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Original Article

In vivo and *in vitro* Evaluation of Polymeric Gel Loaded with Biologically Synthesized Black Tea and *Baliospermum solanifolium* Silver Nanoparticle

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ABSTRACT

Aim: The overall objective of the study was to synthesise and assess a polymeric gel that contained biologically synthesised Silver Nanoparticles (AgNPs). **Materials and Methods:** AgNPs were produced through the reduction of Silver Nitrate ($AgNO_3$) with Black Tea and *Baliospermum solanifolium* extracts separately and evaluated for different parameters. The antibacterial activity of generated nanoparticles against *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli* was assessed. Staphylococcus aureus was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of AgNPs. D-optimal mixture design was used to optimise the formulation. The concentrations of black tea AgNPs, *Baliospermum solanifolium* AgNPs, gelling agent concentration, propylene glycol, and gelling agent type were used as independent factors, and responses for antibacterial activity, spreadability, and viscosity were assessed. The optimised formulation was tested for antifungal capability, *in vitro* release of drugs, *ex vivo* permeation, and *In vivo* wound-healing efficacy (MRSA-infected skin wound model). For its *In vivo* wound-healing activity and antibacterial efficacy, an Optimised formulation of Black tea and *Baliospermum solanifolium* Nano Particles Gel (OBBNG) was assessed against commercially available cream. **Results:** The mean diameters of black tea AgNPs and *Baliospermum solanifolium* AgNPs reported 137.8 and 105.6 nm, respectively. The MIC values for black tea AgNPs and *Baliospermum solanifolium* AgNPs were 512 µg/mL and 256 µg/mL, respectively. The MIC for a mixture of black tea and *Baliospermum solanifolium* silver nanoparticles was 0.25:0.125 mg/mL. **Conclusion:** The OBBNG displayed superior antibacterial properties and wound-healing capability, as well as normal skin appearance and development of hair, as compared to the standard cream.

Keywords: Silver nanoparticles, Black tea, *Baliospermum solanifolium*, Polymeric gel, Optimization, D-optimal mixture design, Wound-healing, Antibacterial activity.

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INTRODUCTION

A wound may first appear to be simple damage to tissue, but the health of a person and other inherent factors may make it highly complicated.[1] When the integrity of the skin is damaged, a wound develops, and its cause may be unintentional, intentional, medically linked, or both. Surgery, an accident, or other events or situations including pressure, shear, diabetes, or vascular illnesses can all result in wounds developing. Acute wounds and chronic wounds are the two categories into which they can be separated. Homeostasis, inflammation, proliferation/re-epithelialization, and remodeling are the four stages that typically occur in succession while a wound heals. Acute wounds may become chronic wounds, which are more difficult to treat, when complications inhibit speedy and efficient healing.[2] A major portion of the population suffers from acute and chronic wounds, which place a heavy burden on the nation's healthcare system.[3] Chronic wounds are more common in older people, which greatly lowers quality of life. Due to the use of dangerous substances and high energy requirements during preparation (using both physical and chemical processes), the production of silver nanoparticles on a large scale is hindered. Therefore, it is obvious that a new, more useful, safe, and morally sound way of producing nanoparticles is required. Biosynthesis of green-synthesized silver nanoparticles is a rapid and environmentally beneficial method. AgNPs created through biosynthesis have more accurate sizes, morphologies, and antibacterial effectiveness.[4] The antioxidant, antibacterial, anti-inflammatory, and wound healing activity of the phytochemicals and Ag+ions is improved by their complementary action. When bio-conjugated with plant extracts, ag-based nanomaterials pose less of a threat to biological systems

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and have improved therapeutic efficacy. The phytochemicals in the plant extract serve as a stabilising and capping agent. Silver nanoparticles, although they are small size, have a huge surface area that can absorb a great quantity of capping agents and have therapeutic effects.[5]

MATERIALS AND METHODS

Materials

All chemicals utilised in this investigation were analytical-grade and acquired from Research Lab Fine Chemicals Ltd., in Mumbai, India.

Biological synthesis of black tea silver nanoparticle

For 2 hr, black tea extract was mixed with silver nitrate. The bright yellow to dark brown colour change of the reaction mixture served as a gauge for reaction progress. Then, at various times, the reaction mixture was examined with UV between 200 and 800 nm.[6] The nanoparticles were then rinsed three to four times with double-distilled water and centrifuged (REMI CM 12) for 15 min at 11500 RPM. Utilising a Lyophilizer (CHRIST ALPHA 1-2 LD), nanoparticles were dried. The prepared nanoparticles were kept in a cold, dry environment.

Biological synthesis of *Baliospermum solanifolium* silver nanoparticle

The phenolic extract from *Baliospermum solanifolium* leaves was added to a solution of silver nitrate, and the mixture was stirred for 2 hr. The colour of the solution changed from yellow to a reddish-brown hue. The silver nanoparticle dispersion was centrifuged and washed with double-distilled water three to four times. Lyophilized and preserved in a cool, dark location.[7,8]

Characterization of synthesized silver nanoparticles

The reaction mixture's colour change was noted at various time intervals by visual observation. Absorbance was measured using UV-vis spectroscopy at various time intervals (30, 60, 90, and 120 min). A particle size analyzer (HORIBA SCIENTIFIC and S2-100) was used to quantify polydispersity and particle sizes.[9,10] Phenolic compounds profile in AgNPs was determined by using HPLC (WATERS ALLIANCE 2695 HPLC WITH PDA)[11] Morphological study of prepared SN was performed using FEI Nova Nano SEM 450.[11,12]

Antibacterial activity of AgNPs

Minimum Inhibitory Concentration (MIC)

The standard agar broth dilution technique was utilised to monitor the detectable bacterial growth in the agar broth to investigate the antibacterial capabilities of silver nanoparticles. The MIC in Nutrient Broth was determined by serial dilutions of AgNPs ranging from 1 to 1024 g/mL with an adjusted concentration of bacteria (108 CFU/mL, 0.5 McFarland's standard). The control contained only inoculated broth. It was incubated for 24 hr at 37°C. The MIC is the lowest silver nanoparticles concentration in which there is no observable growth in the tubes. To confirm the MIC value, the optical opacity within the tubes was checked both before and after incubation.[12,13]

Minimum Bactericidal Concentration (MBC)

Following the MIC of silver nanoparticles, 50 µL aliquots from each tube that revealed no apparent development of bacteria were transferred to on plates containing nutrient agar and cultured for 24 hr at 37°C. An MBC endpoint occurs when 99.9% of the population of bacteria is eliminated at the lowest possible antimicrobial agent concentration.[14]

Formulation of silver nanoparticles loaded polymeric gel

To make AgNPs dispersions suitable for topical applications and to boost patient compliance, gel formulations were developed employing polymer-gelling agents. Using a cold mechanical process, topical gel was produced. To avoid the formation of lumps, the required amount of polymers was sprinkled onto the surface of distilled water while being rapidly swirled (REMI, lab stirrer) and allowed to fully swell overnight. The remaining amount of water was mixed with preservative, propylene glycol, and silver nanoparticles. The resulting mixture was next added to the Carbopol mixture, stirring constantly. Triethanolamine was added to the mixture to change the pH, which resulted in the formation of a gel and called for careful stirring to thoroughly integrate all the ingredients.[13-15]

Optimization of formulation of Black tea and *Baliospermum solanifolium* nanoparticles gel

Table 1 lists the parameter ranges that were chosen for D-optimal mixture design in silver nanoparticle gel formulations. ANOVA is used to analyse data and assess the significance of model terms and their interactions.[16-21] 37 formulations were created using D-optimal design (Deign Expert 13 Trial version).[22]

Evaluation of silver nanoparticles loaded polymeric gel

Homogeneity and colour, the compositions created by visual evaluation.[23] The gel pH at a set temperature was calculated using an automated pH monitor (Equip-Tronics). Aqueous solution at 1% concentration was used for the study.[23] Spreadability was determined by two simple glass surfaces One of the glass surfaces had a circle drawn on it with a 2 cm diameter. A circle of 0.5 g of gel that had been precisely measured was spread. Over the gel was retained a second glass surface. For 5 min, a weight of 500 g was allowed to rest on the upper glass, causing the gel to spread. By determining the diameter of the gel, the gel diameter was computed.[24-26] Viscosity of the gel was measured using a viscometer (LABMAN MODEL NO. LDMV-60). Spindle number

4 was utilised to examine the viscosity of the composition. The study was conducted at speeds ranging from 5 to 50 rpm.[27] Antibacterial activity was carried out by cup plate method activity against MRSA, PA, and EC. Nutrient agar Culture medium was produced and sterilised for 20 min at 121°C in an autoclave. At 40°C, 0.1 mL of bacterial inoculums were added to liquefied media. 20 mL media was added to petri plate and left to harden. A 6 mm cork borer was used to aseptically prepare the wells. Gel that was filled with silver nanoparticles (Formulations 1-37) was carefully poured into wells made in plates. 24 hr of incubation at 37°C for prepared plates. The triplicate study's findings were presented as mean SEM.[28-30]

Antifungal activity of Optimized formulation of Black tea and *Baliospermum solanifolium* nano particles gel (OBBNG)

The agar well diffusion method was used to examine the effectiveness of silver nanoparticle gel against*Aspergillus niger* and *Candida albicans*. Each culture was added into tubes containing 10 mL of Sabouraud Dextrose Broth, On Potato Dextrose Agar plates, cultures were spread out. The wells were drilled using a 6 mm borer and filled with OBBNG. Plated were incubated for 48 hr at 37°C. The diameter of the inhibition zone (in mm) was measured. The results of the triplicate study were reported as mean SEM.[31-33]

In vitro drug release study of OBBNG

In vitro were release study was conducted with vertical Franz diffusion cell (diffusion area 1.76 cm^2) using artificial cellophane membrane (MOLECULAR WEIGHT 12000). The receptor phase was stirred at 300 rpm and contained 15 mL of phosphate solution with a pH of 7.4.[2] OBBNG 1 gm applied to the donor side. For 3 hr, 1 mL of the sample was taken out of the receptor compartment every 30 min and replaced with a fresh volume of receptor fluid. Prior to UV spectrophotometer examination, the aliquots were suitably diluted with receptor media. Measurements were made in triplicates and reported. AgNPs formulation's release kinetics were examined and reported.[34,35]

Ex vivo permeation study of OBBNG

The skin of recently butchered chicken was obtained from the neighbourhood slaughter shop, cleaned, and saline-washed. An appropriately sized piece of chicken skin mounted in a Franz diffusion cell, with the *Stratum corneum* facing the donor compartment and the dermis facing the receiver compartment. The donor chamber was filled with OBBNG. The receptor compartment was filled with 15 mL of phosphate buffer with pH 7.4. mixture was stirred at 37°C plus or minus 0.5°C. with magnetic stirrer for 3 hr. The samples were removed from the receptor cell on a regular interval and subjected to spectrophotometric analysis. The samples were taken out at regular intervals and subjected to spectrophotometric analysis using at wave length of*Baliospermum solanifolium* and black tea silver nanoparticles. Average values of triplicate calculations of the cumulative amount of medication penetrate throughout the skin were made as a function of time.[36,37]

In vivo wound-healing efficacy of OBBNG

Wound healing activity of OBBNG was examined. 30 wistar male Rats of 6-8 weeks with 200-230gm were selected for study (SPCOP/2021-22/288.) The animals were divided into five groups of six animals. Group 1: disease controls, Group 2: OBBNG (0.512 mg/gm), Group 3: BTNG (0.512mg/gm), Group 4: BSNG (0.256 mg/gm), Group 5: 1% SSC (n=6). For premedication, 10 mg/kg of xylazine Hydrochloride (HCl) (Xylazine 2%) and anesthesia, 90 mg/kg of ketamine Hydrochloride (HCl) (Ketamine 10%) were injected intramuscularly into each animal. An aseptically created 1x1 cm spherical skin defect by making an incision in the dorsal area.[38] Body weights were measured on the day of acclimatization, and the weights of all surviving animals were observed on the first day of topical treatment and at weekly intervals thereafter, i.e. on days 7, 14, and 21.40.[42]

Wound healing (contraction)

On days 5, 9, 13, 17, and 21 following wound surgery, wound healing (contraction) was assessed.[39-42] The wound area was measured with a ruler. The maximum perpendicular width is multiplied by the maximum length. The percentage of the wound healing was used to calculate the wound healing:

$$\%\ \text{Wound healing} = \text{Initial wound Size-Specific day wound size} / \text{Initial wound size} \times 100 \quad (1)$$

Bacterial burden in wound

The number of bacteria in the wound was counted on days 1, 5, 9, 13, and 21 after surgery. Based on earlier research, a sterile swab was applied to the wound and then immersed in 1 mL of sterile saline solution (0.9%). After twirling the swab into the liquid to release the bacteria, a sterile saline solution was added to dilute the solution by a factor of 10 (10-fold dilution). A sterile Nutrient agar plate was used to hold 5 µL of the sample. 24 hr was spent incubating the plate at 37°C. After incubation, established colonies were measured to determine the number of living (vegetative) bacteria present at the wound site.[41,43-45]

Histopathological Evaluation

All of the animals that survived the therapy were weighed and slaughtered in accordance with a standard procedure that had been approved. The entire wound and surrounding tissue were excised, kept in 10% Neutral Formalin Buffer (NBF), and then histopathologically processed further.[42]

Table 1: Parameter for the formulation of silver nanoparticle loaded gel.

Code	Independent variables	Low level	High level
A	Concentration of black tea AgNPs (µg/gm gel)	0	512
B	Concentration of *Baliospermum solanifolium* AgNPs (µg/gm gel)	0	512
C	Concentration of gelling agent (%)	0.5	2.5
D	Concentration of propylene glycol (% w/w)	1	3
E	Type of gelling agent	943	940
Dependent variables			
Response 1	Antimicrobial activity		
Response 2	Spreadability		
Response 3	Viscosity		

RESULTS

Analysis Results of synthesized AgNPs

Visual observation

The change in colour of the reaction mixture indicates the formation of silver nanoparticles. The colour of the reaction mixture changed from light brown to dark brown demonstrated the creation of silver nanoparticles. A further change in the color suggested a shift in particle size.[8]

UV-vis Spectrophotometry

The presence of a UV peak at 400 to 470 nm indicates the creation of silver nanoparticles. The greatest absorption of black tea and *Baliospermum solanifolium* silver nanoparticles was measured at 444 nm and 432 nm.

Size of particles and zeta potential

Black tea AgNPs: PDI- 0.278, Average particle size- 137.8nm, Zeta potential- 22.7mV.

Baliospermum solanifolium AgNPs: PDI- 0.42, Average particle size- 105.6 nm, Zeta potential 15 mV.

Phenolic compounds profile in AgNPs

Polyphenol compounds like gallic acid, ellagic acid and catechins were present in black tea nanoparticles and Catechins, ellagic acid, and quercetin extract were discovered in the Baliospermum silver nanoparticles.

FESEM

Figure 1 shown pictures of prepared silver nanoparticles.

Antibacterial activity of AgNPs

The MIC was defined as the minimal antibacterial agent concentration required to block successive dilution-inhibited bacterial growth. The MBC values for black tea and Baliospermum AgNPs against SA were 512 µg/mL and 256 µg/mL, respectively, as indicated in Table 2. The MBC for a mixture of black tea and *Baliospermum solanifolium* silver nanoparticles was 0.25:0.125 mg/mL as shown in Table 3. The blend of black tea and *Baliospermum solanifolium* AgNPs showed the strongest antibacterial impact when compared to the independent silver nanoparticles (black tea AgNPs and Baliospermum AgNPs).

Optimization of silver nanoparticles loaded polymeric gel

In comparison to other optimisation procedures, the D-optimal mixture design is suitable for formulations that include mixes and when the experimental designs have more constraints and limitations. This is due to the fact that fewer optimisation experiments are required.[28,29]

Evaluation of silver nanoparticles loaded polymeric gel

Because of the presence of AgNPs, the formulated AgNPs Carbopol gels (F1-F37) were homogeneous and brownish in colour, whereas the blank Carbopol gels were transparent. The pH range of the gel formulation was 5.20 to 6.47, which was still within the pH range of human skin, according to this test.

Spreadability

Spreadability refers to gel preparation's ability to spread over the skin's surface. The scatter diameter increases the surface area that the gel can contact. A gel with good spreadability will spread uniformly when placed to the skin; good spreadability is between 5-7cm. The spread test results of the gel formulations containing silver nanoparticles reveal a value ranging from 3 to 8.6 cm, showing that the gel was spreadable.

The following polynomial equation was discovered using multiple regression analysis for Spreadability.

$$\text{Spreadability}=+5.81\times A+5.02\times B+4.10\times AB-0.4288\times AC+0.2609\times AD-0.8361 \quad (2)$$

According to statistical polynomial equation 1,

Where, "A" stands for the concentration of black tea AgNPs, "B" for the concentration of *Baliospermum solanifolium* AgNPs, "C" for the concentration of the gelling agent, "D" for the concentration of propylene glycol, "E" for the type of gelling agent, the positive values had a synergistic effect on the response, and the negative values represent an antagonistic effect on the response.

The regression equation obtained from the constructed model was graphically represented by a 3D surface response plot.

As illustrated in Figure 2A, the spreadability of Carbopol gel was significantly affected by increasing the propylene glycol concentration. Propylene glycol has a pH range of 9.3 to 11.5, which boosts the gel's alkalinity, induces polymer swelling, increases viscosity, and limits spreadability.

The concentration of gelling agent increases the viscosity. A rise in surface tension causes an increase in viscosity. Because spreadability is affected by viscosity, higher viscosity makes it more difficult to spread gel over skin (Figure 2B). The grades of polymers also had an effect on gel viscosity. The spreadability of gel reduces when viscosity increases due to greater polymer grades.

Viscosity

The viscosity test findings for gel formulations ranged from 3018.03 to 3805.97cps.

$$\text{Viscosity} = +3350.07\times A+3326.86\times B+110.23\times AB+359.30\times AC +3.96\times AD-29.58\times AE \quad (3)$$

Table 2: MIC and MBC of AgNPs.

Sl. No.	Concentrations of AgNPs (µg/mL)	*Staphylococcus aureus*			
		Black tea AgNPs		*Baliospermum* AgNPs	
		MIC	MBC	MIC	MBC
1	1	+	+	+	+
2	2	+	+	+	+
3	4	+	+	+	+
4	8	+	+	+	+
5	16	+	+	+	+
6	32	+	+	+	+
7	64	+	+	+	+
8	128	–	+	–	+
9	256	–	+	–	–
10	512	–	–	–	–
11	1024	–	–	–	–

Positive (+): Turbidity indicating growth; Negative (–): No turbidity indicating absence of growth.

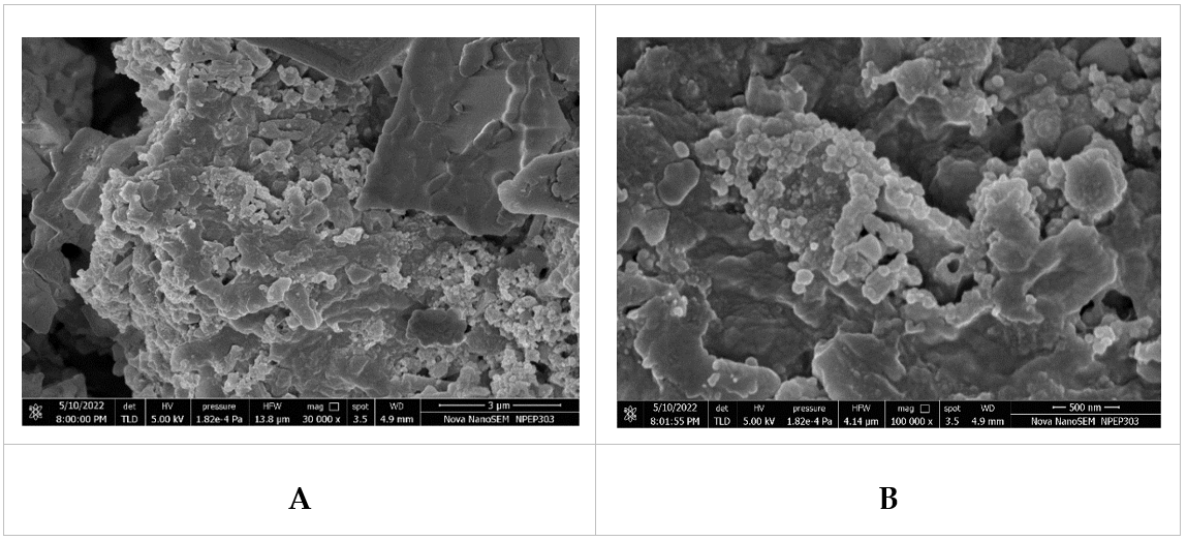


Figure 1: Particle size by FESEM (A) black tea silver nanoparticles (B) *Baliospermum solanifolium* silver nanoparticles.

According to statistical polynomial Equation 2, where "A" denotes the concentration of black tea AgNPs, "B" denotes the concentration of *Baliospermum solanifolium* AgNPs, "C" denotes the concentration of the gelling agent, "D" denotes the concentration of propylene glycol, and "E" type of gelling agent, Positive factors influenced the reaction in a synergistic way, whereas negative values were the opposite impact.

An increase in ionisation, because of increase in electrostatic repulsion between surrounding carboxylic groups and the consequent expanding polymer network, contributes to the increase in viscosity of Carbopol gel (Figure 2C). Figure 2D depicts effect of propylene glycol concentration on viscosity of the Carbopol gel.

Antibacterial study

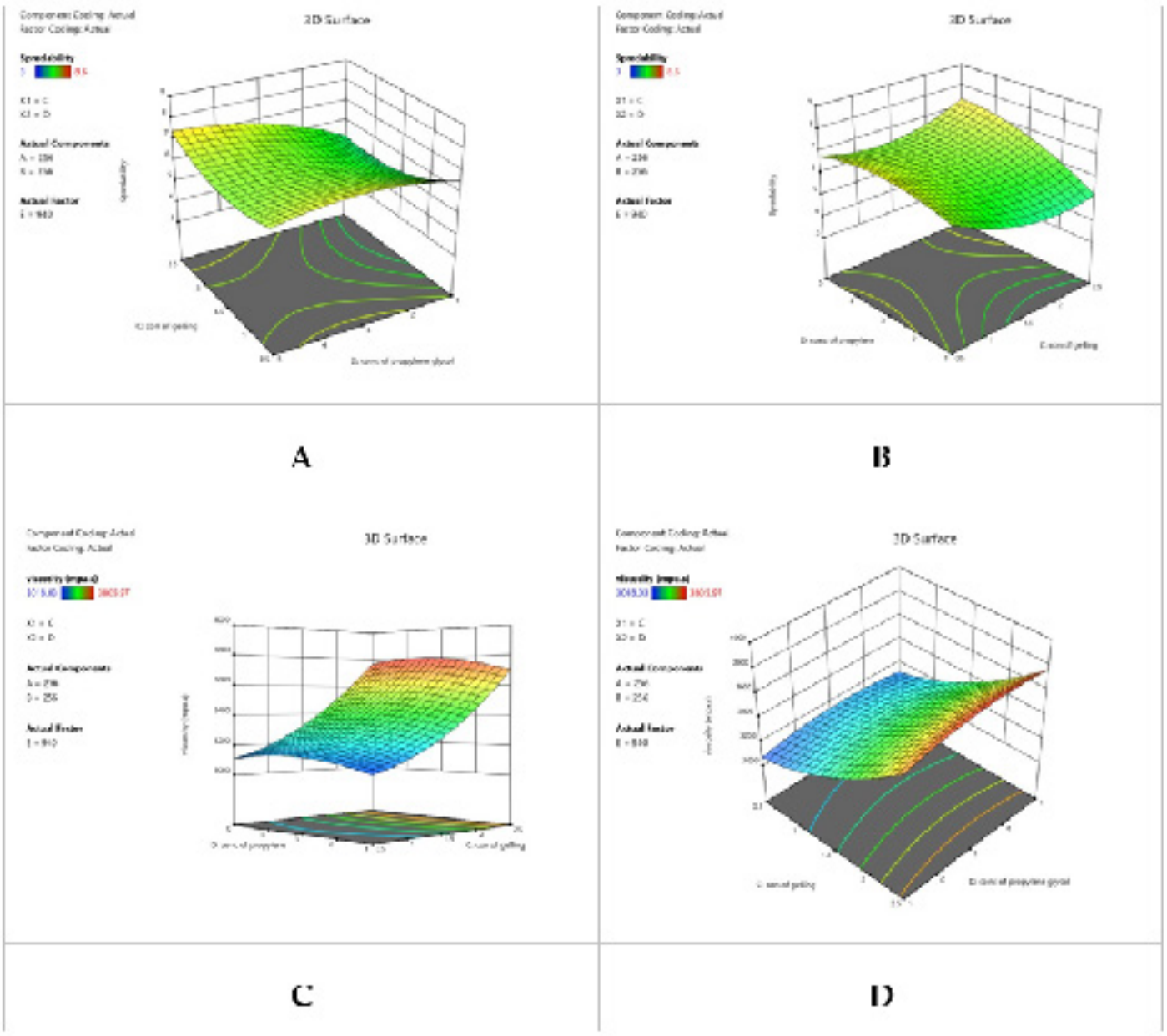
Multiple regression analysis was used to find the following polynomial equation:

MRSA=+15.88×A+22.34×B+17.17×AB+0.5670×AC-3.33×AD-3.30×AE (4)

PA=+15.97×A+21.67×B+5.40×AB+0.3194×AC+0.4496×AD-0.1055×AE (5)

E coli=+9.73×A+18.88×B+17.65×AB+1.30×AC+1.76×AD-1.56×AE (6)

The statistical Equation 4, 5, 6 demonstrated that positive values had a synergistic effect on the response and negative values had an



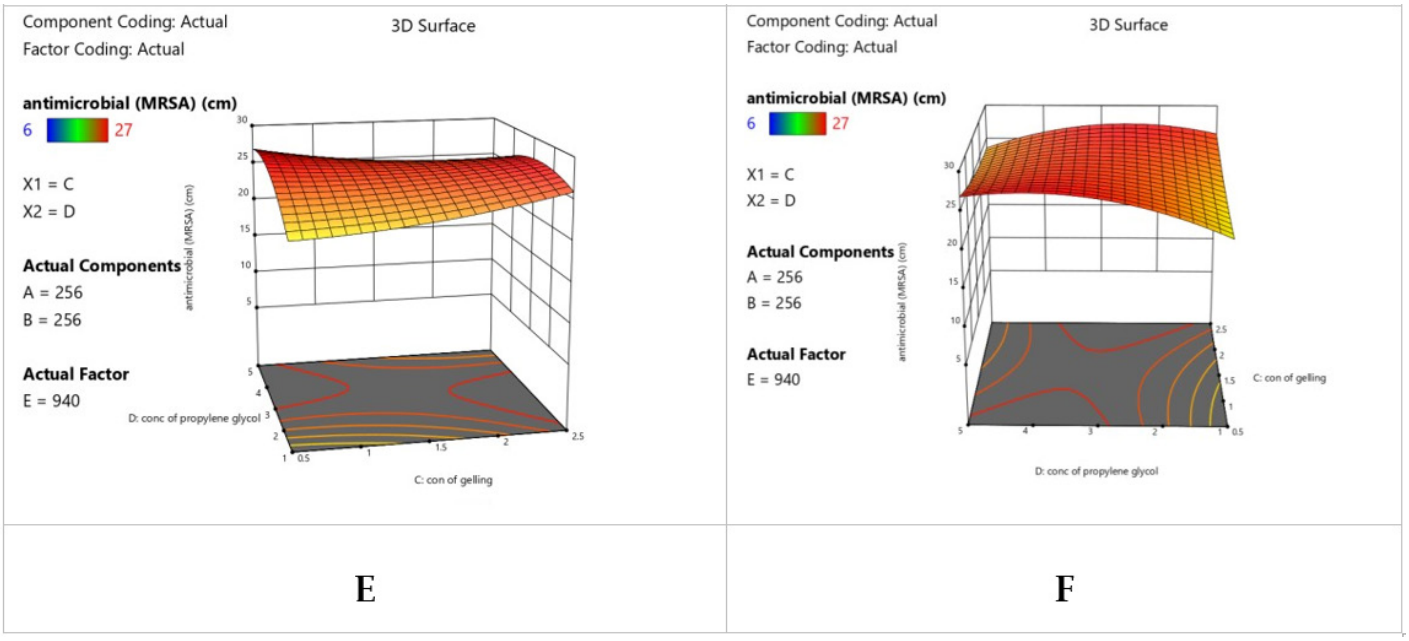


Figure 2: 3D Response surface plot (A) Effect of propylene glycol on spreadability (B) Effect of Carbopol concentration on spreadability (C) Effect of Carbopol concentration on viscosity, (D) Effect of propylene glycol on viscosity (E) Effect of Carbopol concentration on antibacterial activity (F) Effect of Propylene glycol concentration on antibacterial activity.

antagonistic effect on the response, where 'A' is the concentration of Black tea AgNPs and 'B' is the concentration of *Baliospermum solanifolium* AgNPs.

Figure 2E shows effect of polymer grades on the antibacterial activity of gel. Higher-grade polymers have a greater antibacterial impact than lower-grade polymers. The Carbopol 940 dispersion is more acidic than the Carbopol 934 dispersion. This is because the pentaerythritol allyl ether cross linking of Carbopol 940 is more acidic than the sucrose allyl ether cross linking of Carbopol 934. The rate of microbial mortality increased as polymer concentration increased (Figure 2F). The antibacterial activity of gel is affected by its propylene glycol concentration. Propylene glycol can alter the characteristics of microbial cells by replacing or dissolving lipids in microbial cell membranes, resulting in changes in membrane area and fluidity.

Effect of concentration of black tea and *Baliospermum solanifolium* silver nanoparticles concentration on antimicrobial activity are shown in Figure 3. Summary Statistics for all Response Variables are given in Table 4.

Optimized formulation

The D-optimal design optimised factors included 256 g/gm black tea, 256 g/gm *Baliospermum solanifolium* AgNPs, 1 g gelling agent concentration, 3% w/w propylene concentration, and 940 gelling agent type. The Optimised formulation of Black tea and *Baliospermum solanifolium* Nanoparticles Gel (OBBNG) was selected for further *in vivo* experiments (Table 5).

Antifungal activity of Optimized formulation of Black tea and *Baliospermum solanifolium* Nanoparticles Gel (OBBNG)

The agar well diffusion method was used to investigate OBBNG's ability to suppress the growth of pathogenic fungus such as *A. niger* and *Candida albicans*. The wells were filled with OBBNG and inhibition zone was measured to evaluate antifungal activity after 48 hr of incubation at 37°C. The inhibition zone diameters achieved for *A. niger* and *C. albicans*, which can be seen in Figure 3.

In vitro drug release study of OBBNG

In vitro drug release studies were conducted using OBBNG, and cumulative percentages of drug release were estimated by plotting time on the x-axis versus cumulative percentage of drug release on the y-axis. Percentage of release, which was calculated to be 71.51%.

Ex vivo permeation study

Ex vivo skin permeation tests on chicken skin were conducted by OBBNG. For 3 hr, the cumulative percentage of medication absorbed through the skin for gel loaded with silver nanoparticles was reported 68.18%.

In vivo wound-healing efficacy

Body Weight

The body weights of the rats were monitored at various time intervals. During the experimentation period, there was no significant change in groups 2, 5, however rats in groups 3, 4 had a modest decrease in body weight. The results showed that the

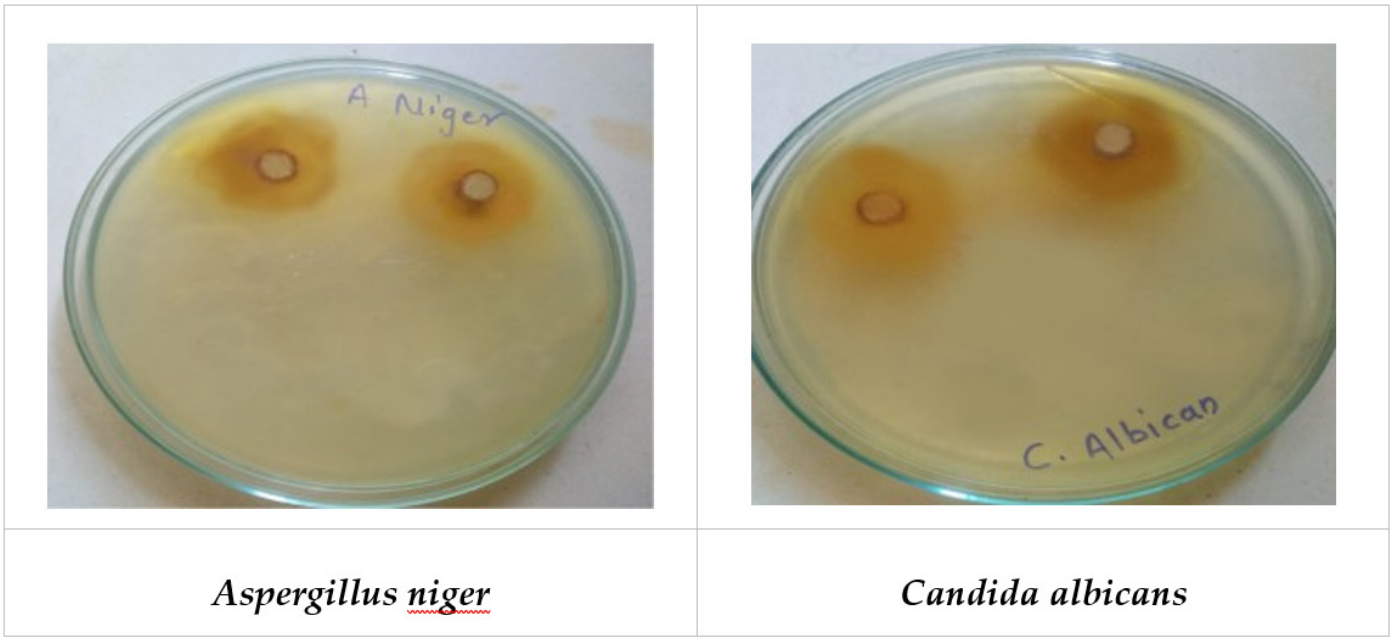


Figure 3: Zone of inhibition by OBBNG.

Table 3: MIC and MBC of Mixture of black tea and *Baliospermum* AgNPs.

Sl. No.	Concentrations of AgNPs (mg/mL)		*Staphylococcus aureus*	
	Black tea	*Baliospermum*	MIC	MBC
1	0.062	0.031	+	+
2	0.125	0.0625	–	+
3	0.25	0.125	–	–
4	0.5	0.25	–	–
5	1	0.5	–	–
6	2	1	–	–
7	4	2	–	–
8	8	4	–	–

Positive (+): Turbidity indicating growth; Negative (–): No turbidity indicating absence of growth.

body weight of the rats in all groups decreased in the first week, indicating that the rats were harmed by the wound induction, but in the second week, the weight of groups 2, 3, 4, and 5 gradually increased. In terms of body weight, the control group was the most affected.

- Clinical Signs Observations and Mortality.
- There was no evidence of morbidity or mortality.

Skin Irritation

After OBBNG application, no signs of redness, dryness, or flakiness.

Determine average lesions score of wound

Wound of Group 1 (Control) was determined to be more serious than all other groups. Erythema was observed at the wound site on the 21st day. In group 2, there was no evidence of a lesion. Groups 3, 4, and 5 had erythema, although not as much as the control group. The wound lesions score was substantially different (**p<0.0048) for all groups when compared to the control. The results of the wound lesion score are reported in Table 2 with standard deviations.

As a result, the wound surface area gradually decreased in all groups. Group 2 had the fastest rate of decrease in wound surface area, followed by groups 5, 4, and 3. The control group, group 1, had a slow rate of decrease in wound surface area (Figure 4). Overall, the data showed that the change in surface area did not follow a consistent pattern. Regardless of treatment method, all groups improved in wound surface area by the end of the research. When compared to group 1, groups 2, 3, 4, and 5 revealed statistically significant differences. When compared to the other groups, Group 2 performed the best.

The Wound recovery was studied between Groups 1, 2, 3, and 4. Wounds dressed with OBBNG or SSC healed significantly faster and showed visible indications of cutaneous repair. The rate of wound healing in the control group was consistently lower. The percentage of wound healing in the OBBNG group was substantially higher than in the BTNG, BSNG, reference standard, and control groups ($p<0.05$).

Bacterial burden in wound

Bacterial burden in wound study data revealed that wounds treated with OBBNG had a faster reduction in bacterial count, allowing for a speedier recovery (Figure 4). The difference between the control and the other groups is significant (***$p<0.001$).

Histological Evaluation of Healed Wounds

According to Figure 4, unhealed skin was clearly visible in skin samples from group 1, where the epidermis was badly injured and no reformation was evident. A varied degree of inflammatory cell infiltration with lymphocyte buildup was detected, and the damage was severe. Furthermore, significant localised cutaneous inflammation with intercellular edema was found. Groups 2 and 5 had a well-stratified epidermis, complete repair of epidermal layers, and complete basal, spinosum, granular, and grain layers. Groups 3 and 4 showed mild epidermal injury and partial recovery. There is no evidence of malignancy in any group.

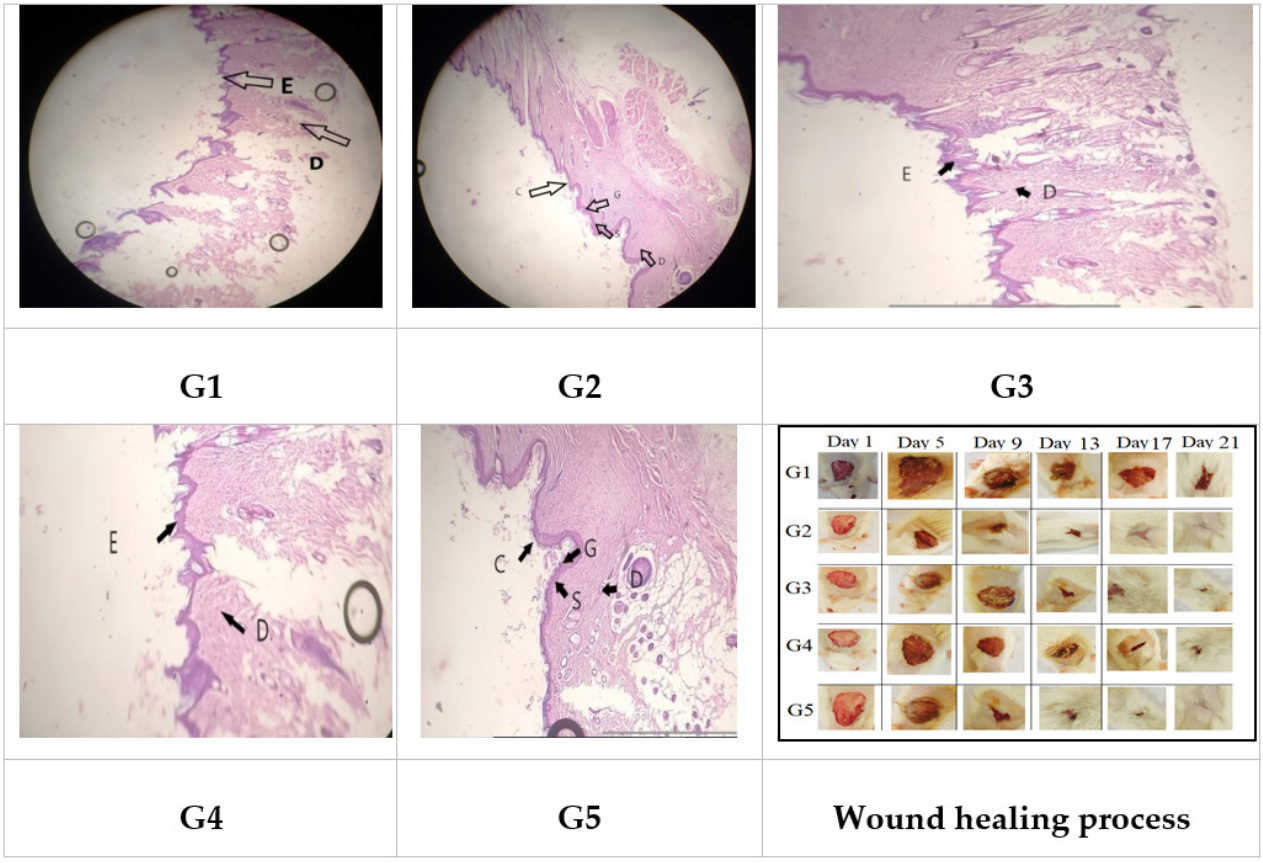


Figure 4: Light microscopy images of representative skin samples (stained with hematoxylin and eosin) after 21 days of treatment (G1) Control group-untreated (G2) OBBNG-treated group (G3) BTNG-treated group (G4) BSNG-treated group (G5) SSC-treated group. Arrows refer to lymphocytes. C, G, S, D, and E represent stratum corneum, stratum granulosum, stratum spinosum, dermis, and epidermis, respectively.

Table 4: Summary Statistics for Response Variables.

Response variable		Predicted model	F value	*p*-value (Prob>F)	R-squared	Adjusted R-squared	Predicted R-squared
Antimicrobial Activity	MRSA	Quadratic	27.66	0.0001	0.9863	0.9506	0.7285
	PA	Quadratic	51.58	0.0001	0.9926	0.9735	0.7136
	E coli	Quadratic	43.65	0.0001	0.9913	0.9686	0.6806
Spreadability (cm)		KCV	7.11	0.0001	0.8007	0.6881	0.5184
Viscosity		KCV	58.20	0.0001	0.9705	0.9538	0.9137

Table 5: Optimized formulation of Black tea and *Baliospermum solanifolium* nanoparticles gel.

Component	Name	Concentration
A	Black tea AgNPs	256.00
B	*Baliospermum solanifolium* AgNPs	256.00
C	Conc of gelling	1.50
D	Conc. of propylene glycol	3.00
E	Type of gelling	940

DISCUSSION

This work successfully demonstrated a quick and environmentally friendly green synthesis of silver Nanoparticles (AgNPs) using black tea and *Baliospermum solanifolium* extract.[7] The distinctive Surface Plasmon Resonance (SPR) peak observed in UV-vis spectroscopy verified the rapid synthesis of stable colloidal AgNPs, emphasizing the potency of the phytocompounds in the combined extract as capping and reducing agents. In particular, it was discovered that the extract of Black Tea had polyphenol chemicals such gallic acid, ellagic acid, and catechins, whereas the extract of *Baliospermum solanifolium* contained quercetin, ellagic acid, and catechins. As a well-known mechanism in green nanotechnology, these plant-derived biomolecules help reduce silver ions (Ag+) to metallic silver (Ag^0) and stabilize the resulting nanoparticles by avoiding their aggregation.

The synthesized AgNPs showed promising antifungal and antibacterial properties.[10,18,25] The phenolic compounds found in the extracts, such as quercetin, gallic acid, ellagic acid, and catechins, may exhibit a variety of antimicrobial effects.[7]

By limiting bacterial infectivity at the wound site, AgNP-loaded gels create a sterile environment that promotes active wound healing. A potential, eco-friendly delivery method for treating bacterial infections and encouraging tissue regeneration is provided by this combination of artificial AgNPs created from natural sources in a hydrogel matrix.[1-5]

In summary, this study develops a simple, easy, and effective green synthesis method for creating stable silver nanoparticles utilizing *Baliospermum solanifolium* and Black Tea extract. The resultant AgNPs showed significant antifungal and antibacterial properties. This implies that they have enormous potential for use in several biomedical fields.

CONCLUSION

Formulation and evaluation of a polymeric gel loaded with silver nanoparticles was the aim of study. Extract from black tea and *Baliospermum solanifolium* was used to reduce silver nitrate and it was also act as capping and binding agent. Silver nanoparticles made from black tea and *Baliospermum solanifolium* extract were added to gel. The phytochemicals and Ag+ ions show better antioxidant, antibacterial, anti-inflammatory and wound healing activity by working synergistically.

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ABBREVIATIONS

OBBNG: Optimised Black tea and *Baliospermum solanifolium* Nanoparticles Gel; **AgNPs:** silver nanoparticles; **RPM:** Revolution per minute; **XRD:** X-ray Diffraction; **RSM:** Response surface methodology; **AgNO3:** Silver nitrate, **FESEM:** Field Emission Scanning electron microscopy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUMMARY

Silver nanoparticles synthesized by biosynthesis method is rapid, environment friendly and poses more accurate sizes, morphologies, and antibacterial effectiveness. Combined action of Ag+ ions and phytochemicals enhances the anti-inflammatory, antibacterial, wound-healing, and antioxidant properties. In this study, polymeric gel containing silver nanoparticles was used for wound healing in a rat model. This formulation demonstrated possible use in treating skin wounds.

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