TẠP CHÍ KHOA HỌC
TRƯỜNG ĐẠI HỌC QUY NHƠN

Tổng hợp màng nhựa sinh học từ cao chiết lá Ngũ trảo dựa trên nền tinh bột Khoai lang và khảo sát hoạt tính kháng khuẩn của sản phẩm

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TÓM TẮT

Ô nhiễm nhựa và an toàn thực phẩm là hai thách thức toàn cầu đòi hỏi các giải pháp bền vững. Nghiên cứu này nhằm phát triển màng nhựa sinh học đa chức năng từ tinh bột khoai lang kết hợp cao chiết lá Ngũ trảo (*Vitex negundo* L.) có hoạt tính kháng khuẩn. Quá trình tối ưu hóa xác định nồng độ thích hợp của chất hóa dẻo glycerol là 10% (w/w), kết hợp với 10% cao chiết tạo ra màng nhựa có đặc tính cơ-lý phù hợp với độ bền kéo đạt 4,26 MPa và khả năng thấm nước thấp. Phân tích FT-IR và SEM xác nhận sự kết hợp thành công của cao chiết vào cấu trúc phân tử của màng, với bề mặt đồng nhất không có vết nứt. Đặc biệt, màng nhựa thể hiện hoạt tính kháng khuẩn đối với cả vi khuẩn Gram dương (*Listeria innocua*) và Gram âm (*Escherichia coli* O157:H7, *Salmonella enterica*) - những tác nhân chính gây ngộ độc thực phẩm. Quan trọng hơn, hoạt tính kháng khuẩn vẫn duy trì sau 3 tháng lưu trữ, chứng minh tính ổn định dài hạn của sản phẩm. Kết quả của nghiên cứu này mở ra triển vọng ứng dụng rộng rãi cho vật liệu bao gói thực phẩm thân thiện với môi trường, có khả năng kéo dài thời gian bảo quản thực phẩm và giảm thiểu nguy cơ nhiễm khuẩn. Màng nhựa sinh học này không chỉ góp phần đưa ra giải pháp cho vấn đề ô nhiễm nhựa mà còn tận dụng được nguồn nông sản sẵn có tại địa phương để sản xuất vật liệu, hướng tới mục tiêu phát triển bền vững.

Từ khóa: *Màng nhựa sinh học, Ngũ trảo, chất hóa dẻo glycerol, hoạt tính kháng khuẩn.*

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Synthesis of bioplastic films from *Vitex negundo* L. leaf extract based on sweet potato starch and investigation of antibacterial activity of the products

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ABSTRACT

Plastic pollution and food safety are global challenges requiring sustainable solutions. This study aimed to develop a multifunctional bioplastic film from sweet potato starch combined with *Vitex negundo* L. leaf extract with antimicrobial properties. Optimization processes determined that 10% (w/w) glycerol plasticizer combined with 10% extract produced films with appropriate physicochemical properties, including tensile strength of 4.26 MPa and low water absorption. FT-IR and SEM analyses confirmed the successful incorporation of the extract into the molecular structure of the film, with a homogeneous surface free of cracks. Notably, the bioplastic film exhibited antimicrobial activity against both Gram-positive (*Listeria innocua*) and Gram-negative bacteria (*Escherichia coli* O157:H7, *Salmonella enterica*) - key foodborne pathogens. Importantly, this antimicrobial activity was maintained after three months of storage, demonstrating the long-term stability of the product. The results of this study reveal extensive potential applications for environmentally friendly food packaging materials capable of extending food preservation time while reducing contamination risks. This bioplastic film not only contributes to providing solutions for plastic pollution but also utilizes locally available agricultural resources for material production, supporting sustainable development goals.

Keywords: *Bioplastic films, Vitex negundo L., glycerol as a plasticizer, antibacterial activity.*

1. INTRODUCTION

Plastic pollution has emerged as one of the most serious environmental challenges of the 21st century. Since the 1950s, approximately 8.3 billion tons of plastic have been manufactured, with around 60% now considered waste in landfills or the natural environment.[1] Annually, an estimated 8 million tons of plastic enter the oceans, resulting in severe repercussions for marine ecosystems and hazards to human health via the food chain.[2,3] Microplastics have been detected in many marine organisms, in the air, drinking water, and the food daily consume.[4–6] Besides the threat of plastic pollution, an even more urgent issue is the dependence of the plastic industry on non-renewable fossil fuels. Today's

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commercial plastic products from petroleum cause considerable greenhouse gas emissions.[7] The long-term sustainability of the plastic industry requires alternatives from renewable and environmentally friendly resources.

Bioplastic films from natural raw materials have been attracting increasing research interest as a potential solution for environmental issues related to traditional plastics.[8] Bioplastics originate from renewable materials and are biodegradable, reducing ecological impact after disposal.[9] Among natural raw materials, plant polysaccharides, especially starch, stand out as an attractive choice for bioplastic production due to their abundance, low cost, and complete biodegradability.[10]

Sweet potato (*Ipomoea batatas* L.) starch is a promising polysaccharide source for bioplastic production. Sweet potatoes are widely grown in many countries, including Vietnam. This crop has a high starch content and is suitable for producing biopolymer materials. Sweet potato starch has an appropriate amylose/amylopectin ratio, affecting the physicochemical properties of the bioplastic films created from it.[11] Sweet potato starch has good viscosity and gelation properties, suitable for producing films with good mechanical strength.[12] However, a significant limitation of starch-based materials is their sensitivity to water and limited mechanical strength compared to synthetic plastics.[13] To improve these limited characteristics of starch-based bioplastic films, many studies have focused on using various plasticizers and additives such as glycerol, sorbitol, and chitosan[14–16] to enhance the properties of bioplastic films. Another promising research direction is the development of bioplastic films with antimicrobial properties for food packaging applications. Several studies have proposed the potential of plant extracts or essential oils in creating antimicrobial bioplastic films.[17] However, using plant extracts with biological activity to enhance the antimicrobial capability of starch-based plastic films remains an interesting field for further research.

Vitex negundo L. is a medicinal plant widely used in traditional medicine in many Asian countries. *V. negundo* leaves contain many biologically active compounds, such as flavonoids, terpenoids, glycosides, and alkaloids.[18–20] Several studies have demonstrated that *V. negundo* leaf extract has good antimicrobial (such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio mimicus*, *Shigella* spp, and *Aeromonas* spp) and antioxidant capabilities.[21–23] Although there have been many studies on the biological activity of *V. negundo* leaves and the potential of sweet potato starch in bioplastic film production, the combination of these two materials to create bioplastic films with antimicrobial potential remains an area that needs to be researched to develop environmentally friendly materials useful for food packaging applications.

2. MATERIALS AND METHODS

2.1. Preparation and extraction of *V. negundo*

V. negundo leaves were collected in Can Tho City and identified by Dr. Pham Ha Thanh Tung (Phenikaa University). After removing damaged leaves, the leaves were washed, dried, and ground into powder to obtain the raw material. The powder was placed in a cloth bag and soaked in 96° EtOH solvent for 72 hours. Then, the extract was dried with Na_2SO_4 and filtered through filter paper. The soaking extraction process was repeated two more times. The extract was concentrated by rotary evaporation to recover the solvent, obtaining the EtOH extract.

2.2. Extraction of sweet potato starch

White sweet potatoes purchased from markets in Can Tho City were washed and ground into a paste. Then, distilled water was added to the sweet potato mixture, and a filter cloth was used to squeeze out the solution containing starch. The filtered starch solution was held at room temperature to allow the starch sediment to settle. After about 5 hours, the water was removed, obtaining the starch in paste form. The

starch was washed 3 times with water, dried at 40°C, and then finely ground.[24]

2.3. Preparation of bioplastic films

5.0 g of sweet potato starch was dispersed in 50 mL of distilled water, adding glycerol or sorbitol at different concentrations (10%, 20%, or 30% w/w). The mixture was stirred using a heated magnetic stirrer (Phoenix Instrument RSM-01HP) for 25 minutes at 75°C until a homogeneous mixture was obtained. It was then blended with *V. negundo* leaf extract at different concentrations (0%, 10%, 20%, or 30% w/w). This mixture was poured into the molds and dried at room temperature for 2 days, then dried at 50°C for 10 minutes. The resulting plastic films were removed from the molds and stored in sealed containers with desiccant for subsequent experiments.

2.4. Investigation of sensory, physical, and chemical properties of bioplastic films

2.4.1. Sensory evaluation and thickness measurement

A sensory evaluation was conducted regarding the feeling upon contact and the texture of the plastic film samples at different extract concentrations.

The thickness of plastic film samples without extract and with extract at different concentrations was measured using an electronic thickness gauge with 0.01 mm accuracy. Thickness measurements were taken at 5 random locations on the plastic film sample.

2.4.2. Water absorption measurement

Water absorption measurement was performed according to the ASTM D570 standard with modifications to suit the current sample condition better. Plastic film samples at different extract concentrations (0, 5, 10, 20, or 30% w/w) were cut into 2 cm × 2 cm samples, then weighed and recorded the dry weight before testing. Those samples were immersed in 20 mL of distilled water. At specific time points, the samples were removed and wiped with a dry cloth before the wet weights were recorded.[24] The experiment was repeated 3 times. The percentage of water absorbed was calculated according to the formula:

% Water absorption = (Wet weight - Dry weight)/ Dry weight ×100.

2.4.3. Tensile strength measurement

The tensile strength of the plastic film was determined based on the ASTM D882 standard. The results were expressed in N.

2.4.4. Fourier-transform infrared spectroscopy (FT-IR) analysis

The plastic films were analyzed by FT-IR spectroscopy using an FT/IR-4600typeA instrument, with spectra recorded from 4000 cm^{-1} to 400 cm^{-1} and 16 scans. The two types of plastic films were measured as film sheets, and the extract was measured as a KBr pellet.

2.4.5. SEM Scanning electron microscopy (SEM) analysis

Scanning electron microscopy (JCM 7000, JOEL, Japan) was used to analyze the surface morphology of the bioplastic films.

2.5. Antibacterial activity testing

2.5.1. Evaluation of the antibacterial activity of V. negundo leaf EtOH extract

The agar well diffusion method is a simple method widely used to evaluate antibacterial activity. Similar to the procedure used in the disk diffusion method, the surface of the agar plate is spread with bacterial suspension across the entire agar surface. After the agar plate has been inoculated, wells with diameters of 8 mm are created using a sterile tool, and an amount of antimicrobial agent or extract at the test concentration is introduced into the wells. Then, the agar plates are incubated under appropriate conditions depending on the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the test microbial strain.

Preparation: TSB (tryptic soy broth) or TSA (tryptic soy agar) bacterial culture media were prepared. The TSB liquid medium was prepared in deionized water at a ratio of 3% (w/v), and the TSA medium was supplemented with 1.5% (w/v) agar. Sterilization was performed at 121°C for 20 minutes.

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Procedure: Bacterial strains were cultured for 24 hours in TSB medium. Then, the bacterial suspension was transferred to a new liquid medium tube to achieve a bacterial density of approximately 0.5 McFarland Units. 100 µL of bacterial suspension was pipetted onto TSA agar plates and spread evenly. After the plates were dried, wells of 8 mm diameter were created, and 100 µL of extract dissolved in 20% DMSO was added to each well. Similarly, 100 µL of tetracycline 0.1 mg/mL (positive control) or 20% DMSO (negative control) was added.

Results were read after 24 hours of incubation at 32°C, measuring the inhibition zones and taking the average value of 3 replicates. The larger the diameter of the inhibition zone, the stronger the antibacterial activity, and vice versa. The diameter of the inhibition zone (DIZ) was calculated according to the formula:

$$DIZ\ (mm) = D - d$$

Where: D: diameter of the bacterial inhibition zone, including the diameter of the well (mm); d: diameter of the well (d = 8 mm).

2.5.2. Investigation of antibacterial activity of bioplastic films

Media preparation: Soft agar consisting of 3% TSB (w/v) and 0.5% agar (w/v), sterilized at 121°C for 20 minutes. After sterilization, the soft agar was maintained at a stable temperature of 50°C before use.

Procedure: 100 µL of bacterial suspension at a concentration of 0.5 McFarland Units (~ 1.5×10^7 CFU/mL) was added to 4 mL of soft agar solution, mixed well, and immediately poured onto a hard agar plate, then waited for the plate to solidify and dry. The pre-prepared plastic film samples with a diameter of 10 mm were placed on the surface of the soft agar. The negative control was plastic film without extract (sample diameter 10 mm) and plasticized film. The agar plates were incubated for 24 hours at 32°C and then observed for the antibacterial ability of the plastic films.

3. RESULTS AND DISCUSSION

3.1. Extraction of *V. negundo* leaf extract and sweet potato starch

From 1.0 kg of dry *V. negundo* leaf powder, approximately 200 g of extract was obtained using 96° EtOH solvent maceration, yielding an extraction efficiency of approximately 20%. To utilize readily available agricultural resources, we selected sweet potatoes from local markets as our starch source in this study. From 10 kg of fresh sweet potatoes, after extraction, approximately 1.8 kg of sweet potato starch was obtained. The extraction yield was approximately 18%.

3.2. Optimization of bioplastic film synthesis conditions

Natural polymers, such as polysaccharides, can form bioplastic films. However, bioplastic films synthesized from starch are typically brittle and easily broken. Hence, plasticizers need to be added to the film structure to improve the mechanical properties of the bioplastic films. Glycerol and sorbitol at different concentrations were selected as plasticizers in this study to investigate the appropriate supplementation concentration on the starch base as a foundation for subsequent studies, combined with *V. negundo* leaf extract.

Table 1. Sensory evaluation and thickness of bioplastic films containing glycerol and sorbitol plasticizers.

Sample		Thickness (mm)	Color	Texture	Sensory Evaluation
Plasticizer	Concentration (%, w/w)				
Glycerol	5	0.21 ± 0.06^{bc}	White	Brittle	Not good
	10	0.19 ± 0.03^{c}		Flexible	Good
	15	0.28 ± 0.04^{ab}		Flexible	Good
	20	0.26 ± 0.03^{abc}		Moist, soft	Not good
	30	0.33 ± 0.06a		Soft, sticky	Not good
Sorbitol	5	0.24 ± 0.02^{A}	White	Brittle	Not good
	10	0.22 ± 0.05^{A}		Flexible	Good
	15	0.25 ± 0.01^{A}		Flexible	Good
	20	0.23 ± 0.04^{A}		Moist, soft	Not good

Values not sharing the same letter are significantly different ($p < 0.05$).

Based on the results shown in Table 1, the film thickness ranged from 0.19 mm to 0.33 mm for glycerol plasticizer and from 0.22 mm to 0.25 mm for sorbitol plasticizer. These results demonstrate that for glycerol plasticizer, as the concentration of plasticizer increased, the thickness of the film also increased. Because glycerol can form hydrogen bonds with water molecules, increasing the volume of the plastic film, the sample thickness increases accordingly when the glycerol concentration increases. Meanwhile, sorbitol primarily forms hydrogen bonds with polymer chains, forming fewer bonds with water than glycerol, so plastic films using sorbitol plasticizers remain of similar thickness while increasing sorbitol concentration.[25] Besides the hydrogen bonding interactions, the observed thickness variations can be attributed to differences in solution viscosity and homogeneity between plasticizers. Glycerol exhibits significantly higher viscosity (~1400 cP) compared to sorbitol (200-300 cP) at room temperature, resulting in slower solvent evaporation and reduced solution spreading during film-making, which leads to thicker films with greater thickness variation. Therefore, glycerol-plasticized films demonstrate significant thickness differences between concentrations ($p < 0.05$), whereas sorbitol films maintain consistent thickness across all concentrations tested.

For bioplastic films containing 5% glycerol or 5% sorbitol, the products formed were brittle and easily broken when subjected to external forces. Films containing 20% and 30% glycerol or 20% sorbitol had soft textures and were easily torn. Therefore, films at those concentrations did not meet the requirements for strength and flexibility for subsequent further studies.

Films containing 10% and 15% glycerol or sorbitol could be bent and had appropriate flexibility; additionally, in terms of sensory evaluation, the film surface maintained dry characteristics upon contact. Therefore, films containing glycerol or sorbitol at these concentrations met the requirements in terms of sensory evaluation. However, when considering the economic aspects of the product, 10% glycerol or sorbitol concentration was determined to be more reasonable.

We propose that films with 10% glycerol or sorbitol are the most suitable for combining with different concentrations of *V. negundo* leaf extract (Table 1). As seen in Table 2, when the extract is incorporated into the film, the thickness of the product increases. In addition, a clear difference can be observed between films using glycerol or sorbitol as plasticizers when the extract is added. The film thickness changes significantly when the extract concentration increases for glycerol films. However, the difference is insignificant when combining extract into films containing sorbitol, although the film thickness increases, as demonstrated by the statistical results in Table 2.

Based on the sensory evaluations of texture, strength, and flexibility of the different types of films, it can be seen that films containing 5% and 10% extract with two kinds of plasticizers (glycerol or sorbitol) meet the sensory requirements as they have flexibility when bent and folded, and dry surface characteristics.

Films containing higher concentrations of the extract showed unacceptable results during sensory evaluation due to either too soft texture or excessively sticky surface characteristics.

Table 2. Sensory Evaluation and thickness of bioplastic films containing 10% glycerol and 10% sorbitol combined with *V. negundo* leaf extract.

Sample		Thickness (mm)	Color	Texture	Sensory Evaluation
Plasticizer	Concentration (%, w/w)				
10% Glycerol	5	0.16 ± 0.01^{b}	Green	Flexible	Good
	10	0.184 ± 0.04^{b}	Green	Flexible	Good
	20	0.23 ± 0.03^{b}	Green	Moist, soft	Not good
	30	0.36 ± 0.11^{a}	Dark green	Moist, soft	Not good
10% Sorbitol	5	0.22 ± 0.02^{A}	Green	Slightly flexible	Good
	10	0.24 ± 0.04^{A}	Green	Slightly flexible	Good
	20	0.26 ± 0.01^{A}	Green	Moist, soft	Not good
	30	0.23 ± 0.02^{A}	Dark green	Moist, soft	Not good

Values not sharing the same letter are significantly different ($p < 0.05$).

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3.3. Results of physical-chemical properties investigation of bioplastic films

3.3.1. Water absorption

Films containing 5% and 10% extract demonstrated the lowest water absorption among all tested bioplastic films, while those without extract (containing only 10% glycerol) exhibited the highest water absorption (Figure 1). The water absorption manner followed a consistent pattern: all films absorbed water rapidly during the first 5 minutes of immersion, followed by minimal additional absorption from minute 10 to 60, as evidenced by the nearly horizontal curve relative to the x-axis, indicating that the films reached water saturation. Despite 60 minutes of water immersion, all films maintained their structural integrity, remaining firm and resistant to tearing or pulling forces.

Similar to glycerol-containing films, the sorbitol-containing films exhibited strong water absorption during the first 5 minutes, followed by minimal absorption from minutes 10 to 60, eventually reaching saturation (Figure 2). After 60 minutes of water immersion, all films maintained their structural integrity, remaining firm and resistant to tearing or pulling forces. Notably, glycerol-containing films showed clear differences in water absorption percentages among formulations, whereas sorbitol-containing films demonstrated more uniform water absorption capabilities across different formulations.

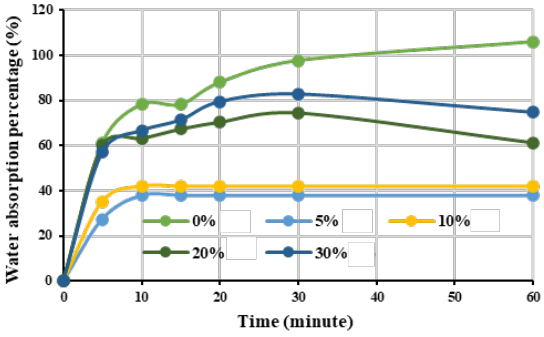

Figure 1. Water absorption percentage of bioplastic films containing 10% glycerol with extract additions from 0% to 30%.

Taken together, bioplastic films using glycerol plasticizer supplemented with 5% or 10% extract had the lowest water absorption percentages, indicating the potential application of these products in food preservation.

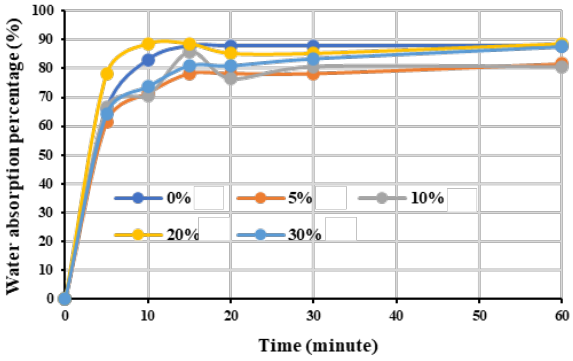

Figure 2. Water absorption percentage of bioplastic films containing 10% sorbitol with extract additions from 0% to 30%.

3.3.2. Tensile strength

Film samples containing 10% glycerol or sorbitol with 10% extract addition were tested for tensile strength using a digital testing device according to the modified ASTM D882 standard. After two months of sample storage, sorbitol-containing films exhibited brittle and easily breakable, so we did not proceed with tensile strength measurements for these samples. At the same time of sample storage, glycerol-containing films still maintained flexibility and elasticity, so tensile strength testing was performed. The results showed that the tensile strength of this film was 4.26 MPa. Compared with results from previous studies, bioplastic films from dialdehyde starch had tensile strengths in the range of 1.63 MPa to 3.06 MPa[26], and bioplastic films synthesized from sweet potato starch combined with glycerol plasticizer had a tensile strength of 2.57 MPa[15]. It can be seen that the films synthesized in this study have better tensile strength than the compared films.

Based on the tensile strength measurement results, we propose further studies only for the most suitable bioplastic film containing 10% glycerol and 10% extract.

3.3.3. Fourier-Transform Infrared Spectroscopy (FT-IR) analysis

Figure 3 shows the FT-IR spectra of three samples: the EtOH extract, the bioplastic film containing 10% glycerol, and the bioplastic film containing 10% glycerol supplemented with 10% extract. All three spectra display corresponding features. In the wavenumber range of 3500 cm^{-1} to 3000 cm^{-1}, the signals are attributed to hydroxyl (OH)

groups. Vibrations from 3000 cm^{-1} to 2800 cm^{-1} are assigned to symmetric and asymmetric stretching vibrations of C-H in the CH_2 or CH_3 groups (sp^3 hybridization). Besides, the addition of EtOH extract, which contains many secondary compounds such as polyphenolics, flavonoids, terpenoids and alkaloids, may have brought about small contributions from aromatic C–H vibrations (~3000 cm^{-1}) or additional aliphatic C–H groups from these compounds, causing a slight shift in the spectral peak of the extract-containing bioplastic film. Wavenumbers in 1400 cm^{-1} to 1300 cm^{-1} show signals of asymmetric C-H vibrations in CH_2 and CH_3 groups, and C–O–H bending vibrations. However, notable differences can be observed among the three spectra. Specifically, the FT-IR spectra of the extract and the bioplastic film containing 10% glycerol supplemented with 10% extract show signals in the range of 1850 cm^{-1} to 1650 cm^{-1}, accounting for the exist of C=O groups and potential C=C stretching from aromatic structures of secondary compounds, whereas the spectrum of the bioplastic film containing only 10% glycerol does not exhibit signals in this region. The differences are likely due to the starch-based film without extract lacking C=O functional groups. At wavenumbers 1300 cm^{-1} to 1000 cm^{-1}, strong absorption signals appear, attributed to C-O groups from starch in both types of films, while the extract shows only weak vibrations in this range. Finally, at wavenumbers 700 cm^{-1} to 900 cm^{-1}, characteristic vibrations of C-O-C groups from starch are observed in both types of films, while the extract does not clearly exhibit vibrations in this range.[12]

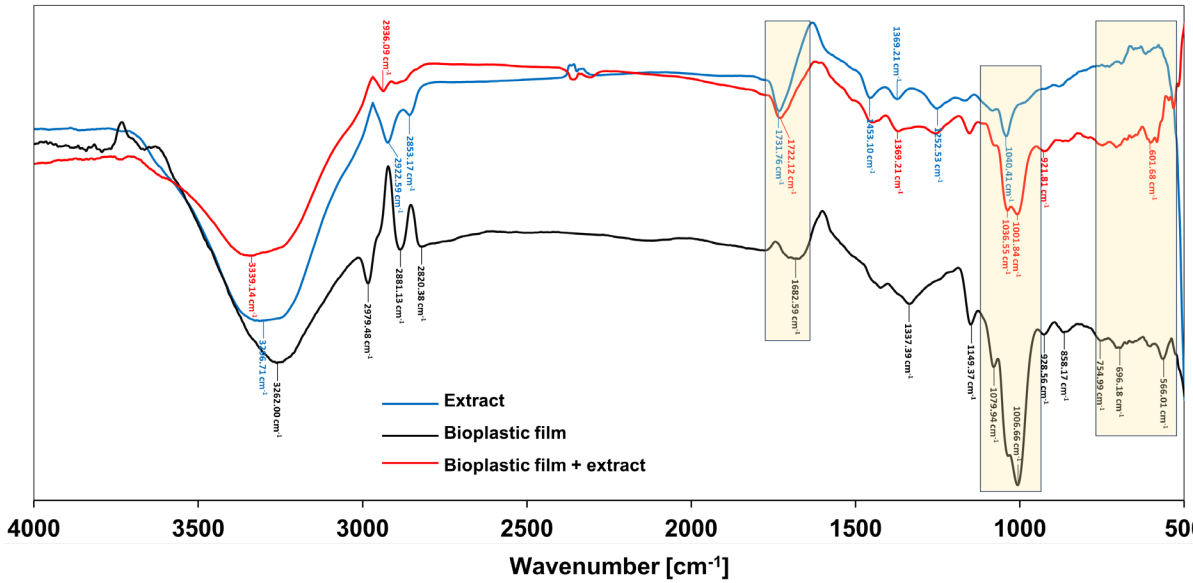

Figure 3. FTIR spectra of *V. negundo* leaf EtOH extract (blue), bioplastic film containing 10% glycerol (black), and bioplastic film containing 10% glycerol with 10% extract addition (red).

3.3.4. Surface morphology analysis by Scanning Electron Microscopy (SEM)

Observation of the SEM images (Figure 4) shows that the surface of the glycerol-plasticized film remains intact when the extract is added, displaying no evidence of tears, fractures, or discontinuities on the film surface.

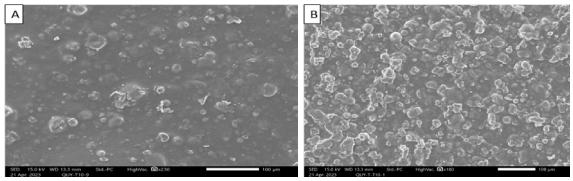

Figure 4. SEM images of bioplastic film containing 10% glycerol (A) at 230× magnification and bioplastic film containing 10% glycerol with 10% extract (B) at 180× magnification.

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3.4. Antibacterial activity results

3.4.1. Antibacterial activity of the extract

Results in Table 3 and Figure 5 demonstrate that *V. negundo* leaf extract at an initial test concentration of 40 mg/mL showed good antibacterial activity against Gram-negative bacteria (*E. coli* O157:H7, *S. enterica* serotype Enteritidis, and *Proteus* sp.). When the extract concentration was increased to 80 mg/mL, the extract exhibited moderate antibacterial activity against other Gram-positive bacteria like *L. innocua* and Gram-negative bacteria (*Campylobacter* sp. and *Citrobacter freundii*). The extract showed weak or no antibacterial activity for some bacterial strains such as *S. aureus, S. enterica* serotype Typhimurium, and S*higella* sp.. The negative control (20% DMSO) did not exhibit antibacterial activity, while the positive control (tetracycline at 0.1 mg/mL) showed antibacterial activity against almost all bacterial strains used in this study.

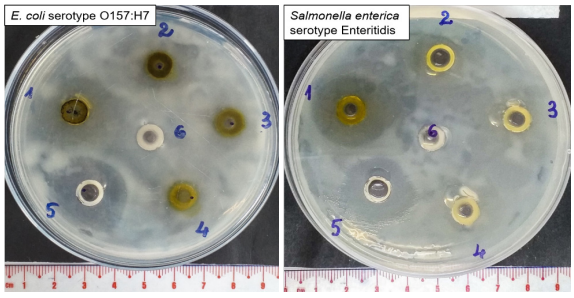

Figure 5. Agar well diffusion results of *E. coli* serotype O157:H7 and *S. enterica* serotype Enteritidis at different concentrations of *V. negundo* leaf extract: (1) 40 mg/mL, (2) 20 mg/mL, (3) 10 mg/mL, (4) 5.0 mg/mL, (5) positive control (tetracycline 0.1 mg/mL), (6) negative control.

Table 3. Antibacterial activity assessment of *V. negundo* leaf extract.

Gram	Bacteria	Inhibition zone diameter (mm)					
		Extract (mg/mL)					Tetracycline (0.1 mg/mL)
		80	40	20	10	5	
(+)	Listeria innocua	6.33 ± 3.51^{a}	4.33 ± 1.16^{ab}	3.00 ± 0.00^{ab}	2.33 ± 0.58^{ab}	NT	6.67 ± 1.53^{a}
	Staphylococcus aureus	+/	–	–	–	NT	+/–
(-)	*Campylobacter* sp.	7.00 ±1.41^{a}	4.00 ± 0.00^{b}	2.50 ± 0.71^{bc}	0.50 ± 0.71^{c}	NT	6.52 ±1.03^{a}
	Citrobacter freundii	4.33 ± 0.58^{b}	0.67 ± 1.15^{c}	0.33 ± 0.58^{c}	0.33 ± 0.58^{c}	NT	22.33 ± 4.93^{a}
	E. coli serotype O157:H7	NT	22.33 ± 3.21^{a}	13.67 ± 5.13^{b}	3.00 ± 1.73^{c}	1.33 ± 0.58^{c}	13.67 ± 2.89^{b}
	Proteus sp.	NT	10.67 ± 1.53^{a}	6.33 ± 1.16^{b}	2.67 ± 0.57^{c}	NT	4.33 ± 2.01^{bc}
	Salmonella enterica serotype Enteritidis	NT	17.0 ± 1.00 ^{a}	5.00 ± 2.65^{b}	3.33 ± 1.53^{c}	3.00 ± 1.00^{c}	13.67 ± 1.16^{a}
	Salmonella enterica serotype Typhimurium	+/–	–	–	–	NT	15.85 ± 1.69
	Shigella sp.	+/–	–	–	–	NT	20.53 ± 2.74

Note: (-) No inhibition zone observed, (+/–) Weak antimicrobial activity or no clear inhibition zone observed, (NT) concentration not tested for activity. In the row, values not sharing the same letter are significantly different ($p < 0.05$).

3.4.2. Antibacterial activity of bioplastic films

Results in Table 4 show that bioplastic film containing 10% extract demonstrated antibacterial activity against all three test bacterial strains s(*L. innocua*, *E. coli* serotype O157:H7, and *S. enterica* serotype Enteritidis) as evidenced by

reduced bacterial growth on the agar surface, particularly exhibiting good antibacterial activity against *L. innocua* and *S. enterica* serotype Enteritidis. Films containing 5% extract showed good antibacterial results against *S. enterica* serotype Enteritidis while demonstrating moderate antibacterial activity against the other two bacterial strains.

Film samples stored for 3 months before testing still maintained antibacterial activity against the test bacterial strains. Negative controls (films without extract and plasticized films) showed no antibacterial activity against all four test bacterial strains.

Table 4. Antibacterial activity assessment of bioplastic films containing extract.

Bacteria	Bioplastic films containing extract			Films after 3 months of storage		Plasticized film
	0%	5%	10%	0%	10%	
L. innocua	−	+	++	−	+	−
E. coli serotype O157:H7	−	+/−	+	−	+/−	−
S. enterica serotype Enteritidis	−	++	++	−	+	−

Note: (++) Good antimicrobial activity, (+) Fairly good antimicrobial activity, (+/–) Weak antimicrobial activity, (-) No antimicrobial activity.

Bioplastic films from sweet potato starch combined with *V. negundo* leaf extract have demonstrated significant potential in simultaneously addressing two issues: plastic pollution and food preservation. FT-IR analysis results indicate effective incorporation of the extract into the film structure through the appearance of characteristic signals for C=O groups (1850 cm^{-1} to 1650 cm^{-1}) in the extract-containing film, while these signals are absent in the starch-only film. These results confirm that the extract is not merely physically dispersed but also chemically interacts with the starch matrix, explaining the stability and ability to maintain antibacterial activity after prolonged storage.[27] SEM images show homogeneous film surfaces without cracks when the extract is added, ensuring the structural integrity necessary for packaging applications.

Regarding antibacterial activity, films containing the extract demonstrated effective inhibition against both *L. innocua* (Gram-positive) and *E. coli* O157:H7 and *S. enterica* (Gram-negative). These three bacteria are dangerous foodborne pathogens, particularly *E. coli* O157:H7 and *S. enterica*, which are common causes of severe food poisoning.[28] The antibacterial effect may be related to flavonoids, terpenoids, and alkaloids in *V. negundo* leaves, allowing the film to gradually release these compounds, creating a protective barrier for food.

The low water absorption capacity of the extract-containing films (as demonstrated in previous experiments) is an important property that helps overcome the major limitation of traditional starch-based bioplastics. The reduced water uptake can be explained by polyphenolic and terpenoid compounds in the extract forming bonds with hydroxyl groups in starch, limiting their interaction with water.[29]

The potential applications of antimicrobial bioplastic films from this research could extend beyond food packaging to other fields such as agriculture (seed coating, fertilizer coating) and healthcare (antimicrobial bandages). Youssef and El-Sayed (2018) reported that antimicrobial packaging materials can extend the shelf life of fresh food by 25% to 50%, reducing food waste and enhancing food safety.[30] However, for commercialization, further research is needed on large-scale production, durability under real conditions, as well as biological safety assessment when in direct contact with food.[31]

3. CONCLUSION

The research has successfully synthesized biodegradable polymeric films from sweet potato starch incorporated with *V. negundo* leaf extract, exhibiting prominent antimicrobial properties. The biopolymeric matrix containing 10% glycerol and 10% *V. negundo* leaf extract demonstrated an optimal equilibrium between

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mechanical characteristics (tensile strength of 4.26 MPa) and aqueous stability. Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM) analyses confirmed the successful molecular integration of starch, glycerol, and phytoextract within the film's supramolecular structure. Notably, the biopolymeric film exhibited efficacious antimicrobial activity against both Gram-positive (*L. innocua*) and Gram-negative (*E. coli* O157, *S. enterica*) bacterial strains - common foodborne pathogens associated with food deterioration and foodborne illness. Significantly, this antimicrobial efficacy persisted after a three-month storage period, exhibiting the long-term stability of the bioactive compounds within the polymeric matrix.

The experimental outcomes elucidate the potential applications of these antimicrobial biopolymeric films in environmentally sustainable food packaging solutions, simultaneously addressing the critical issue of plastic pollution. Subsequent investigations should focus on practical food preservation trials, elucidation of the antimicrobial mechanisms at the molecular level, and process optimization for commercial-scale implementation.

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