addition of *Hypericum* extracts enhanced the antioxidant properties and surface morphology of the films, while maintaining their biodegradability and stability. The soil and water degradation tests confirmed the eco-friendly nature of the films, making them promising candidates for green material applications. Additionally, the antioxidant and cell viability studies suggested potential applications in food packaging and biomedical fields. Overall, this study highlights the feasibility of utilizing keratin and medicinal plant extracts for the development of sustainable biomaterials with enhanced functional properties. Future research should focus on optimizing the film formulations and evaluating their real-world applications in various industries.

DECLARATIONS

Ethics Committee Approval: Not applicable.

Author Contributions: Concept – EC, BKK; Design – EC; Supervision – EC, BKK, IS; Resource – EC; Materials – EC, IS, TKY; Data collection and/or processing – EC, BKK, TKY, IS; Analysis and/or interpretation – EC, BKK, TKY; Literature review – EC, IS; Writing – EC, IS, BKK; Critical Review – EC, BKK.

Conflict of Interest: The authors denied any conflicts of interest related to this study.

Use of AI for Writing Assistance: Not declared.

Financial Disclosure: Not declared.

REFERENCES

1. Fuller R, Landrigan PJ, Balakrishnan K, Bathan G, Bose-O'Reilly S, Brauer M, et al. Pollution and health: a progress update. Lancet Planet Health 2022;6:e535–47. Erratum in: Lancet Planet Health 2022;6:e553. [CrossRef]
2. Wayman C, Niemann H. The fate of plastic in the ocean environment – a mini review. Environ Sci Proc Impacts 2021;23:198–212. [CrossRef]
3. Scotti G, Esposito V, D'Alessandro M, Panti C, Vivona P, Consoli P, et al. Seafloor litter along the Italian coastal zone: an integrated approach to identify sources of marine litter. Waste Manag 2021;124:203–12. [CrossRef]
4. Monteiro RCP, Ivar do Sul JA, Costa MF. Plastic pollution in islands of the Atlantic Ocean. Environ Poll 2018;238:103–110. [CrossRef]
5. Nielsen TD, Hasselbalch J, Holmberg K, Stripple J. Politics and the plastic crisis: a review throughout the plastic life cycle. WIREs Energy Environ 2020;9:e360. [CrossRef]
6. Sharma S, Rostamabadi H, Gupta S, Kumar Nadda A, Kharazmi MS, Jafari SM. Nano/micro-formulations of keratin in biocomposites, wound healing and drug delivery systems; recent advances in biomedical applications. Eur Polymer J 2022;180:111614. [CrossRef]
7. Tesfaye T, Sithole B, Ramjugernath D. Valorisation of chicken feathers: a review on recycling and recovery route—current status and future prospects. Clean Technol Environ Pol 2017;19:2363–78. [CrossRef]
8. Teixeira-Costa BE, Andrade CT. Natural polymers used in edible food packaging—history, function and application trends as a sustainable alternative to synthetic plastic. Polysaccharides 2022;3:32–58. [CrossRef]
9. Sionkowska A. Current research on the blends of natural and synthetic polymers as new biomaterials. Prog Polymer Sci 2011;36:1254–76. [CrossRef]
10. Çakmak E. Chemical and biological characterisation of 3D keratin-fibre chitosan-based films from goose feathers. Europ Poultry Sci 2023;87:376. [CrossRef]
11. Łopusiewicz Ł, Kwiatkowski P, Drozłowska E, Trocer P, Kostek M, Śliwiński M, et al. Preparation and characterization of carboxymethyl cellulose-based bioactive composite films modified with fungal melanin and carvacrol. Polymers 2021;13:499. [CrossRef]
12. Pak ES, Ghaghelestani SN, Najafi MA. Preparation and characterization of a new edible film based on Persian gum with glycerol plasticizer. J Food Sci Technol 2020;57:3284–94. [CrossRef]

13. Bölgen N, Demir D, Yalçın MS, Özdemir S. Development of Hypericum perforatum oil incorporated antimicrobial and antioxidant chitosan cryogel as a wound dressing material. Int J Biol Macromolecules 2020;161:1581–90. [CrossRef]
14. Lopes AI, Melo A, Caleja C, Pereira E, Finimundy TC, Afonso TB, et al. Evaluation of antimicrobial and antioxidant activities of alginate edible coatings incorporated with plant extracts. Coatings. 2023;13:1487. [CrossRef]
15. Santhosh R, Nath D, Sarkar P. Novel food packaging materials including plant-based byproducts: a review. Trends Food Sci Technol 2021;118:471–89. [CrossRef]
16. Mir SA, Dar BN, Wani AA, Shah MA. Effect of plant extracts on the techno-functional properties of biodegradable packaging films. Trends Food Sci Technol 2018;80:141–54. [CrossRef]
17. Silva AR, Taofiq O, Ferreira ICFR, Barros L. Hypericum genus cosmeceutical application – A decade comprehensive review on its multifunctional biological properties. Indust Crops Prod 2021;159:113053. [CrossRef]
18. Caldeira GI, Gouveia LP, Serrano R, Silva OD. Hypericum genus as a natural source for biologically active compounds. Plants 2022;11:2509. [CrossRef]
19. Kaya M, Khadem S, Cakmak YS, Mujtaba M, Ilk S, Akyuz L, et al. Antioxidative and antimicrobial edible chitosan films blended with stem, leaf and seed extracts of Pistacia terebinthus for active food packaging. RSC Adv 2018;8:3941–50. [CrossRef]
20. Martínez-Camacho AP, Cortez-Rocha MO, Ezquerra-Brauer JM, Graciano-Verdugo AZ, Rodriguez-Félix F, Castillo-Ortega MM, et al. Chitosan composite films: thermal, structural, mechanical and antifungal properties. Carbohydrate Polymers 2010;82:305–15. [CrossRef]
21. Salgado-Cruz Ma de la P, Calderón-Domínguez G, Chanona-Pérez J, Farrera-Rebollo RR, Méndez-Méndez JV, Díaz-Ramírez M. Chia (Salvia hispanica L.) seed mucilage release characterisation. A microstructural and image analysis study. Indust Crops Prod 2013;51:453–62. [CrossRef]
22. Archana G, Sabina K, Babuskin S, Radhakrishnan K, Fayidh MA, Babu PAS, et al. Preparation and characterization of mucilage polysaccharide for biomedical applications. Carbohydrate Polymers 2013;98:89–94. [CrossRef]
23. Cerqueira MA, Souza BWS, Teixeira JA, Vicente AA. Effect of glycerol and corn oil on physicochemical properties of polysaccharide films – A comparative study. Food Hydrocolloids 2012;27:175–84. [CrossRef]
24. Schulz H, Baranska M. Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibra Spectroscopy 2007;43:13–25. [CrossRef]
25. Mayachiew P, Devahastin S. Effects of drying methods and conditions on release characteristics of edible chitosan films enriched with Indian gooseberry extract. Food Chem 2010;118:594–601. [CrossRef]
26. Pelissari FM, Grossmann MVE, Yamashita F, Pineda EAG. Antimicrobial, mechanical, and barrier properties of cassava starch–chitosan films ıncorporated with oregano essential oil. J Agricult Food Chem 2009;5:7499–504. [CrossRef]
27. Naqvi SA, Khan MM, Shahid M, Jaskani MJ, Khan IA, Zuber M, et al. Biochemical profiling of mucilage extracted from seeds of different citrus rootstocks. Carbohydrate Polymers 2011;83:623–8. [CrossRef]
28. Salazar Vega IM, Quintana Owen P, Segura Campos MR. Physicochemical, thermal, mechanical, optical, and barrier characterization of chia (Salvia hispanica L.) mucilage-protein concentrate biodegradable films. J Food Sci 2020;85:892–902. [CrossRef]
29. Shavandi A, Carne A, Bekhit AA, Bekhit AEDA. An improved method for solubilisation of wool keratin using peracetic acid. J Environ Chem Eng 2017;5:1977–84. [CrossRef]
30. de Paiva PHEN, Correa LG, Paulo AFS, Balan GC, Ida EI, Shirai MA. Film production with flaxseed mucilage and polyvinyl alcohol mixtures and evaluation of their properties. J Food Sci Technol 2020;58:3030–8. [CrossRef]
31. Choque-Quispe D, Froehner S, Ligarda-Samanez CA, Ramos-Pacheco BS, Palomino-Rincón H, Choque-Quispe Y, et al. Preparation and chemical and physical characteristics of an edible film based on native potato starch and nopal mucilage. Polymers 2021;13:3719. [CrossRef]
32. Hosseini MS, Nabid MR. Synthesis of chemically cross-linked hydrogel films based on basil seed (Ocimum basilicum L.) mucilage for wound dressing drug delivery applications. Int J Biol Macromolecules 2020;163:336–47. [CrossRef]
33. Dick M, Costa TMH, Gomaa A, Subirade M, Rios A de O, Flôres SH. Edible film production from chia seed mucilage: Effect of glycerol concentration on its physicochemical and mechanical properties. Carbohydrate Polymers 2015;130:198–205. [CrossRef]
34. Dou B, Dupont V, Williams PT, Chen H, Ding Y. Thermogravimetric kinetics of crude glycerol. Bioresource Technol 2009;100:2613–20. [CrossRef]
35. Dinh TA, Le YN, Pham NQ, Ton-That P, Van-Xuan T, Gia-Thien Ho T, et al. Fabrication of antimicrobial edible films from chitosan incorporated with guava leaf extract. Prog Org Coatings 2023;183:107772. [CrossRef]
36. Riaz A, Lagnika C, Luo H, Dai Z, Nie M, Hashim MM, et al. Chitosan-based biodegradable active food packaging film containing Chinese chive (Allium tuberosum) root extract for food application. Int J Biol Macromolecules 2020;150:595–604. [CrossRef]
37. Brebu M, Spiridon I. Thermal degradation of keratin waste. J Anal Appl Pyrolysis 2011;91:288–95. [CrossRef]
38. Wang K, Li R, Ma JH, Jian YK, Che JN. Extracting keratin from wool by using l-cysteine. Green Chem 2016;18:476–81. [CrossRef]

39. Zhang X, Feng Y, Yang X. Extraction of keratin from poultry feathers with choline chloride-oxalic acid deep eutectic solvent. Fibers Polymers 2021;22:3326–35. [CrossRef]
40. Ünver E, Çakmak E. Production and characterisation of mucilage-based keratin films using goose feathers. Europ Poult Sci 2023;87:381. [CrossRef]
41. Martucci JF, Ruseckaite RA. Biodegradation of three-layer laminate films based on gelatin under indoor soil conditions. Polymer Degrad Stab 2009;94:1307–13. [CrossRef]
42. Guan Y, Shao L, Dong D, Wang F, Zhang Y, Wang Y. Bio-inspired natural polyphenol cross-linking poly(vinyl alcohol) films with strong integrated strength and toughness. RSC Adv 2016;6:69966–72. [CrossRef]
43. Graupner N, Herrmann AS, Müssig J. Natural and man-made cellulose fibre-reinforced poly(lactic acid) (PLA) composites: An overview about mechanical characteristics and application areas. Composites Part A Appl Sci Manufact 2009;40:810–21. [CrossRef]
44. Rahmah M. Effect of aging on film strength and morphology of natural additive polypropylene packaging film. Mater Sci Eng 2011;10:452–6.