Synergistic evolution of stable bioactivity and better mechanical strength in polyvinyl alcohol and sweet lime peel film

Ritika Arora
Aparna Agarwal
Rizwana Haleem
S.K. Shukla
sarojshukla2003@yahoo.co.in

Delhi University https://orcid.org/0000-0001-8624-4177

Research Article

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Abstract

The present study discloses the evolution of bioactivity with improved chemical stability in the composite film of polyvinyl alcohol (PVA) and sweet lime peel (SLP) powder along with biodegradability and improved hardness due to the structural miscibility between constituents polymer during solution blending. The evolved structure, properties, bioactivity, and synergistic effect between PVA and SLP were established with the help of spectrochemical results, morphological results, and physio-mechanical properties i.e. thickness, chemical stability, water vapor transmission rate, heat seal-ability, and shelf-life. The result reveals the better preservation of the bioactive compound of SLP by 200%, antimicrobial nature against *S. aureus* and *E. coli*, improved tensile strength, and heat stability in the film. Further, the prepared film of SLP with optimum properties and 0.12 mm thickness was used for packing of sprouts as an alternative to currently used non-biodegradable film with comparable life span for seven days.

1. Introduction

Synergism among properties and structure of materials has been the significant driving force towards the development of suitable materials for different devices, and applications with better features. The synergistically develop materials with novel chemical, physical and biological properties are also used as active, passive, and intelligent packaging materials. Thus, the design and development of biodegradable polymer films with tuneable properties need of time to ensure the storage, supply, and availability of different edible and non-edible materials along with controlled environmental hazards [1, 2]. For this purpose, several biopolymers i.e. chitosan, alginate, cellulose and synthetic polymers polyvinyl alcohol (PVA), and polylactic acid are explored for making their composite and chemical modifications. Among the different polymers, PVA is a synthetic polymer with several features and used for packaging applications of medical items, electronic components, and other edible and non-edible after eliminating its basic limitations i.e. more water affinity, poor biodegradability, and limited mechanical strength [3, 4, 5]. The method used to improve the properties of PVA are making composite, grafting, and encapsulating with cellulose, methylcellulose, silver, metal oxides, rice husk, peel powders, and other bioactive ingredients [6, 7].

The bioactive compounds are secondary metabolites present or produced in both plant and animal living bodies, and have currently proven significant relevance in food packaging industry to develop an advanced active and intelligent packaging system [8, 9]. The important sources of bioactive compounds are fruits, seeds, bark, leaves, husk, peel, skin, oilcake, and exoskeletons. Among the different agricultural sources of the bioactive compound, the sweet lime peel (SLP) is perennially available about 50 to 55% of its weight in most part of the world. SLP contains several valuable bioactive components like phenols, flavonoids, essential oil, carotenoids, organic acids, ascorbic acid, vitamins along with pectin and cellulose based dietary fibre [10]. The presence of valuable components have been attracted the scientist for its valorisation into different valuable products like essential oil and pectin. The valorisation of SLP has been reported after using different techniques i.e. solvent extraction, microwave extraction, ultrasonication, soxhlet extraction, and hydro-distillation with their limitation and advantages [11]. The

important report about the use sweet lime powder for packaging films are listed in Table 1 along with properties and limitation.

Table 1
Important applications of SLP are packaging.

SN	Composition	Properties	Limitation	References
1.	SLP, PVA, and Starch	Improved transparency, barrier, tensile strength and elongation at break	Limited study and specific application	[12]
2.	SLP and soya lecithin, sweet lime essential oil	Mechanical properties, barrier properties, antimicrobial properties	Non-cost effective and commercial viability	[13]
3.	SLP, PVA, and Sugarcane bagasse	Enhanced water vapor permeability i.e1.53 ± 0.03 10-7g/Pahm, tensile strength i.e 947.79 ± 46.31kPa and elongation at break is 11.35 ± 0.009%	Focused applications with parameters	[14]
4.	PVA, starch, modified wax and sweet lime pomace	water absorption, textural, biodegradability, higher temperature tolerance and better barrier properties	No Product compatibility, water vapour permeability, no commercial viability	[15]
5.	SLP, sago powder, and gum Arabic	Valorisation and improved mechanical properties, folding endurance, and film strength	Packing applications and individual contribution	[16]
6.	Sodium alginate, titanium dioxide and sweet lime peel extract	Edible coating with extended shelf life extension and better properties	Limited study about coating materials	[17]
7.	PVA and SLP	Synergistic evolution of antimicrobial properties, barrier properties, long term preservation of bioactive compound for sprout packing	Economic consideration	Present work

The above data and discussion is confirming the need for establishing the synergistic relation during valorisation for the development of different SLP-based packaging films for various properties and applications. The impact of SLP on polymer matrix as the filler has been reported along with the use of SPL-derived bioactive compound in developing active packaging film. However, the extraction of bioactive compounds is time-consuming and costly and leaves large residue fractions of primary sources, which also does not fulfil the holistic concepts of valorisation and reusability [18, 19]. The importance of SLP-based composite film has been explored for packaging film with bioactive nature and better mechanical properties even for point-of-care application. But, mutual coherency between structure of SLPs and polymer matrix has not been reported for development of holistic properties as per our

observation [20, 21]. In the context of the above developments, the present manuscript demonstrates the synergistic effect between PVA and powder of sweet lime peel to develop bioactivity, mechanical properties, and long-term stability. Further, the obtained films with improved strength, optimized biodegradability, water vapor transmission rate, and suppressed nature towards microbial growth demonstrated use as a wrapping sheet for sprouts with comparable properties to the existing commercial film.

2. Materials and methods

2.1 Materials

Fresh sweet lime peels (SLP) were procured from a local juice shop, New Delhi, India. Polyvinyl alcohol (60000 to 125,000 and 87–89% hydrolysis), Muller Hinton Agar, and Glycerol (99%) were purchased from CDH Pvt. Ltd, Hi Media, Qualigens, and used without any further purification. However, the used solvents were of analytical grade, and double distilled water was used in the entire investigation.

2.2 Pre-treatment of sweet lime peels

The peels of sweet lime were dried using a tray dryer at 60 C ± 5 C for 6h. The dried SLP was broken into small fractions and then crushed using a laboratory scale grinder (Morphy Richards Icon *DLX*). The obtained powder was then sieved using a sieve with the mesh size of BSS150. The dried SLP powder was kept in a glass vial at room temperature till further use.

2.3 Preparation of packaging films

The PVA/SLP films were prepared using the solution blending method. In brief 1g of PVA powder was dissolved in 30 mL of hot deionized water at 90 C after continuous stirring on a magnetic stirrer working at 1000 rpm. The resultant solution was cooled down to room temperature, then 150 mg of SLP was added along with 0.2 mL of glycerol, and the mixture was further stirred for 30 mins.

The final blended solution was cast into the Petri dishes of 100mm diameter kept as a uniform substrate, and dried at 70 C ± 5 C for 18h in the hot drying oven. The films were peeled off from the petri dishes and kept in airtight bags for further use. The reference film of PVA was also prepared using similar conditions and composition.

2.4 Testing and characterization

The testing and characterization of the PVA and PVA + SLP films was carried out using standard ASTM methods [22].

2.4.1 Dimension

The thickness of films was measured using a digital vernier calliper with the least count of 0.1 mm, after due calibrations. The measurements were performed at five positions and their mean is reported.

2.4.2 Swelling index, Porosity and Solubility

The developed films were subjected to evaluation for swelling index, porosity, and solubility in water solution using standard ASTM methods. Initially, the films were cut into strips form with the dimension of 1×1 cm and weighed with the help of sartorius balance with the least count of 0.1 mg. The film was then immersed in water at room temperature for 24h, then out gently and wiped gently with filter paper to remove surface adsorbed water. After that weight and thickness of the films were also measured after using the same instruments. Further, the swelling index and porosity were calculated using Equations 1 and 2, while the swelling index was assessed by comparing the thickness of the dry and soaked film.

$$Solubility\,(\%) = \frac{W_1 - W_2}{W_2} (1)$$

Where, W_1 represent initial weight before drying and W_2 represent final weight of after drying of the film after immersion in the water

$$Porosity = \frac{W_w - W_d}{AL\delta_w} (2)$$

Where, W_w is the wet weight and W_d is the dried weight of the sample, A is the area of the sample taken, L is the thickness of the sample taken and δ is the density of water.

2.4.3 Hardness

The hardness of the film was measured using a shore A durometer designed as per ASTM D2240. The measurement was performed at five places and its mean was reported.

2.4.4 Water Vapor Transmission Rate

The water vapor transmission rate was determined using a standard weight loss method with time. For this, the films were cut into circular pieces with 1cm diameter, sealed onto the 25ml glass bottles filled with 15 ml distilled water with the help of a side rubber cork, and kept in a glass chamber containing controlled relative humidity. Further, the weight of the bottle was monitored for 10 days at regular intervals of 12 hrs. The change in mass was used to calculate WVTR using Eq. 3.

$$WVTR\left(gh^{-1}mm^{-2}\right) = \frac{x}{\Delta t \times A} (3)$$

x is the weight change; *t* represents the time and *A* area.

2.4.5 Heat Seal ability

The heat seal ability of the film was determined using the heat-sealable machine model Khera KI 351. A circular film was folded into half and was kept under the heat-sealing machine and pressed for 30 s at a temperature of 60–70 C and pressure of 2.5×10^4 Pa.

2.4.6 Spectroscopic analysis

The FT-IR analysis was carried out on the Bruker ALPHA infra-red Spectrometer at resolution of 4 cm^{-1}. The phase and crystallinity of the composite films were determined using an X-ray diffractometer (Bruker, D8 Discover), Cu Kα1 radiation with λ 1.5405 A generated at 40 KV and 30 mA. The thermal behavior of the film was evaluated using STA7300, HITACHI TG-DTA instrument. The measurement was taken from ambient to 500 C at a heating rate of 10 C/min under a static nitrogen flow of 100ml per minute. The surface morphology of the films was evaluated using the JSM 6610LV, JEOL, SEM at different magnifications after coating with thin layer of gold.

2.4.7 Biodegradability

The biodegradability of the films was carried out using a standard soil burial test. The films were buried in a pot filled with moist garden soil. The weight and the appearance of the films were monitored and noted at regular intervals of time.

2.4.8. Antimicrobial Testing

The prepared films were subjected to antimicrobial testing using the agar disc diffusion method. The films were checked against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria. The nutrient agar plates were used for culturing the bacteria. The concentration of the inoculum was adjusted to 10^8CFU/ml using 0.5 McFarland turbidity standard 1ml of inoculum was spread on the Muller-Hinton agar plates. The films were cut into a circular disc of punch hole size and placed on the Muller-Hinton agar plates inoculated with *S. aureus* and *E.coli*. The plates were incubated for 24 hours at 37 C. The inhibition zones were measured by calipers after due incubation [23].

2.4.9 Shelf-life analysis

The shelf life of the sprout samples were analysed by covering them with the PVA, PVA and SLP and commercial film and the samples were checked for texture, color and overall acceptability.

3. Results and Discussion

The mixing of SLP in PVA solution reveals the increase in viscosity of pristine PVA solution by 125% along with the change in colour sequence from whitish brown to light green. These changes confirmed the blending of both matrices, including the structural interaction between SLP with PVA during mixing. The resultant solution was converted into a uniform sheet with a thickness of sub-mm after hot air drying without any significant change in blended structure. The blending mechanism is also supported by a variation of the pH of PVA from neutral to slight acidic i.e. 6 pH. The acidic pH supports the oxidation of matric, which facilitates the loosening of the matrix and supports the electron interaction between both blended polymer matrixes. Based on above observation, the schematic for the development of film is shown in Fig. 1.

3.1 Physical Characterization

The observed physio-mechanical properties of the PVA and PVA + SLP films are listed in Table 2.

Table 2
Physio mechanical properties of film

S.No.	Parameters	Samples	
		PVA	PVA + SLP
1.	Thickness (mm)	0.05	0.12
2.	Swelling Index	0.02	0.01
3.	Water Absorption Capacity	11.11%	18.18%
4.	Porosity	2.8%	40%
5.	Hardness (Mhos)	85	74
6.	WVTR	47.45%	52.83%
7.	Tensile Strength	0.064kg/mm^2	0.068kh/mm^2

The properties are indicating significant improvement in the tensile strength, water vapor transmission rate and comparable hardness of PVA film after incorporating sweet lime powder in the PVA matrix.

3.2 FT-IR Spectroscopy

IR spectra of PVA and PVA + SLP are shown in Fig. 2, while the present peaks positions and their corresponding functional groups are listed in Table 3.

Table 3
Peak positions are corresponding functional groups

S.no.	PVA	SLP	SLP (1week)	SLP + PVA Film	Functional Group
1.	-	1014	1018	1030	C-4-OH (typical for glucose residue of disaccharides)
2.	1230	1241	1242	1254	C(O)–O stretching vibrations and –OH in plane vibrations/amide III (e.g. in aromatic ethers)
		-	-	1320	C-C Stretching
3.	1522	1524	1432	1421	C-H Bending
4.	-	1641	1610	1644	C = C Stretching
5.	1716	1737	1735	-	C = O Stretching

The spectrum of PVA is showing standard reported peaks at 3267, 2932, 1657, and 1446. 1087, 1033, and 851cm-1- for OH, CH, C-O, CH_2, C-O-C, C-O (crystalline), and C-C groups in Table 4 [24, 25]. However, the comparison of peak PVA with PVA + SLP is indicating the redshifts in their position along with changes in intensity and shape. These changes are confirming the progress of some types of interaction between PVA and SLP during blending. However, the evolution of a single peak between 3500 to 3000 cm^{-1} is indicating the evolution-aligned composite structure after hydrogen bonding between PVA and SLP. Furthermore, the peak present in around 1000 cm^{-1} in SLP confirms the presence of pectin, which is an important polysaccharide with potential applications in foods, pharmaceuticals, and other industries [26]. The other use of pectin includes edible films, paper substitute, foams, and plasticizers. The stability of pectin is also important for the long term use of packaging film. Further, in addition to pectolytic degradation, pectins are susceptible to heat degradation during processing, and the degradation is influenced by the nature of the ions and salts present in the system. In the present case the comparison of IR spectra of stored SLP and PVA + SLP is revealing the sustainable presence of pectin in PVA + SLP, while in the pure SLP, pectin degraded due to atmospheric aging. However, its presence in the PVA matrix is due to the non-availability of oxygen and other ingredients [27], which supports the sustainable stability of SLP due to the interaction of PVA.

3.3 X-ray Diffraction

XRD curve of PVA and PVA + SLP is shown in Fig. 4, while the details of information are listed in Table 5.

Table 4
XRD derived crystal data.

Samples	Interpretation		D value
	Peak Position	Intensity	
PVA	1. 19.48	371.55	0.46nm
	2. 22.64	161.30	0.39nm
	3. 40.6	78.21	0.22nm
PVA + SLP	1. 19.52	1222.04	0.45nm
	2. 22.96	688.89	0.39nm
	3. 40.52	579.02	0.22nm

The comparison of peak positions and intensity is revealing a change in positions and intensity of PVA in the PVA + SLP composite. The change in peak position is confirming the interaction between PVA and SLP. However, increasing in intensity of the composite is revealing significant improvements in the crystallinity of the PVA matrix due to the reorientation of the composite matrix by 324%. Further, the shouldered peak of PVA present at 22.64 has significantly loses its intensity in composite, which is confirming the alignment of the matrix at 101/– plane of PVA instead of 200 planes during blending [28].

The structural alignment of the PVA chain in the presence of SLP is responsible for optimized properties suitable for the development of packaging film for different applications.

3.4 TGA

The results of a TGA measurement are displayed in Fig. 5, while the data is listed in Table 5.

Table 6
TG data of PVA and PVA + SLP

Sample		Stages		
		1st	2nd	3rd
PVA	**Temp. range (°C)**	50.34–96.16	96.16-216.43	216.43-322.22
	Weight loss (%)	9.02	24.49	89.80
PVA + SLP	**Temp. range (°C)**	51.13–99.58	99.58–242.20	242.20-357.91
	Weight loss (%)	7.78	34.74	79.25

TG curve of PVA shows weight loss in three stages, i.e., first due to the removal of water molecules, second due to the elimination of water molecules from PVA, and the third stage is because of the decomposition of the carbon chain of polyvinyl alcohol followed by diffused weight loss [29]. However, the thermogram of PVA + SLP also reveals similar peaks with changes in the range, nature, and value of mass loss. The variation is due to the presence of SLP in the PVA matrix and the synergistic interaction between PVA and SLP. Further, the comparison of the third stage degradation indicates a sharper transition than pristine PVA because of the evolution of the composite matrix of PVA and SLP with better miscibility and aligned structure.

3.5 SEM

The SEM images of the samples are shown in Fig. 6. The microscopic image of PVA depicts its porous nature, while the image of PVA + SLP shows non-porous morphology and confirms the presence of SLP in the composite matrix.

3.6 Biodegradability

The soil burial test of the films showed biodegradability as shown in Fig. 7. It showed that PVA film degraded up to 25%, PVA and SLP degraded up to 38% and commercially did not degrade at all in 30 days.

3.7 Antimicrobial Testing

The antimicrobial nature helps enhance the shelf life of the food product. The peels of sweet lime are a rich source of bioactive compounds like phenols, and terpenoids which are responsible for their antimicrobial nature. The prepared PVA + SLP films came out to be more effective against Gram-positive

bacteria as compared to Gram-negative bacteria as depicted in Fig. 8. Due to the protective nature of their comparatively impermeable, lipopolysaccharide-containing outer membrane, gram-negative bacteria are often highly resistant to antibiotics than gram-positive bacteria [30].

3.8 Shelf-life analysis

The sprout samples were kept for shelf-life analysis and the result showed that the uncovered sample dried faster compared to the other samples as shown in Fig. 9. The samples kept covered with PVA and PVA + SLP films stayed fresh for up to 7 days, keeping the texture and color at a good state while maintaining the slightly acidic pH around 6. The samples covered with commercial film stayed fresh for a longer period but lately developed fungal growth with a change in pH towards alkalinity. It can be concluded that the prepared films can enhance the shelf life of the samples for about 7 days.

Discussion

The synergism has been established among naturally occurring SLP and PVA to optimize long time stability of bioactive compound present in SLP with optimized properties of PVA matrix to use for alternate packaging film for sprouts. The factors responsible for synergism are in-situ interactions between PVA and SLP during blending, which produces aligned restructuring in the PVA matrix against 110 plane with improved crystallinity after forming aligned hydrogen bonding. The restructuring in the matrix further controls inherited porosity in the PVA matrix, which is responsible for the evolution of lowering porosity and oxygen permeability in the packaging matrix. Further, the obtained composite matrix with optimized properties promises to be used as a packaging film with the presence of bioactive ingredients as an active film for longer durations. Finally, the claim of the above discussion was confirmed with observation gathered after packaging the film on sprouts with comparable properties to replace currently used non-biodegradable and non-recyclable film for an optimum period of 7 days.

Conclusion

An antimicrobial film with improved mechanical properties has been prepared after dispersing SLP powder in a PVA solution under optimum conditions. The evolved chemical structure, morphology, and decomposition behavior of the composite have been confirmed based on the spectroscopy, microscope, TG analysis, and relevant analytical result. Further, based on the observed antimicrobial nature and improved mechanical properties, the film was explored for wrapping the sprouts for seven days with insured quality. Finally, the study confirms the valorization of perennially available fruit waste to develop biodegradable packaging for sprouts with comparable properties of non-biodegradable film and control the plastic hazards towards the atmosphere and biosphere.

Declarations

Conflict of interest: Authors have no conflict of interest to declare.

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Figures

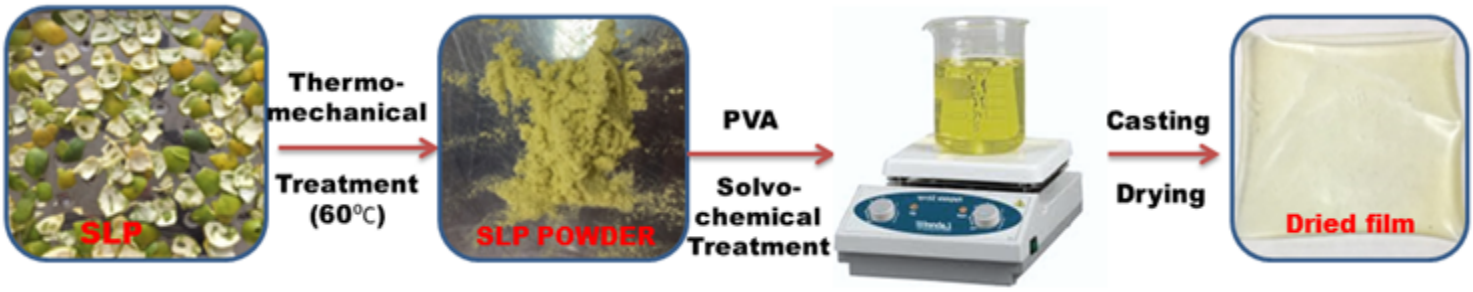

Figure 1

Schematic for preparation of films

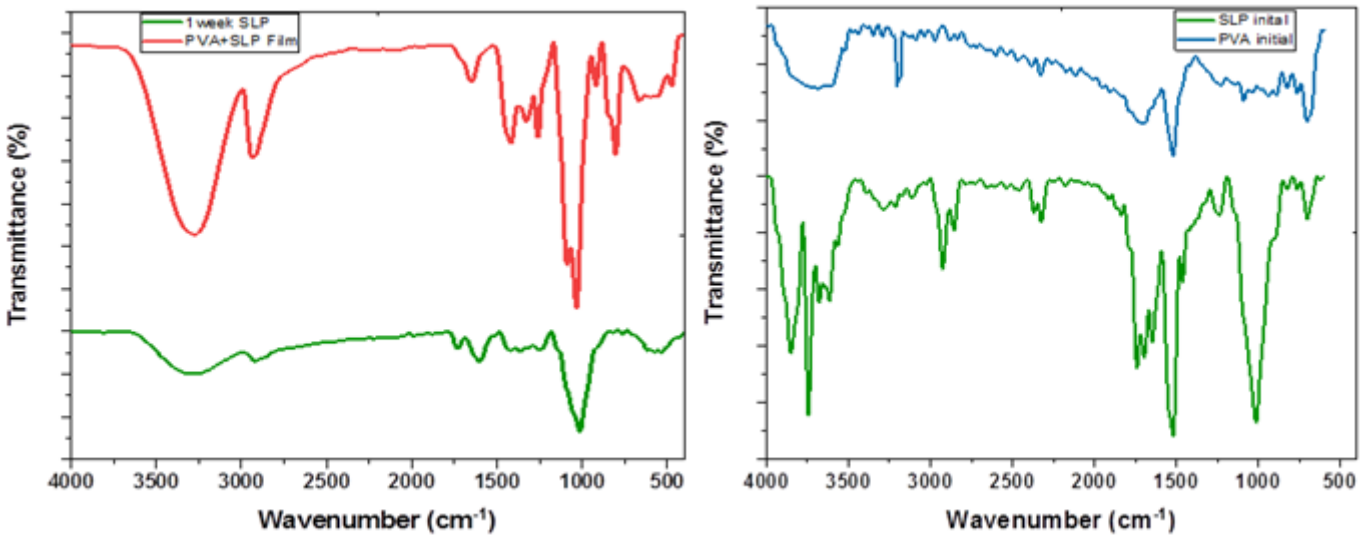

Figure 2

FT-IR spectra of PVA, SLP, and PVA+SLP

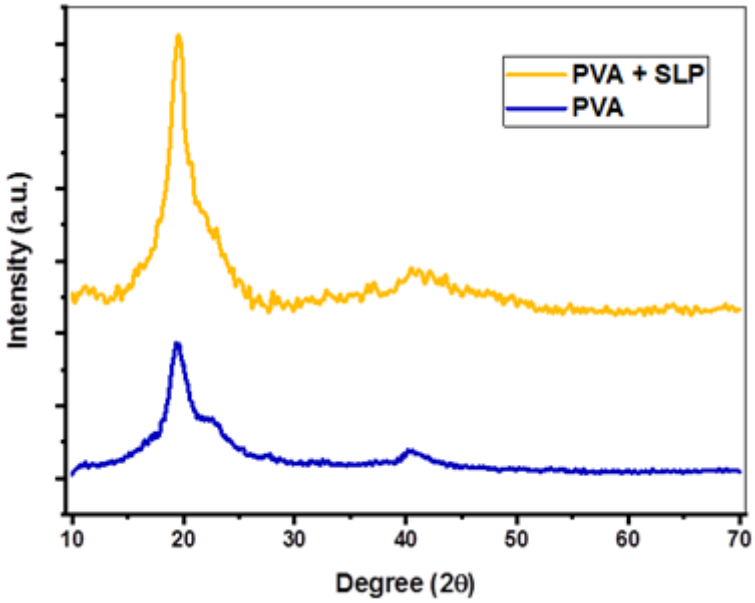

Figure 3

Figure 4 XRD Graph of PVA and PVA+SLP

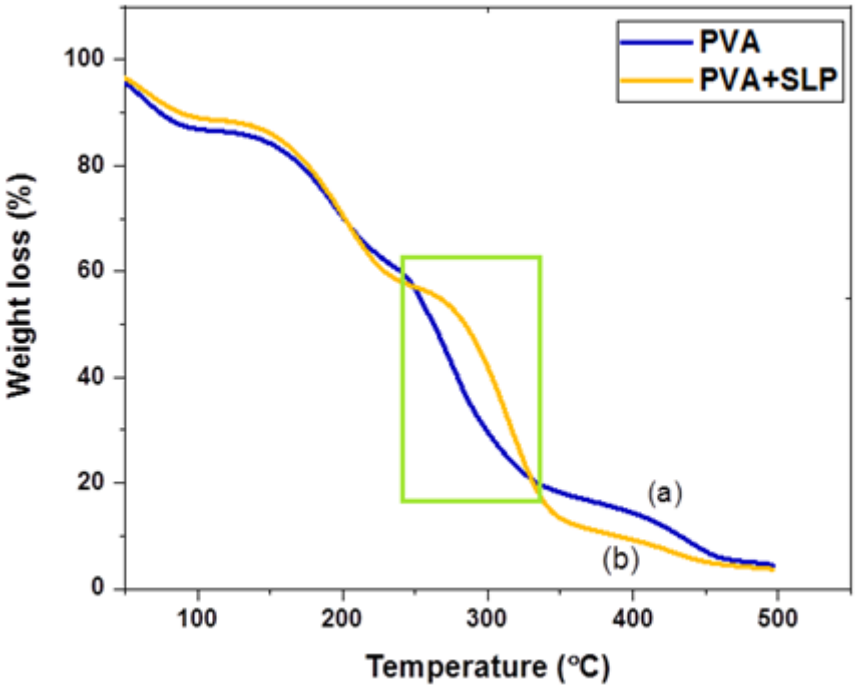

Figure 4

Figure 5 TG curve of PVA and PVA+SLP.

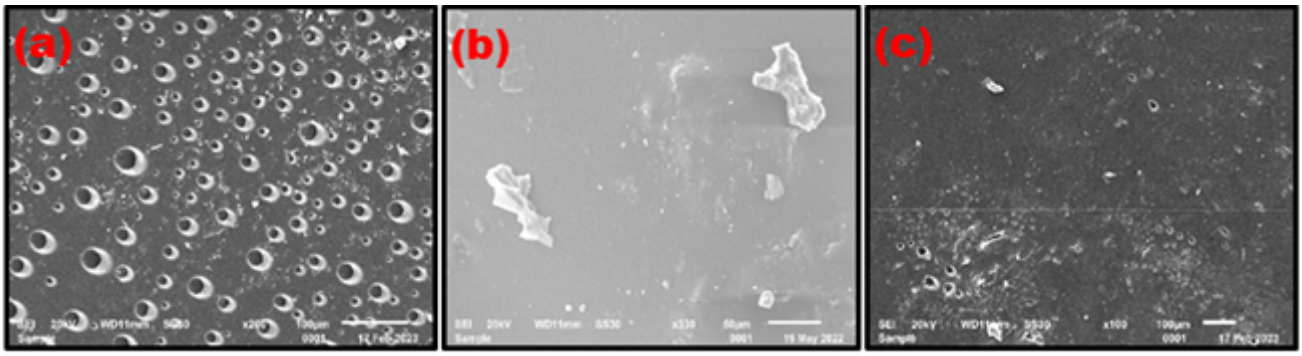

Figure 5

Figure 6 SEM images of (a) PVA (b) SLP (c) PVA+SLP

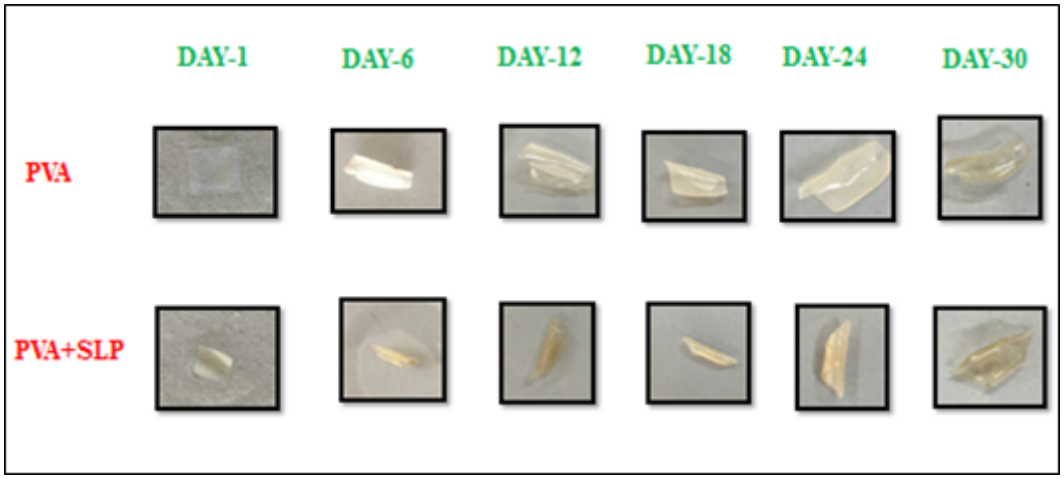

Figure 6

Figure 7 Biodegradability test of PVA and PVA+SLP

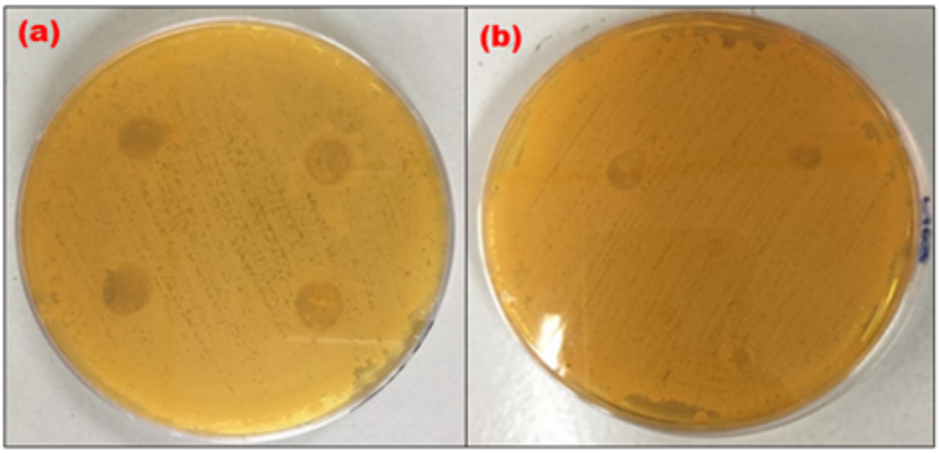

Figure 7

Figure 8 Inhibition zone PVA+SLP (a) Gram-positive *S. aureus* (b) Gram-negative *E.coli*.

Figure 8

Figure 9 Optical photograph of packed sprout with PVA+SLP and commercial film

Supplementary Files

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- GraphicalAbstract.tif