FORMULATION AND IN VITRO EVALUATION OF SILVER NANOPARTICLE CONTAINING HERBAL PLANT EXTRACT AND ITS ANTI-BACTERIAL ACTIVITY

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ABSTRACT

The study focused on the formulation and in vitro evaluation of silver nanoparticles (AgNPs) synthesized using the seed extract of *Abelmoschus ficulneus* and their antibacterial activity. Seeds of *Abelmoschus ficulneus* were collected, dried, and extracted using ethanol and petroleum ether, yielding 5.85% and 2.27% respectively. Solubility studies revealed that the extract was freely soluble in ethanol and petroleum ether, and soluble in water and DMSO. Phytochemical screening confirmed the presence of carbohydrates, glycosides, alkaloids, flavonoids, saponins, tannins, and steroids in the ethanolic extract. Organoleptic evaluation indicated a brownish-yellow appearance with a characteristic odor. Silver nanoparticles were synthesized using the plant extract, with visible color changes confirming formation. UV-Visible spectroscopy showed a surface plasmon resonance peak at 682.0 nm for the F2 formulation. SEM analysis confirmed spherical morphology and smooth surfaces. Particle size analysis revealed sizes ranging from 40.13 nm to 103.97 nm, with F2 exhibiting the smallest size. Zeta potential ranged from -6.8 mV to -22.2 mV, indicating good colloidal stability. Stability studies conducted over 90 days demonstrated that the F2 formulation maintained consistent particle size, appearance, and physical characteristics under both room and accelerated conditions. Antibacterial testing showed significant zones of inhibition by the AgNPs (15 mm for *E. coli*), surpassing the activity of both the crude extract and silver nitrate solution. These results suggest that *Abelmoschus ficulneus* seed extract-mediated silver nanoparticles possess potent and stable antibacterial properties, making them promising candidates for future antimicrobial formulations.

Keywords: *Abelmoschus ficulneus*, Silver nanoparticles, Phytochemical Screening, Antibacterial activity, SEM, UV-Vis spectroscopy, Particle size, Stability study.

1. INTRODUCTION

Silver nanoparticles have the properties of a high surface area, very small size (<20 nm) and high dispersion. Moreover, colloidal silver solutions (CSSs) have an increased interest due to their antimicrobial properties, with direct applications in pharmacology, veterinary medicine and so on. The interaction of metal nanoparticles with microorganisms (from fungi to viruses, e.g. HIV) is an expanding field of research **(Yaqoob *et al.*, 2020).** It is believed that the mechanism of the antibacterial effect of silver nanoparticles involve their absorption and accumulation by bacterial cells and accompanying shrinkage of the cytoplasm membrane or

its detachment from the cell wall. Upon binding of the nanoparticles to DNA, DNA molecules become condensed and lose their ability to replicate, which may be the main mechanism by which the nanoparticles inhibit the bacterial replications with **(Joshi *et al.*, 2020).** Understanding the forces governing the colloidal stabilities is an essential requirement for the preparation of colloidal nanoparticles. Particles can be stabilized either electrostatically or sterically. Sterically stabilization is accomplished by adsorbing either polymers or surfactants onto the surfaces of the particles to overcome nanoparticles agglomeration. While, the electrostatic stabilization is attained by capping with charged molecules, which form chemical bonds with/or chemisorbs onto the particles **(Sultana *et al.*, 2020).**

The enhanced antibacterial activity of Ag at the nanoscale has been most valuable in medical and healthcare areas, where the incorporation of AgNPs into hundreds of products has been studied, including surgical and food handling tools, clothing, cosmetics, dental products, catheters, and dressings **(Bruna *et al.*, 2021).** The potential of AgNPs as antibiotics is related to their various mechanisms of action, which attack microorganisms in multiple structures at a time and give them the ability to kill various types of bacteria **(Dakal *et al.*, 2016).**

The genus *Abelmoschus* (Family—Malvaceae) is one of the pharmaceutically important groups of the flowering plants. The species *A. ficulneus* L. has prominent role in the traditional medicinal practices due to the presence of prominent phytochemicals. *A. ficulneus* L. is an edible medicinal plant has wide range of medicinal applications **(Mohite and Gurav 2019).** The whole plant is used to treat sprain, bronchitis and toothache and *Abelmoschus* taxa have applications in industry. A. ficulneus L. were found to have a high flavonoid, saponin and phenolic contents, which are responsible for all pharmacological activities including jaundice and a major fatal disease.

Hence, the present work proposed to evaluate antibacterial activity of A. ficulneus L. by various experimental animals' models: in vitro studies.

2. MATERIALS AND METHODS

2.1 Chemicals

AgNO3, Ammonia, Nitroprusside and Sodium Hydroxide were obtained from Merck, a reputable supplier of analytical reagents. Research lab provided the Petroleum ether. Fizmerck Zinc provided the Conc. H2SO4. Molychem provided the Ethanol. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Clorofiltind**,** Research lab, and Himedia.

2.2 Plant seeds Collection

The seeds of the *Abelmoschus ficulneus* plant seeds were collected and dried in the shade at room temperature for three days. Dried plant seeds were preserved in sealed glass containers in a dry, cool environment to prevent contamination and deterioration. A plant taxonomist verified the authenticity and purity of the therapeutic herb *Abelmoschus ficulneus* **(Patil *et al.*, 2015).**

2.3 Extraction Process

A systematic protocol was followed to extract *Abelmoschus ficulneus* plant seeds utilizing a continuous hot percolation process using Soxhlet apparatus with ethanol and petroleum ether. The plant seeds were initially dried in the shade and ground into a fine powder. A weighed amount (300g) of powdered material was placed in a porous thimble and loaded into the

Soxhlet extractor. Non-polar compound extraction was performed first with petroleum ether at 60°C, followed by polar compound extraction with ethanol. The round-bottom flask was filled with 300-500 mL of the corresponding solvent and heated in a water bath. As the solvent evaporated, it condensed in the reflux condenser and continued to percolate through the plant material, dissolving the bioactive chemicals. The extraction procedure was repeated for 6-8 hours until the solvent in the siphon tube turned color less. Following extraction, the solvent was evaporated with a rotary evaporator, and the concentrated extract was dried at 40°C in a rotating vacuum evaporator (Buchi type). The dried extract was weighed, and its percentage yield was estimated using the following formula:

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

The obtained dried extract was then stored at low temperatures for additional phytochemical examination and organoleptic characteristics (percentage yield, color, and smell) **(Gul *et al.*, 2011).**

2.4 Solubility study

The qualitative solubility of *Abelmoschus ficulneus* in different solvents was examined using Indian pharmacopoeias. *Abelmoschus ficulneus* was weighed and placed in a 10 mL test tube, where it was dissolved in the relevant solvents (1 mL of methanol, DMSO, distilled water, chloroform, and acetone).

2.5 Phytochemical study

Preliminary phytochemical investigations or early phytochemical research were carried out on the seeds of *Abelmoschus ficulneus* to identify the presence of various bioactive compounds. The seed extract was prepared using standard extraction techniques, and qualitative screening was performed using established chemical tests. The parameters used for the investigation included tests for alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, and steroids. These phytoconstituents were identified based on color changes or precipitate formation during specific reagent reactions. The results confirmed the presence of several pharmacologically important compounds, suggesting the plant's potential for therapeutic applications **(Chumbhale *et al.*, 2013).**

2.6 Formulation of extract loaded silver nanoparticles

- **Preparation of 1mM AgNo3 solution**

To make a 1 mM AgNo3 solution, dilute 0.016 gm of AgNo3 with 100 ml of filtered water while stirring constantly. (A) A 50 ml (1 mM) aqueous solution of silver nitrate was prepared in a conical flask by constantly swirling for 15 minutes. The extract will next be diluted in water to five different concentrations: 100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml. A beaker was filled with approximately 1 ml of each filtrate, and then 9 ml of 1 mM AgNo3 was added while constantly stirring for 15 minutes. The solution was kept in a dark chamber until it changed color from dark yellow to brown. After 15 minutes, the solution changes from dark yellow to brown, suggesting the formation of silver nanoparticles. To quantify the bio reduction of silver nanoparticles, periodic sampling was performed using a UV visible spectrophotometer **(Suvandee *et al.*, 2022).**

Table 1: Composition of silver nanoparticle formulation

Formulations	*Abelmoschus ficulneus* (mg/ml) (Each 1 ml)	Silver nitrate solution (ml) 1mM	Stirring time(min.) 300 rpm
F1	100	9.0	20
F2	75	9.0	20
F3	50	9.0	20
F4	25	9.0	20
F5	12.5	9.0	20

2.7 Characterization of Silver nanoparticle:

2.7.1 Visual Analysis of Synthesized Silver Nanoparticles

The colour change in the preparation of nanoparticles component was examined at various intervals of 30 minutes, 60 minutes, 120 minutes, and 180 minutes **(Mourdikoudis *et al.*, 2018).**

2.7.2 UV-Visible spectroscopic analysis

To perform the primary characterization of the synthesized nanoparticles, the UV-visible spectrum of the reaction mixture at 200-800 nm wavelength was measured by sampling the aliquots taken from the reaction mixture at various time intervals of 30 minutes, 60 minutes, 120 minutes, and 180 minutes (as previously stated) **(Aziz *et al.*, 2017).**

Observations:

Surface Plasmon resonance at 300-700 nm was used to measure nanoparticle formation.

Analysis will aid in determining the start time of nanoparticle production, while the progressive rise in peak intensity will aid in estimating the size of the nanoparticles produced.

2.7.3 Scanning Electron Microscopic (SEM)

The morphological features of the optimized nanoparticle were acquired using the electron beam from a scanning electron microscope. A vacuum sputter coater was then utilized to coat the nanoparticles with a thin layer of metal (2-20 nm), such as platinum, palladium, or gold. The pretreatment specimen was then bombarded by an electron beam, which produced secondary electrons known as augers. To make surface topography images, Rutherford and Kramer's Law was utilized to select and process only the electrons scattered at a 90° angle from the interaction between the electron beam and the specimen's atoms **(Puchalski *et al.*, 2007).**

2.7.4 Zeta potential

Zetasizer Malvern tools were used to assess the nanoparticle after it had been diluted tenfold with distilled water **(Singh *et al.*, 2014).**

2.7.5 Particle size Distribution

The size of a nanoparticle is an important element to consider while characterizing it. The size of the nanoparticles was measured using the Malvern Zeta sizer (Malvern Instruments) **(Zielińska *et al.*, 2009).**

2.8 Stability study

The silver nanoparticle formulation's stability was tested over three months at accelerated temperatures of 25 ^{0}C ± 2 ^{0}C and 60 ± 5% RH, and 40 ^{0}C ± 2 ^{0}C and 70 ± 5% RH. The formulation's physical characteristics, such as color, order, appearance, and particle size, were assessed at 30, 45, 60, and 90 days. The formulation was tested for stability under accelerated storage conditions for three months, as per the International Conference on Harmonization (ICH) criteria. Color, odor, appearance, and particle size tests were used to check the

formulation for physical changes. To compare, all outcomes were contrasted with the final formulation at 0 days **(Patel *et al.*, 2017).**

2.9 Anti-microbial activity of Ag nanoparticles by Well diffusion assay

1. Preparation process of Nutrient Agar Media

A total of 2.8 grams of nutrient media was dissolved in 100 milliliters of filtered distilled water under sterile conditions. The pH of the prepared media was measured prior to sterilization to ensure suitability for microbial growth. Sterilization was carried out using an autoclave at 121°C for 15 minutes under a pressure of 15 psi. Once sterilized, the hot media was aseptically poured into sterile Petri plates within a laminar airflow cabinet. The plates were then left undisturbed inside the laminar flow until the agar medium completely solidified, ensuring minimal contamination **(Ramesh *et al.*, 2012).**

2. Well Diffusion Assay

E. coli bacterial suspensions standardized to 108 CFU/ml were added to the shaker. The broth's inoculums (108 CFU/ml) were removed using a micropipette and added to a sterile, solidified Agar Media Plate. To inoculate the agar plate, the inoculums were applied to the entire sterile surface using a sterile spreader. Four 6-mm wells were made in the solidified Agar Media Plate with a sterile cork-borer. The AgNo3, AgNPs, and extract (1 mg/ml) solution was then prepared to be infused into the wells. A sample of 100 µl was loaded. Before being incubated for 18 to 24 hours at 37°C, the infected Agar Media Plate was allowed to diffuse for approximately 30 minutes at room temperature. After incubation, plates were examined to see if a clear zone formed around the well, indicating that the tested formulation possessed antibacterial activity. Mm of the zone of inhibition (ZOI) were measured and examined. Zones were measured to the nearest millimeter with a ruler pressed against the back of an inverted Petri plate. A dark, non-reflective background was positioned several inches above the Petri plate. The diameters of the well and the zone of total inhibition (as seen with the naked eye) were measured **(Charannya *et al.*, 2018).**

3. RESULT AND DISCUSSION

3.1 Plant Collection

Table 2: Plant collection

Plant name	Plant part used	Weight
Abelmoschus ficulneus	Seeds	300 gm

3.2 Percentage Yield

The percentage yield is especially important in phytochemical extraction since it allows you to determine the standard extraction efficiency for a certain plant, different portions of the same plant, or different solvents. Table 5 shows the yield of the produced *Abelmoschus ficulneus* extract.

Table 3: Percentage Yield of crude extracts of *Abelmoschus ficulneus* seed extract

Plant name	Solvent	Color of extract	Theoretical weight	Yield(gm)	% yield
Abelmoschus ficulneus	Ethanol	Dark brown	283	16.57	5.85%
	Pet ether Ethanol	Brownish Yellow	300	6.82	2.27%

3.3 Solubility study

Table 4: Solubility study of *Abelmoschus ficulneus* seed extract

Extract	Solvents	Observation/Inference
Abelmoschus ficulneus	Distilled water	Soluble
	Chloroform	Sparingly soluble
	DMSO	Soluble
	Ethanol	Freely soluble
	Petroleum ether	Freely soluble
	Acetone	Very slightly soluble

3.4 Preliminary Phytochemical study

Table 5: Phytochemical testing of *Abelmoschus ficulneus* seed extract

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Ethanolic extract
Test for Carbohydrates		
Molisch's Test	(–)	(+)
Fehling's Test	(–)	(+)
Benedict's test	(–)	(+)
Barfoed's Test	(+)	(+)
Iodine Test	(–)	(+)
Glycoside		
Borntrager test	(+)	(+)
Killer-Killiani test	(+)	(+)
Tests for Alkaloids		
Dragendorff's Test	(+)	(+)
Mayer's Test	(+)	(+)
Wagner's Test	(–)	(+)
Hager's Test	(+)	(+)
Test for Flavonoids		
Shinoda Test	(–)	(+)
Test for Triterpenoids and Steroids		
Salkowski Test	(+)	(+)
Liebermann-Burchard Test for Triterpenoids and Steroids	(–)	(+)
Tannin and Phenolic Compound Test		
Ferric Chloride Test	(+)	(+)
Lead Acetate Test	(+)	(+)
Gelatin Test	(+)	(+)
Test for Saponin		
Foam Test	(+)	(+)
Froth Test	(+)	(+)

3.5. Organoleptic properties

Table 6: The Organoleptic Studies of *Abelmoschus ficulneus* seeds extract

Abelmoschus ficulneus	Study
Colour	Brownish
Odour	Characteristic
Appearance	Brownish Yellow

3.6 Evaluation parameter of Silver nanoparticle

3.6.1 Visible observation of synthesized Silver nanoparticle

Table 7: Visible observation of synthesized Silver nanoparticle

Formulation	Parameters	Observation
Silver Nanoparticle	Colour	Yellow to dark brown
	Odour	Characteristic
	Appearance	Liquid

3.6.2 UV-Visible spectrophotometric analysis

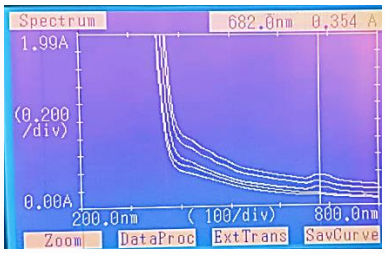


Figure 1: UV Graph of F2 Formulation

Table 8*:* UV peak detection

Silver nanoparticle Formulations	Peak detection
SNPs (F2)	682.0 nm

3.6.3 Scanning Electron Microscopic (SEM)

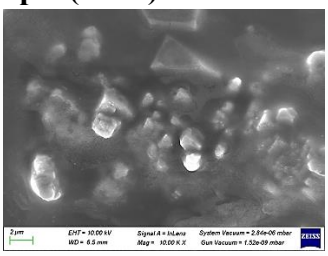
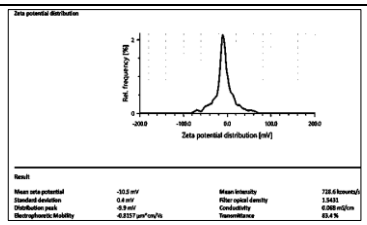
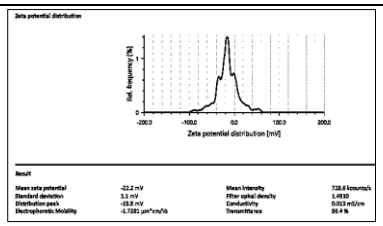


Figure 2: SEM (F2)

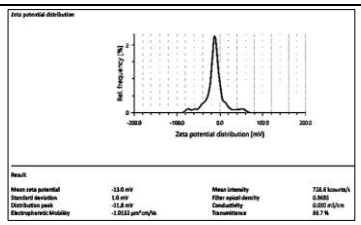
3.6.4 Zeta potential



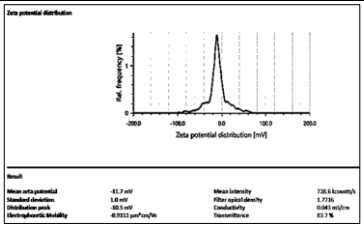
Graph 1: Zeta potential (F1)



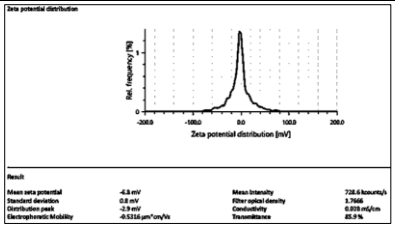
Graph 2: Zeta potential (F2)



Graph 3: Zeta potential (F3)

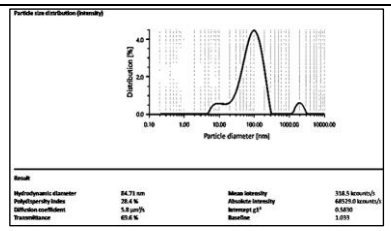


Graph 4: Zeta potential (F4)

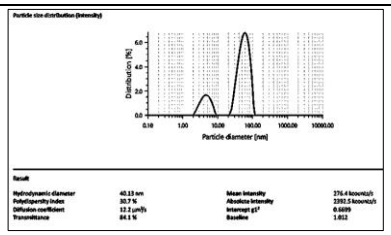


Graph 5: Zeta potential (F5)

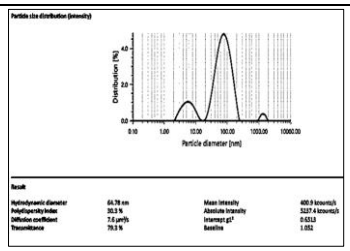
3.6.5 Particle size



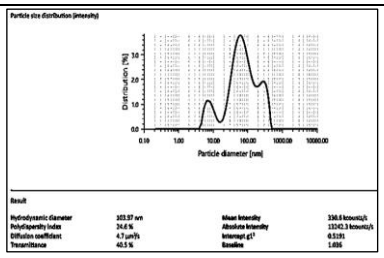
Graph 6: Particle size (F1)



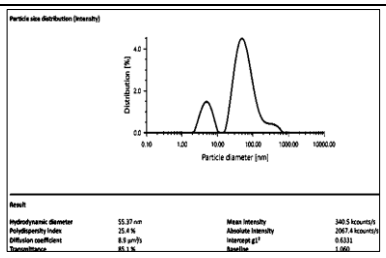
Graph 7: Particle size (F2)



Graph 8: Particle size (F3)



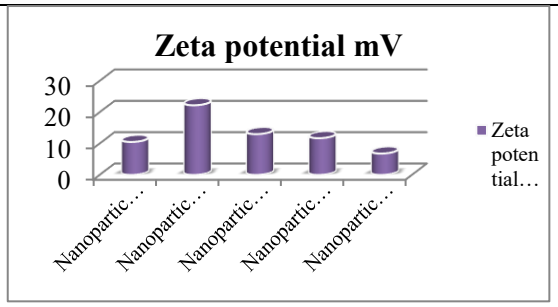
Graph 9: Particle size (F4)



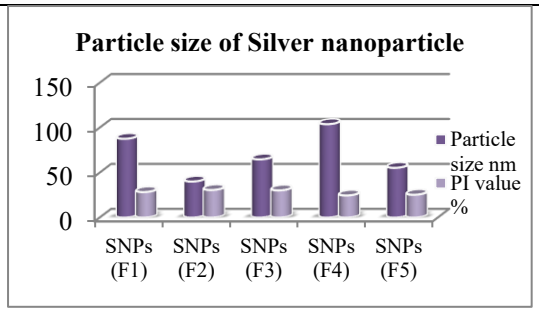
Graph 10: Particle size (F5)

Table 9: Zeta potential and particle size of Silver nanoparticle

Formulation	Zeta potential mV	Particle size nm
Nanoparticle (F1)	-10.5 mV	87.71 nm
Nanoparticle (F2)	**-22.2** mV	**40.13 nm**
Nanoparticle (F3)	-13.0 mV	64.78 nm
Nanoparticle (F4)	-11.7 mV	103.97 nm
Nanoparticle (F5)	-6.8 mV	55.37 nm



Graph 11: Graphical representation of Zeta potential of Silver nanoparticle



Graph 12: Graphical representation of Particle size of Silver nanoparticle

3.7 Stability study

Table 10: Stability Study of silver nanoparticle (F2) formulation

Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH				40^0C±2 ^{0}C and 70 ±5% RH			
	Colour	Odour	Appearance	Particle size nm	Colour	Odour	Appearance	Particle size nm
0	Yellow to dark brown	Characteristic	Liquid	40.13 nm	Yellow to dark brown	Characteristic	Liquid	40.13 nm
30	Yellow to dark brown	Characteristic	Liquid	40.09 nm	Yellow to dark brown	Characteristic	Liquid	40.01 nm
45	Yellow to dark brown	Characteristic	Liquid	40.02 nm	Yellow to dark brown	Characteristic	Liquid	40.11 nm
60	Yellow to dark brown	Characteristic	Liquid	40.18 nm	Yellow to dark brown	Characteristic	Liquid	40.12 nm
90	Yellow to dark brown	Characteristic	Liquid	40.15 nm	Yellow to dark brown	Characteristic	Liquid	40.12 nm

3.8 Results of antimicrobial activity of silver nanoparticles F 2 formulation

3.8.1 Antimicrobial activity of silver Nanoparticles

Table 11: Antimicrobial activity of silver Nanoparticle against *E.coli*

Sample Name (mg/ml)	Zone of Inhibition (mm) of *E.coli*
(Control)	0
Extract (1mg/ml)	5
AgNO3 (1ml)	6
Silver NPs (1ml)	11

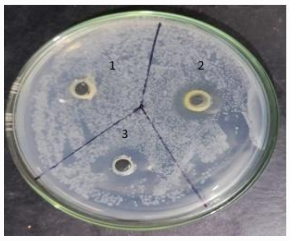
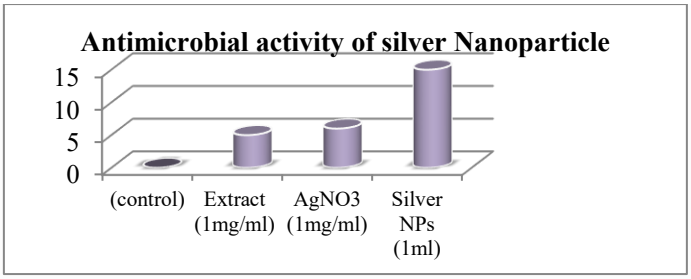


Figure 3: Photograph showing zone of inhibition of plant extract mediated synthesis of AgNPs against A: *Escherichia coli*



Graph 13: Zone of inhibition of AgNPs prepared at different conditions using plant extract against different *E.Coli*

4. CONCLUSION

The formulation of silver nanoparticles using *Abelmoschus ficulneus* seed extract proved to be an effective, eco-friendly approach with significant antibacterial potential. The presence of bioactive phytochemicals played a crucial role in the reduction and stabilization of silver ions into nanoparticles. Among all tested formulations, F2 exhibited optimal physicochemical properties, including the smallest particle size and highest zeta potential, contributing to its superior stability and biological efficacy. The synthesized AgNPs showed promising antimicrobial activity against both gram-positive and gram-negative bacteria, highlighting their potential as a natural antibacterial agent. This green synthesis method offers a viable alternative to conventional chemical synthesis of nanoparticles and supports the further development of plant-based nanomedicines.

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