

**INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY****Biogenic Silver Nanoparticles from *Stereospermum kunthianum Cham* Stem Bark Extract: Synthesis, Characterization, In Vitro Antimicrobial Study and Cytotoxicity Effects Against Brine Shrimp *Artemia*.**\*H. D. Kabiru, K.B. Ahmad, N. M. Bello and C. E. Chiegero  
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**ABSTRACT**

Metal nanoparticles as a category of nanomaterial are receiving interest due to their wide range of applications including in biomedical fields. Among these nanoparticles, silver nanoparticles are the most extensively studied for medical applications because they have been proven to exhibit excellent activity against a wide range of medically significant pathogenic microorganisms, including bacteria, viruses, fungi, and yeasts. In this work, silver nanoparticles were biogenically synthesized using the stem bark extract of *Stereospermum kunthianum Cham* as capping agent. Physicochemical properties, antioxidant activity, antimicrobial properties and in vitro cytotoxicity of the synthesized AgNPs were studied following standard procedures. UV-Vis studies show broad absorbance peak of the nanoparticles between 430 and 450 nm, SEM image shows that some of the synthesized nanoparticles are near spherical shape with many of the particles appear to have irregular shapes while the material average crystalline size from the XRD analysis was 27.878 nm. The synthesized AgNPs exhibits moderate antioxidant activity with IC<sub>50</sub> value of 124.84 µg/mL. Among the microorganism tested, the AgNPs shows more activity against *Staphylococcus aureus* than *Salmonella typhi* and *Candida albicans*. Brine shrimp lethality test of the synthesized AgNPs indicates some level of cytotoxicity with LC<sub>50</sub> of 461.850 µg/mL. using biological materials as reducing agents remains best green approach for the synthesis of silver nanoparticles.

**Keywords:** Silver nanoparticles, Antioxidant, Antimicrobial, Brine Shrimp Lethality.**1.0 INTRODUCTION**

In recent years, the application of nanotechnology is receiving growing interest in various fields, including the field of biomedical research. Nanomaterials are defined as a large class of materials composed of particulate substances that have at least one dimension of less than 100 nm (Khan *et al.*, 2019). These materials find applications in various industrial products such as electronics, sporting goods, sunscreens, tires, and cosmetics. They are also used for a variety of medical purposes, including drug delivery and diagnostic imaging (Luo *et al.*, 2015).

Metal nanoparticles are a category of nanomaterial that contains metals such as silver, copper, gold, titanium, and platinum. Various methods were adopted for the preparation of metal nanoparticles. Physical, chemical, photochemical, and biological

approaches were the common methods for the synthesis of metal nanoparticles. The high cost of operation and possible chemical hazards associated with physical and chemical methods make biological methods the most preferred for metal nanoparticle preparation in recent times. In the biological method, simple green chemistry is adopted as a viable alternative to more complex chemical synthetic processes using plant extracts or microorganisms as natural reducing agents to produce environmentally friendly AgNPs (Natsuki, 2015).

Silver nanoparticles are the most extensively utilized material among all other forms of metal nanoparticles. They have been proven to exhibit excellent activity against a wide range of medically significant sessile-pathogenic microorganisms, including bacteria, viruses, fungi, and yeasts (Oves

et al., 2022). Besides their antimicrobial properties, silver nanoparticles were also found to exhibit robust optical features, which make them suitable for applications in biological sensing and imaging (Hasnain et al., 2019). Silver nanoparticles are widely used in food and cosmetic industries to improve the quality of products and prevent deterioration due to their antifungal and anti-bactericidal activities (Verma & Maheshwari, 2019).

*Stereospermum kunthianum* Cham is a medicinal plant belonging to the *Bignoniaceae* family and is native to tropical Africa. It is known as Sansami among the Hausa of Northern Nigeria, Ayada among the Yoruba of South West Nigeria and Alakiriti among the Igbo of South East Nigeria (Ching et al., 2009). The plant is used extensively in traditional medicine for the management of different ailments and conditions such as wounds, ulcers, gastritis, bronchitis and other pain and inflammatory related health conditions by the Sudano-Guinea people of Africa (Ior et al., 2021; Nwinyi et al., 2021). Scientific evidence has shown that leaves, stem bark as well as root bark of *Stereospermum kunthianum* Cham possess antibacterial, anti-inflammatory, anticonvulsant, antihelmintic, antidiarrhoeal, and analgesic and wound healing activities respectively (Abdou, et al., 2019). Thus, the present study attempted to outline the use of *S. kunthianum* stem bark extract as a reducing agent for the green synthesis of silver nanoparticles and to characterize and evaluate the biological activities of the synthesized silver nanoparticles.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

#### 2.1.1 Sample Collection

Sample of stem bark of *Stereospermum kunthianum* Cham was collected from outskirts of Lokoja metropolis, Kogi State. The sample was dried under shade for three weeks, ground into powder using laboratory mortar and pestle and kept in an air tight container until needed.

#### 2.1.2 Sample Preparation

The preparation of the stem bark of *S. kunthianum* was done following procedure described by (J et al., 2017) with minor modification. 20 grams of the

powdered *S. kunthianum* Stem Bark was transferred in a 500 mL conical flask containing 300 mL of distilled water and boiled for 40 minutes. After cooling to room temperature, the extract was filtered with Whatman No.1 filter paper and the filtrates was kept in refrigerator until needed for the synthesis of silver nanoparticles.

### 2.3 Biosynthesis of Silver Nanoparticles

*S. kunthianum* mediated biosynthesis of silver nanoparticles was achieved by adopting procedure described by (J et al., 2017) with some modification. 100 mL of the aqueous stem bark extract was treated with 1mM aqueous solution of AgNO<sub>3</sub> in a cleaned beaker. The mixture was incubated at room temperature. The formation of silver nanoparticles was confirmed by the formation of a brown-yellow colouration

### 2.4 Characterization of Silver Nanoparticles

The silver nanoparticles formed was centrifuged at 3000 rpm for 25 min, the supernatant was discarded and the resulting pellets was collected and dried in an oven at 100°C for 24 h. The pellet was used for the characterization of the silver nanoparticles.

### 2.5 Ultraviolet-visible spectroscopy

UV-visible spectrophotometer (Biochrom Libra PCB 1500 UV) was used to the absorbance of the nanoparticles. An aliquot of the synthesized silver nanoparticles was taken into a quartz cuvette with a 1cm optical path and wavelength scan was taken at every 30mins interval varying wavelength until a stable absorbance was obtained at maximum wavelength.

### 2.6 Scanning Electron Microscopy (SEM) Analysis

The surface morphology of the synthesized silver nanoparticles was studied using Scanning Electron Microscopy (SEM) Phenom Pro-X Scanning Electron Microscope (Model No.: 800-07334 MVE01570775, Phenom World, The Netherlands).

### 2.7 X-ray Diffraction (XRD) Analysis

The crystal structures of the synthesized silver nanoparticles were examined using X-ray Diffraction (XRD) method with Diffractometer (Model No.: ARL'XTRA X-ray.XRD 197492086, Thermo fisher Scientific Company Switzerland).

### 2.8 DPPH Radical Scavenging Activity Assay

Antioxidant activity of the synthesized nanoparticles was determined according to the procedure described by (Rahman et al., 2015). One ml of freshly prepared 0.3mM DPPH (2, 2-diphenyl-1-picrylhydrazyl) methanol solution was added to the AgNPs or standard (20µg/ml-100 µg/ml) and the mixture was incubated in the dark at room temperature for 30mins. The absorbance of the resulting mixture was then measured at 518 nm using spectrophotometer. A blank solution was prepared with Methanol (1.0 ml) and AgNPs. Solution of L-ascorbic acid used as standard. Radical scavenging activity of the nanoparticles was calculated using the formula;

$$S.A. (\%) = \frac{(A_c - A_s)}{A_c} \times 100$$

Where S.A. is scavenging activity,  $A_c$  is the absorbance of control and  $A_s$  is the absorbance of the samples.

### 2.9 Antimicrobial Properties of the Synthesized Silver Nanoparticles.

Antimicrobial activity of the nanoparticles was determined by adopting the procedure described by (Devanesan et al., 2021). The microorganism used in this study are *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* were kept in nutrient agar slants before use. Well diffusion method was used for the antimicrobial assay and the activity of the nanoparticle was evaluated using the pathogenic organisms at different doses of 12.5, 25, 50, and 100 µg/ml.

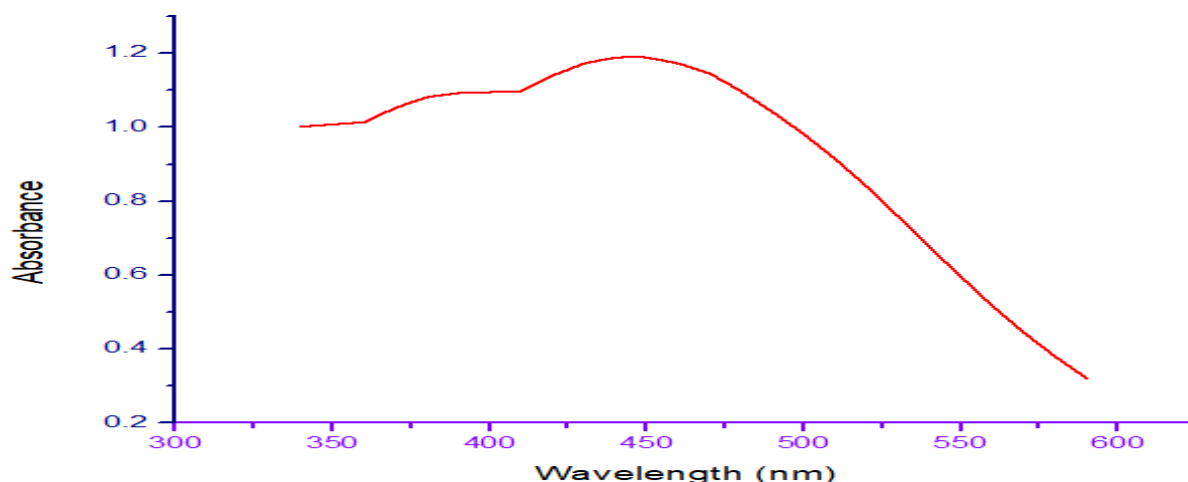
### 2.10 Brine Shrimp lethality Test of the Synthesized Nanoparticles.

The cytotoxic effects of the synthesized silver nanoparticles were measured using in vitro Brine shrimp lethality assay (BSLT). 0.5 g of brine shrimp eggs were hatched using sea water (500 mL) in a tank made of glass dish in the presence of incandescent lamp as source of light. The hatched larvae were observed moving towards light source after 48 hours. ten (10) active nauplii were carefully transferred into 5 sterilized plates each containing 10 mL of sea water. Different concentrations of the nanoparticles were then added to plate. Another plate containing only sea water without the nanoparticles was prepared and used as a control. Numbers of dead and alive nauplii were counted after 24 hours and percentage mortality was calculated using Microsoft excel.

## 3.0 RESULT AND DISCUSSION

### 3.1 UV Analysis of the Nanoparticles.

Aqueous extract of stem bark of *S. kunthianum* was used as bioreductant in the green synthesis of silver nanoparticles. Formation of silver nanoparticles was achieved by adding the aqueous extract of the plant to the solution of silver nitrate ( $AgNO_3$ ). The phytoconstituents in the extract act as reducing agent. Appearance of dark brown color after addition of the extract to the  $AgNO_3$  confirms the formation of AgNPs in accordance with the principle of surface plasmon resonance (Amendola et al., 2010).



**Fig. 1: UV-VIS Peak Values of the synthesized silver nanoparticles**

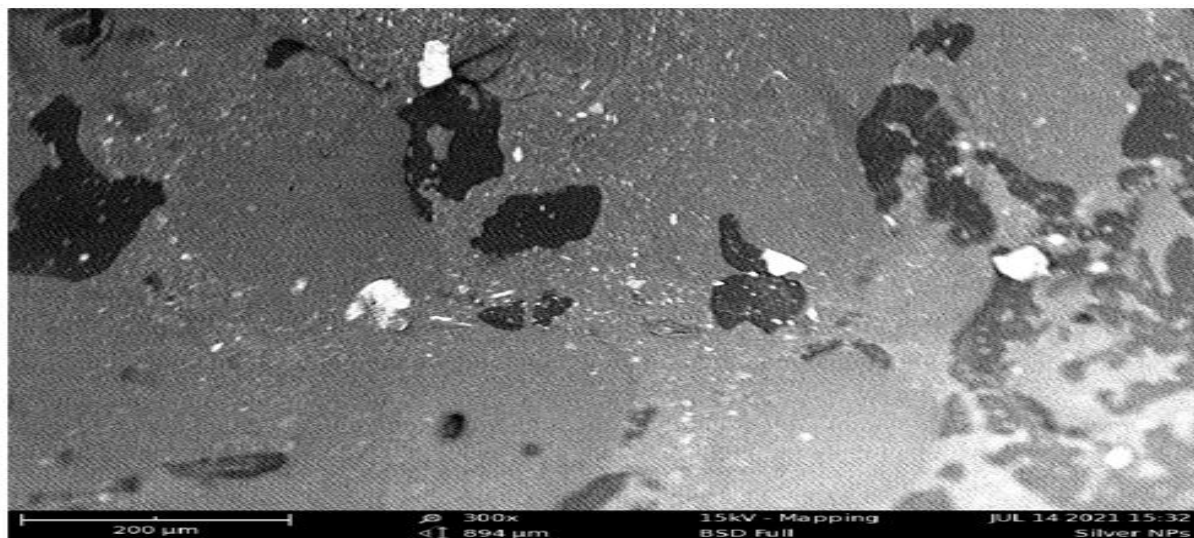
The synthesized silver nanoparticles were characterized using UV-V is spectrophotometer and the result is presented above. Colour change was observed from light brown for the extract to dark brown after the formation of the silver nanoparticles. A broad absorbance peak of the

nanoparticles was observed between 430 and 450nm. This agrees with a well-established phenomenon that synthesized AgNPs exhibit a brown color and maximum absorbance peak between 430 and 450 nm due to surface plasmon vibration excitation (Choi et al., 2021).

### 3.2 Scanning Electron Microscopy

The morphology of the synthesized silver nanoparticles was studied using scanning electron microscopy. The image of SEM analysis of the AgNPs is presented above shows that some of the synthesized nanoparticles are near spherical shape with many of the particles appear to have irregular

shapes. Jagtap & Bapat (2013) observed different sizes from the SEM image of silver nanoparticles synthesized using *Artocarpus heterophyllus Lam.* Seed extract and attributed the presence of irregular shapes to evaporation solvent during sample preparation.

**Fig. 2: SEM Image of the silver nanoparticles**

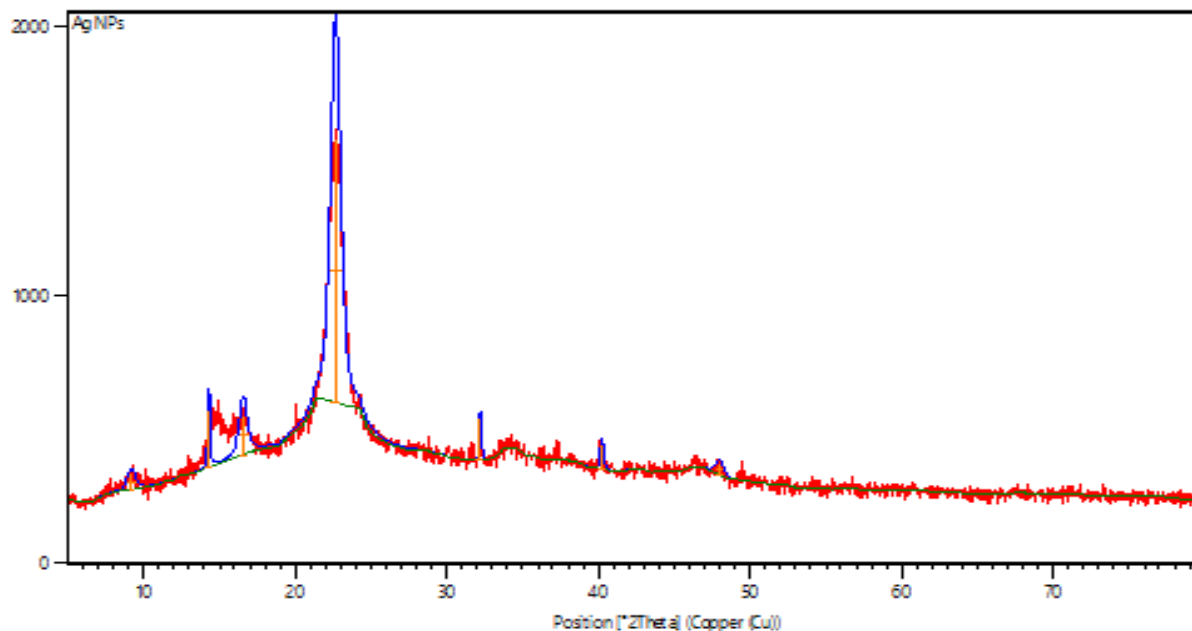
### 3.3 XRD Analysis of the Silver Nanoparticles

XRD pattern of the synthesized nanoparticles is presented in fig. above. XRD technique provides information on the crystallographic structure and other physical properties of a material. X-ray

diffraction (XRD) analysis of the synthesized nanoparticles was performed in the range of 10–80° at 2θ angles and average crystalline size was calculated using the Debye–Scherrer's equation and was found to be 27.878nm.

**Table 1: XRD Peak Values of the Silver nanoparticles**

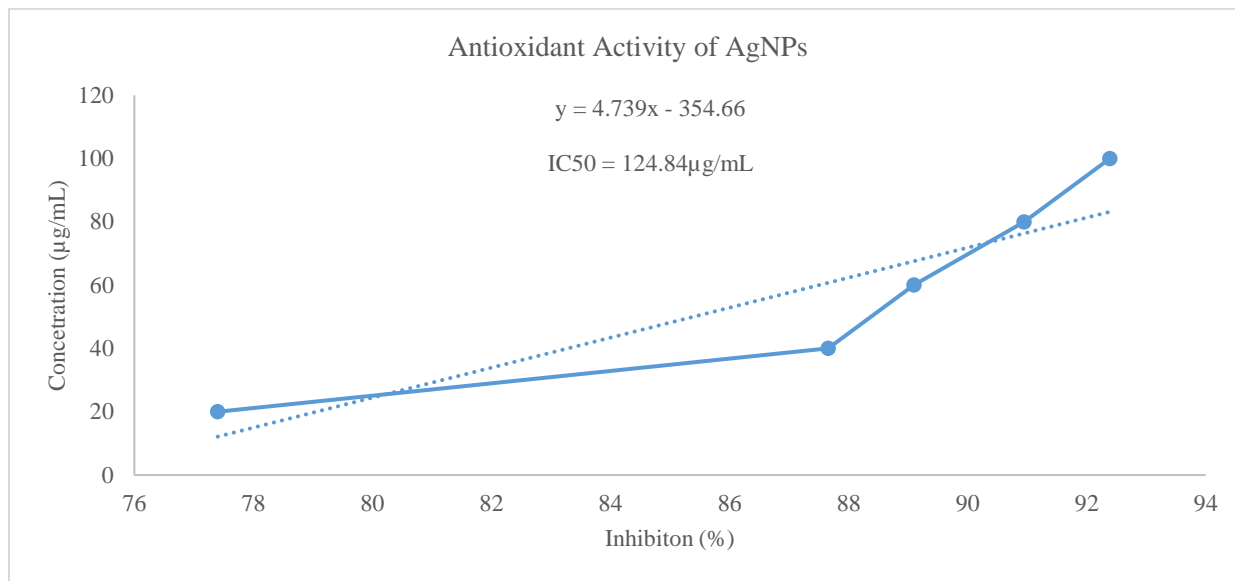
Pos. (°2θ.)	Height (cts)	FWHMLeft (°2Th)	d-spacing (Å)	Rel. Int. (%)
9.2039	53.45	0.6140	9.60875	5.45
14.2966	212.62	0.1535	6.19535	21.68
16.5632	145.95	0.6140	5.35231	14.87
22.6511	980.51	0.7675	3.92568	100.00
32.1919	155.12	0.1279	2.78069	15.82
40.2002	92.41	0.2047	2.24329	9.42
47.9454	40.46	0.6140	1.89745	4.13



**Figure 3: XRD Pattern of the silver nanoparticles**

$$D = \frac{K\lambda}{\beta \cos\theta} \dots\dots\dots\text{Equation 1}$$

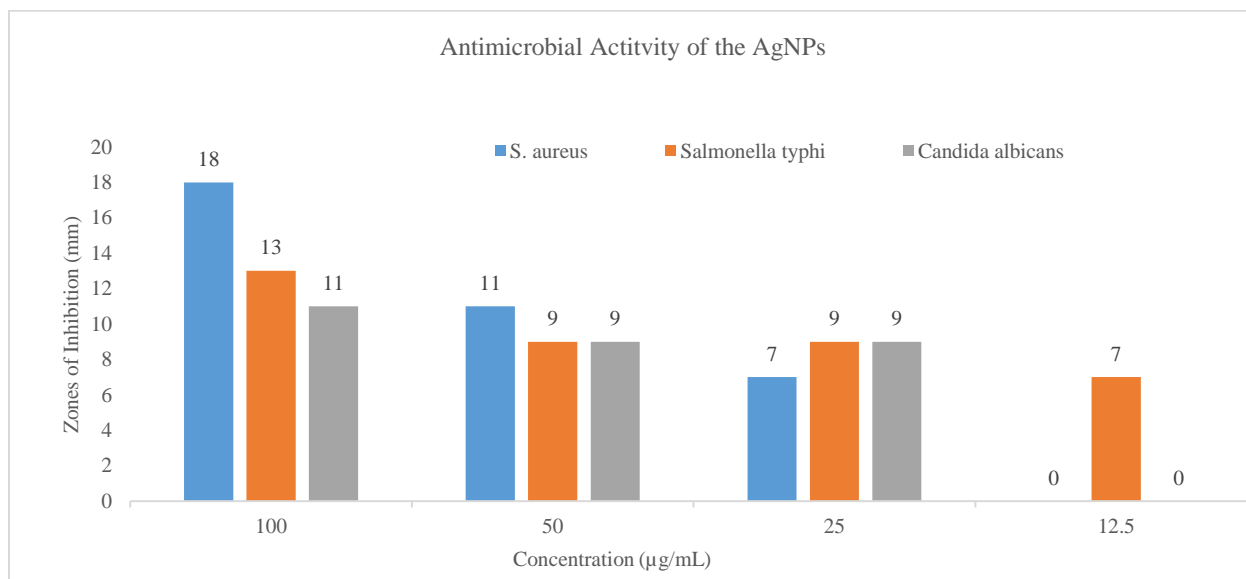
- D = Crystallite size (nm)
- K = 0.9 (Scherrer’s constant)
- λ = 1.5406 Å = 0.15406 nm (wavelength of X-ray source).
- B = FWHM (radians).
- θ = Peak position (radians)



**Fig 4: Antioxidant Activity of AgNPs**

The antioxidant potential of the synthesized AgNPs was evaluated by in vitro DPPH radical scavenging assay using BHT as a standard. The result (Fig. ) shows that synthesized AgNPs possesses moderate antioxidant activity with IC<sub>50</sub> value of 124.84 µg/mL. Various studies revealed that silver nanoparticles from plant materials possess strong antioxidant properties and some researchers

suggested the use of photosynthesized nanoparticles as a source of potential free radical scavenger (Kharat & Mendhulkar, 2016). Application of AgNPs as useful natural antioxidants for health preservation against degenerative diseases associated with different oxidative stress is strongly recommend (Mohanta et al., 2017).



**Fig 5: Antimicrobial Activity of AgNPs**

The synthesized silver nanoparticles were tested for antimicrobial activity against some selected microbial strains using well diffusion method and

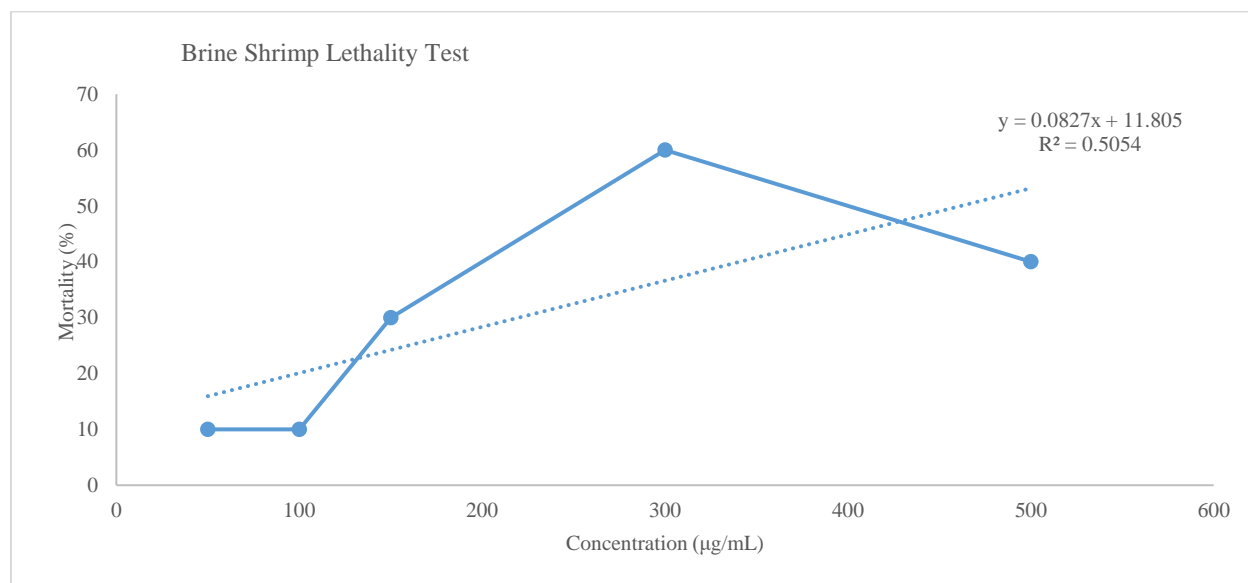
the result is presented in fig. : the result of the antimicrobial activity showed zones of inhibition against the studied microbes at different

concentration of the synthesized AgNPs. Maximum zone of inhibition of 18mm was observed against *Staphylococcus aureus* at 100µg/mL concentration while at 12.5µg/mL the nanoparticles showed no activity against *Staphylococcus aureus* and *Candida albicans*. Silver nanoparticles synthesized from plant materials were reported to show strong antimicrobial activities against different clinical isolates. Mohammadi et al. (2019) reported the antimicrobial properties of silver nanoparticles synthesized using leaves and stems of some medicinal plants from Iran. A strong Inhibition

against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was observed in the silver nanoparticles synthesized using leaf extract of *Carya illinoensis* (Javan bakht Dalir et al., 2020). The synthesized AgNPs from the stem of *S. kunthianum* also showed some antimicrobial activities against the studied microbes with highest activity against *Staphylococcus aureus* followed by *Salmonella typhi*. The findings of this study imply that AgNPs from the stem of *S. kunthianum* can serve as a potential remedy for diseases caused by the tested microorganisms

**Table 2: Brine Shrimp Lethality Test of the synthesized Silver Nanoparticles**

Concentration (µg/mL)	Log conc. (µg/mL)	Number of Nauplii	Number of Nauplii Survivors	Number of Dead Nauplii	% of mortality	LC <sub>50</sub> (µg/mL)
50	1.699	10	9	1	10	461.850
100	2.000	10	9	1	10	
150	2.176	10	7	3	30	
300	2.301	10	4	6	60	
500	2.398	10	6	4	40	



**Fig 4: LC<sub>50</sub> for the Brine Shrimp Lethality Test of the Synthesized Silver Nanoparticles**

Brine shrimp lethality assay was used to assess cytotoxicity of the synthesized AgNPs. Concentrations of 50, 100, 150, 300 and 500µg/mL of the silver nanoparticles were prepared and used for the cytotoxicity study. All the concentrations showed certain level of toxicity which increases with increase in the concentration from 50-

300µg/mL. A highest mortality was observed at 300µg/mL. LC<sub>50</sub> was found to be 461.850 µg/mL using Microsoft excel. Brine shrimp lethality assay is an important preliminary test for anticancer properties and if the test results show some level of cytotoxicity, the cytotoxicity test can be continued on human cancer cells (Nerdy et al., 2021)

#### 4.0 CONCLUSION

Plant mediated synthesized silver nanoparticles are receiving attention for their therapeutic potentials against several disease-causing pathogens. The silver nanoparticles synthesized from the stem bark of *Stereospermum kunthianum Cham* in this work possesses appreciable antimicrobial and antioxidant activities. Characterization of the nanoparticles revealed some physicochemical properties which are very important parameters for possible use of silver nanoparticles in drug delivery systems.

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