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Research Article

PREPARATION AND CHARACTERIZATION OF CHITOSAN-SILVER NANO COMPOSITE FILMS AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The ecological method of plant-mediated synthesis of Ag nanoparticles is an important step in the field of nanotechnology. Chitosan is a biopolymer that is biocompatible and antibacterial. In this work, we synthesized a chitosan-based hydrogel and combined herbal synthesized Ag into nanoscale nanoparticles to form CS-Ag nano hydrogel. These are green-synthesized Ag nanoparticles, which were made from the leaves of the Salix viminalis plant and characterized using a UV-visible spectrophotometer and DLS. This work focuses on the synthesis of CS-Ag nano hydrogel. These hydrogels were characterized by FTIR, XRD, and contact angles. The size distribution of nanoparticles was determined in the range from 1 to 100 nm using DLS, and the optimal wavelength was recorded in the range from 400 to 450 nm using UV-visible spectroscopic examinations. This synthesized CS-Ag nano hydrogel showed good antibacterial properties against *E. coli* (Gram-ve) and *S. aureus* (Gram+ ve) with a maximum inhibition zone of 7 mm.

Keywords: *Salix viminalis*, Ag nanoparticles, chitosan, antibacterial activity.

1. INTRODUCTION

Nanotechnology is one of the main directions of dynamic research in the field of modern materials science. New applications of nanoparticles and nanomaterials are increasing rapidly [1]. The introduction to nano-technology comes from NANOS, the Greek word for dwarf. A nanometer is a fraction of a meter or approximately 1/80000 the diameter of a human hair [2]. Scientific research in this field has grown exponentially in recent years to develop nanomaterials with characteristics and improved properties. Almost all areas of science were influenced by the tools and concepts of nano-technology, and inventions were made in the areas of computer technology, medicine, sensors, energy generation, and environmental protection [3]. To develop various and cheaper methods for the synthesis of nanoparticles, scientists have sacrificed the progress of a relatively new and largely uncultivated field of research based on the biosynthesis of nanomaterials [4]. In recent decades, wound care has been motivated to dry the wound layer to maintain a well-controlled moisture environment [5]. The structure of wound care is at an early stage of development, and most drugs are based on phytomedicine [6]. Plants and their products have great potential for injury. Many plants play a very important role in the healing process.

In 1960 the first hydrogel was used for biomedical applications when poly (hydroxyethyl methacrylate) (PHEMA) was crosslinked for the treatment of eyes [7]. Therefore, the development of modern biomaterials with controlled physical, chemical, electrical and biological properties will be useful to facilitate the formation of functional tissues. Among the various biomaterials, hydrogels are one of the likely applicants because they can simulate the physical, chemical, electrical, and biological properties of most biological tissues [8–11]. Hydrogels (networks of hydrophilic polymers) can capture a large amount of water without losing their three-dimensional structure [12]. These are the most likely materials for biomedical applications with great potential for use as an association. Chitosan (CS) is one of the richest natural aminopolysaccharides and, due to its significant antibacterial activity, is frequently used in the pharmaceutical industry and biotechnology [13]. Chitosan can be considered one of the best-studied materials in recent years. Chitosan is a natural cationic polysaccharide, a copolymer of 2-deoxy-N-acetyl-D-glucosamine, and 2-deoxy-D-glucosamine, which are linked by b-1,4-glycosidic bonds [14]. Chitosan is a natural biomaterial that has been widely used in tissue engineering for ten years. It has received great attention and has been used in medicine and other fields that are

widely used due to its excellent biocompatibility, biodegradability, and wound healing. Chitosan is suitable for bandages due to its antibacterial effect.

The *Salix viminalis* plant belongs to the *Salicaceae* and *Salix* family. This is a well-known hyper accumulator of cadmium, chromium, lead, mercury, Ag, uranium, and zinc [15, 16]. *S. viminalis* is native to Asia, Europe, and the Himalayas [17, 18]. It is usually used to treat arthritis, malaria, bowel disease, gout, etc. This plant contained glycosides, flavonoids, and phenols, Terpenoids [19]. Polyvinyl alcohol is a water-synthetic polymer that can form a stable and crystallizing hydrogel. The hydrogel was used as a biomaterial due to its non-toxic nature, high mechanical strength, biocompatibility, economy, and environmental friendliness. Polyvinyl alcohol has excellent film-forming, mixing, and adhesive properties. It is also stable for oils, fats, and solvents. It has high tensile strength and flexibility, as well as high oxygen content and aromatic barricades. These properties of polyvinyl alcohol are often used in the manufacture of paper, paints, adhesives, clothing, pharmaceuticals, ceramics, and building materials [20]. A polyethylene glycol-based hydrogel is characterized by high biocompatibility, a source of toxic effects on the surrounding tissue and solubility in water, which makes it a good candidate for use in drug delivery systems. PEG is also used as an active ingredient in many pharmaceutical products. Polyethylene glycol can slow the removal of transferred protein from the blood using various protein treatment methods [21]. In this study, the silver nanoparticles synthesized by using plant extract of *Salix viminalis* were mixed into the chitosan-based polymeric hydrogel film solutions and made a combined CS-Ag nano hydrogel and studied in details with all of their properties. In this work, we focused to study the improvement in the antibacterial activity of hydrogel film after the coating with herbal synthesized silver nanoparticles. The formation of Ag-nanoparticles is confirmed by UV in this work. Changes in FTIR and XRD during the study have been used to analyze the absorption of the NPs into the hydrogel films. Disc diffusion assay used to analyze the effect of CS-Ag nano hydrogel on the bacteria.

2. MATERIAL AND METHODS

2.1. Material

In this study, all of the salts and reagents used were of analytical quality. PVA supplied from SD Fine Chemical Limited Mumbai, MW=85000^{-1}, 24,000 g ml^{-1}. CS (Aldrich Chemical) powder, medium molecular weight, MW=161, 000g mol^{-1}, degree of deacetylation, DD= 75.6%, 200-400mPa.s and (1% (w/v) of polymer in aqs. acetic acid solution) were used without further purification. PEG supplied from Thomas Baker Chemicals Pvt. Limited, Mumbai, and MW=1400-1600g ml^{-1}. Glyoxal 98% purified, MW 58.04 g·mol^{-1}, density 1.27 g/cm^3, Merck Specialties Pvt. Limited, Mumbai, India. Leaves of the *S. viminalis* plant were collected in Jammu, India. $AgNO_3$ is supplied by Merck, India. CTAB is supplied by Merck, India. Different types of bacteria *E. coli* and *S. aureus* were collected in the H. Family Hospital, New Delhi, India.

2.2. Preparation of plant extract

In this work, the leaf extracts mentioned above were involved in the synthesis of silver nanoparticles. About 25 g. Fresh leaves of the plant were collected and washed thoroughly first with tap water, then with distilled water to remove dust and harmful substances, and then dried at room temperature. An aqueous extract was prepared by boiling 15 g of cursed plant leaves in an Erlenmeyer flask with 250 ml of distilled water at 30°C for 4 to 5 hours and then filtering. The solution was then filtered through Whatmann filter paper and stored at a temperature of about 4 to 5°C for the further process of nanoparticle synthesis.

2.3. Green synthesis of silver nanoparticles

100 ml of a 0.01 mol/dm^3 $AgNO_3$ solution was prepared in an Erlenmeyer flask, after which 2, 3, 4, and 6 ml of plant extract were separately added to the AgNO3 solution while maintaining a concentration of 0.01 mol/dm^3. Then 1 ml of CTAB (cetyltrimethy lammonium bromide) was added to these solutions while maintaining a concentration of 0.01 mol / dm^3. In this work, CTAB is used as a stabilizer. We observed a decrease in the Ag ion content by measuring the absorption of the reaction mixture in the range of 200 to 800 nm using an ultraviolet spectrophotometer. The synthesis of nanoparticles is also made by changing the color of the solution from transparent to brown.

2.4. Synthesis of CS Ag Nano Hydrogel

Chitosan was dissolved in 1% aqueous acetic acid at room temperature and left overnight in a shaker at a speed of 250 rpm to obtain a 1% (w/v) solution. A viscous light yellow chitosan solution was filtered through filter paper to remove undissolved and contaminated substances. 30 ml of a PVA solution, 30 ml of a chitosan solution and 30 ml of a PEG solution are mixed in a 250 ml beaker

(1:1:1) and stirred for 1 hour at 60°C until a homogeneous solution for a 1 % hydrogel film is obtained. Then a 1% glyoxal solution is mixed into the prepared solution. Similarly, a 2% and 3% hydrogel film with glyoxal as a cross-linking agent is obtained. After confirmation of the synthesis of Ag nanoparticles, the solution of the polymer hydrogel based on chitosan is mixed with the synthesized solution of Ag nanoparticles (1:1) at room temperature with continuous stirring. After completing this continuous mixing for 4 to 5 hours, we received a brown solution of a chitosan-based hydrogel immersed in a solution of Ag nanoparticles. Then the synthesized solution is transferred to petri dishes and allowed to dry for 72-120 hours. at room temperature. After 2-3 days, CS-Ag nano hydrogel was collected from each solution and stored for further analysis.

2.5. Characterization

2.5.1. Characterization of synthesized Ag nanoparticles

2.5.1.1. Optical properties

To distinguish the optical properties of synthetic Ag nanoparticles, which were synthesized in herbs, from *S. viminalis*, the samples were analyzed, whereby the color of the solution together with UV spectroscopic examinations (Hitachi U 3900) at room temperature (b/w 250 and) from clear to dark brown changed 800 nm in the range.

2.5.1.2. Zeta potential

The zeta potential of synthesized plant Ag nanoparticles was observed using dynamic light scattering (DLS spectroscopy) (201). This property gives the average particle size within the sample together with the correlation between the number of particles of a certain size and the size of the nanoparticles.

2.5.2. Characterization of CS-Ag-Nano-Hydrogel

2.5.2.1. Hydrophobicity of hydrogel

The wetting behavior of these CS-Ag nanohydrogels was characterized by measuring the contact angle of water at room temperature using the drop method using an optical contact angle analyzer (SEO Phoenix 150). Cut 10 × 10 mm films and place them on the surface of a slide. 7 µl of a drop of distilled water was carefully injected onto the surface of the films with a micropipette before measurement.

2.5.2.2. FTIR analysis

IR Fourier spectra of these CS-Ag nanofilms were recorded on a Perkin Elmer 1750 FTIR spectrophotometer. The IR spectra of this film were from 500 to 4000 cm^{-1} recorded.

2.5.2.3. X-ray analysis

X-ray diffraction patterns of the samples were measured in the 2θ Range from 5 to 80° recorded on a Phillips X-ray diffractometer at a scan rate of 10 degrees/min. Cu Kα radiation (wavelength 1.54 Å; filament current 30 mA; voltage 40 kV) is used to generate X-rays.

2.6. Antibacterial tests

The antibacterial activity of the synthesized CS-Ag nanohydrogels was examined using two different strains (*E. coli* and *S. aureus)*. The Luria broth was used to cultivate bacteria and then incubated at 35°C for 24 hours. 100 µm Fresh overnight cultures were neatly placed on Luria agar plates to grow bacteria using disc diffusion. In this process, these films are cut into 8 × 8 mm shapes, autoclaved for 30 minutes at 120°C and placed on agar plates. The plates were incubated for 2 days at 37°C in an incubation chamber that supported 5% CO_2 flow and the zone of inhibition was measured.

3. RESULTS AND DISCUSSION

3.1. Optical and ultraviolet studies

The addition of the plant leaf extract to the AgNO3 solution led to a color change from transparent to light yellow and then dark brown due to the formation of Ag nanoparticles, as shown in Fig. 1. These color changes are the result of the excitation of the surface plasmon by the vibration of Ag nanoparticles [22]. We rely on the fact that the formation of an absorption peak with decreasing bandwidth and increasing band intensity is a sign of a spherical shape that is smaller than a particle with a certain agglomeration [23]. A slow but steady rise in temperature is a key sign of an increase in the concentration of Ag nanoparticles. The frequency and breadth of the surface plasmon absorption of the dielectric function of Ag practically disappeared [24]. Ag nanoparticles synthesized in green gave a peak in the range of 400 to 500 nm, as shown in Fig. 2. The absorption peak was observed to indicate the reduction of AgNO3 to Ag nanoparticles. It has also been found that the biological reduction of Ag ions to Ag nanoparticles begins at the start of the reaction and the biological reduction is completed in almost 10 minutes, which indicates a fast green synthesis of silver nanoparticles.

Fig. 1: Green synthesis of Ag nanoparticles from *S. viminalis*: visible observation by changing the color from transparent to brown (from left to right)

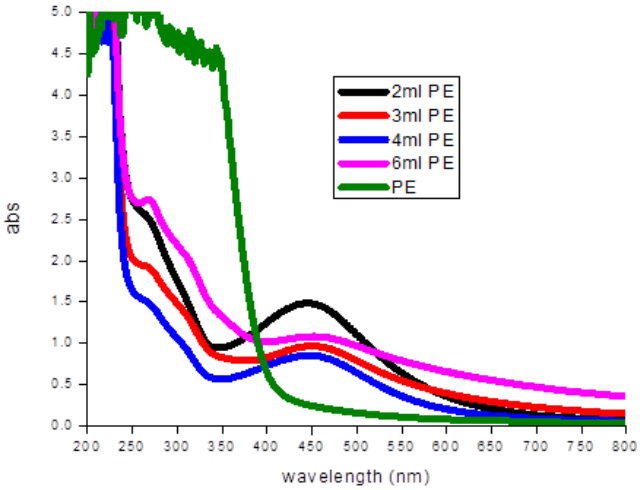

Fig. 2: UV-visible spectra of silver nanoparticles from *S. viminalis*, reaction conditions with constant AgNO3 (10 ml) and constant CTAB (1 ml) with different amounts of plant extract (PE)

3.2. Investigations on dynamic light scattering (DLS)

The DLS diagram shows that Ag nanoparticles synthesized using this method have an average zeta diameter in the range from 1 to 100 nm. The size of these synthesized Ag nanoparticles is 70±9.51 nm. Fig. 3 shows a DLS image of a suspension of Ag nanoparticles, which is obtained using an aqueous extracts S. viminalis leaves were synthesized.

3.3. Visual observation of the synthesized CS-Ag nano hydrogel

In this study, visual observation of this hydrogel film is shown in Fig. 4. This picture showed the perfection of the hydrogel films along with this uniformity. It is clearly shown that the film has a uniform shape and is easy to see with naked eyes.

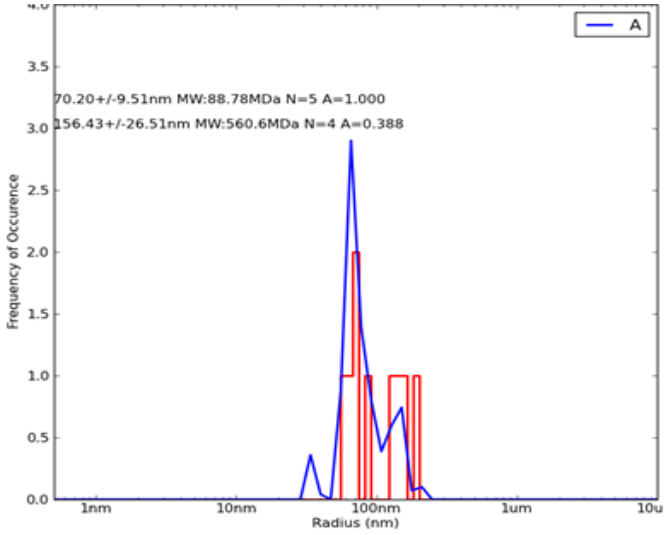

Fig. 3: Particle size distribution curve for green synthesized silver nanoparticles from *S. viminalis*

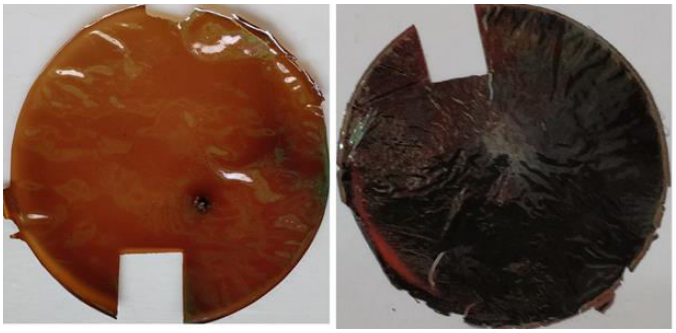

Fig. 4: Visual observations of the synthesized films from CS-Ag nano hydrogel

3.4. Contact angle

Studying the contact angle is the best way to determine the relative hydrophobicity of the substrate. Fig. 5 shows contact angle reports whose angle values are the average of at least two measurements. The contact angles of pure chitosan films with water are around 90° according to the values found in previous studies [25, 26]. A chitosan film is a hydrophobic material, the wettability of which is impaired by mixing with another material or chemical crosslinking. Thus, it was shown in this study that a chitosan-based hydrogel without Ag nanoparticles has a contact angle i.e., 22°, due to the hydrophilic nature of chitosan. Ag nanoparticles had a positive effect on the hydrophobicity and their concentration led to an increase in the contact angle of this hydrogel. In the case of *S. viminalis*, this is an increase in the contact angle to 56°, as shown in Fig. 5.

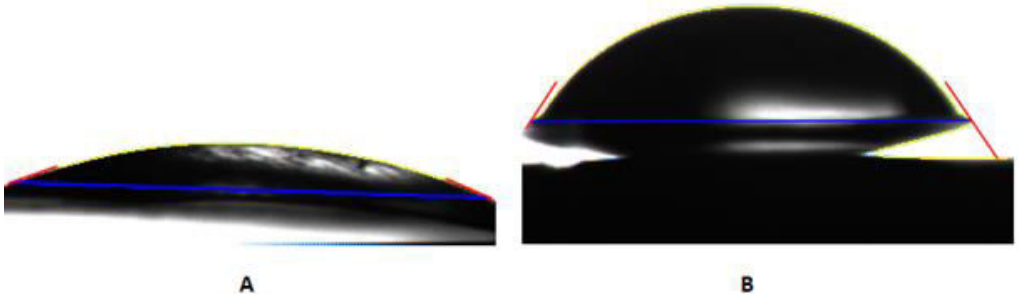

Fig. 5: Contact angle of the synthesized CS (A) film and the CS-Ag nano hydrogel from *S. viminalis* (B)

3.5. FTIR analysis

The FTIR spectrum of the CS-Ag nano hydrogel is shown in Fig. 6. In the case of CS-Ag nano hydrogel from *S. viminalis*, several absorption spectra were recorded at 3207 cm^{-1}, 2968.16 cm-1, 2869.16 cm-1, 1536.98 cm-1, 1406.49 cm-1 observed and 1052 cm-1 as shown in Fig. 6 and Table 1. In this case, each spectrometer-wide range appears in the range from 3000 to 3500 cm-1 with a maximum range of about 3200 cm-1 functional groups of pure chitosan stretching due to the overlap of -OH and - NH_2.

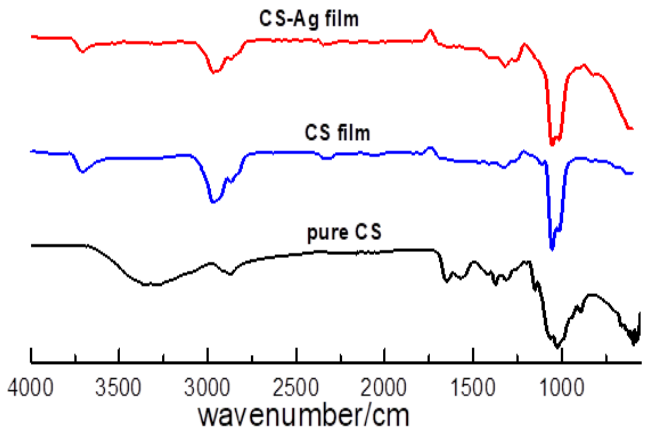

Fig. 6: FTIR spectra of pure CS, CS hydrogel, and CS-Ag nano hydrogel from *S. viminalis*.

Other bands occur in the range of 2960 and 2869 cm^{-1} due to the asymmetrical or symmetrical elongation of CH_2. The absorption bands of the pure CS film at 1644 cm^{-1}, the elongation C=O (amide I), 1536 & 1546 cm^{-1} due to the N-H bend (amide II) or about 1400 cm^{-1} attributed to the OH bend. The absorption band that appeared at about 1052 cm^{-1} is due to the C-O-C extension of the polysaccharide chain. These absorption bands found for the chitosan film are in good agreement with those previously published in the literature [27-30]. A shift in the peaks of pure CS is observed in the FTIR spectrum of CS-Ag nano hydrogel due to the interaction of Ag nanoparticles with chitosan hydrogel. Additional changes are seen in a decrease in the intensity of the hydroxyl peak and an increase in the intensity of the CO stretching, which is consistent with the FTIR spectrum in the previously published literature [31, 32].

Table 1: FTIR absorption bands from synthesized CS-Ag nano hydrogel from *S. viminalis*

CS-Ag nano hydrogel	Wavenumber(cm^{-1})	Functional Groups
S. viminalis	3207.00	-NH_2**:** O-H Stretch
	2869.16	-CH_2 Symmetric
	1536.98	-N–H Bend (amide II)
	1406.49	–OH Bend
	1052.49	-C–O–C stretch

3.6. XRD analysis

An investigation of the crystal size and structure of CS-Ag nano hydrogel was developed by X-ray diffraction. The X-ray diffraction pattern of the CS-Ag nano hydrogel is shown in Figs. 7 and 8. In the case of a CS-based hydrogel film, the number of Bragg reflections with 2θ Values is 19.3°, 28.54°, and 44.1°. On the other hand, in the case of CS-Ag, the nano-hydrogel obtained from *S. viminalis* showed the number of Bragg reflections with 2θ Values of 18.98°, 32.1°, 44.8°, and 61.0°, respectively. These values indicate a spherical structure and crystalline hydrogel films. The areas of the diagram at the CS peaks are also shown in this field. CS peaks were at 2θ Values of 19.9° were found. The change in these XRD peaks is due to the presence of plant extracts in the hydrogel film based on CS. When the concentration of the plant extract is added, the peak intensity reflects, which indicates the distance between the polymer matrices. These results show good compatibility and interaction between different components in hydrogel films [33, 34].

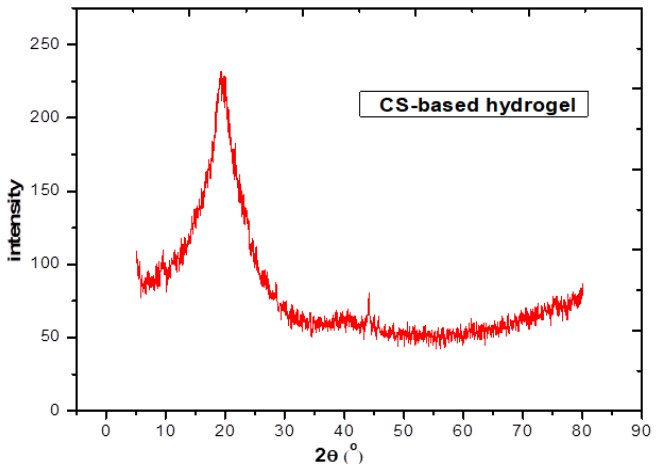

Fig. 7: CS-based hydrogel x-ray

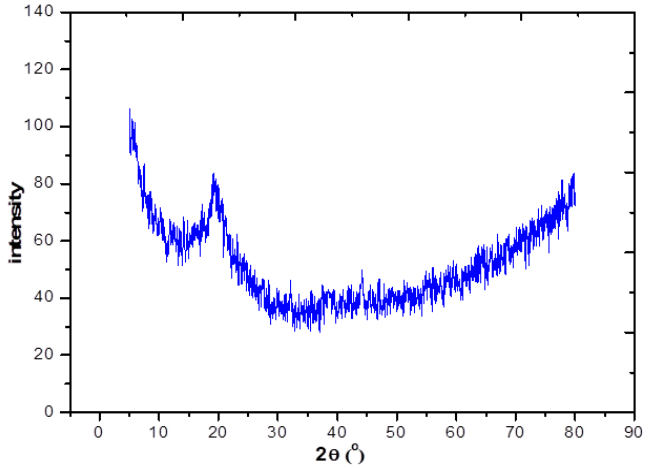

Fig. 8: X-ray of the CS-Ag nanohydrogels by *S. viminalis*.

3.7. Antibacterial studies

The green-synthesized CS-Ag nano-hydrogel showed good antibacterial activity against the growth of the pathogens *E. coli* and *S. aureus* in Fig. 9.

Since ancient times, elemental Ag and its compounds have been used as antibacterial agents and used to maintain water in the body. the shape of the coins Ag/vessels Ag [35-37]. With the green synthesis, CS-Ag nano hydrogel can complement a possible antibacterial agent for the treatment of bacterial infections. In this study, an 8 × 8 mm piece of CS-Ag nano hydrogel was used as the final product for antibacterial analysis. In the case of *E. coli*, the maximum zone of inhibition obtained from CS-Ag nano hydrogel is 7 mm for 3% *S. viminalis* and 6 mm for 1% *S. viminalis*. On the other hand, in the case of *S. aureus*, an inhibition zone was obtained which was significantly less effective than *E. coli*, with a value of 4 mm for 3% *S. viminalis* and 5 mm for 1% *S. viminalis*. The values of this zone of inhibition are shown in Table 2.

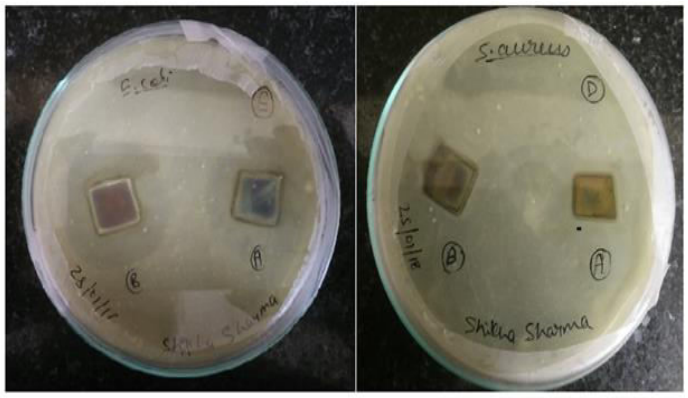

Fig. 9: Antibacterial activity of CS-Ag nano hydrogel against *E. coli* (left) and *S. aureus* (right) from 1% *S. viminalis* (A) and 3% *S. viminalis* (B)

Table 2: Antibacterial activity of CS-Ag nano hydrogel against *E. coli* and *S. aureus* from *S. viminalis*

S. No.	CS-Ag nano hydrogel	Area of a zone of inhibition in mm	
		E. coli	*S. aureus*
1.	3% *S. viminalis*	7mm	4mm
2.	1%*S. viminalis*	6mm	5mm

4. CONCLUSION

This study clearly showed that CS-Ag nano hydrogel is successfully synthesized in a greenway from the leaves of the *S. viminalis* plant. The 10 minute response showed a rapid and economical synthesis of Ag nanoparticles for further research. In the UV-visible spectra, a sharp peak showed the formation of depressions from Ag nanoparticles. The DLS study found that the size of the synthesized Ag nanoparticles is in the range of 1-100 nm. Examination of the contact angle is clear evidence of the hydrophilicity or hydrophobicity of the synthesized CS-Ag nano hydrogel by increasing the film angle. The shift in the CS peaks observed in the FTIR spectrum indicates the formation of CS-Ag nanohydrogel. Together with the sharpness of the peaks, it was indicated that a hydrogel had been formed. X-ray diffraction patterns of CS-Ag nanohydrogel contain no impurity peaks, and the corresponding X-ray

diffraction patterns show the synthesis of CS-Ag nano hydrogel. Antibacterial studies of this CS-Ag nano-hydrogel against both pathogens (*E. coli, S. aureus)* were very effective. It can be seen from all the properties carried out that the CS-Ag nanohydrogel was successfully produced.

5. ACKNOWLEDGEMENT

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