Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14,issue.6s (2025)
https://www.jneonatalsurg.com

"Biogenic Synthesis Of Silver Nanoparticle Of Polemonium Reptans Extract And Its Antimicrobial Activity"

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Cite this paper as Ankit Mishra, Har Govind Garg, Govind Nayak, Priyanka Namdeo, Surendra Kumar Jain..(2025) "Biogenic Synthesis Of Silver Nanoparticle Of Polemonium Reptans Extract And Its Antimicrobial Activity". .Journal of Neonatal Surgery, 14, (6s), 931-939

ABSTRACT

Polemonium reptans, belonging to the family Polemoniaceae is usually known as Jacob's ladder in Indian traditional medicine. It has been found to exhibit anti-microbial properties and used in the treatment of tuberculosis, whooping cough, fever, infertility, epilepsy, endometritis and insomnia. The present study focuses on the green synthesize of silver nanoparticle, which was conducted by Polemonium reptans extract and evaluating their antibacterial activity against E.coli. From all the formulations Formulation 4, was considered as the optimized formulation with 98.2 nm of particle size and -35mv zeta potential. The spectrum of UV–visible of the prepared Ag-NPs shows an absorption peak at 688 nm due to the excitation of surface plasmon resonance (SPR). SEM image showed Ag-NPs have a spherical shape. The result of antibacterial activities showed that Ag-NPs synthesized had an inhibiting activity against E.coli, Where the inhibition zone was 7 mm, 13 mm and 17 mm respectively. The results revealed that Ag nanoparticles were synthesized, formulated and optimized successfully...

Keywords: *Polemonium reptans, Silver nanoparticle, Green synthesize, E.coli, Particle size, SEM Analysis.*

1. INTRODUCTION

"Nanotechnology" is the newest and one of the most promising and active areas of modern research. The technology deals with the design, synthesis, and manipulation of particles size ranging from 1–1000 nm (Mahasneh, 2013). Within this size range, the chemical, physical, and biological properties change in the fundamental way of both individual atoms and their corresponding bulk material. This very small size increases the surface area-to volume ratios of particles. The nanoparticles synthesized using plant extract have gained huge consideration in recent years due to their remarkable properties and wide range of applications in catalysis, plasmonic, optoelectronics, biological sensor, water treatment, pharmaceutical applications, and agriculture and crop protection.

Recent years researchers are interested on developing efficient method for the large-scale synthesis of nanoparticles (NPs). Nanomedicine is a rapidly developing and promising field that makes best use of inert metals like silver, gold and platinum to synthesize metallic nanoparticles with high therapeutic potential for various biomedical applications. Among all metal nanoparticles, silver nanoparticles (AgNPs) have much attention due to the surface plasmon resonance (SPR) (strong absorption in the visible region), which can be easily observed by UV– visible spectrophotometer.

Nowadays, a fast-growing field in science and nanotechnology is the formation of remarkable materials known as nanoparticles. Such materials have a vital role in different human activities due to their unique size properties that are more influential than Ag ions as proved in different

investigations. Nanoparticles can be produced by several chemical and physical approaches,

which have shown high efficiency in the production of nanoparticles. However, such techniques have disadvantages, such as the high cost of large-scale production and expected environmental pollution. Recently, the production of nanoparticles using biogenic materials such as plants, microbes and natural biomolecules, which is known as green chemistry, could be considered as an alternative technique to other methods due to their eco-friendly nature .Different literature has extensively described the ability of plant extracts and microbes for the positive production of AgNPs. **(Ahmed *et al.*, 2023).**

Silver Nanoparticles

Among the various metallic nanoparticles, AgNPs are one of the most promising products in the nanotechnology industry. The development of consistent processes for the synthesis of AgNPs is an important field of current nanotechnology research. AgNPs have a wide range of use because of their unique characteristics such as optical, electrical and magnetic properties, which can be incorporated into antibacterial, antiviral, and antifungal applications, composite fibers, biosensor materials, cosmetic products, food industry uses, and electronic components. The AgNPs are also reported as medical and pharmaceutical agents that have directly encountered by a human system in such products as shampoos, detergents, soaps, toothpaste, and cosmetics. The biomedical use of AgNPs includes their application as antibacterial, antifungal, anti-inflammatory,antiviral, and anti-diabetic agents.

Synthesis of Nanoparticles

The nanoparticles are synthesised by various methods that are categorised into bottom-up or top- down method. A simplified representation of the process is presented in Figure 4.

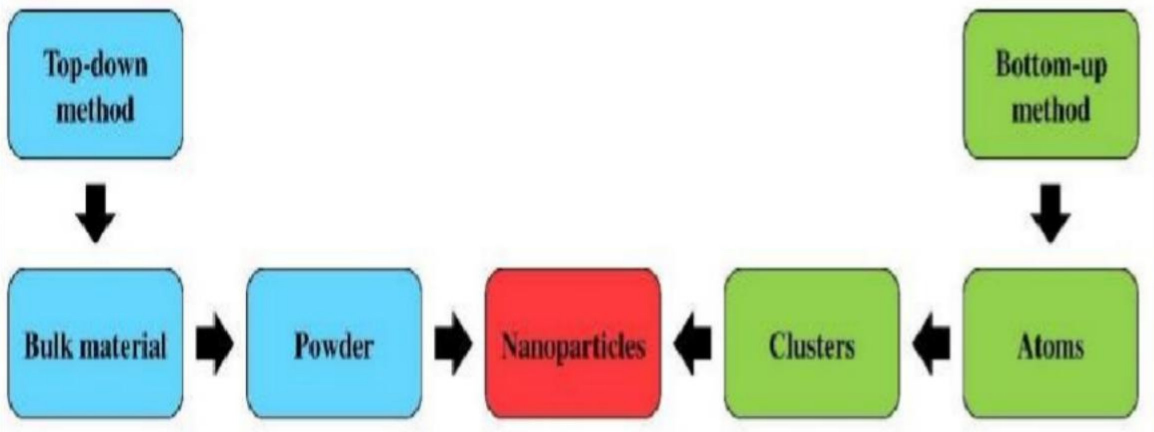

Figure 1: Synthesis of Nanoparticles

Factors Influencing the Synthesis of Silver Nanoparticles

Methods for the production of silver nanoparticles

There are many methods to manufacture nanoparticles, including physical and chemical techniques and biological protocols. Various organic or inorganic chemicals as well as living organisms are used for the synthesis of nanoparticles in these methods.

Temperature

Temperature has been found to be an important factor for the production of nanoparticles. Spherical nanoparticles are synthesized in the presence of elevated temperature. In contrast, nanotriangle formation occurs mostly at lower temperatures **(Rahimi-Nasrabadi*et al.*, 2014).**

PH

Most studies suggest that nanoparticle stability is improved in basic media than acidic. However, a very high pH (pH > 11) was found to have some drawbacks such as the formation of agglomerated and unstable silver nanoparticles **(Tagad *et al.*, 2013).**

Time

Decreasing the reaction time (minutes–hours) is another factor affecting the reduction of ions to a bulk metal with variant shapes. The optimum time period results in higher concentrations of nanoparticles in the medium, indicated by high absorbance peaks.

Shape and size

The shape and size of nanoparticles are crucial in determining their properties. It has been concluded that optimal activities are determined by the shape and size of the nanoparticle and most properties of nanoparticles are size-dependent **(Venil *et al.*, 2020).**

Applications

Antibacterial Activity: - Either killing or reducing the growth of bacteria without affecting surrounding cells is known as antibacterial activity. Ag is preferred as nanoparticle for the reason that it has antibacterial property and non-toxic to human beings. AgNPs are able to overcome the resistance that has been due to antibiotics. **(Baker *et al.*,.2005).**

Antiviral Activity: - In the whole world, viral infections and disease are found to be very common, so it's very important to make antiviral agents that results in showing prominent results. **(Trefry&Wooley 2013).**

Dental materials: - The oral cavity is an active ecosystem. Microorganisms, including pathogenic microorganisms, often colonize the mouth, thus causing contamination of dental materials and implants, as well as increase the risk during the colonization processes **(Pokrowiecki *et al.,* 2017).**

Anti-inflammatory Activity: - Inflammation is the state in which some part of the body becomes swollen, red, hot and sometime painful also and this may occur due to certain injury or sometime infection also.

Anticancer Activity: - Cancer is basically an uncontrolled growth of cells in specific area in a body. It is well known that treatment of cancer includes chemotherapy, surgery, radiation therapy; targeted therapy is very expensive and painful also **(Gurunathan *et al.,* 2015)**

2. MATERIAL AND METHODS

Soxhlet extraction and yield determination of Plant material

Dried and powered whole plant of *Polemonium reptans.* Successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using 30% methanol (hydroalcoholic) solvent system at 40-60ºC temperature of the heating mantle for 8-10 hours. After the extraction process, the extract of sample was filtered and concentrated to dryness. Extracts were collected in air tight container **(Alara *et al.,* 2019).** Extraction yield of all extracts were calculated using the following equation below:

Yield= Actual yield X 100 / Theoretical yield

Phytochemical testing was performed to identify presence or absence of different phyto constituents in extracts of *Polemonium reptans* using standard procedures.

Organoleptic Properties

Organoleptic properties were observed by visual observation. The organoleptic studies of *Polemonium reptans* like general appearance like appearance, color, odor, state etc. were observed/ performed.

Solubility study

Qualitative solubility of *Polemonium reptans* different solvents was determined according to USP NF, 2007 and Indian pharmacopoeia. Approximately 1 mg of *Polemonium reptans* was weighed and transferred into a 10 ml test tube; then, it was dissolved in the respective solvents (1 ml each of methanol, DCM, Distilled water, chloroform and acetone).

Fourier transmission Infra-Red Spectroscopy

FT-IR spectrum of extract was recorded over the range of 4000 to 400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of extract and 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 region (**Chowk, M. I. 2020)**

Formulation of Silver nanoparticle

Preparation of 1mM AgNo3 solution

For preparation of 1mM AgNo3 solution we have to take 0.016gm AgNo3 and dilute it with 100ml of distilled water with continues stirring.50 ml (1mM) aqueous solution of silver nitrate was prepared in conical flask with continuously stirring for 15 minute. Then five dilutions of *Polemonium reptans* extract was prepared in water (100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) About 1 ml of each filtrate was taken into a beaker and 9 ml of 1mM AgNo3 added and continuously stirring for 15 minutes. The solution was kept in dark chamber until solution color changes to dark yellow to brown color. After, 15 min, the solution turns dark yellow to Brown color it indicates the formation of silver nanoparticles. The bio reduction of silver ions was monitored by periodic sampling by the UV visible spectrophotometer.

Table 1: Composition of silver nanoparticle formulation

S. no.	*Polemonium reptans* (mg/ml (Each 1 ml)	Silver nitrate solution (ml)	Stirring (time)
1	100	9.0	15
2	75	9.0	15
3	50	9.0	15
4	25	9.0	15
5	12.5	9.0	15

Characterization of Silver nanoparticle:

Color change

Color change in the preparation of nanoparticle section was monitored at different interval of 30 min, 60 min, 120 min and 180 min.

UV-Visible spectrophotometric analysis

The primary characterization of the synthesized nanoparticles was performed using UV-visible spectroscopy by measuring the UV-visible spectrum of the reaction mixture at 200–800 nm wavelength by sampling the aliquots withdrawn from the reaction mixture at different time intervals of 30 min, 60 min, 120 min and 180 min.

Observations:

Surface Plasmon resonance at 300 to 800 nm was representing nanoparticle synthesis.

Analysis was help to identify the time of nanoparticle synthesis initiation and progressive increase in intensity of peak was help to ascertain the extent of nanoparticles formed.

Particle size of Silver nanoparticle

The particle size is one of the most important parameter for the characterization of nanoparticle. The size of nanoparticle was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette. The size data is documented in Table 11 (**Balla and Goli *et al*., 2020).**

Zeta potential of Silver nanoparticle

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the nanoparticle was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements. The zeta potential data is documented in Table 12**.**

Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the optimized nanoparticle were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pre-treated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography.

Antibacterial activity of Ag nanoparticles by Well diffusion assay

Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

Well Diffusion Assay

The bacterial suspension of *E. coli* was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate **(Mohammadi- Sichani *et al.*, 2012).** The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the AgNo3, AgNPs and extract (1mg/ml) solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well.

Stability study

The silver nanoparticle formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing (25^0 C±2^0 C and 60 ± 5% RH) and (40^0 C±2^0 C and 70 ±5% RH) for 3 months. The formulation was checked for Physical appearance like color, order, appearance, and particle size 30, 45, 60, 90 days (3 month). Formulation was analyzed for the change in Physical appearance like color, Odor, appearance and particle size studies. All Results were compared against final formulation of 0 days as the reference.

3. RESULTS AND DISCUSSION

Percentage yield

Table 2: Percentage yield of extracts

S. No	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Polemonium reptans*	Pet. Ether	Yellow	250	0.531	0.212
		Methanol	Brown	237.64	8.35	3.513

Table 3: Phytochemical analysis of *Polemonium reptans* Extract

S. No.	Experiment	Results	
		Petroleum ether	Methanolic
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	-
2.	Hager's Test	-	-
Test for Terpenoids and steroids			
1.	Salkowski Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	+	+
2.	Alkaline Reagent T	-	+
Test for Tannins and Phenolic Compounds			
1.	FeCl3 Test	+	+
2.	Lead Acetate Test	+	+
Test for Saponins			
1.	Foam test	+	+

Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	-	+

Organoleptic properties

Organoleptic properties of *Polemonium reptans*

The plant extract organoleptic qualities, including color, odor, and appearance was conducted. Extract was discovered to have a reddish-colored to it when tested. Plant extract has an Offensive odor and has a solid state according to research conducted on

Solubility study

The solubility of *Polemonium reptans* extract was determined in various non-volatile or volatile liquid vehicles such as DCM, methanol, Acetone, chloroform, and water shown in Table 6. From the results, it was observed that the drug is freely soluble in methanol and sparingly soluble in chloroform and Soluble in water, DCM.

Fourier transmission Infra-Red Spectroscopy

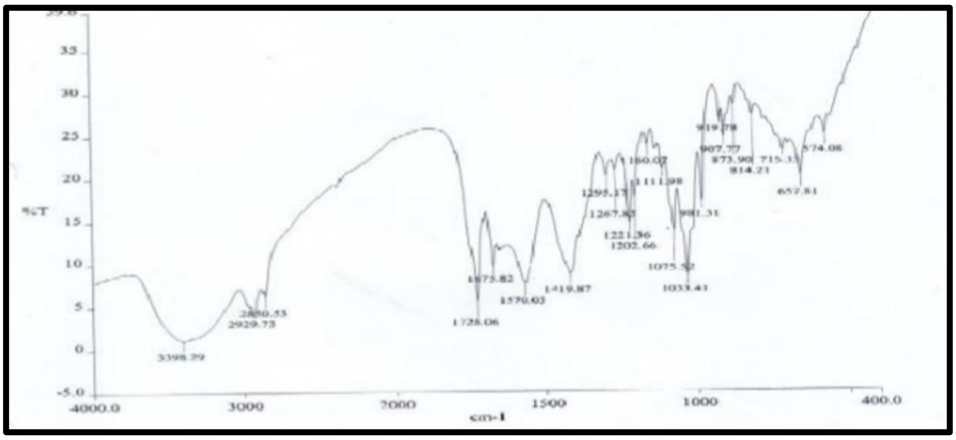

Figure 2: FTIR of extract

UV-Visible spectrophotometric analysis

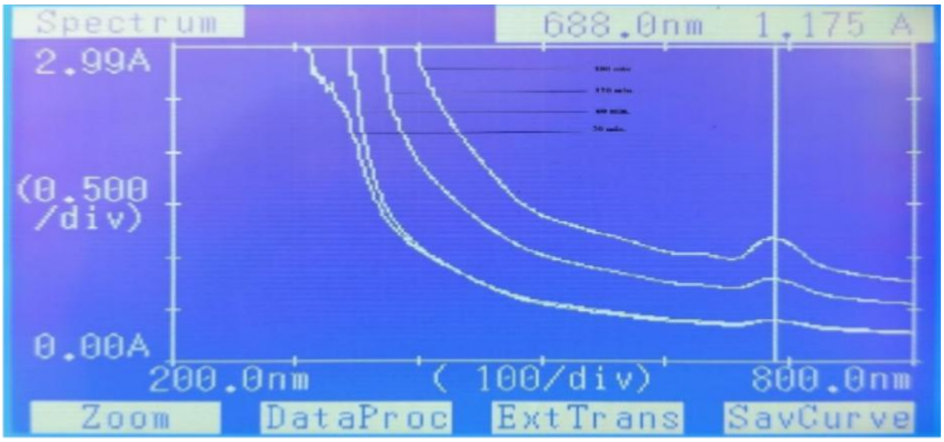

Figure 3: UV Peak detection after (F4)

The synthesized SNPs showed the following absorption spectrum at the wavelength range of 300- 800 nm. The surface Plasmon resonance peak at range 300 to 800 nm will confirm the formation of silver nanoparticle as shown in above Figure UV analysis of silver nanoparticle. Surface Plasmon resonance at 688.0 nm (F4) was representing best nanoparticle synthesis. Analysis was help to identify the time of nanoparticle synthesis initiation and progressive increase in intensity of peak will help to ascertain the extent of nanoparticles formed.

Particle size determination

The particle size is one of the most important parameters for the characterization of nanoparticles. The average particle sizes of the prepared silver nanoparticle formulation were measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of nanoparticles was found to be range between 98.2 to 552.4 nm. These particle size values indicate that the all-formulated nanoparticle is under the range (Below 1000 nm) of nanoparticle and F4 is the lowest particle size of all formulation.

Zeta potential

Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range -16.7to -60.4 mV with peak area of 100% intensity. These values indicate that the all-formulated nanoparticle is table.

Table 4: Zeta potential of Silver nanoparticle

S. No.	Formulation	Zeta potential (mV)
1	Nanoparticle (F1)	-45.6 mV
2	Nanoparticle (F2)	-60.4 mV
3	Nanoparticle (F3)	-37.9 mV
4	**Nanoparticle (F4)**	**-35.0 mV**
5	Nanoparticle (F5)	-16.7 mV

Results of antimicrobial activity of Ag nanoparticles F4 formulation

Antimicrobial activity of Ag Nanoparticle of against *E.coli*

Table 5: Antimicrobial activity of Ag Nanoparticle against *E.coli*

S. No.	Sample name	Zone of Inhibition (mm)
1	AgNO3	7 mm
2	Extract	13mm
3	Silver NPs	17mm

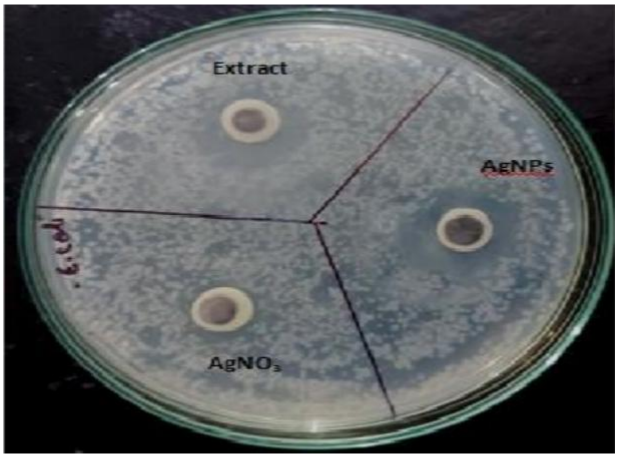

Figure 4: Antimicrobial activity against *E. coli*

The stability study of the silver nanoparticle formulation was conducted over a period of 90 days under two controlled storage conditions: 25 ± 2 °C with 60 ± 5% relative humidity (RH) and 40 ± 2 °C with 70 ± 5% RH, to assess the formulation's physical and particle size stability.

In terms of particle size, the nanoparticles showed only minor and negligible variations, remaining close to the original size of 98.2 nm. At 25 °C, particle size fluctuated slightly between 98.1 nm and 98.6 nm, while at 40 °C, it ranged between 98.0 nm and 98.9 nm. Overall, the results demonstrate that the silver nanoparticle formulation exhibits good stability in terms of physical characteristics and particle size under both ambient and accelerated storage conditions, making it a reliable and stable formulation for potential long- term use.

4. SUMMARY AND CONCLUSION

Ag nanoparticles were synthesized successfully by green synthesis methods From *Polemonium reptans* extract, respectively. The detail characterization of the nanoparticles was carried out using UV-Vis spectroscopy, particle size analysis, Scanning Electron Microscopy (SEM), From Dynamic Light Scattering (DLS) particle size and SEM image analysis, the average particle size was found to be range below 1000 nm Ag. Antibacterial potential of Ag nanoparticles as a function of nanoparticles concentration was tested against four different bacteria like Escherichia coli. The test was performed by both Disc diffusion assay and colony forming unit (CFU) estimation method. From the study, both types of nanoparticles were observed to have strong antimicrobial potential.

The growth study of *Escherichia coli* was carried out in presence of different concentration of both nanoparticles to observe the effect on the growth of the bacteria in liquid media. It was observed that both the nanoparticles strongly affected the specific growth rate of *E.coli*. It was also observed that the growth rate was strongly inhibited by the presence of small concentration of nanoparticles.

Stability Study of silver nanoparticle formulation (F3) for a period of 3 months at accelerated stability conditions (25^0C±2 ^{0}C and 60 ± 5% RH) and (40^0C±2 ^{0}C and 70 ±5% RH). Physicochemical parameters, including color, order, appearance, and particle size were not altered significantly. In conclusion, The 3-month stability study under accelerated conditions showed no significant changes in color, odour, appearance, or particle size, indicating excellent formulation stability. These findings suggest strong potential for safe and effective use in antimicrobial applications..

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