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ORIGINAL PAPER

Characterisation and Antibacterial Properties of Novel Biodegradable Films Based on Alginate and Roselle (*Hibiscus sabdariffa* L.) Extract

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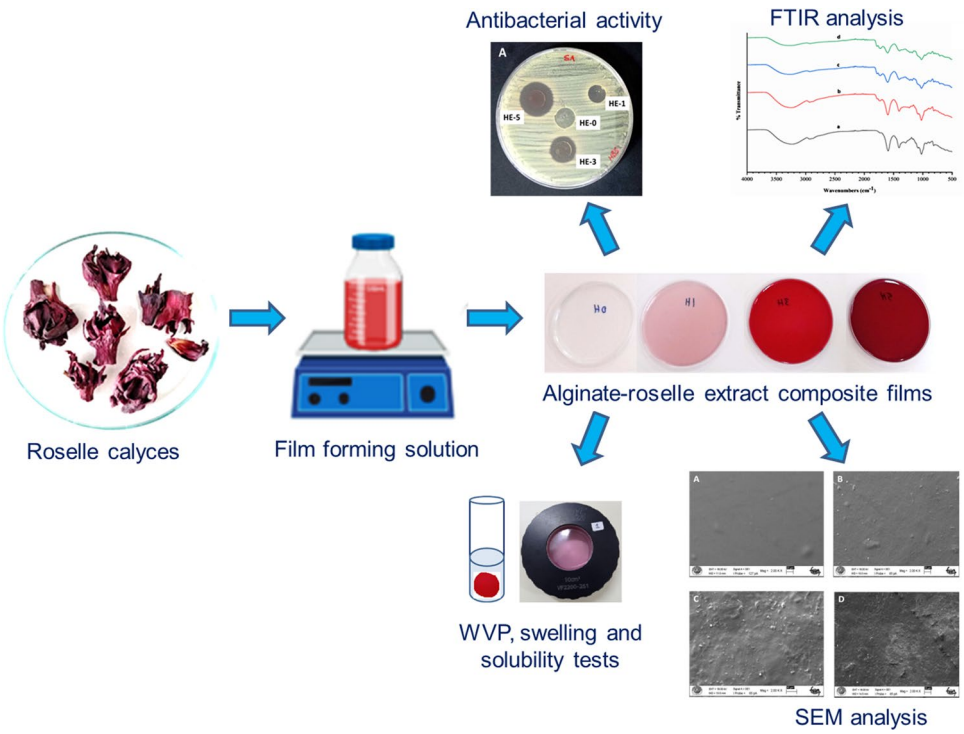
Abstract

Composite films were prepared with alginate and roselle extract (HE) at different concentrations (1%, 3%, and 5% w/v) via solvent casting technique and analyzed in terms of physical, mechanical, and antibacterial properties. The incorporation of HE into alginate films resulted in rough and heterogeneous surface characteristics with increasing concentrations of HE. The thickness and water vapor permeability of alginate–HE composite films were significantly higher ($p < 0.05$) compared to pure alginate films. Moreover, water content, solubility, swelling, tensile strength, and elongation at break value of the composite films decreased ($p < 0.05$) with increasing concentrations of the extract. FTIR spectra revealed shifts and intensity variations in the composite films and the formation of new peaks suggesting a possible interaction between alginate and HE. Alginate–HE films exhibited good antibacterial activity against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria. The antibacterial effect of the films, more pronounced against Gram-positive bacteria, increased with higher amounts of HE. The resulting films may be utilised as new biodegradable, antibacterial films in the food packaging industry to prolong shelf life and preserve food safety.

Graphical Abstract



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Keywords Alginate film · Antibacterial · Food packaging · *Hibiscus sabdariffa*

Statement of Novelty

Global plastic contamination is a severe environmental problem. Biopolymers of natural origin are environmentally friendly, renewable, non-toxic, biodegradable, and biocompatible materials to replace non-biodegradable and non-renewable synthetic polymers for producing active packaging materials. In this context, films were prepared with alginate and roselle extract and antimicrobial, mechanical, and physical properties of the films were evaluated. Plant extracts have been extensively investigated as renewable and natural sources of antimicrobial compounds. Extract of roselle calyces incorporated in alginate films conferred high antibacterial activity against both Gram-negative and Gram-positive bacteria suggesting that these films are promising to be used as a natural and degradable biopolymer-based composite packaging material that may improve the quality and safety of foods. To the knowledge of the authors this is the first report of an alginate-based edible film incorporated with roselle extract.

Introduction

Global plastic contamination is a severe environmental problem, and a tremendous increase is reported in 2020 due to the utilization of personal protective materials for the COVID-19 pandemic [1]. A total of 8.3 billion tons of plastics have been manufactured since the advent of this miracle material. Besides its significant benefits, 6.3 billion tons of synthetic plastics have become waste resulting in serious damage to the environment [2]. The non-biodegradable and non-renewable character of petroleum-based synthetic polymers has motivated the use of natural biopolymers. Biopolymers such as polysaccharides, lipids, and proteins are environmentally friendly, renewable, biodegradable, non-toxic, and biocompatible materials. The eco-friendly nature of these materials renders natural biopolymers good alternatives to synthetic polymers to produce edible and biodegradable packaging materials. The utilization of natural biopolymer-based packaging materials preserves the quality of foods and prolongs their shelf life besides reducing the consumption of petroleum-based plastics [3–5].

Edible films or coatings are materials less than 0.3-mm thick that are used for fresh and minimally processed foods. The films or coatings are formed via the dispersion of biopolymers and various additives in an aqueous media. Complete dissolution and dispersion of the film-forming components are essential for the fabrication of a homogeneous and continuous film matrix. During film preparation, the film forming solutions are usually filtered to remove undissolved solutes and sonicated to remove the bubbles to ensure the homogeneity and continuity of the polymer matrix [6, 7]. The resulting films should meet the main characteristics of traditional packaging such as antimicrobial and antioxidant activity, gas/moisture barrier property, protection against UV light, biodegradability, and barrier against mechanical damage. The appropriate selection of packaging material is crucial to guarantee the safety and quality of food as it affects the physicochemical, functional, and organoleptic characteristics of the food [8, 9]. Biopolymers such as chitosan [6, 10, 11], starch [12], cellulose [13, 14], and alginate [15–17] are among the most studied materials for the fabrication of food packagings.

Among natural polymers, alginate has been considered a film-based material due to its excellent film-forming capacity, non-toxic nature, and unique colloidal properties. It is a natural, linear polysaccharide generally synthesized by brown seaweeds, including *Laminaria hyperborean*, *Macrocystis pyrifera*, and *Ascophyllum nodosum.* Alginate is also produced by bacteria such as *Azotobacter* and *Pseudomonas* with physical properties and chemical structure that is well defined compared to the seaweed-originated alginates [18]. Alginate is a family of linear copolymers composed of β-D-mannuronate (M) and α-L-guluronate (G) residues linked by 1–4 glycosidic bonds. The blocks can contain homopolymeric residues (MMMM or GGGG) or alternating residues (MGMGMG). Alginates obtained from different sources differ in molecular weight, length of each block, M and G contents, and their distribution in the polymeric chain, significantly influencing its chemical, physical, and gelling properties and also the resultant hydrogels' [15]. Alginate hydrogels are generally produced by external gelation using divalent cations (i.e., Ca^{2+}) as cross-linking agents. G blocks of alginate chains aid in intermolecular cross-linking with the cation to form the gel network. Although calcium chloride ($CaCl_2$) is one of the widely used cross-linking agents, its high solubility in aqueous environments causes fast and uncontrolled gelation limiting its extensive use. Calcium sulfate ($CaSO_4$) and calcium carbonate ($CaCO_3$) have lower solubility in aqueous solutions, and they are alternatively used to slow and control the gelation rate. Gradual gelation of the alginate solution ensures higher mechanical integrity and more uniform structures [19].

Alginate films are used in many applications such as drug delivery systems, tissue engineering, food packaging, and wound dressing. In the last decades, alginate-based films have been used broadly as an alternative to synthetic packaging. Increasing consumer demands for more natural and

healthier foods accelerated studies on edible films produced from natural biopolymers [20]. Food spoilage caused by bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* may result in severe foodborne diseases. Therefore, preventing microbial spoilage contributes to ensuring the safety and quality of foods and extending shelf life. Sodium benzoate, sodium nitrite, and sorbic acid are commonly used synthetic antimicrobials to prevent microbial food spoilage. However, public concern about the health risks and long-term toxicity of these agents has been restricted their extensive use [21].

A tremendous amount of research is focused on natural active agents such as bioactive peptides and protein hydrolysates from food proteins, plant extracts, plant-derived essential oils, and bacteriocins as renewable and natural sources of antimicrobial agents [5]. Instead of directly applying antimicrobial compounds to food products, they are incorporated into edible coatings and films to obtain a gradual release of the agents. These controlled release systems regulate the diffusion of the antimicrobial agent to keep a high concentration of active compounds on the surface of food products for a prolonged period. Active agents may prevent bacterial growth and thus microbiological food spoilage [21, 22]. In this context, various studies were conducted to produce alginate-based edible films and coatings containing various plant extracts and/or essential oils as antimicrobial agents. Senturk Parreidt et al. [15] summarized some of these studies and concluded that alginate is an effective matrix to carry the antimicrobial agents. Alginate films and coatings carrying plant extracts such as gallnut extract [21], olive leaves extract [16], ginseng extract [23], *Stryphnodendron adstringens* extract [24], and black chokeberry extract [25] were reported in the literature.

In this study, roselle (*Hibiscus sabdariffa* L.) extract was used to obtain antibacterial alginate films. Roselle, an annual tropical shrub, is a member of the family Malvaceae. It is native to tropical Africa and also cultivated in Central and South America and Southeast Asia [26]. Roselle calyces are used in the food industry to produce hot and cold herbal infusions, fermented beverages, sauces, juices, jellies, jams, and baked goods [27]. The extract of flowers is also used in folk medicine against hypertension, liver diseases, and fever [28]. The extracts are deemed GRAS (generally recognized as safe) as a food additive by the U.S. Food and Drug Administration (21 CFR 172.510). The calyces are rich in bioactive molecules such as anthocyanins, organic acids, alkaloids, phenolic acids, and saponins. These bioactive compounds confer antioxidant, cardioprotective, antidiabetic, anticancer, and antimicrobial properties to roselle extracts [29]. The incorporation of antimicrobial and antioxidant roselle extracts enables the production of natural and biodegradable packaging materials that extend the shelf-life and ensure foods' quality and safety. Previously roselle extract was added to carboxymethylcellulose (CMC) [13], tapioca starch [30], corn starch [26, 31], gum arabic [27], polyvinyl alcohol [14], and chitosan/polyvinyl alcohol [32] based polymer matrices to produce biodegradable films.

This research aimed to examine the antimicrobial potential and functional properties [mechanical, water vapor permeability (WVP), microstructure, and solubility] of films prepared with alginate and roselle extract at different ratios. The potential of the films to be a biodegradable and natural alternative as a food packaging material was evaluated. To the knowledge of the authors, this is the first research on alginate-based edible film incorporated with roselle extract.

Materials and Methods

Materials

Sodium alginate derived from seaweed and $CaCO_3$ were purchased from a local food ingredient supplier (Katki Dunyasi, Turkey). Calyces of roselle (Bagdat Baharat, Turkey), glucono delta-lactone (GDL, Smart Kimya, Turkey), Nutrient broth (Merck, Germany), bacteriological agar (Himedia, India), and glycerol (Sigma-Aldrich, Germany) were utilized in the present study. Distilled water was used throughout the experiments.

Extract Preparation

The extract was prepared by the decoction method described by Rasheed et al. [33] with slight modifications. The roselle calyces were ground using a blender (Sinbo SCM-2914, Turkey) and dispersed in distilled water (10% w/v). The calyces were boiled in hot water for 10 min and subsequently, rest at room temperature for 10 min. After the solution had cooled down, it was filtered twice using standard medium pore filter paper to remove calyx particles and stored at 4 °C until use.

Preparation of Alginate-Based Films

Alginate-based films were prepared via the solvent casting method. The desired amount of HE was added into distilled water to achieve 1%, 3%, and 5% HE solutions and stirred for ten minutes using a magnetic stirrer. Then 1.5% (w/v) sodium alginate was added to each solution, and four film-forming dispersions were obtained, as depicted in Table 1. The solutions were stirred until a homogenous mixture was obtained. Glycerol (0.2 g/g of alginate) was incorporated into the solutions as a plasticizer, and it was mixed for 30 min. Finally, $CaCO_3$ (0.02 g/g of alginate) in combination with GDL (5.4 g/g $CaCO_3$) was added as a source of calcium ions to initiate the gelation under constant stirring. GDL slowly hydrolyzes into gluconic acid and acidifies the

Table 1 Composition of the alginate/roselle extract films

Film	Alginate (%, w/v)	Roselle extract (%, w/v)
HE-0	1.5	0
HE-1	1.5	1
HE-3	1.5	3
HE-5	1.5	5

solution. Decreased pH increases the solubility of $CaCO_3$ and releases calcium ions into the solution. Slow liberation of Ca^{2+} into the alginate matrix ensures a much stronger and more homogenous hydrogel overall. Film-forming solutions without HE were also produced. Each filmogenic dispersion was cast onto plastic Petri dishes and left to dry at room temperature for 48 h. The dried films were stored in zip-lock bags at 4 °C until being used.

Appearance and Thickness of the Films

As the films were detached from the Petri dishes, their flexibility was evaluated qualitatively. The presence of cracks and bubbles in the films and the surface homogeneity were visually examined with the naked eye. Film thickness was determined using a digital micrometer (Mitutoyo, MDC-25SX, Japan) with an accuracy of 0.001 mm. Five different regions of each film formulation were measured, and average values were used in the WVP and mechanical tests.

Mechanical Properties

Tensile strength (TS) and elongation at break values (EB) of the films were determined using a universal testing machine (Shimadzu AGS-X, Japan). Sample films were cut into strips with dimensions of 1 × 5 cm. All the test specimens were conditioned for at least 3 days at room temperature prior to measurements. The initial gauge length was set to 20 mm. Mechanical test measurements were reported as the average of at least four replicates for each type of film.

Water Vapor Permeability

WVP of the films was determined gravimetrically, as described by Benavides et al. [34], with slight modifications. Film samples were sealed onto permeability cups (VF2200 TQC, Netherlands) containing 10 mL of distilled water. The cups were put in a desiccator containing an oversaturated NaCl solution (75% RH) to generate a 75/100% relative humidity gradient across the film. The desiccators were kept at 25 °C, and the weight of the cups were measured periodically (each 2 h, for 8 h and at the end of 24 h) using an analytical balance (±0.0001 g). The measurements were replicated three times for each type of film, and WVP was calculated following Villalobos et al. [35].

Water Content, Solubility, and Swelling Degree

The water content, swelling degree, and solubility of film specimens were determined as described by Jamróz et al. [36]. Three randomly selected discs of 1.5 cm were removed from each type of film and weighed (W_1). Then the discs were dried in an oven (70 °C) for 24 h to get the initial dry mass (W_2). Afterwards, the films were soaked in distilled water and kept at room temperature (25 ± 2 °C) for 24 h. The swelled films were weighed (W_3) after excess water was removed, and they were dried in an oven at 70 °C for 24 h. Lastly, the films were reweighed to determine the unsolubilized final dry mass (W_4). Water content, film solubility, and swelling degree were calculated according to the following equations.

$$\text{Water content} = \left[\left(W_1 - W_2\right)/W_1\right] * 100\%, \quad (1)$$

$$\text{Solubility} = \left[\left(W_2 - W_4\right)/W_2\right] * 100\%, \quad (2)$$

$$\text{Swelling degree} = \left[\left(W_3 - W_2\right)/W_2\right] * 100\%. \quad (3)$$

Scanning Electron Microscopy

The microstructure of the film surfaces was investigated using a scanning electron microscope (SEM; Zeiss EVO 10, Germany). Each sample was coated with a thin gold–palladium film. Secondary electron imaging (SEI) mode was used to investigate the surface characteristics of the produced films.

Fourier Transform Infrared Spectroscopy

FTIR spectra of the film samples were recorded on a Thermo Scientific Nicolet i550 model spectrometer in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. FTIR spectra of the samples were used to determine possible interactions of functional groups in the produced films.

Antibacterial Activity

Antibacterial activity of pure alginate films and HE incorporated alginate films were tested against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), and *Bacillus subtilis* (ATCC 6633) via disc diffusion method. A colony of each bacteria was grown overnight in Nutrient broth and then diluted with saline to 10^6 CFU/mL. A total of 100 µL of diluted bacteria were

spread uniformly on nutrient agar plates, and films cut into circular discs with a diameter of 9 mm were placed on the inoculated plates. The films were sterilized under UV light for 10 min before tests. Nutrient agar plates were incubated at 37 °C for 24 h. Inhibition zones observed around the discs were measured in millimeters, and the experiments were performed in triplicate for all the test strains.

Statistical Analysis

IBM SPSS 22.0 software package was used to analyze the differences between alginate films with and without HE. One-way analysis of variance (ANOVA) was conducted to confirm the variability of the data and validity of the results. The least significant differences (LSD) were calculated at the 5% level ($p < 0.05$) to determine the differences between the mean values of the films' properties. A descriptive statistics test in the same program was used to calculate the standard errors of the means.

Results and Discussion

Film Appearance

Alginate films, prepared with or without HE, were uniform with no cracks or bubbles and were easily detached from the Petri dishes. As the films were cast onto Petri dishes, the surface of all films was smooth; however, a rough surface was observed for the films with HE after they were dried. The surface roughness of the dried films increased with higher concentrations of the extract.

Pure alginate films without HE were transparent, as shown in Fig. 1. The incorporation of HE in the alginate films caused a reddish color and the color deepened with increasing extract content. Color is an essential characteristic of a product that affects its appearance and also uses. Gómez-Aldapa et al. [26] prepared a corn starch film with roselle extract, and they also observed a red color that became more intense with increasing concentrations of the extract. The authors concluded that red-colored, opaque films might protect the food from ultraviolet and visible rays by reducing light exposure and thus delay food spoilage and extend shelf life.

Film Microstructure

Microstructures of pure alginate and HE incorporated alginate films prepared by the solvent casting method are represented in Fig. 2. The interactions between different components of the film-forming solution and how they interact during the drying period affect the microstructure of films. Pure alginate films showed a smooth and homogenous surface, whereas a rough and heterogeneous surface was observed for the alginate–HE composite films. The heterogeneity and roughness of the film surfaces were increased with increasing concentrations of HE. The smooth and regular surface of neat alginate films indicates an ordered arrangement of alginate chains within the film matrix. HE incorporated into the films may have disrupted the arrangement of polymer chains resulting in rough and irregular surfaces. Disruption of the arrangement of alginate chains by incorporation of HE may have also contributed to the increased thickness of the films (Table 2). Mango peel extract incorporated gelatin films [37], grapefruit seed extract reinforced chitosan films [38], and rosemary extract containing starch-alginate composite films [39] also showed heterogeneous and/or rough surfaces upon addition of the extracts into the polymer matrices.

Film Thickness

Thickness is an essential attribute of edible films affecting mechanical and barrier properties. It is influenced by factors such as film preparation methods and drying conditions [40]. The thickness of pure alginate and alginate–HE composite films was between 25.9 and 109.2 µm (Table 2). The results showed that pure alginate films had the lowest

Fig. 1 Physical appearances of alginate/roselle extract films

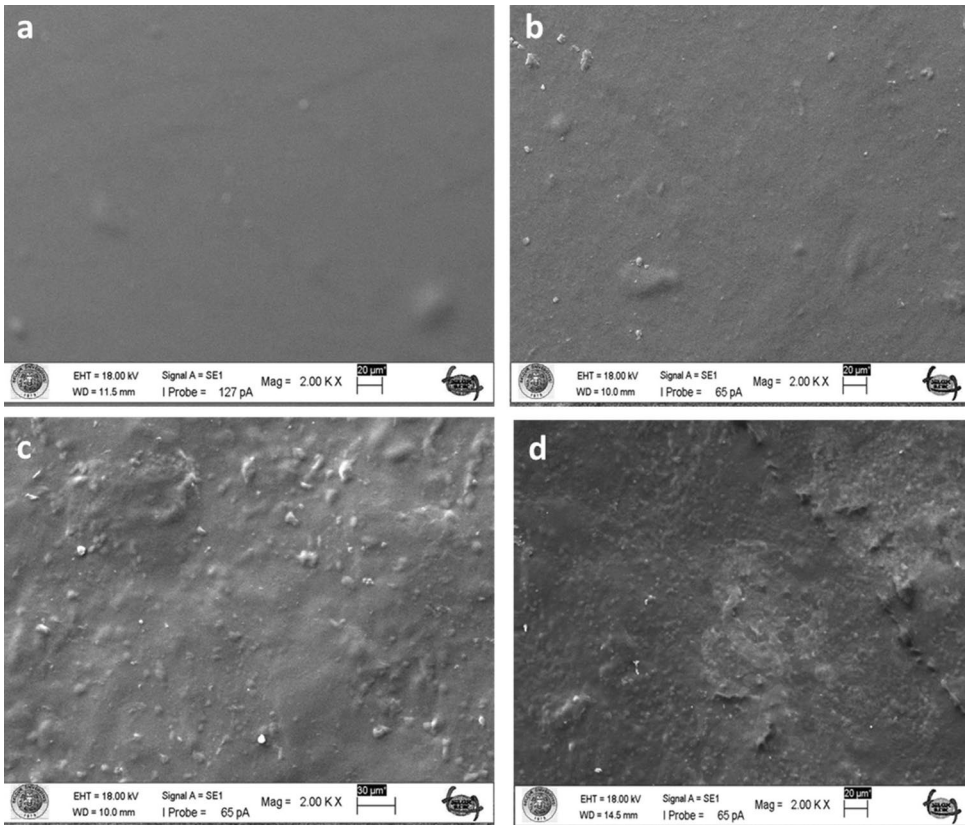

Fig. 2 SEM micrographs of alginate/roselle extract films. **a** HE-0, **b** HE-1, **c** HE-3, and **d** HE-5

Table 2 Physical properties of alginate/roselle extract films

	Film thickness (µm)	Water content (%)	Solubility (%)	Swelling degree (%)	WVP ($\times 10^{-12}$ g/ms Pa)
HE-0	25.93 ± 2.86 a	14.20 ± 0.66 a	62.86 ± 0.99 a	10,046.78 ± 190.14 a	2.49 ± 0.20 a
HE-1	46.53 ± 5.52 b	12.39 ± 0.27 ac	32.22 ± 1.69 b	4591.42 ± 134.31 b	4.70 ± 0.55 b
HE-3	97.66 ± 2.03 c	10.38 ± 0.38 cb	50.92 ± 1.99 c	1320.44 ± 73.54 c	9.45 ± 0.37 c
HE-5	109.20 ± 1.25 d	10.20 ± 1.03 bd	65.76 ± 1.47 a	484.19 ± 74.33 d	9.65 ± 0.10 c

Data shown are the mean ± standard error of the mean (SEM, $n \geq 3$). Similar letters in the same column indicate non-significant differences ($p < 0.05$)

thickness. The incorporation of HE significantly ($p < 0.05$) increased the thickness of the composite films depending on the concentration of the extract. The gradual increase in thickness with increasing HE concentrations may be attributed to the conformational changes of alginate chains by the addition of extract and higher solid content. The compounds in HE may have disrupted the ordered alignment of the alginate chain and subsequently formed protruding structure within the alginate film matrix, as suggested by Adilah et al. [37]. Gómez-Aldapa et al. [26] also observed an increased thickness in the starch-based films containing HE when the HE:water volume ratio in the filmogenic solution was increased from 50:50 to 100:0. The findings of Luo et al. [22] were also in agreement with the results obtained in this study, which showed that the addition of guava leaf ethanolic extract in alginate film resulted in increased thickness. The authors suggested that greater thickness observed for the films with higher extract concentrations was due to the increased amount of solid content.

Water Content, Solubility, and Swelling Degree of Films

The water content of the films varied between 10.2 and 14.2% (Table 2). The water amount in composite films incorporated with 1% HE was similar to the pure alginate film. However, when the HE content was higher than 3%, the water content of films significantly ($p < 0.05$) decreased with the increase of extract content. HE-3 and HE-5 films were more brittle than HE-0 and HE-1 films which could be

related to the lower water content of these films. The higher water amount present in HE-0 film may be due to the abundant carboxyl and hydroxyl groups in the alginate molecules. Hydroxyl groups in HE may interact with the hydrophilic groups in alginate upon adding the extract into alginate, which may limit alginate-water interaction. Similar data have been reported in the literature regarding reduced water content in films incorporated with various plant extracts [22, 23, 41].

An edible film/coating should not solubilize when it is used in humid conditions or utilized for packing foods with high moisture surfaces [42]. Sodium alginate, the most commonly used salt of alginate, is a water-soluble polymer. Applications of pure sodium alginate films are limited due to their high water solubility [42]. Cross-linking alginate films with polyvalent cations such as calcium is a common strategy to enhance physical, mechanical, and barrier properties [34, 43]. Calcium ions induce conformational changes so that G blocks are arranged in a way that calcium ions exist between two sodium alginate chains forming divalent salt bridges. This arrangement is called the "eggbox" model [15, 43]. Cross-linking sodium alginate films with calcium significantly lowers the water solubility of alginate films [44]. Solubility of pure alginate and alginate–HE composite films ranged from 32.2 to 65.8% (Table 2). While the solubility of HE-5 films was similar to HE-0 films, HE-1 and HE-3 films represented significantly ($p < 0.05$) lower solubility with respect to HE-0 films. $CaCO_3$ together with GDL was added to film-forming solutions for internal cross-linking of sodium alginate, and calcium alginate films were produced. When cross-linking was not applied, the films were totally solubilized in water (data not shown). Cross-linking significantly reduced the solubility of alginate films so that solubility of 62.9% was observed for pure calcium alginate films. The incorporation of HE further reduced the solubility of composite films, which was 32.2% for HE-1 films and 50.9% for HE-3 films. Kim et al. [45] reported a decrease in solubility of red ginseng residue protein films containing 1% HE, which is in good agreement with the results obtained in this study. Similarly, Adilah et al. [37] reported that the solubility of fish gelatin films incorporated with mango peels extract was decreased with increasing concentrations of the extract. The authors attributed the higher water resistance of the films to high protein–polyphenols interaction leading to a stronger film network structure. The interaction between HE polyphenols and alginate may have also led to the formation of a strong network, reducing alginate–HE films' solubility. Increased water resistance observed in these films indicates that they may be appropriate for the storage of foods.

The swelling of alginate–HE films was significantly lower ($p < 0.05$) with respect to pure alginate films (Table 2). Swelling of the composite films significantly decreased ($p < 0.05$) upon increasing concentrations of HE. Roger et al. [46] suggested that carboxyl groups of alginate strongly interact with water molecules, affecting alginate's swelling behaviour. HE polyphenols interacting with carboxyl groups of alginate may have reduced the availability of these groups to water molecules. Thus increasing concentrations of HE may have limited the swelling ability of alginate–HE films.

Water Vapor Permeability of Films

WVP is an essential property for edible films/coatings, and a low water vapor transmission is desired for these products to ensure safe and prolonged food storage [47]. WVP of pure alginate films and alginate–HE composite films are represented in Table 2. The results revealed that WVP increased with increasing concentrations of HE. Several studies are available in the literature reporting increased WVP upon the addition of plant extracts into polymer matrices [3, 23, 25, 39]. In contrast to these reports, some studies reveal reduced WVP for the films incorporated with plant extracts [21, 22, 42, 45, 47]. The intermolecular forces between the components of the films influence the WVP. Incorporation of plant extract might weaken the intermolecular interactions in the film network, and these changes in the internal structure of the films may favor solvent mobility and facilitate water diffusion in the polymeric matrix resulting in increased WVP [3, 23, 25]. Increased WVP observed in this study might also be attributed to the weakened intermolecular forces within the composite film matrix upon the addition of HE.

FTIR Spectra of Films

FTIR analyses were carried out to monitor specific functional groups and determine the possible chemical interactions between alginate and HE, which could help better understand the differences observed in the mechanical and physical properties of the films. FTIR spectra of pure alginate film and alginate films incorporated with different HE concentrations are given in Fig. 3. Pure alginate film exhibited five characteristic absorption bands around 3235 cm^{-1}, 2929 cm^{-1}, 1592 cm^{-1}, 1404 cm^{-1}, and 1021 cm^{-1}. The broad band at 3235 cm^{-1} was attributed to the stretching vibration of the OH group, which participated in hydrogen bond formation [22]. The peaks observed at 1592 cm^{-1} and 1404 cm^{-1} were attributed to the asymmetric and symmetric stretching vibration of the COO groups, respectively [48]. These absorption bands can be overlapped with the band associated with the vibration of the CH group observed at 2929 cm^{-1} and 1404 cm^{-1} [24, 48]. A band at 1021 cm^{-1} representing C–O–C glycosidic bond stretching was also observed [17, 22]. The spectra of the films with HE displayed similar transmittance peaks as pure alginate film. However, the absorption bands were significantly decreased and slightly shifted as the concentration of HE increased.

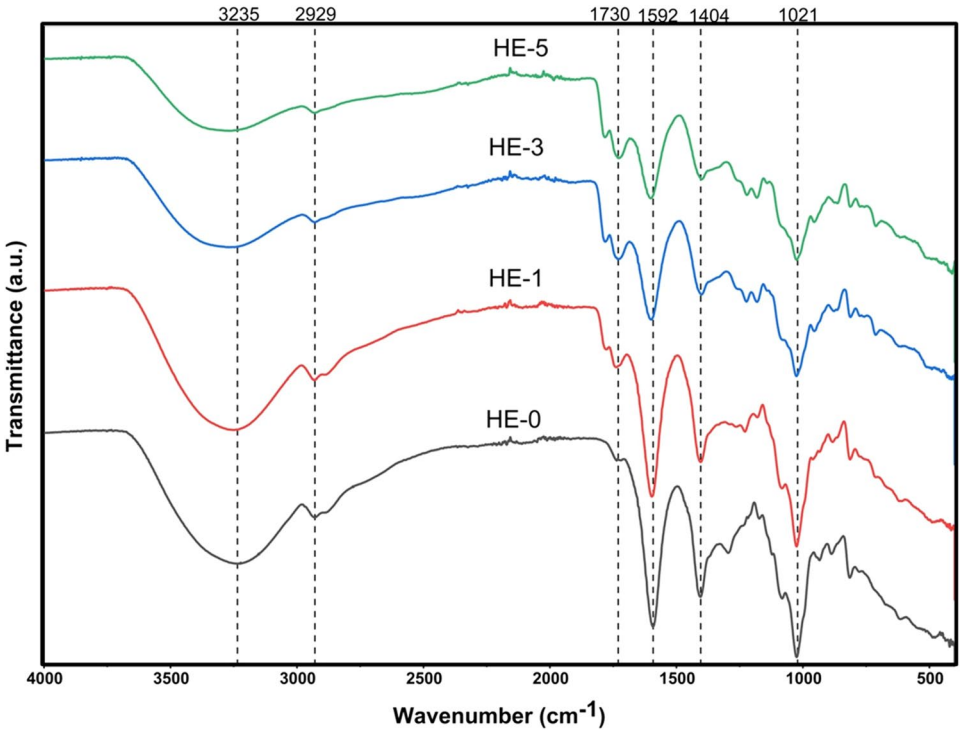

Fig. 3 FTIR spectra of alginate/roselle extract films

Table 3 Mechanical properties of alginate/roselle extract films

Film	TS (MPa)	EB (%)
HE-0	39.78 ± 7.81 a	3.5 ± 0.50 a
HE-1	9.64 ± 1.49 b	1.17 ± 0.05 b
HE-3	5.58 ± 2.08 b	0.83 ± 0.1 b
HE-5	NA	NA

Data shown are the means (± SEM, $n = 4$). Similar letters in the same column indicate non-significant differences ($p < 0.05$)

NA not available

Previous studies attributed these changes to the establishment of hydrogen bonds between polyphenols and alginate [22]. In addition to the shifts and intensity variations observed in the alginate–HE films, two new peaks around 1730 cm^{-1} appeared, suggesting a possible interaction between alginate and HE.

Mechanical Properties of Films

TS and EB are two significant characteristics of food packaging materials that influence the capacity of films to endure external stress and keep integrity and barrier properties during handling, transportation, processing, and storage of food products [21]. Additives including water and plant extracts incorporated into polymeric films interact with the polymer and affect the TS and EB of the films [17]. Mechanical properties of alginate-based films are given in Table 3. For the pure alginate film, TS was 39.78 MPa. Although biopolymer films have poor mechanical properties compared to synthetic films, TS of pure alginate films obtained in this study was higher compared to cross-linked poly(vinyl alcohol) (PVA) membrane (27 MPa), which is a widely used synthetic polymer [49]. On the other hand, EB value for pure alginate film was 3.5% which was lower than the synthetic-based polymers, PLA membrane (8%) [50], and Matrimid®5218 dense membrane (24%) [51].

Compared to pure alginate films, the TS values of films with HE were significantly reduced ($p < 0.05$). However, there was no significant difference between HE-1 and HE-3 films. Increased HE content in the films caused a brittle structure, and it was not possible to measure the mechanical properties of HE-5 films as it was broken during attachment to the test machine. In accordance with the findings of this study, Kim et al. [45], Gómez-Aldapa et al. [26], and Zhai et al. [41] observed reduced TS upon addition of HE into red ginseng residue protein films, corn starch films, and starch/polyvinyl alcohol films, respectively. The changes observed in TS of alginate–HE films are in agreement with the results of WVP and microstructure analysis. SEM images revealed heterogeneous and rough surfaces of films incorporated with HE, which indicated a disrupted polymer chain arrangement. On the other hand, higher WVP observed for alginate–HE films suggested weakened intermolecular interactions in the film network. The disrupted arrangement of alginate chains and the weak intermolecular interactions in the film network due to the addition of HE may be responsible for the

decrease in TS values. The EB values of HE-1 and HE-3 films were significantly lower ($p < 0.05$) compared to alginate films without HE. Contrary to these findings, EB was increased in red ginseng residue protein films [45], corn starch films [31], and starch/polyvinyl alcohol films [41] incorporated with HE. Zhai et al. [41] suggested that HE improved the compatibility of starch and PVA resulting in more homogenous starch/PVA films with increased extensibility. Gómez-Aldapa et al. [26] attributed the increased flexibility of corn starch films incorporated with HE to the interruption of the crystallinity of starch due to disruption of intermolecular H bonds within starch molecules, and the formation of H bonds between the extract and starch chains. On the other hand, Augusto et al. [52] observed a 23% reduction in EB values for alginate films incorporated with *Codium tomentosum* seaweed extract, which is in accordance with the results of this study. The authors suggested that an equilibrium between the degree of polymer cross-linking and extract addition is vital for better film characteristics. Pereira et al. [53] also reported reduced EB value for alginate film incorporating *Aloe vera* gel at 12%. A high concentration of *A. vera* extract was reported to increase the brittleness of the produced films, which was also observed in this study. The water content of alginate–HE films was lower than ($p < 0.05$) pure alginate films (Table 2), which may have contributed to the fragile structure of the HE incorporated alginate films. FTIR analysis indicated an interaction between the components of HE and alginate. The interaction between these compounds may have also caused a reduction in elongation of alginate–HE composite films.

Antibacterial Activities of Films

Antibacterial activities of pure alginate and alginate–HE films against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *K. pneumoniae*) test bacteria determined via disc diffusion method are represented in Table 4. Pure alginate film did not display any inhibitory effect on the test bacteria. These results are in accordance with those reported by de Oliveira Filho et al. [54] Moreover, alginate films incorporated with HE revealed good antibacterial activity that gradually increased with increasing HE concentrations. The growth-limiting effect of alginate–HE composite films was more pronounced against Gram-positive bacteria. The antibacterial activity of HE-3 and HE-5 films against *S. aureus* and *B. subtilis* was significantly higher ($p < 0.05$) than pure alginate films. On the other hand, the antibacterial effect of the composite films against *E. coli* and *K. pneumoniae* was significantly higher ($p < 0.05$) with respect to pure alginate films only when HE was incorporated at a concentration of 5%. The common mechanism underlying the antibacterial effect of plant extracts is the penetration of the active compounds into the lipid bilayer of the cell membrane, which causes an increased membrane permeability with subsequent leakage of vital cell contents [55, 56]. Gram-negative and Gram-positive bacteria differ in their cell wall structure, affecting the interaction of these bacteria with antimicrobial compounds. Cell walls of Gram-positive bacteria are composed of a peptidoglycan layer. In contrast, Gram-negative bacteria possess an additional lipopolysaccharide layer, the so-called outer membrane. The outer membrane surrounds both the cytoplasmic membrane and the peptidoglycan layer and restricts the penetration of small molecules like antibiotics [56, 57]. The lipopolysaccharide layer acting as a permeability barrier renders Gram-negative bacteria more resistant to plant-originated antimicrobials. The peptidoglycan cell wall of Gram-positive bacteria, although mechanically robust, poorly restricts diffusion of small molecules resulting in higher susceptibility of these organisms to plant extracts [56, 58].

Table 4 Inhibition zone diameter (including the 9 mm diameter of film discs) of alginate/roselle extract films

Film	Inhibition zone diameter (mm)			
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*
HE-0	9.00 ± 0.00 a	9.00 ± 0.00 a	9.00 ± 0.00 a	9.00 ± 0.00 a
HE-1	10.00 ± 1.00 a	10.66 ± 1.66 a	9.00 ± 0.00 a	9.00 ± 0.00 a
HE-3	19.33 ± 0.33 b	19.00 ± 1.00 b	11.00 ± 2.00 ab	11.00 ± 2.00 ab
HE-5	22.00 ± 1.00 c	21.66 ± 1.33 b	14.00 ± 0.57 b	14.00 ± 0.57 b

Data are average values ± standard error of the mean (SEM, $n = 3$). Means in the same column followed by different letters are significantly different ($p < 0.05$)

Gómez-Aldapa et al. [26] investigated the antibacterial activity of corn starch films incorporated with aqueous extract of *H. sabdariffa* against four common foodborne pathogenic bacteria. They reported higher antibacterial activity depending on increasing HE concentration in accordance with the current study results. Portillo-Torres et al. [59] reported hibiscus acid to be one of the main antibacterial molecules in *H. sabdariffa* calyces. They suggested that hibiscus acid disrupted the membrane integrity and increased the permeability of cells to solutes resulting in an antibacterial effect against enterohemorrhagic *E. coli* EHEC A and *Salmonella* C65. In this study, hibiscus acid and other antimicrobial compounds in HE may have contributed to the antibacterial effect observed in alginate–HE composite films.

Conclusion

Alginate-based composite films incorporating HE as an antimicrobial agent were successfully produced via solvent casting technique. The FTIR spectra of the films verified that

HE was immobilized into the alginate matrix. SEM investigations revealed a more rough and heterogeneous surface structure for the alginate–HE composite films compared to pure alginate films, which is possibly due to the disruption of the arrangement of alginate chains by incorporating HE. The water content, solubility, and swelling degree of the composite films significantly reduced with the increasing amounts of HE. The lower solubility of HE-1 and HE-3 films suggested that these films may be used in humid conditions or utilized for the packing of foods with high moisture surfaces. Moreover, alginate-based films showed a good antibacterial activity against Gram-negative and Gram-positive bacteria depending on the content of HE. The properties of the alginate/roselle extract films suggest that the films are promising to be used as a natural and degradable biopolymer-based composite packaging material for fresh vegetables and fruits. These materials produced from renewable sources may replace synthetic plastics that cause severe environmental pollution. Despite the eco-friendly nature of biopolymer-based packaging materials, their production costs are higher than conventional plastics limiting their widespread use. Competitiveness of these materials with synthetic plastics can be achieved by strategies such as improving the materials' properties or developing new production technologies.

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Data Availability Data will be available on request.

Declarations

Conflict of interest The authors declare no conflict of interest in publishing this manuscript.

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