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Research Article

Preparation, characterization and antimicrobial activity of curcumin loaded chitosan and alginate biocomposite edible films impregnated with silver nanoparticle

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Abstract: The development of excellent biocomposite edible films are produce from nontoxic, biocompatible, biodegradable and low coast biopolymers as have a versatile biological activities and provided plenty of opportunities for further development of functional biomaterials in various field. The biopolymers chitosan and alginate used in this study. They are abundantly available natural polysaccharides it can be used in various applications. In this present study, the curcumin (Cu) loaded chitosan (Ch) and alginate (Al) impregnated with silver nanoparticles (AgNPs) films are prepared by solvent casting method. The prepared composite films were characterized by UV visible spectroscopy (UV), Fourier transform infrared spectroscopy (FTIR), and Transmission electron microscopy (TEM). The morphological, mechanical and optical properties of the composite films are identified by using conventional methods. The obtained results, showed that the increase of chitosan content in the composite result in the decrease of water absorption capacity. The results have shown, the Cu-Ch-Al-AgNPs composite films shows high level of antibacterial property when compared to the control Al-AgNP films. This study suggests that Cu-Ch-Al-AgNP film may be potential candidate as a packaging material for food packaging application.
Keywords: Chitosan; Alginate; Curcumin; Silver nanoparticle; biocomposite film; packaging material

INTRODUCTION

The developments of biodegradable edible films from natural polysaccharides and incorporated in strongly flavoured antioxidants are allowed and obtained new functionalized and characterized materials [1]. Edible films have been considered as one of the potential technologies that can be used to increase the storability of foods and to improve the existent packaging technology [2]. The health concerns of the consumers and environmental problems are focused on the current research use of natural preservatives in biodegradable packaging materials [3]. It is reduces the need of synthetic polymers, and remove the negative effects of the environment, their permeability and mechanical are producing some adverse effect as restricted in their use of food additives [4]. Some natural antioxidants are alternate to synthetic antioxidants of butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) [5]. Curcumin is a naturally occurring phytochemical and an extract of mango ginger (*Curcuma amada Roxb*) as have antibacterial, antioxidant, antiproliferative and antiinflammatory activities [6, 7]. To improve the antioxidant property, chitosan is mixed with polyphenolic agent curcumin to form various complexes like curcumin alginate-chitosan-pluronic composite [8] and curcumin loaded dextran sulphate chitosan nanoparticles [9]. Proteins and carbohydrates made edible antioxidant films strongly linked to their oxygen permeability, and excellent barriers to oxygen, because of their tightly packed, ordered hydrogen-bonded network structure [10]. Some nanocomposite films as have a limited mechanical and barrier properties (water vapor and oxygen permeability) of biopolymers can be significantly enhanced by the use of strengthening fillers to create nanocomposite films. Nanocomposite films extend the food shelf-life, and also improve food quality as they can serve as carriers of some active substances, such as antioxidants and antimicrobials [11].

Chitosan and Alginates are non-toxic, hydrophilic, biocompatible, biodegradable and low cost polymers [12, 13]. Alginate is a natural polysaccharide derived from brown algae and seaweed [14].Chitin is isolated from the exoskeleton of shrimps and crabs. Chitin is the second most abundant natural polysaccharide after cellulose [15]. Chitin is insoluble in most of the solvents, because of its compact structure. Therefore, the chemical modification of common derivative is chitosan that is derived by partial

deacetylation of chitin. This partially deacetylated chitin (chitosan) is soluble in acidic medium [16]. To improve the mechanical property, chitosan-based composites have been developed with different types of polymers, such as alginate [17], cellulose [18] hyaluronic acid [19], collagen [20] and sago starch [21]. In these composites to improve the antimicrobial property, alginate is mixed with different metal ions to form various complexes like sago starch-alginate-Ag^o complex [21] alginate-chitosan-Au^o and Ag^o [22].Among different metal nanoparticles considerable attention is focused on silver nanoparticles, because of their unique antimicrobial activity, which provide one of the most cost effective alternatives for the development of new antibacterial agents [23]. Chitosan films have been successfully used as a packaging material for the quality of preservation of foods [24]. These (Cu-Ch-Al-AgNP) nanocomposite films containing curcumin encapsulated chitosan-alginate impregnated with silver nanoparticle was not prepared earlier.

The main objective of the present study is to prepare a novel biocomposite film containing chitosan and alginate with improved mechanical properties. This composite was also encapsulation of curcumin and impregnated with silver nanoparticles with the aim of getting antioxidant and antimicrobial property to the end products. Another objective of this present is to evaluate this biocomposite material Cu-Ch-Al-AgNP as a drug releasing materialon food packaging.

MATERIALS AND METHODS

Materials

Shrimp (*Litopenaeus vannamei*) shells were collected from cuddalore fish market, mango ginger was purchased from nearby retail market and other chemicals used in this study were purchased from Merck Specialities PLtd., Mumbai, India and were analytical grade.

Isolation of chitosan from shrimp shell (Litopenaeus vannamei)

The shrimp shells were washed under running tap water to remove all other soluble and insoluble impurities. Followed by the shells were dried in oven at 65^o C for 6 h and chitin was extracted from the pulverized sample by demineralization and deproteinization. The powder of shells was treated with 1.0 MHCl (1gram in 10 ml) for 24 h to remove the mineral content and then treated with 1.0 M NaOH (1 gram in 20 ml) at room temperature for 24 h, repeat the process for two times to remove all proteins. At the end of this process the material was filtrated, washed and dried. The dried matters are treated with acetone (1 gram in 10 ml w/v) for 10 min, filtered, dried for 2h at room temperature. After, the dried matters bleached with 0.5% $NaClO_2$ for 5 min at 1:10 (w/v) ratio. The decoloured chitin was washed with distilled water for to obtain purified chitin. The extracted chitin was treated with 40% NaOH (1 gram in 50 ml),the mixture was fixed at 120^o C for 3 h. The solid was filtered, washed with water and 80% (v/v) alcohol until the filtrate was neutral. Then the solid was dried at oven 60^o C for 12 h. At end of the process given in partially deacetylated chitin derivative chitosan.

Isolation of curcumin from mango ginger (Curcuma amadaRoxb)

The shade dried mango ginger were powdered and then passed through 40 mesh sieve. Powdered mango ginger were defatted by packing 40 g of dried material into a Soxhlet apparatus and extracting with acetone at 80^oC for 6 h. After extracts were distillate 80^oC for 30 mingets crude curcumin, and stored at 4^oC till further use. The total phenolic content was measured by according to the method of Singleton and Rossi [30]. Briefly, the extracted curcumin (mango ginger) were diluted in different concentration, mixed with 3 mL of Folin-Ciocalteu reagent kept at room temperature for 10 min and 1 mL of 5% sodium bicarbonate solution was added to the mixture, samples were incubated at 90 min. The absorbances of the samples were read at 725 nm. The total phenolic content was expressed and the standard phenol was used as a control.

Preparation of silver nanoparticles using alginate (Al-AgNP) solution

2 g of sodium alginate and 0.05 g calcium chloride was dissolved in 100 mL of distilled water and mixtures were heated with 80^o C for uniform complete dissolve.1mL of 2% sodium alginate solution and 1 mL of aqueous 1 mM $AgNO_3$ solution was added followed by stirring for 1 h. To this solution, 0.2 mL of 1.0 M $NaBH_4$ (dissolved in 0.3 M NaOH) was added drop wise followed by stirring till the solution attained golden brown colour. At this stage addition of $NaBH_4$ was stopped, the stirring was continued for another fifteen minutes, observed in the formation of silver nanoparticles. This solution was stored at 4^oC till further use.

Preparation of curcumin loaded chitosan (Ch-Cu) solution

Isolated chitosan was dissolved in 100mL of 2 % Acetic acid 1:10 (w/v) solutions. The extracted curcumin was dissolved in 98 % ethanol (1 mg per mL). Curcumin was loaded by incorporating the required volume of chitosan solution into the calcium chloride solution. To remove free curcumin, the solution was centrifuged at 3000 rpm for 10 min, and the clear yellowish solution was carefully removed. Separated nanocomposite was stored at 4^o C till further use.

Optimization of chitosan and alginate film

As the film formed by drying chitosan solution was very brittle, glycerol was used to get the flexibility to the film. Keeping the amount of chitosan and alginate solution constant, the amount of glycerol added was varied and the resultant solution were poured into

polythene trays having 10 ×10 cm, and dried at clean place at room temperature. After drying, 30 ml of 2% $CaCl_2$ solution was poured onto the dried film for 30 s and re-dried again at 40^oC until films were formed that could be easily removed from the tray. The dried films were stored in polythene covers for further use.

Optimization of Cu-Ch-Al-AgNP composite film

The stoichiometric ratio which gave better mechanical property to the chitosan and alginate films was used for the preparation of Cu-Ch-Al-AgNP composite. Keeping the composition of chitosan: glycerol and alginate: glycerol constant, the amount of Curcumin-Chitosan/Alginate-AgNP solution was varied and these solutions were poured into polythene trays having 10×10 cm, and dried at clean place at room temperature. After drying, 25 ml of 2% $CaCl_2$ solution was poured onto the dried composite film for required time and redried again at same condition until films were formed that could be easily removed from the tray, and dried films were stored in polythene covers for further use.

Characterization

Ultraviolet visible Spectroscopy (UV)

UV-vis spectra was characterized by curcumin loaded chitosan,alginate impregnated silver nanoparticles and curcumin loaded chitosan alginate impregnated silver nanoparticle composite as compare with standardcurcumin and silver nitrate controls respectively. Samples were analyzed by Hitachi U-2910 Spectra, Japan.The analysis was performed by continuous scanning from 300-800 nm. The curcumin and silver nanoparticles were showed at maximum absorbance at 400-450 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of the sample prepared were taken using FTIR Perkin Elmer 1760X by preparing a 500 mg KBr pellet, containing 2-6 mg of the sample. FTIR spectra were recorded from 4000 to 400 cm^{-1}range.

Thermal property

Thermo gravimetric analysis was carried out with a Perkin Elmer TGA as the over temperature range of 30-700^0 C at a heating rate of 20^0 C per minutes under nitrogen atmosphere.

Optical property (Transparency and opacity)

The film samples were cut into rectangles (5x50 mm) and placed directly in a spectrophotometer cell and measurement was performed using air as the reference in another empty cell. The light barrier properties of the film samples were measured at wavelengths between 200 and 800 nm using a UV spectrophotometer according to the method of Han and Floros[31]. The results have been expressed as percentage transmittance. The transparency at 600 nm (T600) was obtained using the following equation-

$$T600 = - \log \%T / b$$

Where, %T is percentage transmittance and b is film thickness (mm). The opacity of the films were measured by the following equation according to the method described by [25]

$$\text{Opacity} = \text{Absorbance at 500 nm} \times \text{Film thickness (mm)}$$

Filmthickness

The thickness of the films was measured by using a calibrated thickness gauge with 0.01 mm accuracy according to the method of [26]. Five thickness measurements were taken of each film, one at the center and four around the perimeter. Average values were used in the calculations.

Tensile strength

Three dumbbell shaped specimens of 4mm width and 10 mm length were cut out from the prepared films. The film strip was hold at one end by a fixed clamp and at another end by a moveable one.Mechanical properties such as tensile strength (Mpa) and percentage of elongation at break (%) of the film samples were measured according to the method of [27] using an Instron 4501 tensile testing system at an extension rate of 100 mm/min.

$$\text{Tensile strength} = \text{Break load} / \text{Strip cross- Sectional area}$$

Water absorption capacity of the composite film

The water absorption capacity of these films was determined by swelling small piece of each sample of known weight swelled in distilled water at room temperature according to the method of [28]. The swollen weights of the samples were determined by first blotting the samples with filter paper followed by accurately weighing the samples. The weight of the swollen pieces was record after 24 hrs. The percentage swelling of the sample at a given time was calculated from the formula-

$$S = Ws – Wo / Wo \times 100$$

Where, W_S is the weight of the sample [moist] at a given time, W_O is the initial weight of the sample and S is the percentage of swelling at a given time.

Transmission electron microscopy

For transmission electron microscopy image were noted on a TEM as operate at an accelerating voltageof 120 kV (Phillips CM 200 field emission gun). Samples were prepared by spin coating of the precursor nanocomposite solutions on carbon coated copper grids using a microprocessor controlled spin coater (Model GP3-8 Spincoat) and dried under air at room

temperature before transferring to the electron microscope.

Application of nanocomposite edible film

Antimicrobial activity

The antibacterial and antifungal activity was followed by the standard disc diffusion method. The prepared nanocomposite films were performed by antibacterial and antifungal activity was done against human pathogenic bacteria obtained from Rajah Muthaiya Medical college, Annamalai University, Chidambaram. These pathogens were maintained on nutrient agar slants at 4^0C. The bacterial stains were inoculated in nutrient and sabouraud dextrose broth respectively. After 24 hour at 37^o C incubated bacterial suspensions were lawn cultured by using sterile cotton swab in Muller Hinton. The prepared film was cut by 0.5cm cork borer and the film discs were placed in to surface of the agar plates. The bacterial plates incubated at 37^o C for 24h.

RESULTS

UV visible spectroscopy

The UV visible spectrum showed that the curcumin and silver nanoparticles at 400-450 nm. The mango ginger isolated curcumin was loaded with chitosan. Hence, colourless clear chitosan changed to yellow colour and UV spectra absorbance band optimized at 432 nm and compare with correspond standard curcumin control. The light yellow colour clear alginate solution was converted to brown colour solution till the addition of silver nitrate and $NaBH_4$. This colour change was clearly indicating the silver nanoparticle synthesis. The UV- spectra absorption band optimized at 446 and 429 nm peaks corresponds to alginate AgNPs and curcumin loaded chitosan-alginate AgNPs composite solutions (Figure 1) clearly indicating the formation of silver nanoparticles.

Fourier Transform Infrared Spectroscopy

FTIR spectra show the presence of chitosan, curcumin and curcumin incorporated chitosan (Figure 2). The characteristics peaks were absorbed in $1116cm^{-1}$, $3373cm^{-1}$, $3400cm^{-1}$. The presence of alginate and silver nanoparticles were identified by the presence of characteristic peaks at 3481 cm^{-1} to 3483 cm^{-1} were related to the stretching vibrations of O-H groups. The bands around 1619 cm^{-1} to 1637 cm^{-1} were corresponding to alkane C=O stretching. The band around 1425 cm^{-1} corresponds to C-OH groups (Figure 3).

Transmission electron microscopy

TEM images showed that the size and shape of the silver nanoparticles of Al-AgNPs and Cu-Ch-Al-AgNPs composites. The size of the AgNPs was found to be 13-37 nm and the shape of the nanoparticles was found to be spherical(Figure 4).

Water absorption capacity

Water absorption capacity is an important property for the food packaging material. As it absorbs food exudates and ensures the organoleptic qualities of food and the film capability to fight against dehydration and rehydration. Keeping this study, water absorption studies of the composite edible films were prepared and recorded. Ch film has shown increased water absorption capacity with increase in time, a maximum of [43.53 %] water absorbing capacity was absorbed after 24 h. Ch-Al film also shown increased water absorption with increase in time [54.76 %] but the water absorption capacity was slightly higher than Ch film. .

Moisture content

Moisture content provides information about the interaction between the each polymeric chain. It affects the water affinity of edible film. The Ch, Al and Al-Ch films moisture content were recorded; Chitosan and alginate films not shown the significant results. But Al-Ch composite films had shown the significant result with high moisture content, when increasing the alginate. The Chitosan without alginate composites have showed the decreased moisture content. Hence, it is clear the increasing of alginate increase the moisture content of the films.

Transparency and opacity of the composite films

The important role of packaging material, it should be protect food from the effect of light, especially UV radiation and photoradiation, such as a powerful oxidizing agent in lipid food. To determine the optical properties of the films were scanned at wavelength ranging from 300 to 400 nm and the percentage of light transmittance was recorded (table- 2). The Cu-Ch-Al-AgNP films with increase alginate impregnated with silver nanoparticles had percentage of transmittance was decrease compared with control film (chitosan). The Cu-Ch-Al-AgNP composite films with have good barrier to ultraviolet light. Kanatt et al., 2012 reported that chitosan poly vinyl alcohol films contain the aqueous mint extract (ME) and pomegranate peel extract (PE) have increased protection against UV light.

Antimicrobial activity of composite film

The Cu-Ch-Al-AgNP composite film was exhibited excellent antimicrobial activity against the human pathogens by showing the clean zones around the wells with bacterial growth on petri plates by well diffusion method (Table 1).

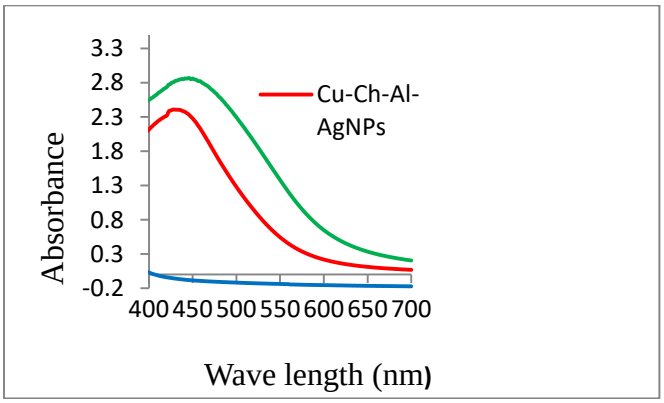


Fig-1: Shows UV spectra of Al-AgNP and Cu-Ch-Al-AgNP

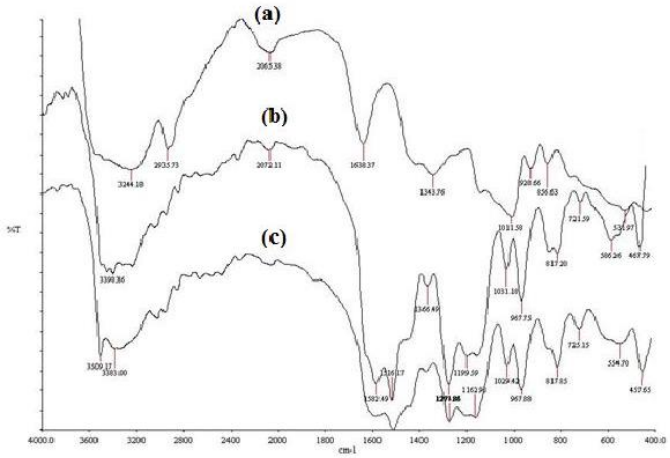


Fig-2: FT-IR spectra of (a)Chitin (b) Curcumin loaded on chitin (c) Pure curcumin

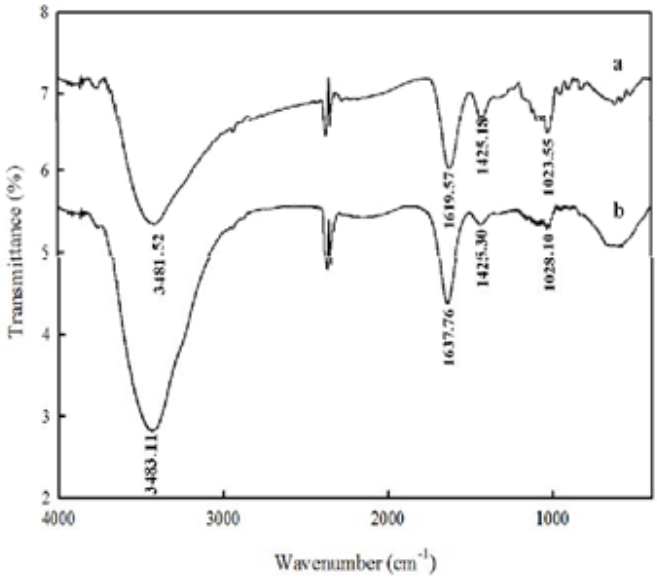
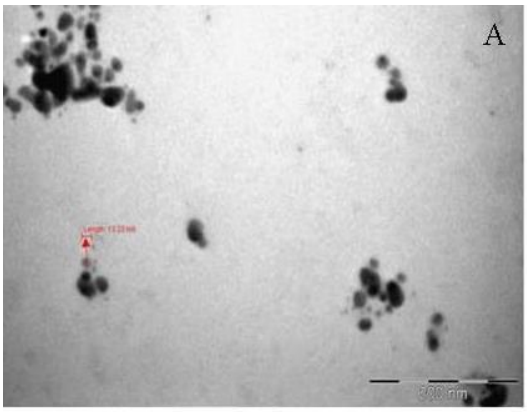
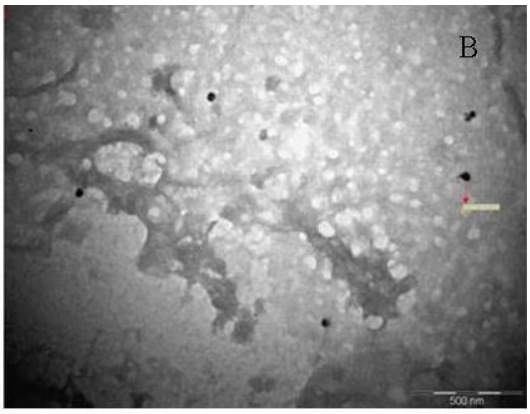


Fig: 3: FT-IR spectra of (a)Chitosan (b) Alginate



A. AL-AgNPs, nanoparticles showing spherical shape



B. Cu-Cs-AL-AgNPs, nanoparticles showing spherical shape

Fig- 4: TEM images of AL-AgNPs and Cu-Cs-AL-AgNPs

Table-1: Shows antibacterial activity of Cu-Cs-AL-AgNPs and AL-AgNPs

S.No	Human pathogens	Cu-Cs-AL-AgNPs	AL-AgNPs
		Zone of Inhibition level (mm)	
1	Staphylococcus aureus	5	2
2	Micrococcus luteus	4	1
3	Streptococcus pyogenes	5	3
4	Bacillus subtilis	4	-
5	Enterobactor faecalis	3	-
6	Escherichia coli	4	-
7	Klebsiella pneumoniae	3	3
8	Shigella sonnei	4	2
9	Salmonella typhimurium	2	2
10	Vibrio cholerae	3	-

DISCUSSION AND CONCLUSION

Recently many researchers have developed variety of packaging materials in order to improve the mechanical, optical, antioxidant, and antimicrobial property. Those materials with good mechanical, optical, antimicrobial, antioxidant and drug releasing property can be applied in the food packaging. In the present study, composite films of curcumin (Cu) loaded chitosan (Ch) and alginate (Al) impregnated with silver nanoparticles (AgNPs) were prepared by solvent casting method. Fig- 1 shows the typical photograph taken in the chitosan-alginate composite film as a brown coloured, minimal light transparent and good flexible nature.

Mechanical property is most important property for the food packaging edible films, but chitosan and alginate films were have poor mechanical property; therefore, a plasticizer was added to get desired mechanical property to the film. In this present study, glycerol was used to increase the flexibility of the chitosan and alginate films. Mikkonen et al. [32] reported that addition of plasticizers such as glycerol to polysaccharides can significantly improve their flexibility. Among the various compositions studied, sample no 5 and 3 of chitosan and alginate film have a better tensile strength and elongation property. The better tensile strength was obtained up to the addition of 1.0 and 0.5 mL of glycerol in chitosan and alginate respectively. The excess of glycerol molecules impregnated between the chitosan and alginate molecules may be leads to reduce the tensile strength and promote the elongation at break. It was also observed that the tensile properties of poly ethylene glycol based composite films were significantly improved with an increase of chitosan loading [29] similarly, Kanatt et al., [33] reported the reinforcing property of chitosan with glycerol plasticized polyvinyl alcohol film. The composition of sample no 5 and 3 (Table- 1) was used to prepare Cu-Ch-Al-AgNP composites. Among various Cu-Ch-Al-AgNP composites prepared, the sample no-3 contain 20 % of alginate gave a better mechanical property. Hence sample no-3 was used for further characterization studies such as mechanical, optical, thermal, morphological, invitro antimicrobial and drug release studies. The increases in tensile strength of the biodegradable films, with the increase of chitosan are due to the formation of intermolecular cross linkage between NH_4 of the chitosan backbone binds with

calcium ions mediated alginate and also to produce polyelectrolyte composite film. The improvement of TS attributed due to good interfacial interaction between the polymers. The increased chitosan content lead to decrease the elongation at break, but total soluble matter and film thickness was increase up to the addition of alginate. The average thickness of the chitosan film was 0.24 ± 0.02 mm. The various compositions of Ch-Al films thicknesses were recorded. The chitosan and alginate control films were varied by the chain length when compared to the Cu-Ch-Al-AgNP composite film. This study confirmed that Cu-Ch-Al-AgNP composite film having very good activity against human pathogens and it can be used as packaging material.

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