

Preparation and Characterization of Cellulose-Chitosan Composite Hydrogel Films from Coconut Coir Fibers

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Abstract

The development of cellulose-chitosan composite hydrogel films is important in repair and regeneration of tissue. In this study, cellulose was extracted from coconut coir fiber via acid hydrolysis, alkali treatment and chlorite bleaching. Chitosan was prepared from crab shell by demineralization, deprotenization and deacetylation. The lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc) solvent system was used to obtain cellulose-chitosan composite hydrogel solutions. It was converted to the cellulose-chitosan composite hydrogel films by phase inversion method without cross-linker. XRD, SEM, FT IR and TG-DTA analyses were utilized to characterize the properties of prepared composite hydrogel films. Antimicrobial activity of cellulose-chitosan composite hydrogel solutions was tested for the purpose of using composite hydrogel films in biomedical applications.

Keywords: cellulose, chitosan, composite hydrogel films, phase inversion method, antimicrobial activity

1. Introduction

New bio-based composite materials that are renewable and biodegradable are of scientific and industrial interest due to their biocompatibility and notable performance improvement. Therefore, a large number of research studies dealing with composite materials based on renewable resources have emerged. Renewable resources, e.g. cellulose, chitosan, and starch are known to have potential to be key components of biocomposites. These renewable resources can also be easily composted after their intended use without harming the environment. Consequently, the use of bio-based composites provides wide opportunities for tissue and organ repair and regeneration (Faisal *et al.*, 2017)

It is widely accepted that blending of polymers may be the best method of improving the chemical and physical properties of polymer-based composites. In a common solvent, polymer blends may form homogeneous solutions. The formation of homogeneous polymer blends can be attributed to strong interaction via hydrogen bonds between the functional groups of the blend components (Almeida *et al.*, 2010).

Cellulose and chitosan are biodegradable and biocompatible polymers; however, it is difficult to process them by dissolving into common solvents. This difficulty is mainly due to the presence of intra- and inter-molecular hydrogen bonding and molecular close chain packing.

The purpose of this study was to prepare composite films mixed with cellulose and chitosan; specifically, cellulose and chitosan which were dissolved in 8 % LiCl/DMAc solution. The LiCl/DMAc solvent system has become very popular due to its advantageous associated with direct dissolution of the cellulose and chitosan that are being faster, easier and more

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reproducible. Furthermore, the composite films were characterized by their physicochemical properties and antimicrobial activity (Zhang *et al.*, 2012).

2. Materials and Methods

2.1 Materials

Crab shells were obtained from Maungmagan Sea Beach, Launglon Township, Tanintharyi Region. Coconut coir fibers were collected from Kanyone Quarter, Dawei Township, Tanintharyi Region.

2.2 Preparation of Chitosan from Crab Shells

A total of 100 g cleaned and dried crab shells were soaked for 2 days with 1 L of 2 M hydrochloric acid at room temperature to remove the mineral content. The crab shells were washed to neutral and treated with 1L of 2 M NaOH at 100 °C under constant stirring to remove protein. Then they were washed with distilled water to neutral state. The alkali and acid treatment was repeated again. After washing them to neutralize the reaction, the chitin was dried in oven at 80 °C. The white chitin was obtained.

50 g of chitin were treated with 500 mL of 50 % (w/v) aqueous sodium hydroxide at 110 °C under constant stirring. Then, it was filtered with filter paper and washed with water until pH neutral (pH, 7) to obtain the chitosan. The chitosan was dried in oven at 60 °C.



Figure 1. Showing the photos of (a) chitin (b) chitosan

2.3 Preparation of Cellulose from Coconut Coir Fibers

Cellulose was prepared from coconut coir fibers using sulphuric acid, sodium hydroxide and sodium hypochloride using chemical method (Soe Mi Mi *et al.*, 2022)

2.4 Preparation of Cellulose Hydrogel Solution

Cellulose hydrogel solution (CHS) was prepared by using 8 % LiCl/DMAc solvent system (Soe Mi Mi *et al.*, 2022)

2.5 Preparation of Cellulose Hydrogel Film

Cellulose hydrogel film (CHF) was prepared using phase inversion method (Soe Mi Mi *et al.*, 2022)

2.6 Preparation of Cellulose-Chitosan Composite Hydrogel Solution

Firstly, 5 % (w/w) chitosan/cellulose were used and stirred in 300 mL of distilled water at room temperature for 36 h using a magnetic stirrer. The fibers that swell in water were obtained

and then filtered by a Buchner funnel under a vacuum pump. Secondly, the swollen fibers were added to 300 mL of ethanol for solvent exchange and stirred for 36 h at room temperature. The fibers that swell in ethanol were obtained and then filtered. After filtration, the swollen fibers were stirred in 300 mL of DMAc solvent at room temperature for 36 h. Finally, 8 % LiCl/DMAc solvent system was added to the swollen fibers and stirred at room temperature for 5 days until viscous solutions were obtained. The cellulose fiber-chitosan composite hydrogel solution was obtained and coded as S_1 . Similarly, 10 % and 15 % (w/w) chitosan/cellulose were used and prepared using the same procedure to obtain cellulose-chitosan composite hydrogel solutions and coded as S_2 and S_3 .

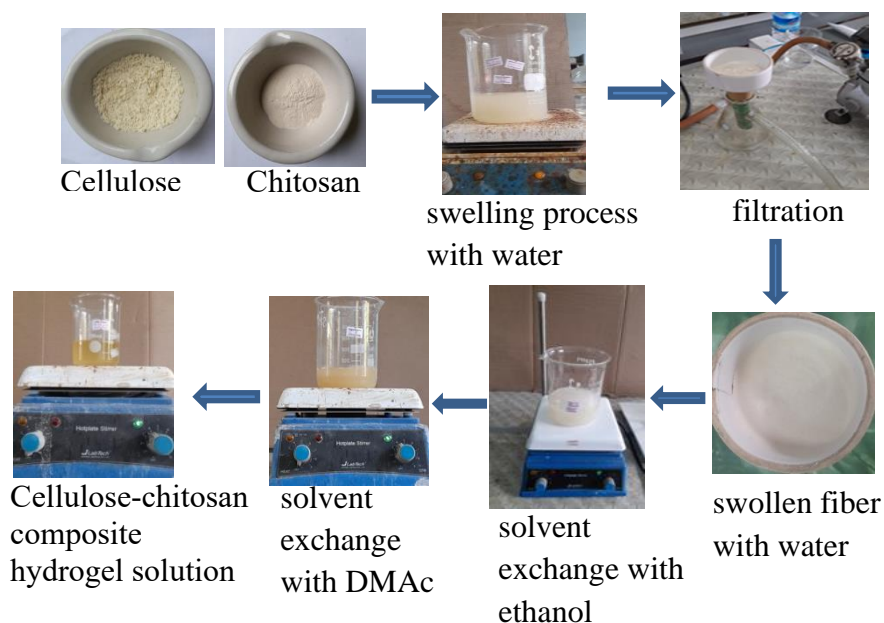


Figure 2. Preparation of cellulose-chitosan composite hydrogel solution

2.7 Preparation of Cellulose-Chitosan Composite Hydrogel Films by Phase Inversion Method

The cellulose-chitosan composite hydrogel solution (S_1) was poured into petri dishes (10 cm diameter) and kept for 24 h in plastic container filled with 40 mL of ethanol. In this step, coagulation of cellulose-chitosan composite hydrogel solution was gradually progressed in the ethanol vapour at room temperature. Finally, the cellulose-chitosan composite hydrogel film (C_1) was obtained by phase inversion method from liquid to solid gels. The resultant composite hydrogel film was washed with distilled water and then placed in distilled water for 24 h to remove DMAc. In the same way, composite hydrogel films C_2 and C_3 were carried out. The obtained composite hydrogel films were kept in plastic container filled with distilled water for further experiments. The composite hydrogel films are very soft, flexible and it has pale yellow colour.

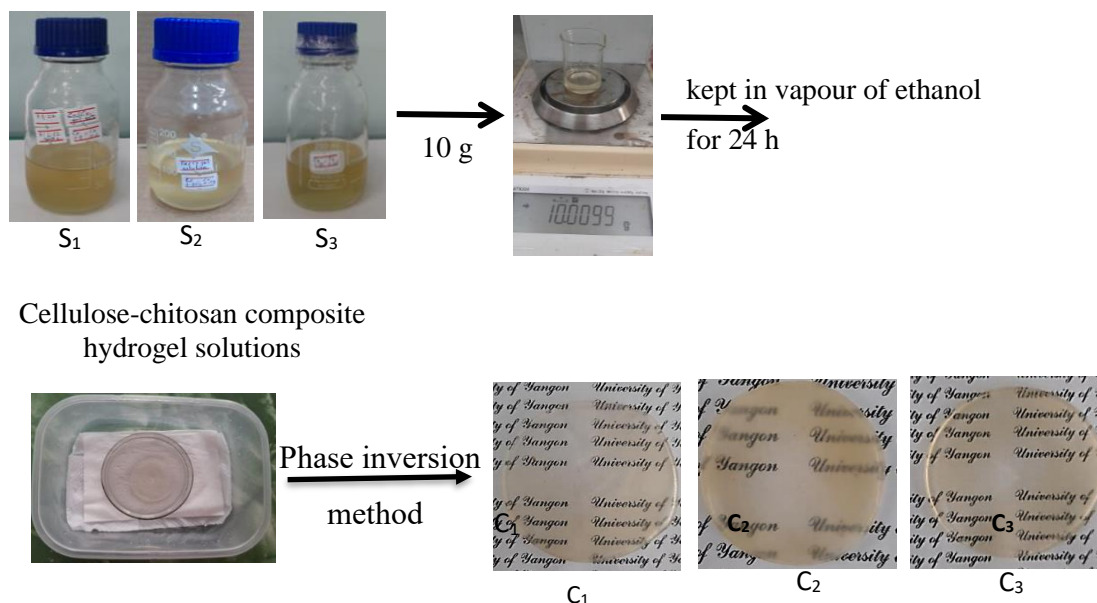


Figure 3. Preparation of cellulose-chitosan composite hydrogel films (C₁, C₂ and C₃)

2.8 Characterization

The physicochemical properties such as pH and viscosity of composite hydrogel solutions and water content of composite hydrogel films (C₁, C₂ and C₃) were measured. The characteristics of the prepared films were investigated by XRD, SEM, FT IR and TG-DTA analyses.

2.9 Study on antimicrobial activity

The composite hydrogel solutions were tested with *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus lutes*, *Pseudomonas fluorescens*, *Staphylococcus aureus* to investigate the nature of antimicrobial activity. In antimicrobial activity tests, nutrient agar was prepared according to agar well diffusion method described by (Cruickshank, 1975). Nutrient agar was boiled and 20-25 mL of the medium was poured into a test tube, plugged with cotton wool and autoclaved at 121 °C for 15 min. Then the tubes were also added to the dishes. After that, about 0.5 mL of samples were introduced into the 8 mm agar well and incubated at 37 °C for 24 h. The inhibition zone appeared around the agar well (diameter of inhibition zone ranging from 10 to 18 mm), indicating the presence of antimicrobial activity.

3. Results and Discussion

3.1 Physicochemical properties

The physicochemical properties of cellulose hydrogel solution (CHS) and composite hydrogel solutions (S₁, S₂ and S₃) were presented in Table 1. The viscosity of composite hydrogel solutions (S₃) was the highest. The water content of cellulose hydrogel film (CHF) and composite hydrogel films (C₁, C₂ and C₃) were shown in Table 2. The values of water content decreased from 93.54 % to 81.32 % with the increment of chitosan content. Therefore, water

contents of the composite films became lower when the chitosan content was higher. It may be due to polymer network formation in composite films.

Table 1 Physicochemical Properties of CHS, S₁, S₂ and S₃

Samples	pH	Viscosity (cP)
CHS	5.7	106.9
S ₁	6.4	132.7
S ₂	5.6	181.9
S ₃	5.5	298.6

Table 2 Water Content of CHF, C₁, C₂ and C₃

Samples	Water content (%)
CHF	93.54
C ₁	86.42
C ₂	83.55
C ₃	81.32

3.2 XRD analysis

From the comparison of X-ray diffractograms, there were no significant changes for the diffraction patterns of all hydrogel films but the diffraction peaks of composite hydrogel films were higher intensity than cellulose hydrogel film. This result may be the reformation of hydrogen bonds between cellulose and chitosan during dissolution and regeneration processes (Lin *et al.*, 2012).

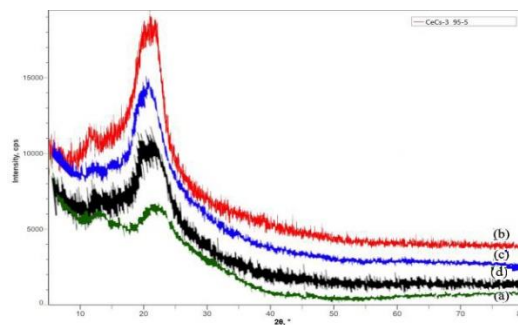


Figure 4. XRD patterns of (a) CHF (b) C₁ (c) C₂ and (d) C₃

3.3 SEM analysis

Figure 5 (a) shows the morphology of cellulose hydrogel film and it looks smooth in nature. When the combination of cellulose with chitosan to form composite films, there were significant changes in their morphologies as shown in Figures 5 (b) to (d). It may be the formation of cellulose-chitosan network structure in composite films. According to SEM images, composite film (C₂) was the most network formation among the three composite films. SEM morphologies of composite films clearly pointed out that C₁, C₂ and C₃ were cellulose-chitosan composite films.

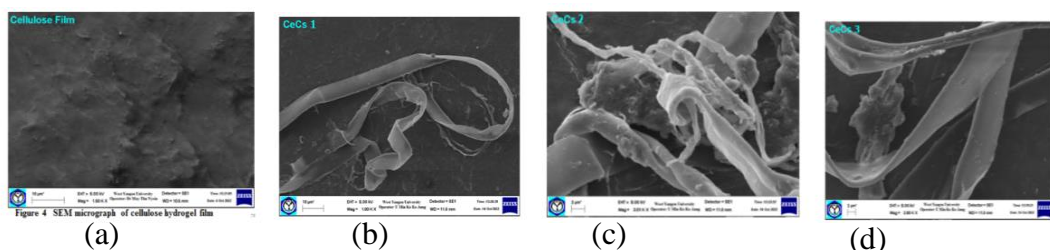


Figure 5. SEM micrographs of (a) CHF (b) C₁ (c) C₂ and (d) C₃

3.4 FT IR analysis

The FT IR spectra of cellulose hydrogel film and composite films were shown in Figures 6 (a) to (d). In Figure 6 (a), the band at 3338 cm^{-1} represent O-H stretching vibration of alcohol group and in Figures 6 (b) to (d), the broad bands at 3298 cm^{-1} to 3333 cm^{-1} attributed to the O-H stretching, which may overlap with N-H stretching in the same region. Asymmetric and symmetric C-H stretching vibration peaks were found to be at 2918 cm^{-1} and round about 2851 cm^{-1} . The band around 1640 cm^{-1} represented C=O stretching vibration. The bands at 1420 cm^{-1} to 1370 cm^{-1} represented C-H bending and 1016 cm^{-1} to 1019 cm^{-1} showed the C-O stretching (Romainor *et al.*, 2014).

From the comparison of FT IR spectra, the peaks around 1539 cm^{-1} are absent in cellulose hydrogel film. But the peaks around 1539 cm^{-1} are present in composite hydrogel films due to the cross-linking between the primary amino groups of chitosan and the carbonyl groups of cellulose (Faisal *et al.*, 2017)

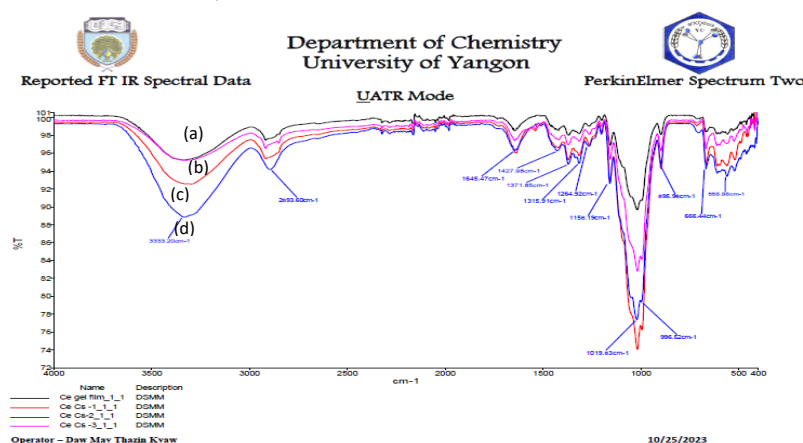


Figure 6. FT IR spectra of (a) CHF (b) C₃ (c) C₁ and (d) C₂

3.5 TG-DTA analysis

TG-DTA thermogram profiles of cellulose hydrogel film (CHF) was presented in Figure 7(a). In the first stage, the temperature ranged between $40\text{ }^{\circ}\text{C}$ to $200\text{ }^{\circ}\text{C}$ accompanied with 17.03% and the first weight loss was due to the dehydration of surface water. The second stage ranged between $200\text{ }^{\circ}\text{C}$ to $400\text{ }^{\circ}\text{C}$ with 56.7% weight loss. The second weight loss was due to decomposition of cellulose. The third stage ranged between $400\text{ }^{\circ}\text{C}$ to $600\text{ }^{\circ}\text{C}$ with weight loss 24.3% . This final weight loss was due to degradation of polymer backbone and complete combustion.

As seen in Figure 7(b), the thermogram of C₁ possess three stages of distinct weight loss between $40\text{ }^{\circ}\text{C}$ to $600\text{ }^{\circ}\text{C}$. The first stage ranged between $39\text{--}230\text{ }^{\circ}\text{C}$ with 12.26% of weight loss. The first weight loss was due to the dehydration of surface water. The second stage ranged between $230\text{ }^{\circ}\text{C}$ to $340\text{ }^{\circ}\text{C}$ and there was about 61.26% weight loss. The second weight loss was due to decomposition of cellulose. The third state of weight loss started at $340\text{ }^{\circ}\text{C}$ to $600\text{ }^{\circ}\text{C}$ with 24.51% of weight loss. The final weight loss was due to degradation of polymer backbone and complete combustion.

C₂ as shown in Figure 7(c), had weight loss in two stages. The first stage had the temperature range of 40 °C to 140 °C and about 23.7 % weight loss. The first weight loss was due to the dehydration of surface water; and the second stage the temperature range of 140-400 °C and about 41.6 % weight loss. The second weight loss was due to decomposition of cellulose.

As shown in Figure 7(d), C₃ had weight loss in three stages. The first stage was found at the temperature range between 39 °C to 235 °C with 10.95 % of weight loss. The first weight loss was due to the dehydration of surface water. The second stage was found at the temperature range between 235 °C to 350 °C with 54.73 % of weight loss. The second weight loss was due to the decomposition of cellulose. The third stage had the temperature range of 350 °C to 600 °C and about 25.53 % weight loss. This final weight loss was due to degradation of polymer backbone and complete combustion (Wahid *et al.*, 2018).

The total weight loss of CHF is 97.27%. The total weight loss of C₁ is 98.04 %. The total weight loss of C₂ is 65.45 % and the total weight loss of C₃ is 91.24 %. According to TG-DTA thermogram, C₂ had more thermal stability than the others.

Figure 8(a) shows the screening antimicrobial activity of cellulose-chitosan composite hydrogel solutions (S₁, S₂, S₃) and 8 % LiCl/DMAc solution. Figure 8(b) shows the position of test samples. Each petri dish represented to test results of antimicrobial activity against eight microorganisms including *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus lutes*, *Pseudomonas fluorescens*, *Staphylococcus aureus* respectively. According to the results, cellulose-chitosan composite hydrogel solutions showed more activity in eight microorganisms than LiCl/DMAc solution.

4. Conclusion

Cellulose-chitosan composite hydrogel films were successfully prepared by phase inversion method using LiCl/DMAc solvent system. The composite hydrogel films were flexible and they had pale yellow colour. The prepared hydrogel films were characterized by FT IR, XRD, SEM and TG-DTA analyses. According to FT-IR data, it revealed that composite hydrogel films had cross-linking structures between the primary amino groups of chitosan and carbonyl groups of cellulose. XRD data results indicated that the cellulose-chitosan composite hydrogel films showed amorphous nature that changed from the crystalline nature of cellulose. SEM images indicated that composite hydrogel films had variable morphologies due to network structures of cellulose and chitosan. Based on the TG-DTA analysis, the thermal stability of C₂ was higher than that of C₁ and C₃. The antimicrobial activity of cellulose-chitosan composite hydrogel solutions (S₂) showed more activity in eight microorganisms than that of S₁ and S₃. So, the cellulose-chitosan composite hydrogel film (C₂) is suitable to be utilized in biomedical applications.

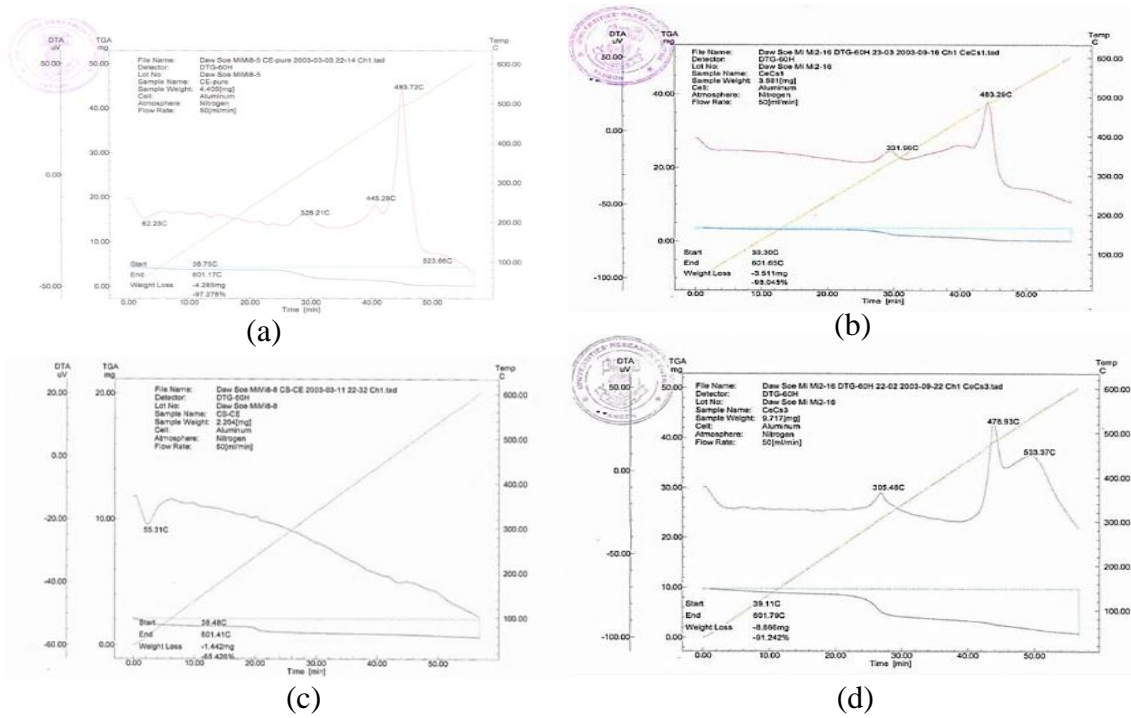


Figure 7. TG-DTA analysis of (a) CHF (b) C₁ (c) C₂ and (d) C₃

Table 3 Antimicrobial activity results for test samples

Samples	Inhibition Zone Diameter (mm) for Tested Organisms							
	<i>A. tumefaciens</i>	<i>B. pumilus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>M. lutes</i>	<i>P. fluorescens</i>	<i>S. aureus</i>
S ₁	15	12	10	11	16	16	14	13
S ₂	16	13	13	14	18	16	18	13
S ₃	12	12	11	13	15	16	16	12
LiCl/DMAC	12	12	10	11	14	14	13	10

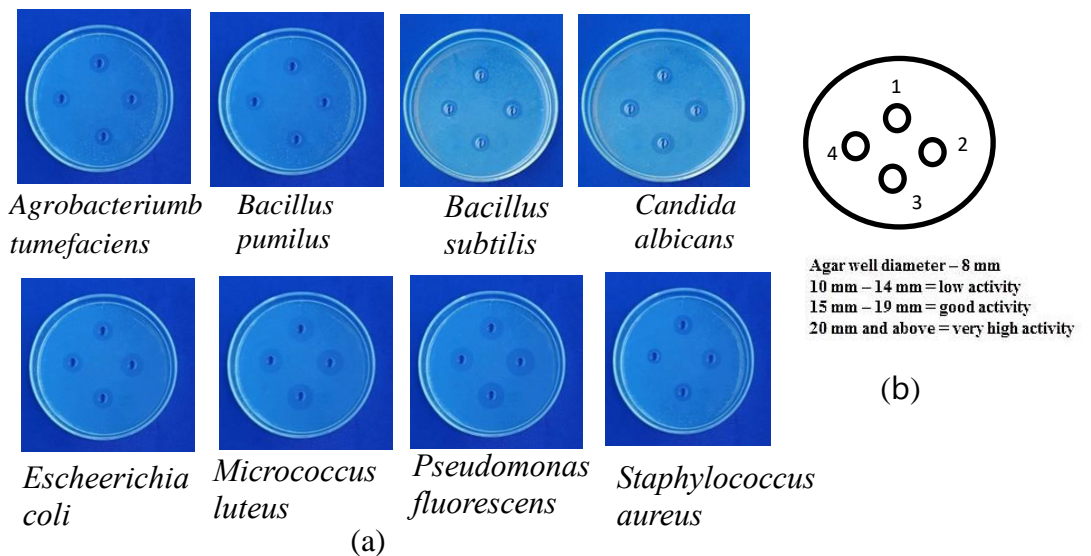


Figure 8. (a) Screening antimicrobial activity of (1) cellulose-chitosan composite hydrogel solution S₁ (2) S₂ (3) S₃ and (4) LiCl/DMAC solution and (b) the position of test samples

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