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# BIOGENIC SYNTHESIS AND EVALUATION OF SILVER NANOPARTICLE CONTAINING HERBAL PLANT EXTRACT AND ITS ANTIMICROBIAL ACTIVITY

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## ABSTRACT

This study reports the green synthesis and evaluation of silver nanoparticles (AgNPs) using aqueous *Abelmoschus ficulneus* extract as a reducing and stabilizing agent. Phytochemical screening revealed the presence of proteins, flavonoids, carbohydrates, and polyphenols, which facilitated the bioreduction of silver nitrate into stable AgNPs. A distinct color change from colorless to brown indicated nanoparticle formation, which was confirmed by UV–Vis spectroscopy with a characteristic surface plasmon resonance (SPR) peak at 677 nm. Optimization experiments demonstrated that increasing plant extract concentration (12.5–100 mg/mL) and process parameters such as stirring (300 rpm) accelerated nanoparticle synthesis, reducing reaction time from 3 hours to 30 minutes under alkaline conditions. Purification through repeated centrifugation successfully eliminated excess plant metabolites, as confirmed by the absence of peaks in the 200–400 nm range. Characterization revealed hydrodynamic particle sizes ranging from 150.6 to 168.2 nm, with size variations influenced by pH and stirring conditions. Stability studies indicated that the AgNPs remained chemically and physically stable for up to three months under both accelerated (40 ± 2°C, 70 ± 5% RH) and ambient (25 ± 2°C, 60 ± 5% RH) conditions without significant changes in physicochemical properties. These findings confirm that *A. ficulneus* extract is an effective green source for producing stable, biocompatible AgNPs with promising potential for biomedical applications.

**KEYWORDS:** Silver nanoparticles; *Abelmoschus ficulneus*; Green synthesis; Antimicrobial activity; Surface plasmon resonance; Stability studies; Nanomedicine.

## 1. INTRODUCTION

Silver nanoparticles (AgNPs) have become one of the most extensively studied nanomaterials due to their unique physical, chemical, and biological properties. With sizes typically ranging between 1–100 nm, these nanoparticles exhibit a high surface area-to-volume ratio and strong surface plasmon resonance, which contribute to their wide range of applications. AgNPs are widely used in medicine, pharmaceuticals, food packaging, textiles, and water treatment because of their stability, catalytic activity, and remarkable antimicrobial properties **(Beyene *et al.*, 2017).**

Conventional methods of synthesizing silver nanoparticles involve chemical and physical approaches, which often require toxic reagents, high energy input, and generate harmful by products. As result, interest has shifted toward eco-friendly and sustainable synthesis methods, particularly biological or "green" synthesis using plants, microorganisms, and natural products. These approaches not only minimize environmental hazards but also utilize natural biomolecules as reducing and stabilizing agents in nanoparticle formation **(Nguyen *et al.*, 2023).**

*Abelmoschus ficulneus* (commonly known as white wild musk mallow) belongs to the Malvaceae family and is traditionally used in herbal medicine for its anti-inflammatory, antioxidant, and antimicrobial properties. Its phytochemical composition makes it a suitable candidate for nanoparticle synthesis. This study focuses on synthesizing AgNPs using *A. ficulneus* extract, characterizing them, and evaluating their antimicrobial activity against selected pathogens **(Onakpa, 2013).** Pharmacological studies have reported that *A. ficulneus* exhibits antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties. These bioactive compounds not only support its medicinal applications but also make the plant a promising candidate for use in nanotechnology, particularly in the green synthesis of

nanoparticles. Plant extracts are considered effective natural reducing and stabilizing agents, and the phytochemicals present in *A. ficulneus* can facilitate the eco-friendly production of stable nanoparticles such as silver nanoparticles. Given its diverse phytochemical composition and medicinal relevance, *Abelmoschus ficulneus* extract holds significant potential for biomedical and pharmaceutical applications. Exploring its role in nanoparticle synthesis and evaluating the biological activity of the resulting nanomaterials could provide valuable insights into developing sustainable alternatives to conventional chemical methods **(Al-Malki and Alharbi 2025).**

The present study aims to synthesize and evaluate silver nanoparticles using Abelmoschus ficulneus plant extracts and assess their antimicrobial efficacy against selected bacteria and fungi.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

AgNO3, Nitroprusside, Sodium Hydroxide, and Ammonia reagent was produced from Merck. Chloroform, Conc. HCl and 95% Alcohol, was received from Clorofiltind. Petroleum ether was acquired from Researchlab. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Fizmerck, Molychem, Himedia and Rankem.

### 2.2 Plant Material Collection

*Abelmoschus ficulneus* plant Flower were gathered. in local area Bhopal (M.P), along with they were dried for 3 days in shade, at normal temperature. Dried plant components were kept in sealed glass containers in an arid, cool atmosphere to avoid contamination and deterioration. A plant taxonomist confirmed the identity with therapeutic plant's purity *Abelmoschus ficulneus.*

### 2.3 Plant Material Extraction process

The extracting process of *A. ficulneus* plant Flower using a continuous heated percolation method by Soxhlet apparatus with Petroleum ether and ethanol was completed after a standardized protocol. The foliage of plant was first dried off in the shadows and grind into fine powder. A weighed amount (300g) of powdered material was placed inside a porous thimble and kept into the Soxhlet extractor. Petroleum ether was used first at 60°C non-polar compound extraction, followed by Methanol for polar compounds. The flask with a circular bottom was packed with 300–500 millilitre of the respective solvent and warmed with water bath. Since the solvent evaporated, it condensed in the reflux condenser and repeatedly percolated through the plant matter, causing bioactive substances to dissolve. Six to eight hours were spent about the extraction procedure until the solvent in the siphon tube had lost its color. After extraction, using a rotating evaporator, as solvent was eliminated and concentrated extract was dried even more under at 40°C in rotary vacuum evaporator (Buchi type). Weighing dried extract, and extract's % yield was computed using formula that follows:

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

The prepared dry extract was maintained at cold temperatures for further examination of phytochemicals and organoleptic characters (percentage yield, colour and odour) **(Khandelwal, 2008).**

### 2.4 Preliminary phytochemical investigations

Preliminary phytochemical investigations are the methodical analyses the chemical components discovered in plants called phytochemicals, that are in charge of their medicinal, nutritious, or poisonous qualities. These investigations were conducted to look into and determine the effective constituents like tannins, Phenolics, alkaloids, flavonoids, and saponins of extracts of Flower.

### 2.5 The Organoleptic Studies of *A. ficulneus*

Visual observation was hired to evaluate organoleptic qualities. Organoleptic test of *Abelmoschus ficulneus*, such as general appearance, color, odor, and condition, were conducted and observed **(Assi *et al.*, 2017).**

### 2.6 Solubility study

The qualitative solubility of *Abelmoschus ficulneus* in various solvents was examined utilizing Indian pharmacopoeia. Abelmoschus ficulneus was weighed together placed on10 ml test tube, where it added to proper solvents (1 ml each of methanol, DCM, distilled water, chloroform, and acetone) **(Dashputre and Bandawane 2021).**

### 2.7 Biosynthesize of Ag nanoparticles

Take 0.016g of AgNo3 and dilute it with 100ml of filtered water, stirring constantly, to get 1mM solution of AgNo3. (A) conical flask was packed with 50 milliliters silver nitrate (1 mM) solution, which was mixed continuously for 30 min. The excerpt will next be diluted into water to 5 different concentrations (100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml). About 10 ml each filtrate was put in beaker, and 90 ml of 1mM AgNo3 was added while stirring constantly for 30 minutes. The concoction was kept within a dark chamber until it shifted color from dark yellow to brown. After 30 minutes, the solution becomes dark yellow or brown, showing silver nanoparticles are forming. Periodic sampling an ultraviolet visible spectrophotometer was accustomed to gauge the biodegradation of silver nanoparticleions.

**Table 1: Composition of silver nanoparticle formulation.**

| Formulations | *Abelmoschus ficulneus* (mg/ml) (each 1 ml) | Silver nitrate solution (ml) 1mM | Distilled water | Stirring time (min) 300 rpm |
|---|---|---|---|---|
| F1 | 100 | 90.0 | 100 | 30 |
| F2 | 75 | 90.0 | 100 | 30 |
| F3 | 50 | 90.0 | 10 | 30 |
| F4 | 25 | 90.0 | 10 | 30 |
| F5 | 12.5 | 90.0 | 10 | 30 |

### 2.8 Characterization of silver nanoparticle

#### 2.8.1 Visual observation

The shift in hue during the nanoparticle preparation process portion was examined at different intervals of 30 minutes, 60 minutes, 120 minutes, and 180 minutes **(Mourdikoudis *et al*., 2018).**

#### 2.8.2 UV-Visible spectrophotometric analysis

The primary nanoparticles characterization was finished by sampling the aliquots taken from the combine reactions at various points in time of 30 minutes, 60 minutes, 120 minutes, and 180 minutes (as previously mentioned). This allowed for the response's measurement mixture's UV- visible spectrum at Water wavelength: 200–800 nm **(Paramelle *et al.*, 2014).**

#### 2.8.3 Scanning electron microscopic

The optimized nanoparticle's morphological characteristics were produced by employing scanning electron microscope's electron beam. Sputter coater in vacuum was then utilized to coat particles that possess thin layer (2–20 nm) of metal, like palladium and platinum, or gold. Following that, pretreatment specimen was struck by beam of electron, producing secondary electrons called augers. Rutherford and Kramer's Law was accustomed to choose and process just the electrons scattered at a 90° angle from this interplay between electron beam and the specimen's atoms regarding to produce surface topography photographs **(Ahmed *et al.*, 2020).**

#### 2.8.4 Zeta potential

To locate the particle charge alog with the velocity at which particles moved zeta potential electric field within measurement was made. Zetasizer Malvern instruments were hired to evaluated the nanoparticle after it had been diluted ten times Using purified water **(Balla *et al.*, 2020).**

#### 2.8.5 Particle size

Among characteristics of particles their size characteristics to think about. The nanoparticle's size was ascertained using the Malvern Zeta sizer (Malvern Instruments) **(Jain *et al.*, 2011).**

### 2.9 Silver nanoparticles of Anti-microbial activity through Well diffusion assay

- **Preparation of nutrient agar media**

In 100 milliliters of purified water, 2.8 grams of Nutrient Media were dissolved. The media's pH was measured prior to sterilization. To sanitize the media, it was autoclaved about 15 min at $121^{o}$ Celsius with 15 psi. Plates containing nutritional medium were kept in laminar air flow until agar get solidified.

- **Well diffusion assay**

Standardized to 108 CFU/ml of bacteria E. coli along with S. aureus bacterial suspension were installed in the shaker. After that, 100µl of the broth's inoculums (108 CFU/ml) were removed utilizing a micropipette and inoculated onto a fresh, sterile, solidified Agar Media Plate. The entire sterile agar surface was encased in the inoculums utilizing a sterile spreader to inoculate agar plate. Using sterile cork-borer, four 6-mm wells were borde into the solidified Agar Media Plate. The AgNo3, silver NPs with extract (1 mg/ml) next, a solution was produced for inoculation into the wells. A sample about 100 µl was loaded **(Mohammadi-Sichani *et al.*, 2012).**

- **Incubation period for observe ZOI on agar plates**

The infected Agar Media Plate was permitted to permeate at standard temperature for approximately thirty minutes prior to incubation for 18 to 24 hrs at 37°C. Following during incubation was done on the plates examined to determine clear zone had developed around well, indicating the tested formulation's antibacterial efficacy. Millimeters were used to measure and examine the zone of inhibition. Zones were measured using connection with use ruler to nearest millimeter that was positioned on reverse side of Petri plate. A couple of inches up to Petri dish was a black, non-reflective background. The well's and zone of total inhibition's (as seen using the unaided eye) dimensions were measured **(Manandhar *et al.*, 2019).**

### 2.10 Stability study

Three months were spent testing silver nanoparticle's stability formulation at accelerated temperatures of $25^{0}$ C±$2^{0}$ C and $40^{0}$ C±$2^{0}$ C, 70 ± 5% RH, and 60 ± 5% RH. When the days are 30, 45, 60, & 90 (3 months), the formulation's physical characteristics—such as color, order, appearance, and particle size—were assessed. Following the guidelines set forth by ICH, International gathering on harmonization, assessed the concept for stability under accelerated storage circumstances about 3 months. Using tests for color, odor, appearance, and particle size, formulation was inspected for physical alterations. As a comparison, all outcomes were contrasted with the final formulation at 0 days **(Khan *et al.*, 2020).**

## 3. RESULTS AND DISCUSSION

### 3.1 Plant Collection

**Table 2: Plant Collection.**

| Plant name | Plant part | Weight |
|---|---|---|
| *Abelmoschus ficulneus* | Flower | 300 gm |

### 3.2. Percentage Yield

In phytochemical extraction, the percentage yield is especially significant since It aids in figuring out the typical extraction efficiency for a given plant, various plant sections, or various solvents. Table 6 displays *Abelmoschus ficulneus* extract yield that was obtained.

**Table 3: Percentage Yield of *A. ficulneus* crude extracts.**

| Plant name | Solvent | Color of extract | Theoretical weight | Yield (gm) | % yield |
|---|---|---|---|---|---|
| *Abelmoschus ficulneus* | Pet ether | Greenish yellow | 300 | 4.51 | 0.79% |
| | Methanol | Green | 278 | 5.43 | 2.63% |

### 3.3 Preliminary Phytochemical study

**Table 4: Phytochemical testing of extract.**

| Experiment | Presence or absence of phytochemical test | |
|---|---|---|
| | Pet. Ether extract | Methanolic extract |
| **Alkaloids** | | |
| Dragendroff's test | Absent | Present |
| Mayer's reagent test | Absent | Present |
| Wagner's reagent test | Absent | Present |
| Hager's reagent test | Absent | Present |
| Glycoside | | |
| Borntrager test | Present | Present |
| Killer-Killiani test | Present | Present |
| **Carbohydrates** | | |
| Molish's test | Present | Present |
| Fehling's test | Present | Present |
| Benedict's test | Present | Present |
| Barfoed's test | Absent | Present |
| Iodine Test | Absent | Present |
| **Flavonoids** | | |
| Shinoda Test | Absent | Present |
| **Tannin and Phenolic Compounds** | | |
| Ferric Chloride test | Present | Present |
| Lead Acetate Test | Absent | Present |
| Gelatin Test | Absent | Present |
| **Saponin** | | |
| Foam test | Present | Present |
| Froth Test | Present | Present |
| **Test for Triterpenoids and Steroids** | | |
| Salkowski's test | Present | Absent |
| Libbermann-Burchard's test | Present | Absent |

### 3.4. Organoleptic properties

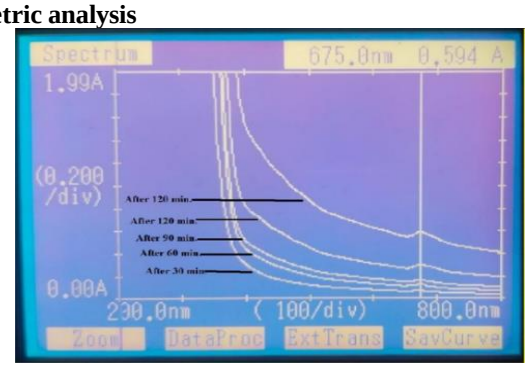
**Table 5: The Organoleptic Studies of *Abelmoschus ficulneus* Flower extract.**

| *Abelmoschus ficulneus* | Study |
|---|---|
| Colour | Greenish |
| Odour | Musky |
| Appearance | Greenish brown |

### 3.5 Evaluation parameter of silver nanoparticle

### 3.5.1 Color Observation

### 3.5.2 UV-Visible spectrophotometric analysis

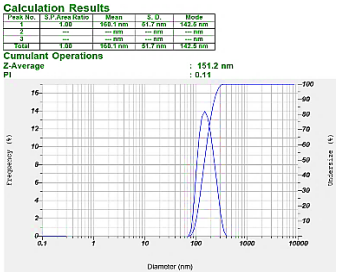



**Graph 1: UV Graph of F3 Formulation.**

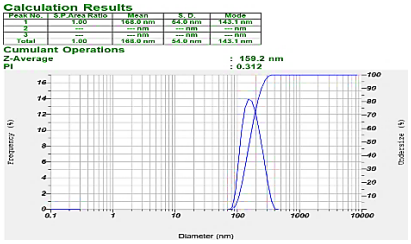
**Table 6: UV peak detection.**

| Silver nanoparticle Formulations | Peak detection |
|---|---|
| SNPs (F3) | 675.0 |

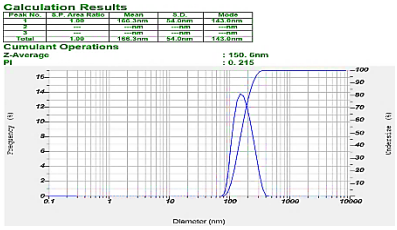
### 3.5.3 Particle size



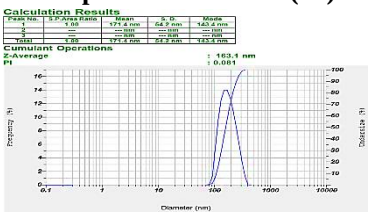

**Graph 2: Particle size (F1)**



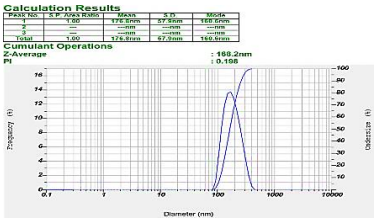

**Graph 3: Particle size (F2)**




**Graph 4: Particle size (F3)**



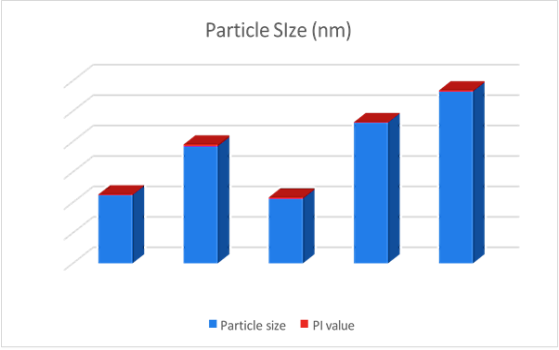

**Graph 5: Particle size (F4)**




**Graph 6: Particle size (F5)**

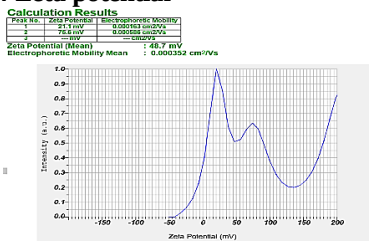
**Table 7: Particle size of silver nanoparticle.**

| Formulation | Particle size | PI value |
|---|---|---|
| SNPs (F1) | 151.2 nm | 0.110 |
| SNPs (F2) | 159.2 nm | 0.312 |
| **SNPs (F3)** | **150.6 nm** | **0.215** |
| SNPs (F4) | 163.1 nm | 0.081 |
| SNPs (F5) | 168.2 nm | 0.198 |

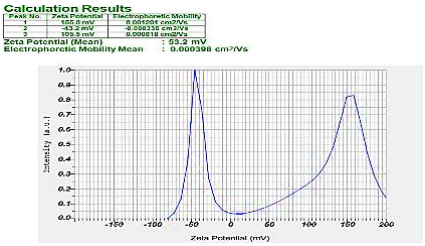



**Graph 7: Particle size represented graphically.**

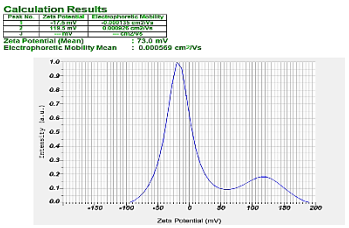
### 3.5.4 Zeta potential



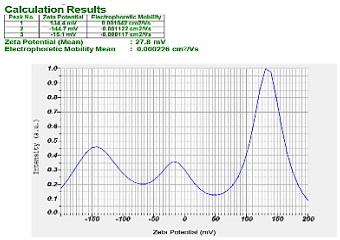

**Graph 8: Zeta potential (F1)**



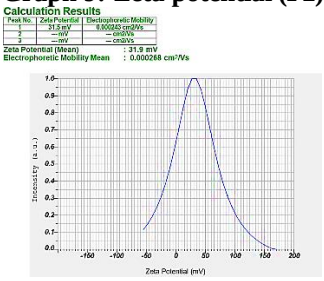

**Graph 9: Zeta potential (F2)**




**Graph 10: Zeta potential (F3)**



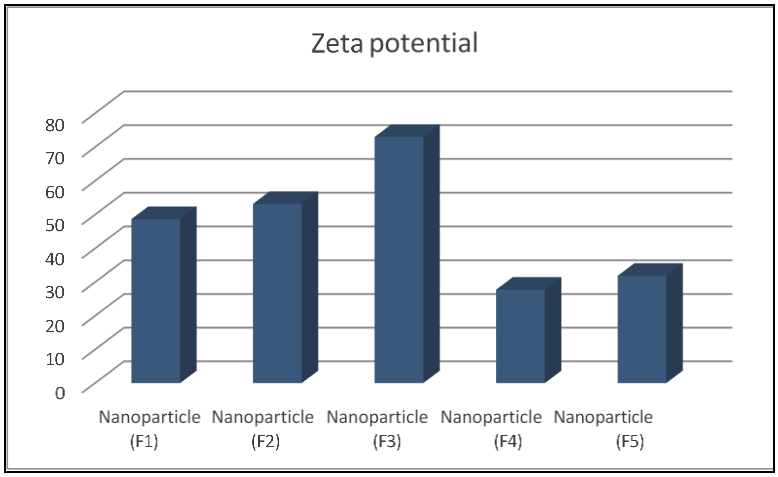

**Graph 11: Zeta potential (F4)**




**Graph 12: Zeta potential (F5)**

**Table 8: Zeta potential of Ag nanoparticle.**

| Formulation | Zeta potential |
|---|---|
| Nanoparticle (F1) | 48.7 mV |
| Nanoparticle (F2) | 53.2 mV |
| **Nanoparticle (F3)** | **73.0 mV** |
| Nanoparticle (F4) | 27.8 mV |
| Nanoparticle (F5) | 31.9 mV |




**Graph 13: Graphical representation of zeta potential of all formulations.**

### 3.5.5 Scanning Electron Microscopic or SEM

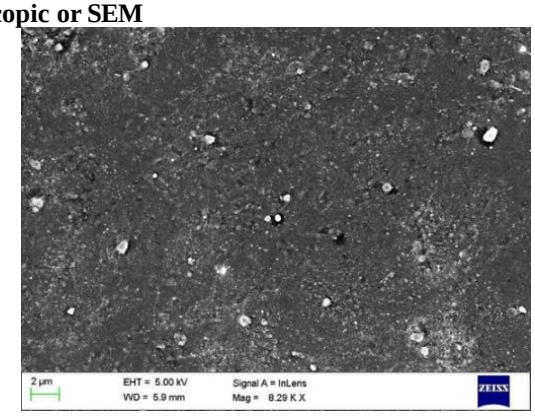



**Figure 2: SEM (F1).**

### 3.6 Antimicrobial activity of silver nanoparticles formulation

#### 3.6.1 Ag nanoparticle antimicrobial activity

**Table 9: Ag nanoparticle of antimicrobial activity against E.coli and S. aureus.**

| Sample Name (mg/ml) *E.coli* | zone of Inhibition (mm) of *E.coli* | Sample Name (mg/ml) *S.aureus* | zone of Inhibition (mm) of *S.aureus* |
|---|---|---|---|
| (control) (C) | 0mm | (control) (C) | 0mm |
| $AgNO_3$ (Placebo) (C1) | 1 mm | $AgNO_3$ (Placebo) (F1) | 1.5 mm |
| Extract (1mg/ml) (C2) | 4 mm | Extract (1mg/ml) (F2) | 5 mm |
| Silver NPs (1ml) (C3) | 11 mm | Silver NPs (1ml) (F3) | 8 mm |

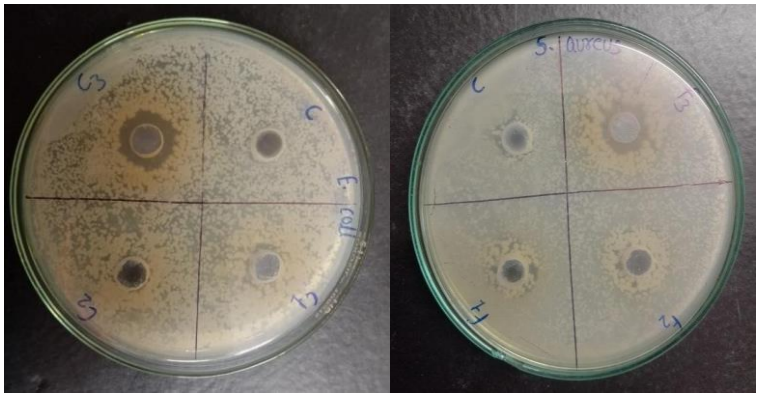
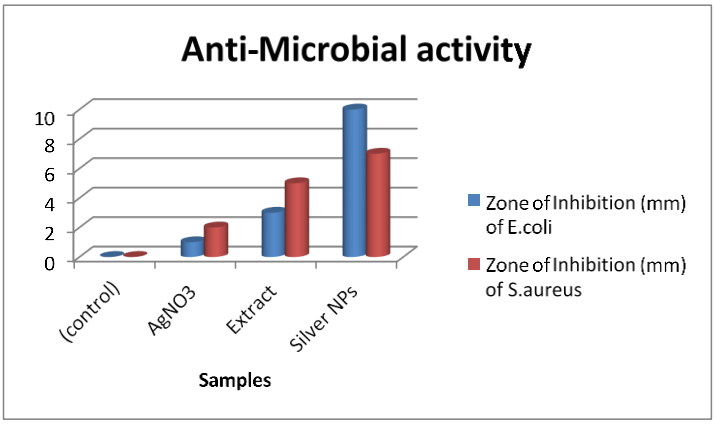


**Figure 3: Photograph showing zone of inhibition of plant extract AgNPs induced synthesis against A: *Staphylococcus aureus*, B: *Escherichia coli.***




**Graph 14:- Zone of inhibition of AgNPs made using plant extract under version circumstances against microorganism.**

#### 3.6.2 Stability study

**Table 10: Stability study of silver nanoparticle (F3) formulation.**

| Time (Days) | $25^0C \pm 2\ ^0C$ and 60 ± 5% RH | | | | $40^0C \pm 2\ ^0C$ and 70 ±5% RH | | | |
|---|---|---|---|---|---|---|---|---|
| | Colour | Odour | Appearance | Particle size nm | Colour | Odour | Appearance | Particle size nm |
| 0 | Yellow to dark brown | Slightly offensive | Liquid | 150.6 nm | Yellow to dark brown | Slightly offensive | Liquid | 150.6 nm |
| 30 | Yellow to dark brown | Slightly offensive | Liquid | 150.3 nm | Yellow to dark brown | Slightly offensive | Liquid | 150.4 nm |
| 45 | Yellow to dark brown | Slightly offensive | Liquid | 150.4 nm | Yellow to dark brown | Slightly offensive | Liquid | 150.6 nm |
| 60 | Yellow to dark brown | Slightly offensive | Liquid | 150.5 nm | Yellow to dark brown | Slightly offensive | Liquid | 150.7 nm |
| 90 | Yellow to dark brown | Slightly offensive | Liquid | 150.5 nm | Yellow to dark brown | Slightly offensive | Liquid | 150.9 nm |

## 4. CONCLUSION

As mentioned by the countries Health Organization, microorganisms are in charge of almost 25% of total 57 million fatalities that occur globally each year; in course of countries, this ratio is noticeably greater. Infections of skin and infections related to burn wounds are a few of the principal causes people die and require treatment.

According to current research, AgNPs may be a more potent and superior topical antibacterial versus group of microorganisms (*Staphococcus aureus* with *Escherichia coli*) that cause burns and skin infections. Abelmoschus Ficulneus possesses capacity to be green source for AgNP production owing to the reality that antioxidant properties.

A distinct zone of inhibition shows that silver Ag nanoparticles are present could be beneficial antibacterial agent. Metal nanoparticles produced by Abelmoschus Ficulneus were combined to establish nanosystem (1.0mm). Therefore, by encouraging development between cells and reducing pain, the developed AgNPs, could potentially be more efficient and superior topical antibacterial in genotypes antibiotic-resistant with the intention of treating burn infections.

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