First-Time Investigation of Bombax ceiba Stem Crude Extract’s Role for Autoclave-assisted Silver Nanoparticle Synthesis from Silver Sulphate and Silver Nitrate: Multifaceted Characterization, Catalytic and Antibacterial Investigations

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Abstract: The Bombax ceiba plant stem extract is selected for the first time in synthesis of silver nanoparticles from silver sulphate and silver nitrate. The pH 6.5 and autoclave conditions facilitated the synthesis of low sized stable SBAN-AgNP and SBAS-AgNP in presence of 3% and 2% v/v SB (Stem extract of Bombax) from silver nitrate (AN) and silver sulphate (AS) at 0.6 and 0.4mM concentrations respectively. The formation and characterization of nanoparticles are performed by employing UV-Vis spec, XRD, FTIR, DLS and FESEM-EDS. These studies revealed that the nanoparticles are crystalline in nature with capping of functional groups from plant crude extract. The FESEM results ensured that the nanoparticles formed are spherical in shape with average particle size of obtained using silver nitrate (SBAN-AgNP) and silver sulphate (SBAS-AgNP) salts are 20.6 nm and 19.4 nm respectively. Both the SBAN-AgNP and SBAS-AgNP showed 94.6% and 87.3% catalytic reduction efficiency of 4-nitrophenol within 11 and 12 mins and are completely eliminated the synthetic dye methylene blue from water in presence of NaBH4 as reducing agent within 10 and 12 mins respectively. The synthesized SBAS-AgNP showed significant MIC, MBC, anti-biofilm and TKK activities with 31.27 ± 2.04 µg/ml MIC value against Bacillus megaterium.

Keywords: Bombax ceiba, Green synthesis, AgNPs, MIC and FESEM-EDS.

1. Introduction

Nanomaterial science is emerging rapidly in various fields due to their efficient applicability in all the fields. However, nanoparticles are available from ancient time. The best example is the “Lycurgus cup” preserved in London Museum, which is made up of with the peculiar type of glass consisting of gold and silver particles at specific ratio. The first reported nanoparticles are gold nanoparticles synthesized by Faraday and he stated, the size of the particle is affecting the color of glass made up of with these particles (Ijaz et al., 2020). Expansion of Nanomaterial interventions in the health sector has tremendously increased from the past several years. In biomedical applications nanomaterials are used
for the development of biosensors, quantum dots, new robust biochemical assays and therapeutic application as drug delivery systems due to its biocompatibility, bioavailability, and biodegradability (Kaddouri et al., 2021). The enhanced surface area of nanomaterials potentially augmented their efficacy with reduced toxicity due to nano sized particles altering their fundamental properties such as chemical, optical and biological etc. (Jayeoye et al., 2020, Rai et al., 2013, Kalpana et al., 2018).

The first used metals for nanoparticle synthesis are silver and gold as these metals are most widely used in the ancient medicinal practices in Ayurveda, Unani etc. Silver based nanoparticles are extensively studied due to its antimicrobial, anti-cancer and anti-viral properties. In the medical field Ag-NP inhibit the binding of HIV-1 to the host cell (Pillay et al., 2010, Kokura et al., 2010, Shegokar et al., 2011). Use of pricey hazardous chemicals, release of toxic byproducts and laborious processes turn the researchers from chemical synthesis to biological synthesis of nanoparticles (Rana et al., 2020).

Biologically fabricated nanoparticles have shown tremendous advantages over other means of synthesis, as bio-based nanoparticles are highly stable, biocompatible, nontoxic, cost effective, non-laborious and eco-friendly etc. (Vanlalveni et al., 2021, Andreescu et al., 2007, Chokkareddy et al., 2018; Barakat et al. 2013). Plant based nanoparticles are showing promising physical, chemical and biological properties, as a vast variety of organic bio-molecules are involved in reduction and stabilization (Milczarek et al., 2013). Further, these nanoparticles have significant biological properties due to the capping of antimicrobial organic molecules present in the plant material (Krithiga et al., 2015). Previously various researchers reported that green synthesized silver nanoparticles have shown potential catalytic and synthetic dye degradation activities (Arya et al., 2017).

In general silver nanoparticles synthesis is exploited very well for green synthesis from the past several years due to its peculiar physical, biological properties and its use in many industrial applications. Especially antimicrobial properties of silver nanoparticles fabricated using extracts of different plant parts reported well (Martin et al., 2011, Cruz et al., 2018).

Several plant species with medicinal importance are still not exploited, Bombax ceiba is one of them. Nanoparticle synthesis using this plant is not reported till date and in the present investigation this plant is selected for green synthesis of silver nanoparticles. In ancient India the species Bombax ceiba is known by the generic name Salmalia which is derived from the Sanskrit language and a common name is red silk cotton tree (Ayurveda and Unani books).

**Classification**

**Kingdom:** Plantae  
**Division:** Magnoliophyta  
**Class:** Magnoliopsida  
**Order:** Malvales  
**Family:** Malvaceae/Bombacaceae  
**Genus:** Bombax  
**Species:** ceiba  
**Botanical name:** Bombax ceiba, Bombax malabarica, Salmalia malabarica  
**Common name:** cotton tree/ red silk cotton tree.

An international code of botanical nomenclature [ICBN] provided the universally accepted names for world taxonomists. The book “Species plantarum” by Linnaeus has given highly accepted scientific names to the plant’s taxon. He coined the genus name Bombax for the first time based on the character
xylon which means silk. Hence that the plant is called Bombax religiossum. L. Hassan Dt. Considered the name Bombax for the neotropical variety [Bombaxopsis] this is an Asiatic spieces previously called Salmalia scopularum. Later in 1976 Saldana & Nicolson in their work with Bombax ceiba separately called the Asiatic species as Salmalia scopularum this is how the same plant got two scientific names as synonyms, its classification, flowering of plant and stem is given below (Santosh Kumar et al., 2018, Madhava Chetty et al., 2013).

2. Materials and Methods

2.1 Chemicals and Reagents

Silver Nitrate, p-Nitrophenol, Methylene Blue and Silver Sulphate is purchased from FINAR and SDFCL respectively. Sodium Borohydride from LOBA Chem. Pvt. Ltd. Luria Bertani Broth from HIMEDIA and Crystal Violet from Fisher Scientific are purchased. The bacterial cultures Bacillus Megaterium (MTCC 428), Pseudomonas Aeruginosa (MTCC 2453), Staphylococcus Aureus (MTCC 96) and Escherichia coli (MTCC 739) are obtained from Department of Microbiology, AN University, Guntur.

2.2 Plant Material

The stem of Bombax ceiba tree is collected from the bank of Godavari River in West Godavari district, Andhra Pradesh. The plant stem and flowers are collected and confirmed as Bombax Ceiba (Salmalia Scopulorum) by the botanist with taxonomic details and the confirmation certificate provided in the supplementary Figure S1.

2.3 Preparation of Bombax ceiba stem crude extract

The Bombax ceiba is an indigenous plant, found on the bank of Godavari River in West Godavari district, Andhra Pradesh. The collected stem is cleaned with MilliQ water for 3 times, shadow dried for 1 week then cut into small pieces and stored in a glass jar at room temperature (Vanlalveni et al., 2021). The dried stem crude extract is prepared using water as solvent with Soxhlet apparatus at water boiling temperature by running about 30 cycles (Ujang et al., 2013). The collected crude is evaporated to concentrate using Rotary evaporator (Heidolph, 100 rpm, at 50oc) under vacuum and suspended in MilliQ water to attain 1:20 w/v stem extract of Bombax (SB) and stored at 10oc.

2.4 Qualitative Phytochemical Screening of Bombax ceiba stem crude extract

Biochemical composition of stem crude is estimated by performing the phytochemical analysis. The plant extract is diluted in different solvents such water, alcohol, chloroform and petroleum ether and part of the diluted samples are boiled. The both boiled and non-boiled samples are used to find out the presence and absence of alkaloids, flavonoids, glycosides, cardiac glycosides, steroids, triterpenoids, carbohydrates, fixed oils and fats, amino acids and proteins, tannins, phenols and saponins as per the standard protocols (Iqbal et al., 2015, Senguttuvan et al., 2014, Dubale et al., 2023, Daoudi et al., 2022) mentioned in the Table 1.

2.5 Bombax ceiba stem crude stabilized AgNPs synthesis

Silver nanoparticle synthesizing efficiency of SB crude is determined in presence of two precursors such as silver sulphate (AS) and silver nitrate (AN) at room temperature (RT). All the reactions are carried out by preparing a final volume of 10 ml reaction mixture solution where 5, 10, 15, 20% v/v SB (SB crude of 1:20 w/v) crude and 1ml of AN and AS precursor from the stock of 10
mM is added to get a final concentration of 1 mM in the Millipore water independently at RT (Jafarizadeh-Malmiri et al., 2017). The blank reaction mixtures are setup with the same concentrations of SB crude and 1 ml precursors separately in Millipore water. The colour change of the reaction mixtures from brown to black are analyzed under UV-Vis spectrophotometer for SPR peaks (Shameli et al., 2013).

Table 1. Various methods employed for the phytochemical analysis of Bombax ceiba stem crude.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s reagent; Dragendroff’s Reagent;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hanger’s reagent; Wangner’s reagent</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>Shinoda Test or Magnesium Ribbon Test</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>Borntrager’s Test</td>
</tr>
<tr>
<td>4</td>
<td>Cardiac glycosides</td>
<td>Keller-Killiani Test</td>
</tr>
<tr>
<td>5</td>
<td>Steroids and terpenoids</td>
<td>Salkowski test and Lieberman-Burchard Test</td>
</tr>
<tr>
<td>6</td>
<td>Phenols and tannins</td>
<td>Ferric Chloride Test</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>Frothing Test</td>
</tr>
<tr>
<td>8</td>
<td>Fixed oils and fats</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Amino acids and proteins</td>
<td>Millon’s test</td>
</tr>
</tbody>
</table>

2.6 Optimization of silver nanoparticle green synthesis

Modifications in reaction conditions are significantly affect the size, shape and morphologically uniform nanoparticle synthesis. Different parameters such as pH, autoclave conditions, concentrations of stem extract of Bombax (SB), and precursors AN and AS were studied for low size, stable and uniform silver nanoparticle synthesis. The reaction mixtures of 10 ml are prepared as described in previous section with modifications presented in the Table 2 (Iravani et al., 2014). The obtained AgNPs during optimization process are (SBAN-AgNP and SBAS-AgNP) pellet down by centrifuging at 10,000 rpm for 10 min at RT and washed thrice with MilliQ water. The dried AgNPs (in a hot air oven at 50oC for 5h) grinded using mortar and pestle and stored at RT in the dark for further analysis (Medina-Ramirez et al., 2009, Yugay et al., 2021, Olfati et al., 2021, Ghorbani et al., 2013).

2.7 Characterization of AgNPs

The formation of the silver nanoparticles preliminary confirmation is determined by measuring the SPR peaks using UV-Vis Spectrophotometer (Shimadzu, model UV 1780 having high resolution up to 0.5 nm with 5 spectral bandwidth wavelengths range from 190 nm to 1100 nm). The precursor, crude and synthesized nanoparticles of 100 µl were diluted to 3 ml in double distilled water and samples are measured for SPR peak by running a scanning program with settings of 200 to 600 nm with 2 nm band width (Rautela et al., 2019, Mulvaney et al., 1996). The structure, physical form and shape of the synthesized nanoparticles are estimated by using PXRD (Buker D8 Advance Powder XRD with advanced Lynx-Eye Detector with 0.5% quantitative accuracy and 5% qualitative accuracy). The X-ray diffraction pattern of AgNPs is performed by applying X-ray radiation with the settings of Kα, 0.154065 nm, 40 kV voltage and 30 mA current. Data was scanned at a speed of 1.2°/min and 20 between 0-80° and the Scherer formula is used to determine the size of the crystal (Rautela et al., 2019):

$$L = \frac{K\lambda}{\beta\cos\theta}$$

Where λ is the wavelength of the X-ray radiation, Kα is 0.154065 nm, k constant is 0.89
Table 2. Optimization steps followed for synthesis of stable and reproducible silver nanoparticle synthesis.

<table>
<thead>
<tr>
<th>Optimization steps for AgNPs synthesis</th>
<th>Percentage of SB v/v (Stem crude)</th>
<th>AN (Silver nitrate)</th>
<th>AS (Silver sulphate)</th>
<th>pH &amp; Temperature</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial synthesis</td>
<td>5%, 10%, 15% &amp; 20%</td>
<td>1 mM</td>
<td>1 mM</td>
<td>RT</td>
<td>5% SB with 1mM precursor is selected</td>
</tr>
</tbody>
</table>

**Optimization of silver nanoparticle synthesis**

<table>
<thead>
<tr>
<th>Effect of pH and autoclave conditions</th>
<th>5%</th>
<th>1 mM</th>
<th>1 mM</th>
<th>pH – 4, 6.5, 8, &amp;10 and autoclave conditions</th>
<th>pH – 6.5 and autoclave is selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of crude concentration</td>
<td>1%, 2, 3, 4 &amp; 5%</td>
<td>1 mM</td>
<td>1 mM</td>
<td>pH – 6.5 at autoclave condition</td>
<td>AN at 1%, 3% SB and AS at 2% SB are selected</td>
</tr>
<tr>
<td>Effect of precursor concentration</td>
<td>1%, 3% (AN) and 2% (AS)</td>
<td>0.2, 0.4, 0.6, 0.8 and 1mM</td>
<td>0.2, 0.4, 0.6, 0.8 and 1mM</td>
<td>pH – 6.5 at autoclave condition</td>
<td>0.6 mM AN with 3% SB and 0.4 mM AS with 2% SB at pH – 6.5 and autoclave condition is optimized as final</td>
</tr>
<tr>
<td>Scale up production (100 ml batch)</td>
<td>3% (AN) &amp; 2% (AS)</td>
<td>0.6 mM</td>
<td>0.4 mM</td>
<td>pH – 6.5 &amp; autoclave condition</td>
<td>Low sized SBAN-AgNP and SBAS-AgNP synthesized.</td>
</tr>
</tbody>
</table>

The size and zeta potential of SBS-AgNP and SBAN-AgNP are estimated by using DLS (dynamic light scattering) instrument (Horiba Zeta sizer Nano ZS, model ZS-100ZV2) with double distilled water as a dispersant. The FESEM-EDS is performed to determine the surface morphology and type of elements present in the synthesized nanoparticles. The AgNPs sample is dehydrated and is mounted on a carbon coated grid. The samples are examined under Scanning Electron Microscope (Hitachi-S520, Japan Oxford Link ISIS-300UK FESEM-EDAX with 20X to 30,000 X magnifications) at required magnifications of 133 EV (EDAX) resolutions, 100 nm (SEM) ± 0.5 resolution (Rautela et al., 2019). FTIR is performed to identify functional groups attached to nanoparticles that are vital to understand the involvement of stem extract (SB) during synthesis and stabilization of the AgNPs. The synthesized NPs are milled with KBr and pressed to get the KBr-sample pellet. Thermo Nicolet 38 FTIR with 7800-350 cm-1 spectral range along with KBr Beam splitter (Thermo Fisher Scientific Inc., USA) is used to determine the presence of active functional groups (Rautela et al., 2019).

2.8 Catalytic performance of AgNPs for 4-nitrophenol

The green synthesized SBAN-AgNP and SBAS-AgNP are tested for catalytic activity against the aromatic compound 4-nitrophenol. The reaction mixture stock is prepared with the aqueous solution of 100µl of 10 mM 4-nitrophenol and 200µl of 100 mM sodium borohydride in 2.7 ml deionized water. Three reactions are set up, where the first reaction contains the reaction mixture stock (2 mM 4-nitrophenol) and 100µl of 5 mg/ml SBAN-AgNP and 5 mg/ml SBAS-AgNP in 2.4 ml deionized water.
independently. Second reaction is carried out with reaction mixture stock without silver nanoparticles and the third reaction is done with 2 mM 4-nitrophenol alone. The redox reaction of 4-nitrophenol to 4-aminophenol is monitored in UV-Visible spectrophotometer (scan 200nm to 600nm) at 25 oC (Arya et al., 2017, Murali Krishna et al., 2015, Mahiuddin et al., 2020, Wadaani et al., 2016).

2.9 Deterioration efficiency of AgNPs for synthetic dye

The deterioration rate of organic dyes is enhanced in the presence of a catalyst, which can be integrated in the wastewater treatment process of efficient removal of organic toxins (Forgacs et al., 2004; Aaddouz et al., 2023). Removal of methylene blue dye using silver nanoparticles from water is determined by Methylene blue dye degradation assay, performed by setting up of reactions, where 10 mM MB is incubated in presence and absence of 5 mg/ml SBAN-AgNP and SBAS-AgNP with and without 100 mM NaBH4 independently. The resultant reaction mixture is periodically scanned in a UV-Vis spectrophotometer (200 to 750 nm) till 12 min to determine the decrease of MB peak at 666 nm.

2.10 Biological Applications of Bombax ceiba assisted AgNPs

The synthesized nanoparticles antimicrobial activity is tested by employing agar well diffusion method. Three different concentrations 100, 500, & 1000 µg of silver nanoparticles SBAN-AgNP (1% and 3 % SB) and SBAS-AgNP (2% SB) are dissolved in sterile double distilled water. Nanoparticles are loaded into the wells of LB agar plates which are inoculated with 0.6 OD of pre-activated pathogenic bacteria Bacillus Megaterium, Pseudomonas Aeruginosa, Staphylococcus Aureus and Escherichia coli individually. Zone of inhibition is calculated after incubating the plates for 24h at 37oC in the bacteriological incubator. Streptomycin is selected as the positive control. The best bioactive nanoparticles are selected for the bulk production (100 ml per batch) of silver nanoparticles (Vanlalveni et al., 2021, Krithiga et al., 2015, Alsamhary et al., 2020, Ali et al., 2022, Shakibaie et al., 2015). The SBAN-AgNP and SBAS-AgNP biological applications MIC, MBC, Anti-biofilm and TKK are performed (Martin et al., 2011, Mulvaney et al., 1996, Mahiuddin et al., 2020, Alsamhary et al., 2020, Basuliman et al., 2023, Orouadi et al., 2022) and detailed methodologies are presented in supplementary data.

3. Results and Discussion

3.1 Preparation of Bombax ceiba stem crude extract

Plant stems are extracted using the Soxhlet method at boiling temperature of water. By keeping the rotary evaporator’s water bath at 50°C, the obtained extract is evaporated under vacuum. According to Figure 1, the crude extract is seen to be semi-solid to oily, yellowish to dark brown in colour, and freely soluble in water. By dissolving crude in sterile MilliQ water, the 1:20 w/v stem crude extract solution (SB) is prepared. The phytochemical analysis of Bombax ceiba stem crude revealed the presence of phytochemicals such as alkaloids, glycosides, carbohydrates, fixed oils and fats and phenols as shown in the Table 3, which may play a vital role in reduction of silver salts and stabilization of silver nanoparticles.

3.2 Bombax ceiba stem crude stabilized AgNPs synthesis

Two different precursors, silver sulphate and silver nitrate are taken for fabrication of silver nanoparticles.
Figure 1. Process flow of silver nanoparticle synthesis starting from collection of *Bombax ceiba* stem, drying of stem pieces, crude extraction by soxhlet apparatus, reduction of silver nitrate and silver sulphate to silver nanoparticles formation.

Table 3. Presence or absence of phytochemicals in the *Bombax ceiba* plant stem crude extract.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical</th>
<th>Name of the test</th>
<th>Solvent used</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s Reagent</td>
<td>Chloroform</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alcohol</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Dragendroff’s Reagent</td>
<td></td>
<td>Chloroform</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alcohol</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hager’s Reagent</td>
<td>Dragendroff’s Reagent</td>
<td>Chloroform</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alcohol</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Wagner’s Reagent</td>
<td>Hager’s Reagent</td>
<td>Chloroform</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Alcohol</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates</td>
<td>Lieberman-Burchard test</td>
<td>Chloroform</td>
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<tr>
<td></td>
<td>Test for flavonoids</td>
<td>Magnesium ribbon test</td>
<td>Ethanol</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Test for glycosides</td>
<td>Borntrager’s test</td>
<td>Water</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Test for cardiac glycosides</td>
<td>Keller-Killiani test</td>
<td>Water</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Test for steroids</td>
<td>Lieberman-Burchard test</td>
<td>Chloroform</td>
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<tr>
<td>6</td>
<td>Test for triterpenoids</td>
<td>Salkowski test</td>
<td>Chloroform</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lieberman-Burchard test</td>
<td>Chloroform</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Carbohydrates</td>
<td>Molish Reagent</td>
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<td>–</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Water</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s Reagent</td>
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<td>+</td>
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<tr>
<td></td>
<td></td>
<td>Barford’s Reagent</td>
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<td>–</td>
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<td></td>
<td>Benedict’s Reagent</td>
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</tr>
<tr>
<td>No.</td>
<td>Component</td>
<td>Reagents/Tests</td>
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<tr>
<td>-----</td>
<td>--------------------</td>
<td>----------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Fixed oils and fats</td>
<td>Petroleum, Ether</td>
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<tr>
<td>9</td>
<td>Amino acids and proteins</td>
<td>Millon’s Test, Water, Alcohol, Ninhydrin Test, Water, Alcohol</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>Tannins</td>
<td>Ferric Chloride Test, Water, Alcohol</td>
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</tr>
<tr>
<td>11</td>
<td>Phenols</td>
<td>Ferric Chloride Test, Water</td>
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<td></td>
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<tr>
<td>12</td>
<td>Saponins</td>
<td>Frothing Test, Water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3.5 Optimization of the crude concentration for AgNPs synthesis

The Crude optimization is performed with different concentrations such as 1% to 5% of SB with 1% increment in presence of 1 mM precursors AN and AS, the reactions are carried out in autoclave independently. The SBAN-AgNP and SBAS-AgNP formed are shown SPR peaks below 430 nm as shown in the Figure 2e & 2f. The nanoparticles showing SPR peak at low wavelength will have very small size. Even though both AgNPs synthesized at autoclave temperature and pH 6.5 showed a broad peak at 413 nm, the SBAN-AgNP and SBAS-AgNP formed when 1% SB, 3% SB and 2% SB crude used in presence of 1 mM silver nitrate and silver sulphate respectively (at pH 6.5) in autoclave are selected as they shown sharp SPR peaks at 421 nm. These selected conditions of AgNP synthesis are further optimized to get higher yields. Logeswari et al. reported that their synthesized silver nanoparticles showed SPR peak at 420 nm and discussed about the previous literature where silver nanoparticles had a characteristic SPR peak at around 430 nm (Logeswari et al., 2013). Similarly, in the present study the SABN-AgNP and SBAS-AgNP formation is clearly evident by the SPR peak observed at 421 nm. This demonstrates that 1% to 3% SB concentration at pH 6.5 and autoclave temperature are optimum conditions for stable nanoparticle synthesis using silver salts AN and AS.

### 3.6 Determination of optimal precursor concentration for stable AgNPs synthesis

The Precursor optimization is performed with the selected crude concentrations and autoclave temperature as discussed above. The silver nanoparticle synthesis is performed at varied AN precursor concentration from 0.2 mM to 1 mM with 0.2 mM increment by using 1% (Figure 2g) and 3% (Figure 2h) crude SB. The synthesized SBAN-AgNP has shown SPR peaks at same wavelength 421 nm in presence of 0.4- and 0.6-mM precursor concentration with one or two exceptions as shown in the Figure 2g. However, 3% crude SB efficiently synthesized the stable SBAN-AgNP with higher yields in presence of 0.6 mM AN.

The optimization of SBAS-AgNP using AS precursor is performed simultaneously along with silver nitrate. SBAS-AgNP showed a similar SPR peak pattern as SBAN-AgNP, however, AS is reduced efficiently at low concentration of SB (2%) as shown in the Figure 2i. At 0.4 mM AS the stable and reproducible SBAS-AgNP (SPR peak 421 nm) are synthesized as shown in the Figure 2i. Stable silver nanoparticles synthesized optimal condition such as 0.6 mM AN and 0.4 mM AS precursor using 3% and 2% crude SB respectively at autoclave conditions at pH-6.5 are used for scaleup process. These results suggest that low concentration of crude is sufficient for synthesis of silver nanoparticles from silver sulphate precursor than silver nitrate precursor. However, irrespective of the crude concentration both the precursors synthesized the significantly identical silver nanoparticles and showed SPR peaks at exactly 421 nm.
3.7 Effect of scale up process on size of AgNPs

Bulk production of AgNPs is performed in autoclave at larger volumes of crude and precursors without altering their optimum concentrations. After bulk production the SBAN-AgNP (3% SB) and SBAS-AgNP (2% SB) showed the SPR peaks at 415 nm and 412 nm respectively as shown in the Figure 2j. This indicates that the precursors are reduced efficiently during bulk production with increased yield. Aboutorabi et al. observed that the silver nanoparticle formation is affected by reaction temperature and they demonstrated that the silver nanoparticles formed using Safflower flower extract showed the absorbance peak at 415 nm (Aboutorabi et al., 2018). Similarly, Bertoglio et al. demonstrated that the chemically synthesized silver nanoparticles using silver fluoride and silver nitrate as precursors showed absorbance peaks at 412 nm at alkaline pH 10 to 11 (Bertoglio et al., 2020). In the present study our plant reduced the silver sulphate and silver nitrate precursors and showed 412 nm and 415 nm at autoclave conditions without the aid of chemicals in less reaction time. These SBAN-AgNP (3% SB + 0.6 mM AN) and SBAS-AgNP (2% SB + 0.4 mM AS) are further analyzed for characterization, catalytic activity and antimicrobial potential.

![Graphs showing SPR peaks at different concentrations and pH levels](image-url)
Figure 2. Silver nanoparticle synthesis initiated at RT: Initial synthesis is performed at RT with different crude concentrations 5% to 20% SB in presence of 1 mM silver nitrate (a) and 1 mM silver sulphate (b). The temperature and pH optimization: (c) Nanoparticles synthesized with 5% SB crude and 1 mM silver nitrate at various pH at autoclave temperature and at crude pH 6.5 showed SBAN-AgNP peak at 413 nm. (d) At similar conditions in presence of silver sulphate showed SBAS-AgNP peak at 413 nm. Crude concentration optimization at autoclave condition: (e & f) SBAN-AgNP and SBAS-AgNP synthesis is performed at various crude concentrations from 1% to 5% in presence of 1 mM each precursor at autoclave conditions. Precursor concentration optimization: (g & h) During crude optimization 1% and 3% crude has showed SBAN-AgNP peak at 421 nm in presence of 1 mM silver nitrate, which is further optimized with the various concentrations of precursor silver nitrate from 0.2 mM to 1 mM. (i) Similarly, silver sulphate at same concentrations reduced to SABS-AgNP at autoclave conditions in presence of 2% SB crude. Bulk production of stable AgNPs at optimized conditions: (j) In the scaleup production at 100 ml volume the 3% SB with 0.6 mM AN and 2% SB with 0.4 mM AS synthesized silver nanoparticles showed SPR peaks at 415 nm & 412 nm respectively.

3.8 XRD diffraction pattern of AgNPs

Bulk production The X-ray diffraction data obtained after powder XRD analysis to determine the physical form of the synthesized silver nanoparticles showed various peaks as shown in Figure 3. Diffracted Bragg’s reflection peaks are observed which correspond to 111, 200, 142, 220, and 311 fcc lattice (JCPDS file No. 04-0783) of the standard silver nanoparticle database. The XRD pattern of SBAN-AgNP and SBAS-AgNP both have shown similar peaks at 20 values of 32.179, 38.059, 46.175, 54.763, and 57.436 with varying intensities and small deviation (Tian et al., 2018, Swanson et al., 1962). These peaks suggested that the AgNPs reduced in presence of Bombax ceiba stem extract are pure crystalline in nature. The XRD pattern of green synthesized silver nanoparticles reported by Olfati and co-workers showed similar peaks further, they have reported one unassigned peak at 210 is diffracted at 28.11° angle and this is due to the presence of silver oxide (Olfati et al., 2021). Similarly, SBAN-AgNP and SBAS-AgNP showed a peak at 27.777° and 27.810° respectively, which may suggest the presence of silver oxide nanoparticles in the synthesized AgNPs. However, slight variations
are inevitably compared to the standard reported peaks. The mean average crystalline size of the synthesized AgNPs is calculated by using the Scherer formula from the XRD data, the average calculated crystalline size of SBAN-AgNP is 22.8 nm and SBAS-AgNP is 22.7 nm.

![Figure 3](image)

**Figure 3.** XRD chromatograms of SBAN-AgNP (a) and SBAS-AgNP (b) showed similar peaks with different intensities.

### 3.9 FTIR analysis of AgNPs

The synthesized SBAN-AgNP and SBAS-AgNP FTIR analysis spectra are presented in Figure 4 and the stem crude extract of *Bombax ceiba* FTIR is shown in Figure 5. The crude has shown a strong broad peak at 3335.27 cm$^{-1}$ and a medium sharp peak at 1633.33 cm$^{-1}$ which indicate that the crude has hydroxyl group molecules from alcoholic compounds and the presence of intermolecular alcohol and aromatic compounds. Whereas the medium and week peaks such as 2360.51, 2340.54, 2131.08, 1735.83, 1716.52 and 13650.04 cm$^{-1}$ indicate the presence of alkynes, conjugated alkene and alkynes, aliphatic ketones, cyclic alkene, phenol and amine group compounds. The presence of this functional group compounds in crude is confirmed by the phytochemical analysis (Table 3). The FTIR spectrum of SBAN-AgNP and SBAS-AgNP nanoparticles has similar types of functional groups due to the capping of aromatic and sugar molecules from plant crude extract.

### 3.10 Zeta Size and Potential of AgNPs

The synthesized silver nanoparticles size and potential are determined in aqueous media using zeta sizer (DLS - dynamic light scattering) instrument (Horiba Zeta sizer Nano ZS, model ZS-100ZV2). Fissan et al. demonstrated that the nanoparticle size obtained in DLS analysis is larger than the other analytical methods like SEM. They explained that the agglomerated particles scattered light is strongly overlap the smaller particles. It measures the hydrodynamic particle diameter which includes biomolecule, stabilizing agents, hydration layer and core shell of the particles leads to the larger particle size (Fissan et al., 2014). Similarly in the present study the nanoparticles SBAN-AgNP and SBAS-AgNP have showed average zeta sizes of 266.3 nm and 277 nm respectively as shown in the Figure 6 with acceptable polydispersity index and size of the particles are higher than the SEM and XRD analysis. Sayes Christie et al. demonstrated that the nanoparticles showed a minimum or less than -30 mV zeta potential are highly stable (Sayes Christie et al., 2020). The zeta potential of SBAN-AgNP and SBAS-AgNP showed -49.2 mV and -62.3 mV respectively, indicating the highly stable and strong anionic charged nanoparticle formation.

Figure 4. FTIR chromatograms of (a) SBAN-AgNP and (b) SBAS-AgNP both have showed similar pattern of FTIR peaks with similar functional groups attached.

Figure 5. FTIR analyses of Bombax ceiba stem crude solution of 1:20 (w/v).

Figure 6. FTIR Size and potential of synthesized silver nanoparticles (a) SBAN-AgNP showed a sharp peak with average Zeta size is 266.3nm (b) SBAN-AgNP showed a net negative charge with Zeta Potential of -49.2mV (c) Zeta size of SBAS-AgNP is 277nm and (d) a net negative potential of silver nanoparticle (SBAS-AgNP) is -62.3mV.
3.11 Morphology and Size of AgNPs by FESEM-EDS

The synthesized SBAN. The size and shape of the synthesized AgNPs are determined by FESEM analysis. SBAN-AgNP and SBAS-AgNP both FESEM images have mono dispersed spherical shaped particles as shown in Figure 7. The size of the AgNPs is calculated using Image J software and both SBAN-AgNP and SBAS-AgNP nanoparticles sizes obtained are 20.6 and 19.4 nm respectively.

Figure 7. SEM image of silver nanoparticles synthesized using Bombax ceiba stem extract in presence of two different silver salt precursors: (a and b) SEM image of SBAN-AgNP and size is determined by ImageJ software and showed an average size of 20.6. (c) EDS of SBAN-AgNP which shows the percentage of elements present in the nanoparticle. (d and e) SEM image of SBAS-AgNP and size determined by ImageJ software showed 19.4 nm. (f) EDS of SBAS-AgNP which shows the percentage of elements present in the nanoparticle.
Elemental analysis (EDS) showed the Ag, Cl, C and O elements presence in AgNPs. Elements Cl, C and O presence in AgNPs may suggest that they incorporated from biochemicals present in the plant crude extract. Olfati et al. demonstrated that the average size of the silver nanoparticles synthesized using the extract of plant Calendula officinalis is 18-24 nm (Olfati et al., 2021). Further, they explained that the silver nanoparticles synthesized using silver nitrate have a low size and homogeneous spherical shape with more antimicrobial effect rather than NPs from silver sulfate. However, contrary to this in the present study SBAS-AgNP synthesized using silver sulfate showed significant antimicrobial activity and low size spherical NPs than SBAN-AgNP (synthesized using silver nitrate). This could be due to the presence of high silver ions from silver sulfate at higher temperatures as it has two silver atoms compared to silver nitrate which is easily soluble with one silver atom per molecule.

3.12 Synthesis of 4-aminophenol from 4-nitrophenol using AgNPs as a catalyst

The catalytic activity of green synthesized SBAN-AgNP and SBAS-AgNP against 4-nitrophenol in presence of sodium borohydride is studied. A wavelength of 316 nm was observed for yellow colored aqueous 4-nitrophenol and yellowish-green color 4-nitrophenolate ion formation is observed at 401 nm wavelength after the addition of aqueous sodium borohydride. A wavelength shift from 401 nm to 296 nm is observed after the addition of SBAN-AgNP and SBAS-AgNP due to the reduction of the 4-nitrophenolate ion to 4-aminophenol. The intensity of the 4-nitrophenolate ion decreased with a simultaneous increase of 4-aminophenol till 11 and 12 min in presence of SBAN-AgNP and SBAS-AgNP respectively. However, the percentage reduction is higher for SBAN-AgNP compared to the SABS-AgNP with 94.6% and 87.3% respectively; this indicates that the AgNPs could effectively reduce 4-nitrophenol as shown in the Figure 8.

![Figure 8](image)

**Figure 8.** Catalytic activity of synthesized AgNPs is determined by the reduction of 4-nitrophenol to 4-aminophenol. SBAN-AgNP reduction of 4-nitrophenol completed at 11 min (a). SBAS-AgNP reduction of 4-nitrophenol completed at 12 min (b).

3.13 Augmented rate of toxic dye degradation in presence of AgNPs

The nanoparticles SBAN-AgNP and SBAS-AgNP demonstrated 100% Methylene blue (MB) dye degradation in the course of 10 and 12 min of incubation time, respectively as illustrated in Figure 9. Artificial colors developed commercially are resistant to degradation. Synthetic colors are frequently...
decolorized by reducing agents like ozone, hydrogen peroxide, etc. Optimization of decolorizing agents is important to removal of dyes, for environmental safety concerns. Kuo, 1992 showed that the presence of metal ions like Fe(II) with H2O2 increased the decolonization of anthraquinone-2-sulfonic acid sodium salt (Forgacs et al., 2004). Mahiuddin et al. demonstrated that the green synthesized silver nanoparticles reduced the methylene blue dye present in the water became colorless within 8 min (Mahiuddin et al., 2020). Similarly, the green synthesized SBAN-AgNP and SBAS-AgNP nanoparticles act as a catalyst and greatly accelerated the degradation of methylene blue dye when NaBH4 is used as the reducing agent. In a short incubation time, the SBAN-AgNP and SBAS-AgNP potentially degraded the MB with 100% hydrogenation of MB dye.

Figure 9. Catalytic SBAN-AgNP degradation of MB dye completed at 10 min (a). SBAS-AgNP degradation of MB dye completed at 12 min (b).

3.14 Potential antibacterial activities (Zone of Inhibition)

The synthesized AgNPs with higher yields are selected for antimicrobial activity by employing the agar well diffusion method. SBAN-AgNP (1% & 3% SB) and SBAS-AgNP (1% & 2% SB) are tested against two gram-positive and two gram-negative bacterial pathogens Staph aureus, B. megaterium, Pseudomonas aeruginosa, and E. coli respectively. The SBAN-AgNP and SBAS-AgNP synthesized by 1%, 3% SB, and 2% SB respectively have shown significant antimicrobial activity against the tested pathogens as shown in supplementary STable 1 & Figure 10 (a to d). The pathogen B. megaterium is more sensitive to the synthesized AgNPs compared to other pathogenic bacteria.

Figure 10. Zone of inhibition of synthesized SBAS-AgNP (100 µg/ml): Plate showing zone of inhibition of silver nanoparticles against Pseudomonas aeruginosa (a). Zone of inhibition of nanoparticles against Staphylococcus aureus (b). Silver nanoparticles showing zone of inhibition against E. coli (c). ZOI against Bacillus megaterium (d).
Based on the antimicrobial activity the SBAN-AgNP and SBAS-AgNP synthesized by 3% SB and 2% SB respectively are selected for further biological activities. Rautela et al. demonstrated that the silver nanoparticles prepared using seed extract of Tectona grandis showed zones of inhibition at 50 and 100 µg/ml concentration against *S. aureus*, *E. coli*, and *B. cereus* (Rautela et al., 2019). Similarly, the synthesized SBAN-AgNP and SBAS-AgNP are effective against *B. megaterium* at 100 µg/ml.

3.15 Determination of MIC & MBC concentration of AgNPs

The minimal inhibitory concentration of the synthesized SBAN-AgNP (3% SB) and SBAS-AgNP (2% SB) against gram-positive and gram-negative bacteria is determined by employing the macro dilution method (NCCLS protocol). The two nanoparticles synthesized using silver nitrate and silver sulfate showed comparable MIC values with respect to standard streptomycin. The SBAS-AgNP showed significant activity against Bacillus megaterium with a MIC value of 31.27 ± 0.6 µg/ml compared to the rest of the pathogens among the tested nanoparticles. Out of four pathogenic bacteria, Bacillus megaterium is more sensitive to all the synthesized AgNPs with MIC values ranging between 31.27 ± 0.6 to 78.59 ± 1.87 µg/ml as shown in Table 4. Masum et al. evaluated the antimicrobial activity of synthesized silver nanoparticles using fruit extract and found 62.41% inhibition at 20 µg/ml (Masum et al., 2019). Similarly, SBAS-AgNP showed 90% inhibition at 31.27 µg/ml against *B. megaterium*. The minimum bactericidal concentration (MBC) of the synthesized AgNPs is simultaneously determined. All the silver nanoparticles synthesized have bactericidal activity ranging from 0.1 to 1 mg/ml as shown in Table 4. Out of the tested silver NPs SBAS-AgNP showed significant MBC activity at 0.1 mg/ml concentration against *Bacillus megaterium*. SBAN-AgNP (synthesized using 3% SB) also showed significant MBC activity with the IC50 values ranging between 10.58 to 121.36 µg/ml concentrations as shown in Table 4. SBAN-AgNP synthesized using 3% SB showed comparable activity with streptomycin. Silver nanoparticle synthesized using silver sulfate as precursor i.e SBAS-AgNP has shown potential anti-biofilm activity with an IC50 value of 27.3 µg/ml against *B. megaterium*. SBAN-AgNP (synthesized using 3% SB) also showed significant anti-biofilm activity with the IC50 values ranging between 10.58 to 121.36 µg/ml concentrations as shown in Table 4. Masum et al. 2019 demonstrated that the silver nanoparticles inhibited the biofilm formation by 66.64% at 20 µg/ml (Masum et al., 2019). Similarly in the present investigation, green synthesized silver nanoparticles showed 50% biofilm inhibition at 23.7 µg/ml.

3.16 Anti-biofilm activity of AgNPs

Silver nanoparticles synthesized from silver sulfate and silver nitrate in presence of stem extract of *Bombax ceiba* showed significant antimicrobial activity in the form of bactericidal and bacteriostatic against both gram-positive and gram-negative pathogens. Extracellular polymeric substances produced by some bacterial species act as a defense mechanism to protect them from external bactericides. Several synthesized antimicrobial compounds experience difficulty in crossing the biofilm. It is imperative to estimate the anti-biofilm activity of the synthesized particles. AgNPs anti-biofilm activity is performed and SBAS-AgNP showed potential anti-biofilm activity with an IC50 value of 27.3 µg/ml against *B. megaterium*. SBAN-AgNP (synthesized using 3% SB) also showed significant anti-biofilm activity with the IC50 values ranging between 10.58 to 121.36 µg/ml concentrations as shown in Table 4. SBAN-AgNP synthesized using 3% SB showed comparable activity with streptomycin. Silver nanoparticle synthesized using silver sulfate as precursor i.e SBAS-AgNP has shown potential anti-biofilm activity against gram-positive pathogen BM, a similar efficacy pattern was observed in MIC and MBC determination against BM. This indicates that the silver sulfate precursor is efficiently reduced by the crude extract of a plant stem and synthesized the potential antimicrobial active nanoparticle this could be due to either binding of the antimicrobial plant compound to the nanoparticle or may be due to the smaller size of the nanoparticle. Chen Xiu et al. 2018 demonstrated that the development of drug resistance of bacterial pathogens is primarily due to biofilm formation (Chen Xiu et al., 2018). Masum et al. 2019 demonstrated that the silver nanoparticles inhibited the biofilm formation by 66.64% at 20 µg/ml (Masum et al., 2019). Similarly in the present investigation, green synthesized silver nanoparticles showed 50% biofilm inhibition at 23.7 µg/ml.
Table 4. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Anti-biofilm activity values of synthesized AgNPs against different bacterial pathogens.

<table>
<thead>
<tr>
<th>AgNPs</th>
<th>Minimum Inhibitory Concentration (MIC in µg/ml)</th>
<th>E.C&lt;sup&gt;c&lt;/sup&gt;</th>
<th>B.M&lt;sup&gt;d&lt;/sup&gt;</th>
<th>P.A&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>SBAN-AgNP (3% SB)</td>
<td>92.85 ± 0.54</td>
<td>85.35 ± 0.89</td>
<td>78.59 ± 1.44</td>
<td>80.71± 0.38</td>
</tr>
<tr>
<td>SBAS-AgNP (2% SB)</td>
<td>85.88±2.84</td>
<td>79.87±4.42</td>
<td>31.27± 2.04</td>
<td>84.13±5.68</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>65.32±0.93</td>
<td>13.2 ± 2.43</td>
<td>15.98±2.44</td>
<td>74.53±1.24</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>AgNPs</th>
<th>Minimum Bactericidal Concentration (MBC in µg/ml)</th>
<th>E.C&lt;sup&gt;c&lt;/sup&gt;</th>
<th>B.M&lt;sup&gt;d&lt;/sup&gt;</th>
<th>P.A&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBAN-AgNP (3% SB)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>SBAS-AgNP (2% SB)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
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</table>

<table>
<thead>
<tr>
<th>AgNPs</th>
<th>Anti-Biofilm activity IC50 (µg/ml)</th>
<th>E.C&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P. A&lt;sup&gt;a&lt;/sup&gt;</th>
<th>B.M&lt;sup&gt;d&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>SBAN-AgNP (3% SB)</td>
<td>102.2251</td>
<td>75.56027</td>
<td>19.99427</td>
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<tr>
<td>SBAS-AgNP (2% SB)</td>
<td>54.20286</td>
<td>92.39957</td>
<td>23.70207</td>
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<tr>
<td>Streptomycin</td>
<td>2.86732</td>
<td>88.34851</td>
<td>10.75447</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> – Pseudomonas aeruginosa; <sup>b</sup> – Staphylococcus aureus; <sup>c</sup> – Escherichia coli; <sup>d</sup> – Bacillus megaterium

3.17 Killing Kinetics of AgNPs

Dose-dependent growth reduction is observed with the incubation time when BM is treated with SBAN-AgNP (synthesized using 3% SB) and SBAS-AgNP (synthesized using 2% SB). These nanoparticles reduced the growth of BM from 8h of incubation when compared to the control (Figure 11). A regular growth pattern is observed in the control however, a high reduction of growth is observed from the initial incubation time in presence of AgNPs.

Figure 11. Time Killing Kinetics study of synthesized silver nanoparticles SBAN-AgNP and SBAS-AgNP against Bacillus megaterium. The BM showed negligible growth in presence of SBAN-AgNP and SBAS-AgNP at their respective MIC and 2xMIC concentration till 20h. Further incubation a slight growth of BM is observed; however, when compared to the control the growth is significantly low. SBAS-AgNP nanoparticles showed potential growth reduction capacity in comparison with SBAN-AgNP.
This reduction is continued till 24h where negligible growth is observed in presence of AgNPs at MIC and 2xMIC concentration. Further, incubation at 36h and 48h slight increase and decrease in growth observed simultaneously. The AgNPs have potentially reduced the growth of BM from the starting stage and the highest reduction is observed in presence of 2xMIC concentration than MIC concentration. Further, SBAS-AgNP showed the highest reduction compared to SBAN-AgNP as shown in Figure 11. Qidwai et al. and Das et al. demonstrated that the green synthesized Ag nanoparticles showed an efficient reduction of bacterial growth with respect to concentration and time. They reported that initially, reduction is slow and later the reduction pattern is stabilized (Qidwai et al., 2018, Das et al., 2017). However, in the present study, SBAN-AgNP and SBAS-AgNP both efficiently reduced the growth of B. megaterium from the 10h of incubation, even though a slight increase in growth is observed at 36h but the growth is restricted to less than 50% which indicates that these AgNPs are significantly controlled the growth of the pathogen.

**Conclusion**

Several researchers employ silver salts as precursors to assess the effectiveness of the natural product they used in the fabrication of nanoparticles. There are numerous papers indicating that environmentally friendly silver nanoparticles made using green synthesis have promising catalytic and antibacterial properties. Yet, studies in the literature have revealed that silver nanoparticles made with silver sulfate as a precursor have less antibacterial activity than those made with silver nitrate, and vice versa. This might be a result of the green manufacturing of silver nanoparticles using various biological components. Hence, for the synthesis of silver NPs in the current investigation, both silver sulfate and silver nitrate salts are chosen. The synthesis of stable nanoparticles depends on optimizing the reaction independent variables such as pH, temperature, precursor, and crude concentration. The green synthetic AgNPs (SBAN-AgNP & SBAS-AgNP) prepared from Bombax plant stem extract are small-sized, stable, and nontoxic with potential catalytic, dye degradation and antibacterial activity through their significant biofilm inhibition potential. Moreover, compared to silver nitrate-based NPs, silver nanoparticles with a precursor of silver sulphate are smaller and more efficient against bacterial infections. Synthesis of 4-aminophenol from 4-nitrophenol and 100% elimination of MB dye in the presence of NaBH4 (reducing agent) from water, both silver NPs demonstrated remarkable catalytic efficiency, so could be used for many pharmaceutical applications.

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**Compliance with Ethical Standards:** This article does not contain any studies involving human or animal subjects.

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