ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

SILVER NANOPARTICLE BASED HYDROGELS OF *TULSI* EXTRACTS FOR TOPICAL DRUG DELIVERY

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ABSTRACT

The present study aimed to formulate Silver Nanoparticle based Hydrogel of *Tulsi* (*Ocimum Sanctum*) extract for Topical delivery. The Silver nanoparticles were prepared using 1mM Silver Nitrate solution and *Tulsi* extract. Prepared nanoparticles were characterized for particle size and zeta potential. The optimized silver nanoparticles were incorporated into three different carbopol-940 solutions 3%, 5% and 10% carbopol-940 solution to produce Gel A, Gel B and Gel C respectively for improving convenience in superficial application. In vitro and vivo drug penetration studies of nanoparticles gels were determined using dialysis membrane. The particle size was found around 14nm to 28nm. The viscosity of the nanoparticle gels were found around 7500 centiPa.S to 7900 centiPa.S respectively. The poly dispersibility value was found very low indicating uniformity of droplet size of the formulations. The drug content in gels was found in between 97.2% to 98.26%. The drug release was found to be 71.33 % to 73.6% after 6 h. The results indicated that nanoparticle based Hydrogel C is a promising vehicle for Topical delivery of *Tulsi* Extract.

KEYWORDS: Tulsi Extract, Silver Nanoparticle, Silver Nanoparticle Based Hydrogel, Carbopol.

INTRODUCTION

Hydrogels loaded with metal nanoparticles have received great importance for many applications in the biomedical and biotechnological fields. Hydrogel are biodegradable, biocompatible and non-toxic formulations and thus provide solutions to some of the challenges in biology, medicine and material science. To enhance Hydrogels unique ability of wound care, a number of researchers have introduced inorganic metals. Because inorganic nanoparticles can easily functionalize with biomaterials, as a resulthydrogels have become a center of attraction for biomedical and biotechnological fields. Recently, hydrogels with silver nanoparticles have attracted attention for their potential applications as antimicrobial materials.[14] They are prepared by chemical, photo induced and microwave - assisted reduction methods, but the chemical reduction methods are the most common. The reduction of nanoparticles procedures depends on toxic chemicals. To overcome this problem, many researchers have introduced the green process. In the green process, plant leaf extracts was used as a reducing agents for metal nanoparticles preparation, green method is cost-effective and also utilize ambient condition for reduction reaction. Therefore, the development of metal nanoparticles based on natural extracts is considered the most appropriate method for obvious environmental reasons. In view of the importance of the above work, the present investigation involves the development of silver nanoparticles in carbopol based hydrogels by reducing the silver nitrate, using *Tulsi* leaf's extracts.[8]

The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burns and open wound. Silver ions (Ag+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells.[1]

Ocimum sanctum is a grassy annual plant originated from Iran, Afghanistan and India. Studies indicated that the reducing phytochemicals in the Tulsi (*Ocimum sanctum*) leaf consisted mainly phenolic, flavonoid, and carotenoid compounds. It was found that these reducing components also served as stabilizing agents in addition to reduction as revealed from FTIR studies. The major advantage of using the *Tulsi* leaves is that it is a commonly available medicinal plant and the antibacterial activity of the biosynthesized silver nanoparticle might have been enhanced as it was capped with the *Tulsi* leaf extract.[8]

Silver has been known to possess strong antimicrobial properties both in its metallic and nanoparticle forms hence; it has found variety of application in different fields. Silver sulfadazine shows better healing of burn wounds due to its slow and steady reaction with serum and other body fluids.[1]

Dressings with silver nano crystals, creams and gels effectively reduce bacterial infections in chronic wounds. The silver nanoparticle containing poly vinyl nano-fibers also show efficient antibacterial property as wound dressing. Reports suggest that silver nanoparticles show better wound healing capacity, higher cosmetic appearance and scar less healing power when tested on animal models.[16]

Use of plant sources offers several advantages such as method is eco-friendly, cost-effective and the

eliminate the need of high pressure, energy, temperature, and toxic chemicals which are necessary in the traditional synthesis methods.[8]

Fourier transforms infrared (FTIR) spectroscopy andX-ray diffraction (XRD) were performed to study structural and morphological properties of prepared silver nanoparticles. The content and distribution of silver nanoparticles in hydrogels were determined by transmission electron microscopy (TEM) and Dynamic light scattering (DLS). The effect of silver nanoparticles on the antibacterial activity of the hydrogels were studied.

The main objectives of this study were to

1. Synthesize the silver nanoparticles using aqueous extract of *Tulsi* leaves.
2. Preparation of three different Carbapol-940 Hydrogel preparations Gel A (3%), Gel B (5%) and Gel C (10%) respectively.
3. Analysis and characterization of silver nanoparticle hydrogels for antimicrobial properties against gram-positive and gram-negative bacteria and Characterization by using UV-Vis spectroscopy, SEM-EDX.

MATERIALS AND METHODS

PREPARATION

Preparation of *Tulsi* (*Ocimum sanctum*) leaf extract

Fresh leaves of *Tulsi* (*Ocimum sanctum*) were collected and were washed thoroughly. Then 25g of the leaves were cut into fine pieces and taken in a 100 ml beaker containing 20 ml methanol and 80 ml distilled water. The beaker was boiled over a hot plate for 30 minutes. The mixture was allowed to cool to room temperature and the contents were filtered and stored at 4°C.

Preparation of 1mM $AgNO_3$

1mM Silver Nitrate solution was prepared by adding 0.1699 g of $AgNO_3$ to 1L distilled water.

Green Synthesis of silver nanoparticles

50ml of *Tulsi* (*Ocimum sanctum*) leaf extract was taken in a 500 ml conical flask. 300 ml of freshly prepared 1mM $AgNO_3$ solution was added to the flask. The flask was incubated in dark for 24 hrs at room temperature.

Preparation of base hydrogels

Three base hydrogels were prepared –

1. Gel A (3%)[carbopol-940 0.3g in 100ml distilled water]
2. Gel B (5%)[carbopol-940 0.5g in 100 ml distilled water]
3. Gel C (10%)[carbopol-940 1.0g in 100 ml distilled water]

Procedure

Disperse weighed quantity of carbopol-940 in distilled water under mild stirring and allow to swell for 24 hr.Add 12g of glycerine and neutralize with Triethyl amine until a transparent gel appears.Store gel for 24 hr at room temperature to stabilize.

Preparation of final silver nanoparticle loaded hydrogels

Three final formulations were prepared

1. Gel A (3%)
2. Gel B (5%)
3. Gel C 10%)

Procedure

Silver Nanoparticle containing *Tulsi* (*Ocimum sanctum*) leaf extract were Incorporated into gels by using slow mechanical mixing (25rpm) for 10min.

- **EVALUATION**

Evaluation of silver nanoparticles

UV–vis spectroscopy

Samples of prepared silver nanoparticles were periodically subjected to UV spectroscopy to study the optical properties of prepared silver nanoparticles. Samples were analysed at room temperature operated at a resolution of 1 nm between 250 and 800 nm ranges.

Atomic absorption spectroscopy

To study the conversion of Silver nitrate into silver nanoparticles Atomic Absorption Spectroscopy (AAS) was performed. At various stages of reaction samples were collected and centrifuged. Then concentration of silver ions present in supernatant was analysed by AAS. Amount of silver ions in silver nitrate solution are much smaller and hence would not be separated on centrifugation, whereas silver nanoparticles are in metallic form and can be easily separated by centrifugation at around 14,500–15,000 rpm.

Transmission electron microscopy

To study the size and shape of prepared nanoparticles Transmission electron microscopy (TEM) was performed. First the sample of was sonicated for 15 min. A drop of this solution was loaded on carbon-coated copper grids, and solvent was allowed to evaporate under Infrared light for 30 min. TEM measurements were performed on Philips model CM 200 instrument operated at an accelerating voltage at 200 kV.

X-ray diffraction measurements

X-ray diffraction (XRD) measurements of film of prepared silver nanoparticles solution cast onto glass slides were done on aneMMA diffractometer operating at a voltage of 40 kV and current of 20 mA with Cu K(a) radiation of 1.54187 nm wavelength. The scanning as done in the region of 2h from 20 to 80 at 0.02 /min and the time constant was 2 s.

Dynamic light scattering

Dynamic light scattering was performed to measure the size distribution of prepared silver nanoparticles. From DLS measurement, the mean size of particles inside the sample was obtained along with the correlation between the number of particles of a particular size versus the size of the nanoparticles.

Fourier transform infrared spectroscopy

To detect the interaction between *Tulsi* (*Ocimum sanctum*) leaf extract and silver nitrate Fourier transform infrared spectroscopy (FTIR) was performed. After complete reduction of silver nitrate by *Tulsi* extract to form silver nanoparticles the reaction mixture was centrifuged at 15,000 rpm for 15 min to separate silver nanoparticles from bio mass or other unwanted compounds. The silver nanoparticles pellet obtained after

centrifugation were redispersed in water and washed with distilled water for three times. Finally, the samples were dried and grinded with KBr pellets and analyzed on a Nicolet IR 200 (Thermo electron corp) model.

Evaluation of formulated gel

Physical examination and pH measurement

The semi-solid formulations were physically examined for colour, homogeneity, and consistency. The pH was also re-evaluated (before each use) to make sure that it was stable within the skin pH of 5.5.[2]

Rheological evaluation

To determine the viscosity of prepared hydrogels Brookfield viscometer was used. Due to the viscous nature of the formulations, 1, 3 and 5 g quantities of the semi-solid formulations were dissolved in 25 ml of purified water for 24 h and their viscosities determined.[2]

Spreadability

Spreadibility test was performed using wooden block and glass slide apparatus. First an glass slide was fixed on the surface of wooden block then 2g of prepared hydrogel was placed on it. Gel was sandwiched by using another glass slide which was provided with hook. Weight (100 g) was placed upon the upper slide for 5 minutes to remove entrapped air and to form a uniform thin gel layer between slides. The weight was removed and the excess gel from the edges was scrapped off. Now without disturbance slides were fixed to a stand in such a manner that only upper slide can move or slip off freely by the force of weight tied to it. A 20 g weight was tied to upper slide carefully. The time taken for the upper slide (movable) to travel the distance of 6 cm and separate away from the lower slide (fixed) under the direction of weight was noted. The determinations were carried out in triplicate and the average of three reading was recorded.[12]

Extrudability

It is a useful empirical test to the measure the amount of force required to extrude hydrogel or any semisolid preparation from a tube. Delivery of desired quantity of gels from jars and tubes directly depends on packing of formulation thus measurement of extrudability become an important aspect. Pfizer hardness tester was used for this test. First an aluminium tube was taken then 15gm of gel was weighed and filled into.Then pressure of 1kg/cm2 was applied for 30 sec and amount of extruded gel was weighed. The % of gel extruded was calculated; and accordinglygrades were allotted (+ + + Excellent, + + Good, + fair). Whole procedure was done at three equidistance places for all three tubes. Test was carried out in triplicates.[2]

Drug content analysis

About 0.5 g of hydrogel was dissolved in 10 ml of water, centrifuged at 1000 rpm for 30 min, filtered through a Whatman No. 1 filter paper, adequately diluted and the concentration of *Tulsi* (*Ocimum sanctum*) leaf extract was determined spectrophotometrically at 412 nm.[2]

In-vitro drug diffusion study

Drug diffusion rate from different gel formulations were studied by Franz diffusion cell using cellophane membrane as a barrier. Diffusion membrane was immersed in receptor compartment having Ethanol: Water (1:1) as diffusion medium, maintained at 37^0C for 24hr for equilibrium. Diffusion cell was assembled on magnetic stirrer along with diffusion membrane, which separates donor and receptor compartments. Gel (2g) was kept on membrane in donor compartment. The contents were stirred using magnetic stirrer at 50 rpm and aliquots each of 5 ml were withdrawn from the release medium at time intervals of 10, 20, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes. Withdrawn samples were replaced by equal volumes of same fresh medium. Absorbance of these samples was measured spectrophotometrically at 412 nm by UV-Visible double beam spectrophotometer. Cumulative release (%) of *Tulsi* (*Ocimum sanctum*) extract from different gel formulations was calculated and analysed.[5]

Determination of antimicrobial activity

The antimicrobial activity of the hydrogels was tested against two microorganisms - Escherichia coli and Staphylococcus aureus by agar diffusion technique. This method depends on the diffusion of antimicrobial agent from holes perforated in the microbe-seeded agar. The agar plates were prepared following manufacturer's specifications and seeded with each bacterial isolate. The agar plates were allowed to set and a sterile cork borer (8 mm diameter) was used to bore holes in the seeded agar medium. Using a sterile instrument, a definite volume of hydrogels were used to fill the holes. The plates were allowed to stand at room temperature for 15 min to enable prediffusion before incubating at 25 ± 1°C for 48 h. Growth was examined after incubation and the diameter of each inhibition zone was measured.[10]

RESULT

Evaluation of silver nanoparticles

UV–vis absorbance studies

After addition of O. sanctum leaf extract to silver nitrate due to the production of silver nanoparticles solution resulted in colour change of the solution from transparent to dark yellow. These color changes arise because of the excitation of surface Plasmon vibrations with the silver nanoparticles [11]. The surface Plasmon resonance (SPR) of silver nanoparticles produced a peak centred near 413 nm. UV–vis absorbance of reaction mixture was taken from 0 till 8 min (Fig. 1). It was observed that absorbance peak was centred near 413 nm indicating reduction of AgNO3 into silver nanoparticles. It was also observed that bio reduction of silver ions into nanoparticles started at the start of reaction and bio reduction was completed at almost 8 min indicating rapid biosynthesis of silver nanoparticles. An absorption band at 260 nm is clearly visible and is attributed to electronic excitation in tryptophan and tyrosine residues in proteins[3]. This indicates the release of extracellular proteins in the colloidal solution and their possible mechanism in bio reduction process [4].

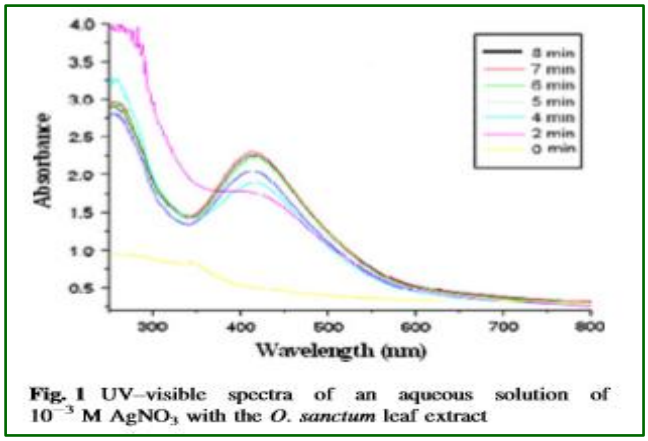


Fig. 1 UV–visible spectra of an aqueous solution of 10^{-3} M $AgNO_3$ with the *O. sanctum* leaf extract

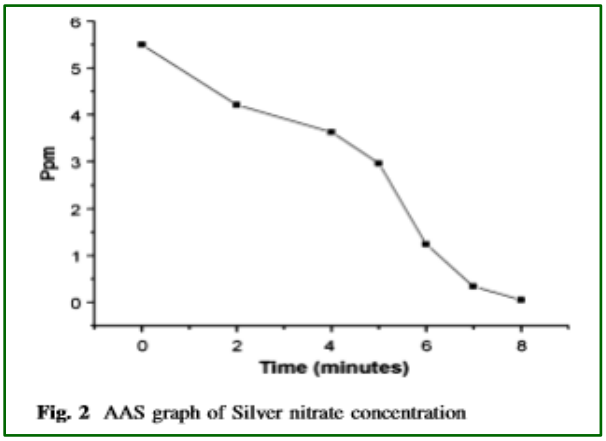


Fig. 2 AAS graph of Silver nitrate concentration

AAS analysis

To analyse Silver ion concentration AAS was performed which as a result conversion of Ag ions into Ag nanoparticles was observed. Initially, standard solution of 5.5 ppm of AgNO3 was prepared and analysed with AAS at 0 min. Now, Ag ion concentration in the reaction solution, after adding leaf extract, was monitored at different time intervals. The result showed decrease in concentration of Ag ions (5.5, 4.21 3.63, 2.96, 1.24, 0.34, and 0.05 ppm at 0, 2, 4, 5, 6, 7, and 8 min, respectively) indicating the conversion of Ag ions into Ag nanoparticles (Fig. 2).

TEM analysis

Fig 3 and fig 4 shows TEM images of prepared silver nanoparticles at 50 and 5 nm scales respectively. From these images shows that the prepared silver nanoparticles were evenly distributed in sample and are circular in shape and the maximum particles size was within 4-30 nm with mean diameter of 14.31 ± 2.5 nm. Particle size distribution histogram determined from TEM is shown in Fig. 5.

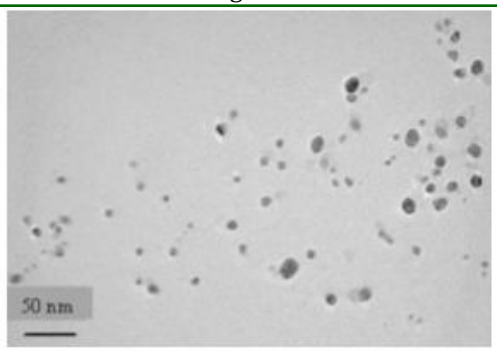


Fig. 3 TEM image of biosynthesized silver nanoparticles using *O. sanctum* leaf extract at 50 nm scale

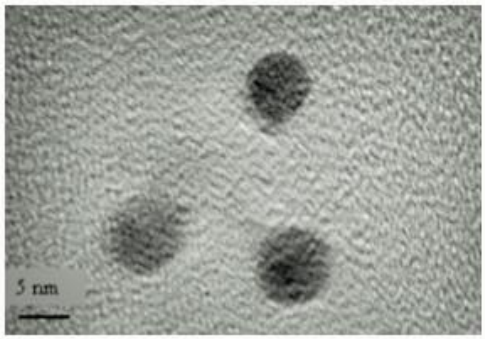


Fig. 4 TEM image of biosynthesized silver nanoparticles using *O. sanctum* leaf extract at 5 nm scale

XRD analysis

Diffraction peaks were observed during XRD analysis corresponding to fcc structure of silver. Intense peaks were observed at 38.098,44.154,64.674, and 77.544 (Fig. 6), corresponding to 111, 200, 220, and 311 Bragg's reflection, respectively. The formation of nanoparticles was indicated by broadening of Bragg peaks. To determine the average particle size Full width at half maximum (FWHM) data were used with Scherer's f ormula[7]. Estimated average particle size was approximately 15 nm.

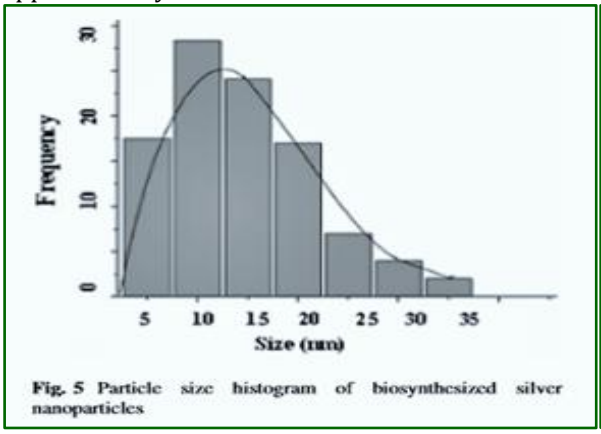


Fig. 5 Particle size histogram of biosynthesized silver nanoparticles

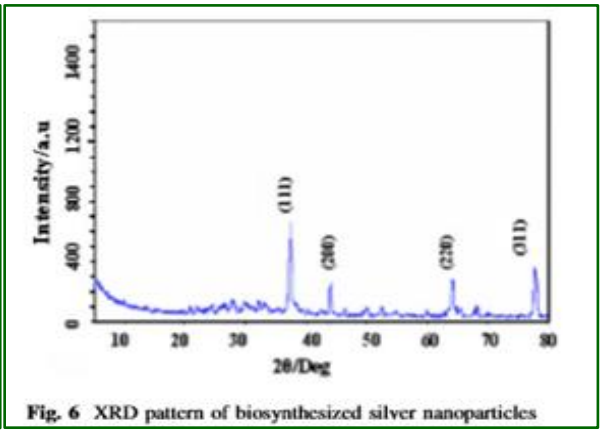


Fig. 6 XRD pattern of biosynthesized silver nanoparticles

DLS Analysis

The DLS pattern shows that synthesized silver nanoparticles have a Zeta average diameter of 22.38 ± 3.56 nm and polydispersity index (PDI) of 0.310 (Fig. 7). As the dynamic light scattering (DLS) method measures the hydro dynamic radius thus the particle size was slightly bigger as compared to the particle size measured from TEM micrographs. [6]

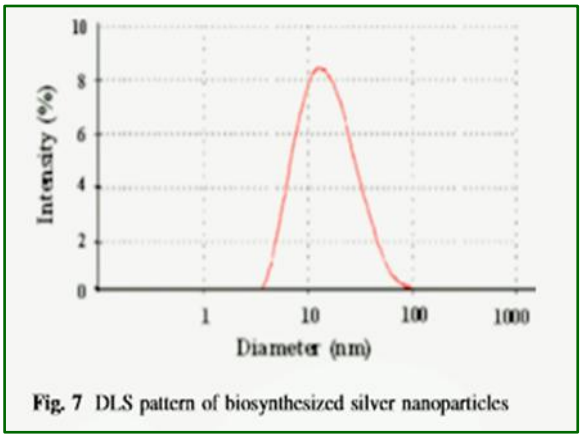


Fig. 7 DLS pattern of biosynthesized silver nanoparticles

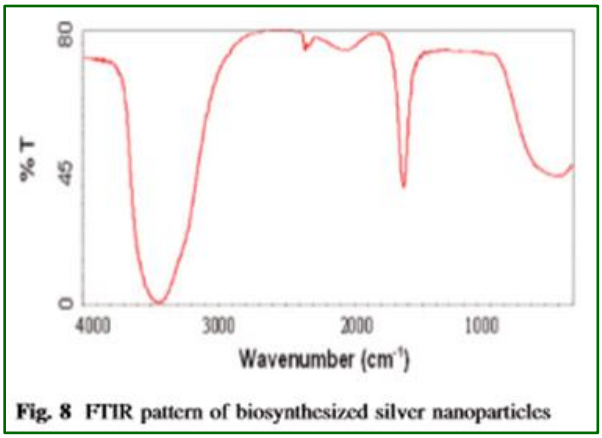


Fig. 8 FTIR pattern of biosynthesized silver nanoparticles

FTIR analysis

To detect the possible interaction between proteins and nanoparticles FTIR measurements were carried. As a results of sharp absorption peaks were observed at 1635 and 3430 cm-1 (Fig. 8). Absorption peak at 3430 cm^{-1} was assigned to OH stretching in alcohols and phenolic compounds and peak at 1635 cm^{-1} was assigned to the amide I bond of proteins arising due to carbonyl stretch in proteins. This peak was close to that reported for native proteins [9] which suggest that proteins and nanoparticles are interacting and but their secondary structure were not affected during or after binding with Ag nanoparticles [4]. The study confirmed that carbonyl group of amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nanoparticles and acting as capping agent to prevent agglomeration and providing stability to the medium[15]. These results confirm the presence of possible proteins acting as reducing and stabilizing agents for silver nanoparticles.

Evaluation of formulated gels:

Physical examination and pH measurement

Results of Physical examination and pH measurement are shown in table no.1. Gel formulations were found to be translucent in nature with ethanoic odour and homogenous. The pH of hydrogels gels were under the acceptable limits.

Spreadibility and Extrudability

Results of the Spreadibility and Extrudability testing are shown in table no.1. All the prepared gels using different polymers in different concentrations were spreadable on the skin surface. Data in table no.1 revealed that increasing the concentration of the gelling agents was associated with a decrease in the Spreadibility and extrudability.

Viscosity

Results of viscosity studies are given in table no.1. The data represent that with increase in the concentration of polymer viscosity was increased.

Drug content

The content of *Tulsi* leaf extract in all the gels was found to be within permissible limits (> 97%). This indicates that the nanoparticles were uniformly distributed throughout the formulations.

Table no.1

Formulation	Homogeneity	pH	Spreadibility (gm.cm/sec)	Viscosity (centipoises)	Extrudability	Drug content %
Gel A	Good	7.1	67	7500	+ +	97.3%
Gel B	Very good	7.3	54	7800	+ +	99.5%
Gel C	Very good	7.3	46	7900	+ +	98.2%

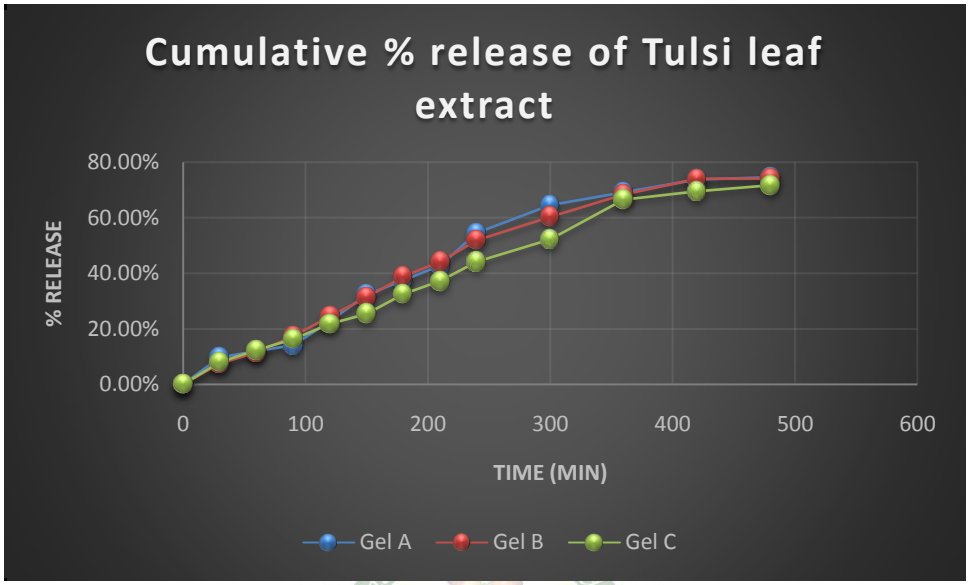
In-vitro drug diffusion study

All three formulated silver nanoparticle loaded hydrogels were analyse by in-vitro release studies across cellulose membrane. Release profiles of *Tulsi* (*Ocimum sanctum*) leaf extract from various gel formulations shows that drug release decrease with increase in concentration of the gelling agent. It was observed that gel prepared using Carbopol-940 (3%) [Gel A], Carbopol-940 (5%) [Gel B], and Carbopol-940 (10%) [Gel C] gave CPR of 74.58%, 74.17%, and 73.42% respectively after 6 hrs.

Table no.2

Time (min)	Gel A	Gel B	Gel C
0	0.00%	0.00%	0.00%
30	09.62%	07.46%	07.96%
60	11.96%	11.53%	12.32%
90	14.07%	17.30%	16.26%
120	22.85%	24.53%	21.83%

150	32.26%	31.22%	25.36%
180	37.50%	38.89%	32.56%
210	42.75%	44.19%	37.21%
240	54.49%	51.91%	44.16%
300	64.59%	60.28%	52.20%
360	69.20%	68.35%	66.54%
420	73.76%	73.99%	69.48%
480	74.58%	74.17%	71.69%

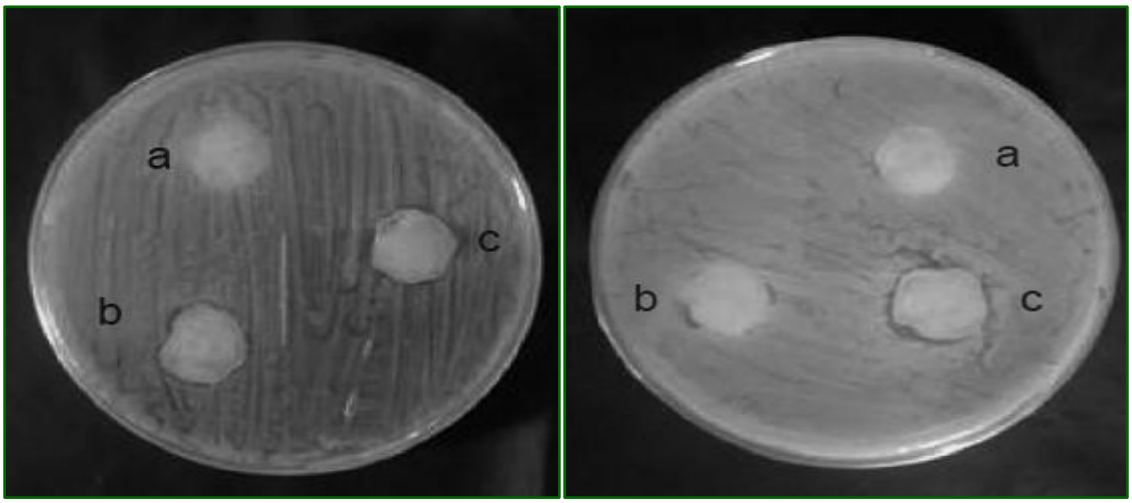


In vitro antibacterial activity

The inhibitory zone diameterIZD) for all three hydrogel formulations is given in Tables 3. The in vitro antibacterial activity of all three formulations were compared with each other. The results indicate that all three *Tulsi* leaf extract nanoparticle loaded hydrogels produced very significant zones of inhibition against Gram positive organism (S. aureus) and Gram negative organisms (E. coli) used in the study.

Table no.3

Formulation	Zone of inhibition for S.aureus	Zone of inhibition of E.coli
Gel A	7 mm	5 mm
Gel B	10 mm	8 mm
Gel C	9 mm	6 mm



E.coli **S.aureus**

CONCLUSION

The most satisfactory formulation was Gel B. From results it is observed that Hydrogel formulation Gel B was free of irritation and have better, spredbility, extrudability, viscosity, pH, antimicrobial activity, better % drug release and higher % drug content than other two i.e., Gel A and Gel C. Thus Silver Nanoparticle based Hydrogel containing

Tulsi leaf extract made from carbopol 940(5%) was found to the most appropriate formulation can be used as an effective topical antimicrobial agent.

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Cite this article as:
Anirban Saha, Navneet Kumar Giri, S.P.Agarwal. Silver Nanoparticle Based Hydrogels of Tulsi Extracts for Topical Drug Delivery. International Journal of Ayurveda and Pharma Research. 2017;5(1):17-23.
Source of support: Nil, Conflict of interest: None Declared

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