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Research Article

Poly (Lactic Acid) Cellulose Biocomposite Films as Potential Antimicrobial Food Packaging Material

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Abstract: Polylactic acid (PLA)-cellulose bio-composites were developed by incorporating *Aloe vera* (*A. vera*) leaf gel at ratios of 100/50 and 50/50. This study aimed to evaluate their physico-chemical, barrier, and antibacterial properties for potential application in sustainable food packaging. Different composite films were prepared and characterized by Fourier-transform infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM), mechanical testing, water absorption, and Ultraviolet (UV) transmission measurements. Their antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus* was assessed through agar diffusion and broth dilution assays. The presence of cellulose significantly affected the properties of the composite films. The 50/50 blend exhibited better chemical resistance and barrier properties compared to the 100/50 blend, highlighting the influence of cellulose content. Both ratios demonstrated promising antibacterial activity against all three target bacteria, while the 50/50 ratio showing pronounced inhibition against *E. coli*. These PLA-cellulose bio-composite films, with their combined UV-protective and antibacterial properties, present a sustainable and potential alternative to conventional food packaging materials, contributing to a reduced environmental pollution.

***Keywords*:** PLA composite, *A. vera* cellulose, bio-derived, antimicrobial film, food packaging

1. Introduction

Microbial contamination of food surfaces is a major cause of food spoilage and a significant challenge in maintaining sterility in various applications. The last century saw the emergence of drug-resistant infectious agents, a testament to the remarkable adaptability of microorganisms to changing environmental conditions.[1,2] To combat food contamination and spoilage, several methods have been employed, including the addition of antimicrobials (such as antibiotics or organic compounds like sorbate, propionate, benzoate, acetate, and lactate), reduction of pH and water activity (through acidification or dehydration), and thermal treatments like pasteurization, sterilization, and heating. While these methods have proven effective, however, consumer concerns regarding the potential health risks of

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chemical additives in processed foods have been growing. This has spurred research into novel preservation methods, aiming to achieve longer shelf lives for minimally processed foods.[3]

Driven by their affordability and ease of processing, polymers like polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS) have become dominant as conventional packaging materials.[4] However, widespread reliance on these non-degradable plastic films has triggered severe environmental pollution due to their throw-away nature.[5] This makes the development of eco-friendly, biodegradable films an urgent necessity. Extensive research has focused on alternative packaging materials to mitigate the environmental impact of petroleum-based options. Studies have shown that bio-polymer-based materials can significantly reduce packaging waste generation, addressing the critical issue of waste disposal to a substantial degree.[6,7,8]

PLA has emerged as a leading contender in the field of biodegradable polymers, offering a promising alternative to conventional non-biodegradable thermoplastics and thermosets.[9] PLA's appeal lies in its unique combination of being both biodegradable and semi-crystalline thermoplastic aliphatic polyester derived from renewable resources. Its susceptibility to hydrolysis grants it biodegradability, while its thermoplastic nature makes it easily processable. Meanwhile, biodegradable films derived from hydrocolloids like starch excel in their ability to control the movement of moisture, oxygen, carbon dioxide, and lipids, acting as effective barriers. As these bio-based materials continue to grow in cost-effectiveness[10] and performance, they hold the potential to eventually displace the dominant reign of oil-based polymers.

A. vera holds the distinction of being the most commercially cultivated species. Leaf pulp processing has blossomed into a thriving global industry, with its applications extending to the realm of functional foods and various food products. While over 75 active components have been identified in the inner gel, the precise therapeutic benefits of each remain an area of ongoing research.[11,12] Nonetheless, the complex composition of *A. vera* is believed to be the source of its diverse pharmacological and therapeutic potential. Additionally, its inherent antimicrobial properties and ability to enhance vitamin bioavailability in humans make it a promising candidate for biopolymer film applications. This unique blend of ingredients contributes to *A. vera*'s impressive range of potential pharmacological and therapeutic activities. Scientists hypothesize that the very heterogeneity of its composition unlocks a synergy of healing effects. Among these promising properties is: Antimicrobial activity: Certain components within the gel exhibit the ability to combat harmful bacteria and fungi, making it potentially useful for wound healing and hygiene applications. Enhanced vitamin bioavailability: Studies suggest that *A. vera* can improve the absorption of certain vitamins when co-administered, potentially boosting their beneficial effects. Biocompatibility for film applications: The inherent properties of *A. vera*, including its antimicrobial nature and biodegradability, make it a well-suited candidate for developing eco-friendly biopolymer films with potential uses in food packaging and medical applications.

Owing to the above-mentioned reasons, the goal of this work is to develop high-strength, long-shelf-life biodegradable films prepared from renewable resources. Thus, the novelty of this work is to use *A. vera*-based cellulose with PLA for a possible food packaging application with antimicrobial properties, which were not taken into consideration until known to our knowledge.

2. Materials and methods

2.1 *Materials*

PLA was purchased from Ingio™ Biopolymer 3052D Nature Tech India Pvt Ltd. and MMT Clay from Sigma Aldrich. The density of PLA is 1.24 g/cm^3. Benzene, acetone, ethyl acetate, chloroform, diethyl ether, and petroleum ether were obtained from SD Fine Chemical Company, India. The cellulose fibers were extracted from *A. vera* pulp through the Soxhlet extraction process.

2.2 *A. vera extraction*

Freshly harvested *A. vera* leaves were thoroughly washed with double-distilled water to remove any dirt or surface impurities. The leaves were then filleted, and the inner gel was carefully separated from the outer rind. This gel constitutes the desired raw material for extraction. The extracted gel was then physically processed to increase

surface area and facilitate subsequent steps. This could involve manual chopping, blending, or passing through a grinder depending on the desired particle size. If removing lipids is desired, the milled pulp can be subjected to Soxhlet extraction[13] using a suitable solvent like ethanol. This step is helpful if you want to focus on the polysaccharide fraction of *A. vera* for your bio-composites. The extracted pulp, with or without defatting, was then dried thoroughly. This can be done using a gentle oven drying at approximately 40 °C to preserve the biomolecules. Finally, the dried pulp was pulverized into a fine powder using a mortar and pestle or a suitable mill. This final powder form is often used for further processing and incorporation into the bio-composite films.

2.3 *Sample preparation*

The *A. vera*-based films' natural cellulose fiber was produced by solvent casting as follows: A transparent solution was produced by mixing 2.0 g of pure PLA in 100 ml of chloroform solvent and stirring constantly for 2 hours at room temperature. The PLA-derived product is then blended with various percentages of *A. vera* (100/50 and 50/50 percent). The supernatant was poured into the PLA (Table 1) and subjected to 2 hours of vigorous sonication to achieve a homogenous solution. The resultant mixture was put into Petri dishes to create thin films and then dried at room temperature for another 24 hours while the solvent slowly evaporated (Figure 1).

Table 1. Composition of PLA and *A. vera* cellulose with solvent

Systems	PLA/Wt % of *A. vera*	Solvent
A	100/50%	Chloroform [100 ml]
B	50/50%	Chloroform [100 ml]

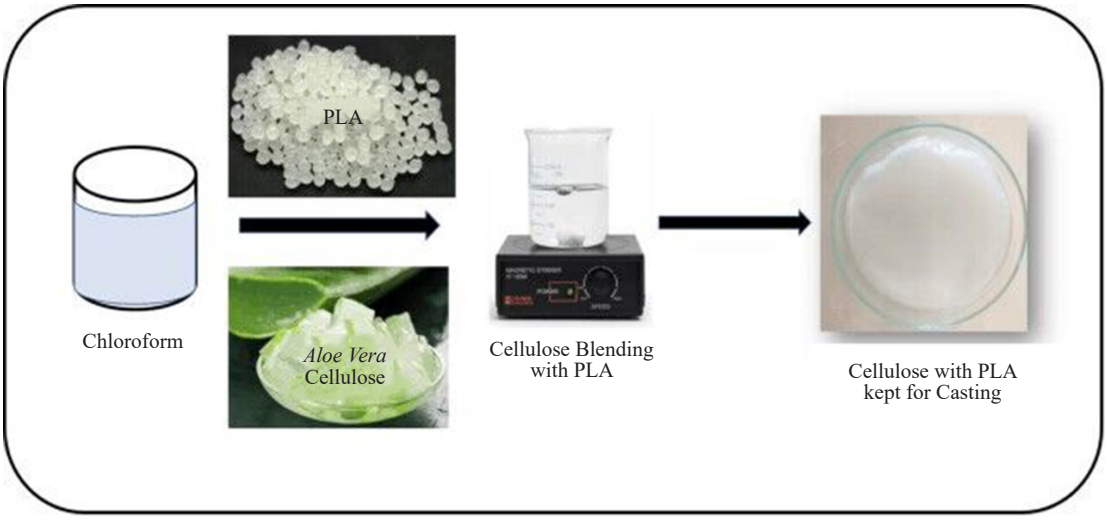

Figure 1. Development of highly compatible biopolymers and PLA-based film for food packaging applications

3. Characterization of PLA and cellulose composite films

3.1 *Ultraviolet-visible spectroscopy*

The UV spectra of the 100/50 and 50/50 PLA-cellulose composite films were measured using Shimadzu UV-1600, Tokyo, Japan, at 200-800 nm.

3.2 *Fourier transform infrared spectroscopy*

The Fourier transform infrared (FT-IR) spectra of the 100/50 and 50/50 PLA-cellulose composite films were determined using FTIR spectroscopy (Bruker Vector 26 spectrophotometer) in the infrared range of 4,000-500 cm^{-1}.

3.3 *X-ray diffraction (XRD) spectroscopy*

The XRD analysis was carried out using an X-ray diffractometer (*Bruker D8 Advance X-ray powder diffractometer*) with Cu Kα at a current of 200 mA. Scanning in the range of 2θ = 10° to 70° at a scanning rate of 4 degrees/min at a 45 kV accelerating voltage yielded the XRD spectra. Scanning electron microscopy (SEM) micrographs were collected with a 15 kV accelerating voltage to study the surface condition of PLA-cellulose composite films at various ratios.

3.4 *Water absorption*

This test was carried out as per ASTM D570 procedure. The results are presented based on the percentage of water absorbed by the film.

3.5 *Chemical resistance*

The ASTM D543 method was used to conduct this test. The results are expressed as a percentage of solubility in the given medium. Many methods for determining the worth of films were employed.

3.6 *Antibacterial activity*

Antibacterial tests were performed using the disc diffusion method. The discs used in antibacterial experiments have controlled dimensions (diameter 6 mm ± 0.05) and their stabilization was performed using UV-Chamber for 30 minutes. Nutrient agar was chosen as the culture medium for the bacterial strain. The whole culture medium was sterilized by autoclaving at 121 °C for 20 min. The bacterial strains *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were used along with ampicillin as a control.

4. Results and discussion

The PLA-*A. vera* composite film was synthesized using a method described in a previous research paper.[14] Accordingly, PLA and cellulose-based films at the ratios 100/50 and 50/50 were selected for further study and characterized for their application as packaging materials for food industry.

4.1 *Ultraviolet-visible spectroscopy*

UV-vis absorption was measured at the wavelength of 400-800 nm for cellulose-based PLA film prepared with *A. vera* at the ratios 100/50 and 50/50, and the results are displayed in Figure 2. From the results of UV, it is quite interesting to observe that the UV transition of the PLA/Cellulose 100/50 composition occurred in the UV region with a λ max value of 256 nm, while the electronic transition of PLA/Cellulose fibre composite film 50/50 occurred in the visible region with λ max values ranging from 420 to 800 nm.

Film A (100 PLA/50 cellulose), has weaker and broader in the visible range (400-800 nm) compared to Film B. This suggests that the lower cellulose content in Film A contributes less to the overall UV absorption in the visible range. Some absorption might still be present in the UV region due to the inherent absorption of cellulose, even though it was masked by the dominant PLA contribution. Film B (50 PLA/50 cellulose) has a broad peak spanning the visible range of (400-800 nm). This implies a significant contribution from cellulose towards the UV absorption in Film B due to its equal proportion. The broader absorption shape compared to pure cellulose spectrum could arise from interactions between PLA and cellulose. Such interactions potentially affecting the electronic transitions are responsible for absorption. The differences in the UV[15] spectra of Films A and B reflect their varying PLA and cellulose compositions.

Film B with equal parts of both components exhibits a more prominent and broader absorption in the visible range due to the stronger contribution of cellulose. This unique behavior exhibited by PLA/cellulose composite film 50/50 may be attributed to the following reasons:

Food packaging material is essentially transparent in nature to visible light, and in the UV region, it must be opaque to protect the food from the oxidative degradation, flavour loss, and discoloration caused by the UV radiation.

Due to the presence of the OH and C = O groups, which lead to n-->π^* transition.

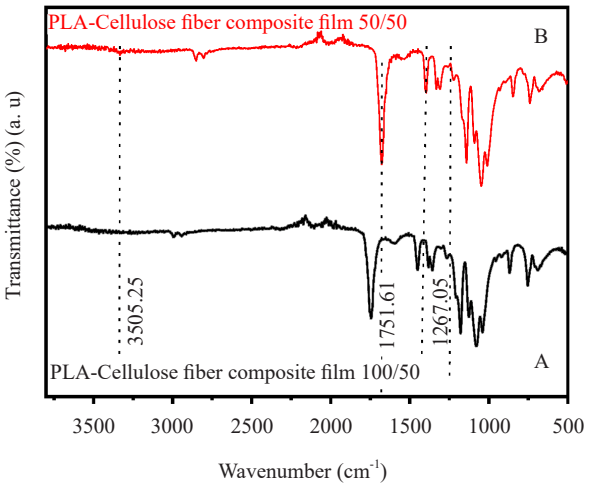

Figure 2. Ultraviolet-visible spectrum of PLA and cellulose composite film at ratio A) 100/50 and B) 50/50

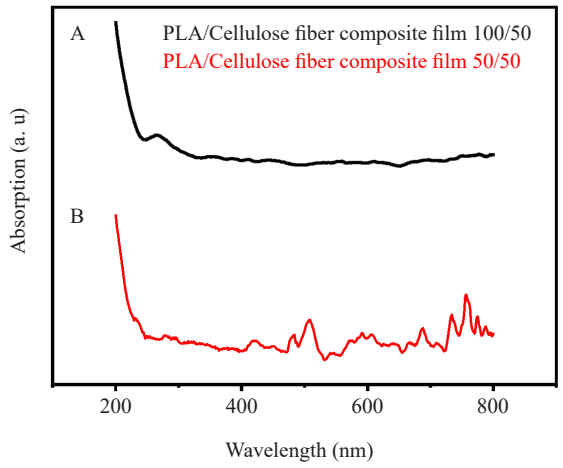

Figure 3. Fourier transform infrared spectra of PLA and cellulose composite film at ratio A) 100/50 and B) 50/50

4.2 *Fourier transform infrared spectroscopy*

The FTIR spectrum is a tool used to identify and analyze the functional groups present in a material based on their characteristic absorption bands in the infrared region. Figure 3 shows the FTIR spectrum of both Film A and Film B of the composite, which exhibit characteristic peaks in their FTIR spectra. Specifically, the peaks around 1,740 cm^{-1} and 1,250 cm^{-1} are noteworthy. These peaks are indicative of the presence of hemicellulose in the films. Furthermore, the spectra suggest that the intensity of the peaks related to hemicellulose gradually decreases as the cellulose content in the

composite films increases. This observation implies a partial removal of hemicellulose content from the cellulose fibers incorporated into the composite. When the composite films have an equal ratio of PLA and cellulose, certain peaks in the FTIR spectrum widen. Specifically, the peaks representing the presence of hydroxyl groups observed around 3,400 cm^{-1}, exhibit an increased width. This widening indicates interactions between PLA and cellulose.[16-18]

4.3 *X-ray diffraction spectroscopy*

In Figure 4, x-ray diffraction patterns of natural cellulose fiber films of PLA (100/50) ratio and (50/50) ratio of PLA film (Figure 4) are reported. To investigate the impact of different materials on the structure of natural cellulose fiber films of PLA, XRD traces for natural films have been included. The structure of natural cellulose fiber film[19] was characterized by three major reflection peaks of 2θ = 16.48, 17.18, and 18.25, while the XRD pattern of lignin natural cellulose fiber film containing cross-linking networks was lacking in the stable super-molecular structure. The natural cellulose fiber film showed two major peaks located at 2θ = 17.2, 24.54.

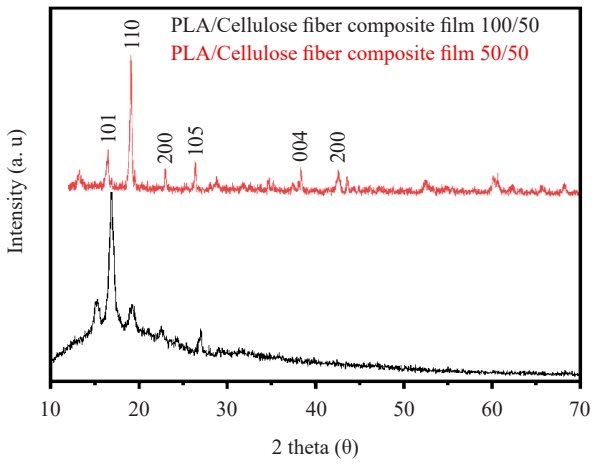

Figure 4. X-ray diffraction spectrum of PLA and cellulose composite film at ratio 100/50 and 50/50

4.4 *Water absorption*

ASTM D570 was used to measure water absorption. The samples were sliced into 1 cm × 1 cm square samples and immersed in distilled water. The weight increase was measured over a period of one week at an internal rate of 24 hours.

Table 2. Water absorption test result of PLA and cellulose composite at ratio PLA + A (100/50) and PLA + B (50/50)

Film type	Initial weight	After 24 hrs (g)	After 48 hrs (g)	After 72 hrs (g)	After 120 hrs (g)	Weight (%)
PLA + A	0.054	0.066	0.090	0.063	0.063	16.7
PLA + B	0.059	0.063	0.065	0.063	0.060	1.69

As Table 2 mentions, hydrogen bonds likely form between *A. vera* and PLA, hindering water molecule penetration into the composite material. This interaction effectively creates a hydrophobic barrier against water. Pure PLA: Shows the highest water absorption. PLA + A (100/50): This blend, with the highest *A. vera* content, exhibits the lowest water

absorption compared to pure PLA (16.6%). PLA + B (50/50): with reduced *A. vera* content, the water absorption decreased slightly (1.69%) compared to PLA + A. The reason behind the PLA matrix is to improve its hydrophobic nature. The Aloe vera and PLA combine to produce highly packed cellulose morphology that enhances the strong interaction of hydrogen bonding, reducing water absorption.

4.5 *Chemical resistance*

The chemical resistance of the synthesized samples was verified with the aid of ASTM D543. Similar to water absorption test, the films were cut into 1 cm × 1 cm squares and soaked in two different chemical systems, and the weight loss was checked every 12 hours. Table 3 and 4 provide more quantitative information showing the percentage reduction in the properties of the films when immersed in chemical solutions for different durations. These tables likely offer a detailed breakdown of the chemical resistance performance over time for both Film A and Film B.

Table 3. PLA and cellulose composite film immersed in different chemical solutions A (100/50)

Immersed time (day)	12	24	36	**48**
Chemical solution	The percent reduction/%			
Acetone	0.0629	0.0635	0.0678	**0.0694**
Ethanol	0.0756	0.0764	0.0789	**0.0805**
HCl	0.0904	0.0943	0.0956	**0.0978**
Benzene	**0.1352**	**0.1845**	**0.1976**	**0.2095**

Table 4. PLA and cellulose composite film immersed in different chemical solutions B (50/50)

Immersed time (day)	12	24	36	**48**
Chemical solution	The percent reduction/%			
Acetone	0.0534	0.0578	0.0523	**0.0590**
Ethanol	0.0523	0.0508	0.0543	**0.0586**
HCl	0.0603	0.0637	0.0656	**0.0659**
Benzene	**0.0755**	**0.0764**	**0.0859**	**0.0957**

The study involved immersing polymer and composite samples in a chemical solution to assess their chemical resistance. Figure 5(A and B), were generated to visualize the observed behaviors. The Figures indicate that the chemical resistance of the immersed samples varies based on the polymer structure. Specifically, the PLA and *A. vera* compositebased film, represented in the Figures, displayed different behaviors. The composite film with a 50/50 ratio of PLA and *A. vera* (Film B) showed higher resistance compared to the film with a 100/50 ratio (Film A).

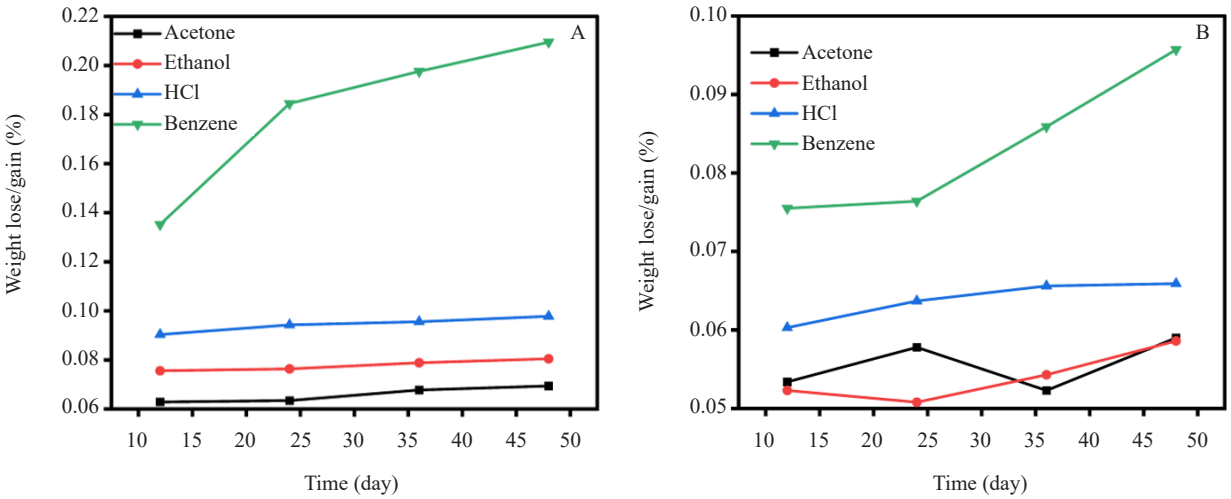

Figure 5. Chemical resistance ability of PLA and cellulose composite film at ratio A) 100/50 and B) 50/50

4.6 *Contact angle*

The main purpose of contact angle determination is to measure the wettability of the PLA films. The contact angle between 0° and 90° implies completely wettable, while above 90° is considered not (Figure 6). The wettability of natural cellulose fiber-based PLA films at ratios of 100/50 and 50/50 shows a contact angle of wetting below 90°.

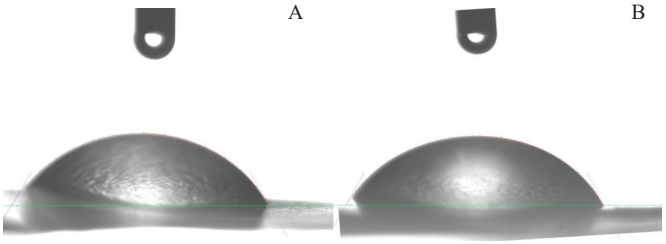

Figure 6. Contact angle of PLA/cellulose composite film at ratio A) 100/50 and B) 50/50

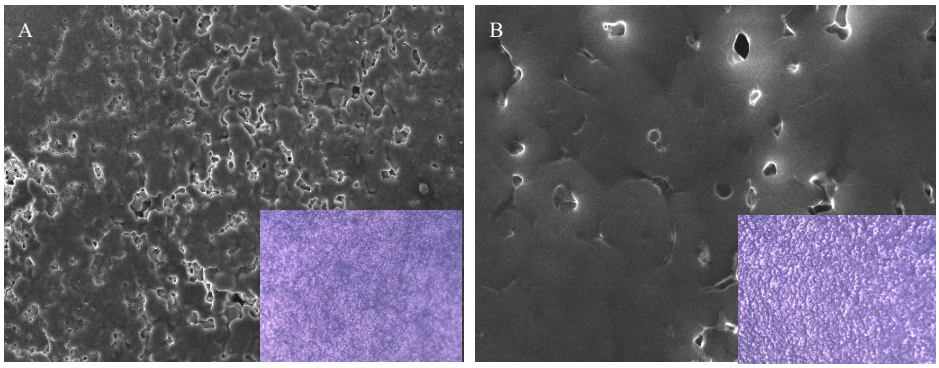

Figure 7. Scanning electron microscope and optical image of PLA/cellulose composite film at ratio A) 100/50 and B) 50/50

The contact angle of a solid surface is measured in this test, and the higher the contact angle, the better the hydrophobicity of the film, which is ideally needed for food packaging applications. Figure 6 showcases the wettability of the prepared composite films. Notably, samples 100/50 and 50/50 exhibit water contact angles of 67.15° and 69.98°, respectively. These values surpass the threshold value 65°[20] for hydrophobicity, indicating that the inclusion of *A. vera* in the PLA/cellulose fiber blends (100/50 and 50/50) renders their surfaces more water-repellent. Furthermore, the increasing weight percentage of *A. Vera* within the blend appears to further enhance this hydrophobicity, which is likely due to the combined effect of *A. vera*'s and the supportive role of long cellulose fiber side chains.

4.7 *Scanning electron microscopy (SEM)*

SEM micrographs illustrated in Figure 7 indicate that the surface morphology changed severely when *A. vera* was added to PLA. The development of smooth surface morphology from rough surfaces with the addition of *A. vera* is shown in Figure 7. This smooth morphology is closely related to the interaction and coordination between *A. vera* and PLA due to cross-linking. This indicates the uniform distribution of *A. vera* particles within the PLA matrix.[20] Interestingly, the surface of *A. vera*-PLA showed characteristic changes such as less brittleness and the absence of particle agglomeration, indicating their homogeneity.

4.8 *Antimicrobial activity*

The antibacterial efficiency of PLA-cellulose-based films was evaluated and summarised in Figure 8. The neat PLA was established to be entirely inert against the following bacteria: *E. coli*, *P. auriginosa*, and *S. aureus*. However, *E. coli* showed a higher zone of inhibition compared to the other two bacteria.

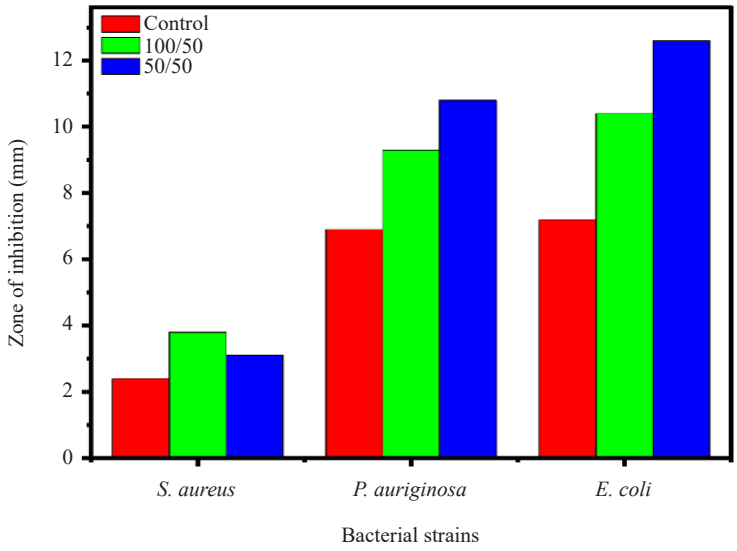

Figure 8. Antibacterial activity of PLA/cellulose bio-composite film

The 50/50 ratio of PLA/cellulose composite shows an excellent antimicrobial activity than the 100/50 ratio.[21] Thus, by incorporating *A. vera* into the PLA matrix, the antibacterial properties of PLA improved drastically.

5. Conclusions

The PLA/cellulose composite derived from *A. vera* leaf gel was reported for the first time. Accordingly, this work

focused on the development and characterization of PLA-cellulose bio-composite films at different concentrations. The physical properties, including optical characteristics, morphology, chemical resistance, and water solubility, of PLA/cellulose bio-composite films were evaluated and found to be superior to those of virgin PLA. The bio-composites in both the 100/50 and 50/50 ratios resulted in an uniform dispersion concerning the application. Moreover, both PLA-cellulose bio-composite films exhibited excellent antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *P. auriginosa*) bacterial strains, besides acting as good UV-light barriers. Notably, these biocomposites showed remarkable antibacterial activity against *E. coli*. Hence, we conclude that this study was conducted as proof of confirming the importance of PLA-cellulose bio-composite films for an active bio-packaging applications, which can be an ideal alternative material to replace the single-waste synthetic materials of pollution-causing nature.

Author contributions

The author's contribution to this manuscript is as follows: Gowsalya Venkatesan (GV) holds an M.Sc. and an M.Phil. in chemistry, and she is pursuing a Ph.D. (Chemistry) in the field of energy. Her contribution to the manuscript is conception, design, and data collection. The author, Dr. Saraswathi Umavathi (SU), holds a Ph.D. degree and is currently pursuing a postdoctoral in the field of botany. Her contribution to this manuscript is antimicrobial testing. The author, Dr. Kavitha Chandramohan (KC), holds a Ph.D. and is currently working as an Assistant Professor in the field of chemistry. Her contribution is manuscript Corrections. The author, Dr. Ananda Kumar Srinivasan (AKS), holds an M.Sc. and Ph.D. in the field of Polymer Chemistry and is currently working as a professor (Associate) in Anna University is a corresponding author for this manuscript. His contribution to the manuscript is material arrangement and characterization. The author, Dr. Sundar Natesan (SN), holds an M.Sc. and Ph.D. in the field of chemistry and is currently working in Research and Development in the private sector. His contribution to this manuscript is organizing the manuscript concerning figures and tables.

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Conflict of interest

The authors declare that they have no conflict of interest.

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