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Synthesis of Silver(AgNP) Nanoparticles using *Blighia Sapida* Peel Extract:

An unexplored Approach for Antimicrobial, Anticoagulant, and Thrombolytic Activities.

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ABSTRACT

Silver(AgNP) Nanoparticles is gaining significant attention for versatile applications,particularly in the medical domain. This study introduces a novel approach and presents a green and sustainable method using *Blighia Sapida* Peel extract .It aims to explore the biogenic synthesis of silver nanoparticles using the peel extract of *Blighia sapida* (isin) and evaluation of the nanoparticles for antimicrobial, anticoagulant and thrombolytic activities. The AgNPs were characterized by UV–Vis spectroscopy and Fourier transform and evaluated for antibacterial, antifungal, anticoagulation, and thrombolytic activities. The biosynthesis of the AgNPs reaction was carried out using 1 mM and 5mM of silver nitrate and peel extract of *Blighia sapida*. The continuous stirring of the reaction mixture at room temperature for approximately one hour resulted in the successful formation of AgNPs. A development of a yellowish brown color and dark brown confirmed the formation of AgNPs. The development of AgNPs was confirmed by the characteristic peaks of UV–Vis and Fourier transform infrared (FT-IR) spectroscopy spectra. These AgNPs also showed potent antibacterial and antifungal activity. AgNPs prevented the coagulation of blood, and also showed thrombolytic activities. Microscopic examination of the lyzed blood clot supported the thrombolytic activities. The result of this study revealed that the biosynthesized AgNPs can serve as effective antimicrobial, anticoagulant and thrombolytic agent highlighting and emphasising its eco-friendly sustainable,therapeutic applications.

Keywords: *Blighia Sapida*,Nanoparticles,Silver(AgNP), spectroscopy spectra, Thrombolytic.

Introduction

Nanotechnology is an emerging area with increased surge of interest in various fields of science, like environmental science, material science, medical science. It is the science of the synthesis of nanomaterials, and the investigation of their applications with their nanometre dimensions (Behravan *et al*., 2019). Recently, the synthesis of nanomaterials has gained massive consideration across the world because of their intensive applications, ranging from target drug delivery, cancer treatment, biomedicine and environmental remediation to antimicrobial progress (Zulfiqar *et al.*, 2019). The most significant perspectives that characterize its appropriateness for these applications are the synthesis procedures.Conventional methods of AgNP synthesis often involve the use of chemical reagents that raises concern about environmental impact and human safety. Consequently, there is an emerging concern to develop green, proficient, innocuous, non-destructive, ecological and biological approaches for the synthesis of nanoparticles (NPs), which will be environmentally friendly (Kaur *et al*., 2020). In particular, among the various NPs, silver nanoparticles (Ag NPs) are the most commercialised nanomaterials, and the production of these nanoparticles is supposed to escalate in the next few years (Godlewska *et al.*, 2019).

Silver nanoparticles ($AgNO^3$) can be generated through several methods, but the most common include physical, chemical and biological components (Singh *et al*., 2020). Although it is true that the green synthesis has three main components: a metal precursor, a reducing agent and a stabilizing agent, it could be said that it is a derivative of both methods (chemical and biological). One of the fundamental pillars of biosynthesis is to obtain extracts with high antioxidant power such as polyphenols, reduced sugars, nitrogenous bases and amino acids; capable of reducing cations in a metal salt solution (Alavi, 2022). Silver nanoparticles show great reactivity with enzymes, DNA and RNA, caused by the interactions with thiol, carboxylate, phosphate, hydroxyl, imidazole, indole or amine groups, triggering a series of reactions that prevent the formation of

microbial processes. It is important to recognize that there is a wide variety of biological resources that can be used for green or ecological synthesis, such as microorganisms (bacteria, fungi, and algae), plants and their derivatives, animal metabolites and even organic waste which opens up a range of possibilities to generate nanoparticles as well as to apply them in various areas (Elegbede and Lateef, 2021).

Ackee (*Blighia sapida* K.D. Koenig; Family: Sapindaceae) is a herbaceous, biennial plant. It is native to West Africa and is also cultivated in India and the American tropics. It is well-distributed throughout Nigeria and is found in drier forests of the savannah region. Ackee seeds contain bioactive substances such as saponins, flavonoids, tannins, terpenoids, alkaloids, steroids, and anthraquinones (Adebayo *et al*., 2020). These bio-constituents contribute to its antioxidant, anti-inflammatory, anti-diarrheal, and antimicrobial activities. Ackee provides medicinal value for traditional healers in Nigeria and across Africa for the treatment of several ailments. Ackee fruit is rich in essential fatty acids, vitamin A, zinc, and protein (Odeniyi *et al*., 2020).

The conventional methods of silver nanoparticle synthesis often involve hazardous chemical reducing agents, emphasizing the need for sustainable and environmentally friendly alternatives. *Blighia sapida* peel, an underutilized agricultural waste product, may contain bioactive compounds suitable for nanoparticle synthesis, yet this potential remains largely unexplored. There is a scarcity of research investigating the use of *Blighia sapida* peel extract in the synthesis of silver nanoparticles, leaving a significant knowledge gap in the field of nanotechnology. The pressing issues of antimicrobial resistance, coagulation disorders, and thrombosis necessitate innovative solutions, and the exploration of *Blighia sapida* peel extract as a nanoparticle synthesis agent could offer promising avenues for addressing these challenges.

2.0. Methods for Synthesis of Silver Nanoparticle

Physical Approaches

In physical approach of synthesis of AgNPs evaporation and condensation has major importance. Temperature gradient play important role in cooling of vapors at desired rate. A chance of contamination by solvent has been removed by physical approach as no solvent has been used in physical method and uniform distribution of particle size precisely obtained (Devi *et al*., 2018). Minimum inhibitory concentration in toxicity studies can be easily achieved by production of nano scale nanoparticles in high concentration(Devi *et al*., 2018). One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique. Wide range of material can be synthesized in nanoparticels by physical method such as Au, Au and PbS (Demirezen *et al*., 2018). The nanoparticles formed in a solution of high surfactant concentration are smaller than those formed in a solution of low surfactant concentration. One advantage of laser ablation compared to other conventional method for preparing metal colloids is the absence of chemical reagents in solutions. Therefore, pure colloids, which will be useful for further applications, can be produced by this method (Demirezen *et al*., 2018).

Chemical Approaches

Chemical reduction is the most frequently applied method for the preparation of AgNPs as stable, colloidal dispersions in water or organic solvents (Ali *et al*., 2018). Most commonly used reductant is citrate. In aqueous solution reduction of silver occurs and nanosize colloidal silver ions are generated. Stability of any colloidal dispersion has prime importance and which could be achieved by stabilizing agent (dodecanethiol) which adsorbed on surface and produce protective

sheath. It can avoid agglomeration and crystal growth of the system. During the synthesis of AgNPs minute changes in parameters (Polymers) makes drastic changes in size, shape, morphology, polydispersibility index, self-assembling and zeta potential (Stability). Frequently used ingredients in synthesis of AgNPs and AuNPs are glycol derivatives. Polyvinyl pyrrolidone (PVP) and Polyethylene glycol (PEG). Polyacrylamide play dual function such as reducing and stabilizing agent in synthesis of AuNPs (Kumar *et al*., 2019). Surfactants containing functional groups such as amines, thoils and acids play important role in stability of colloidal dispersion which protects the system from crystal growth, coaleseces and agglomeration.

Biological Approaches

Biotechnology is an emerging tool to develop biological synthesis of AgNPs. Besides this magnetic nanoparticles has great antibacterial potential due to improved surface area to treat raised microbial resistant against many antibiotics and medicines (Suresh *et al*., 2018). Currently green chemistry is rapidly growing technique utilized for synthesis of AgNPs with naturally occurring stabilizing, reducing and capping agents to synthesize AgNPs without toxic adverse effects. (Suresh *et al*., 2018).

Synthesis of Silver Nanoparticles by Fungi

High production yield AgNPs synthesized by fungi obtained when compared to bacteria due to fungi secret higher amount of proteins that directly responsible for increased production. Higher production rate is mainly due to silver ions entered in to fungal cell wall which leads to reduction of silver ions by fungal enzymes such as naphthoquinones and anthraquinones. Slower rate and process is only disadvantage associated with fungal synthesis of AgNPs hence green synthesis approach is more preferred over the other techniques (Vergheese and Vishal, 2018).

Synthesis of Silver Nanoparticles by Bacteria

Pseudomonas stutzeri which is the first strain of bacteria form which AgNPs were synthesized and isolated form Ag amine. Many of the bacterial strains and microorganism developing resistance to metal at lower concentration. Resistance mainly produced due to efflux, change in solubility, toxicity via oxidation/reduction and precipitation of metals. There are evidences that at lower conc. Microorganisms are alive but once exposed to high conc. Metal ions leads to microbial death. In biosynthesis of silver enzyme nitrate reductase convert nitrate to nitrite (Mohammed *et al*., 2018).

Synthesis of Silver Nanoparticles by Plants

Green synthesis is an excellent tool that can be utilized for synthesis of AgNPs as it uses natural origin medicinal herbs and its extracts which contain wide range of metabolites specifically water soluble flavones, quiones causes rapid rapid and quick reduction of silver when compared to fungi and microbes. Green chemistry approach is safe, cosat efficient, easily scalable to mass productions, easily availability of raw materials at cheaper coast. Phytochemicals directly take part in reduction process of the silver ions during synthesis of AgNPs (Gargi *et al*., 2018).

Synthesis of AgNPs Using Algae

The aqueous extract of *Pithophora oedogonia* was used for the synthesis of AgNPs. The synthesis process was considerably more rapid, and the generation of silver was reached for a few minutes. In the UV-vis spectrum, the maximum absorbance peak was observed at 445 nm. The SEM and DLS analysis of colloidal AgNPs revealed a size of 34.03 nm. The EDX spectroscopy revealed strong signals in the silver region and confirmed the presence of Ag. The FTIR analysis of the nanoparticles indicated the presence of protein, which was regarded as a capping agent surrounding the AgNPs (Gitishree *et al*., 2019). Fresh *Caulerpa racemosa*, marine algae, was used

in the synthesis of AgNPs. The reduction of silver nitrate was conducted at room temperature by the extract. The surface plasmon absorbance peak observed at 413 nm was revealed by UV-visible absorption spectroscopy. The FT-IR analysis revealed the possible functional groups responsible for the reduction and stabilization of the nanoparticles. The particles are crystalline in nature and 5–25 nm in size, as found by XRD and TEM analyses. Spirogyra was also used for the synthesis of AgNPs. In UV-vis absorption spectrum the peak was observed at 430 nm. The synthesized AgNPs were uniform and 17.6 nm in size, as revealed by SEM. AgNPs can act as a powerful antibacterial agent against various pathogenic bacteria, which was confirmed by MIC and MBC results (Sonika *et al*., 2021).

2.1 AgNP's Antimicrobial Mode of Action

When AgNP reaches toward cell they release Ag+ ions. These released ion then interact with sulfur and phosphorus containing compound present in cell wall. This lead to disarranged cell wall formation and small pits forms in the cell wall. Formed pit gives access to entry of ions and other foreign material to entry into cell. This increase intracellular osmotic pressure. As pressure built up in the cell, it begins to swell. Finally all these event lead to bursting of cell wall and cell lysis take place. This type of antimicrobial activity is more in gram −ve cell than gram +ve cell. As gram +ve cell have more cross linked peptidoglycan layer and teichic acid in their cell wall. The gram −ve cell have less or no peptidoglycane layer and have more lipopolysaccharide in their cell wall. So the AgNP's easily interact with gram −ve cell due less barrier (Mathur *et al*., 2018).

2.2 AgNP's Anticancer Mode of Action

When pit formation takes place in the cell wall, the Ag+ ions released by AgNP's get entered into cell. Then they reaches to mitochondria where they interact with thiolgoups and bind to NADPH dehydrogenase enzyme and liberates ROS. These formed ROS in mitochondria

interacted with respiratory enzymes damage ATP formation and respiratory cycle of cell. Formed ROS also interact with protein, sulfur and phosphorus containing cell constituent. Also these formed ROS also bind to phosphorus elements of DNA and RNA which lead to inhibit cell replication and protein synthesis. Due to binding with DNA aggregation of damage protein sysnthesis which lead to cell death. Another possible action is by autophagy. AgNP's have ability to induce autophagy by accumulation of autophagolysosmes in human ovarian cancer cell. This autophagy work by mainly 2 ways; at lower level they increases cell life i.e. surviving rate, but when its level increase it lead to cell death (Gitishree *et al*., 2019).

2.3 Anti-inflammatory Activity of Metallic NPs

Inflammation is a localized phenomenon that occurs as a result of injury, infection and stress by multiple mechanisms like recruitment of macrophages, killer cells cytokines like IL-1, IL-1β, and TNF-α to the desired site and develops the onset of inflammation (Zhang *et al.*, 2020). Conventionally, steroidal and nonsteroidal anti-inflammatory drugs are administered for inflammation but the side effect exerted by the drugs had an adverse effect. Nano-based herbal formulation is proved as a pioneer in developing anti-inflammatory drugs. Numerous articles emphasize the metallic NPs synthesized from plant extracts endowed with anti-inflammatory properties. Recently, a study concluded that silver NPs generated from *Selaginella myosurus* demonstrated the antiinflammatory potential under in vivo and in vitro conditions.

2.4 Larvicidal Activity of Nanoparticles

Plants like *Azadirachta indica, Nicotiana tabacum, Ocimum basilicum, Cinnamomum osmophloeum* and plant bases oils have proved the potential as larvicides (Balaji *et al*., 2020). At the present scenario, numerous plants and their derivatives have been excelled as a botanical insecticide and some of them are commercialized. As the progress of science and technology,

researchers drive their research by formulating nano formulated herbal drugs. It is very indeed at this juncture by combining plants and its products in the nano module to counteract the larvicidal populations (Balaji *et al*., 2020).

2.6 Antiviral Activity of Metallic Nanoparticles

Another important research to be addressed by the researchers is the antiviral properties of virus. In the crusade to develop fabricated nano drugs for antiviral therapy, plant-based metallic nanoparticles have open up the potential to combat viral diseases (Gulbagca *et al*., 2019).

2.7 Leishmanicidal Activity of Nanoparticles

Leishmaniasis is another important life-threatening disease caused by the parasite *Leishmania* transmitted by the sandfly *Phlebotomus* species (Tugarova *et al*., 2018). Naturally, plants bear the active ingredients to perform antagonistic activity against bacterial and viral infections. Ullah *et al.* (2018) synthesized silver NPs through the chemical and biological method from the aqueous extract of *Teucrium stocksianum* and evaluated for antileishmanicidal activity (Ullah *et al.*, 2018).

3.0 MATERIALS AND METHODS

3.1 Sample Collection

Blighia sapida were collected from Ojaoba Market, Ilorin, Kwara State. The peels were thoroughly washed, rinsed and oven-dried at a temperature of 60°C. The oven-dried peels were then blended and extracted using methanol.

3.2 Preparation of Extract

The extract was prepared by dissolving 6 g of *Blighia sapida* powder into 100mls of distilled water. The suspension was heated in a water bath at 60 ^{0}C for 1 hour. It was then filtered using Whatman No 1 filter paper and then centrifuged for 20 min at 4,000 rpm. The clear filtrate was poured out and kept for further use while the residue was discarded. For 1mM AgNo3 in 500ml of distilled water. 0.085 of the salt was weighed in 500ml of distilled water. For 5mM AgNo3 in 500ml of distilled water. 0.4255 of the salt was weighed in 500ml of distilled water.

3.3 Synthesis of AgNP

The synthesis was done by preparing 12.5 of the extract by weighing 6g of sample powder into 100mls of water and put in the shaking water bath at 60^{0}C for 1hour and will be added 500ml of each of the 1mM and 5mM $AgNO_3$ solution and monitored for colour change.

3.4 Characterization of the Plant Extract Silver Nanoparticles

3.4.1 UV–Visible Spectroscopy

The bio-synthetized *Blighia sapida* silver nitrate nanoparticles (BS-AgNO^{3}NP) were characterized using UV-visible spectroscopy. The reduction of AgNO3 to Ag+ by the plant extract was verified using an UV-visible spectrophotometer (CE7400; AQUARIUS). The absorption spectra of the samples were recorded at intervals of 24–72 hours.

3.4.2 Fourier Transform Infrared (FTIR)

The Fourier-transform infrared analysis (FTIR) analysis of the BSAgNO^{3}NP was performed using a potassium bromide (KBr) pellet (Perkin Elmer, Waltham, USA) in transmission mode. Transmission spectra were obtained using 64 scans at a resolution of 8 cm−1 in the spectral range of 400–4000 cm^{-1}.

3.5 Antimicrobial Assay

3.5.1 Antibacterial Assay

The antibacterial activity of the synthesized nanoparticles was assayed using the broth culture method. 0.5 McFarland standard was prepared for each isolates including: *Escherichia coli, Klebsiella oxytoca, Streptococcus pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*. 9.5g of Mueller Hinton was diluted with 250ml of distilled water and 100ml of each isolates was added into a solidified agar medium, swap and was allowed to dry. 6mm well was bored on the agar and 100ml of each graded concentrations 5, 8, 10, 12, and 15 mg/mL was added to the plates and were incubated at 37 ^{0}C for 24 h. After incubation, the diameters of the fungal growth were measured in millimeters and recorded.

3.5.2 Antifungal Assay

Poisoning method was used for the antifungal assay. Three different concentration was used: 50, 100 and 150 mg/mL. 6 mm agar plugs of 48 hour culture of *Aspergillus flavus* and *Aspergillus niger* were placed on the PDA plates containing the nanoparticles. PDA plates inoculated with each fungal agar plug without nanoparticles were used as control. All plates were incubated at 28 ± 2 ^{0}C for 72 hr. The diameters of the fungal growth were measured in millimeters and recorded (Lateef *et al*., 2017).

$$\% \text{ Growth inhibition} = \frac{\text{Dcontrol - Dtest}}{\text{Dcontrol}} \times 100$$

3.7 Anticoagulant and Thrombolytic Assay

The anticoagulant assay was conducted in line with previously established protocols Lateef *et al.* (2017), by adding 1 ml of colloidal 100 microliter/milliliter AgNPs to blood freely donated by a healthy volunteer in equal proportion. While blood collected into EDTA bottle was used as a positive control, blood samples collected in a clean Eppendorf tubes, and those mixed with the

individual extracts and AgNO3 solution served as negative controls. The materials were left for up to 4 h, after which visual observation was made for the detection of coagulation of blood. As a proof, all the Eppendorf tubes were thereafter inverted to show anticoagulation or otherwise. Furthermore, contents of the tubes were smeared on slide and observed with optical microscope to visualize the red blood cells (Lateef *et al.*, 2017).

Thrombolytic activity was carried out following the improved methods of Lateef *et al*. (2017) for both qualitative and quantitative assays. In this method, 0.5 ml of blood was dispensed into a previously weighed clean Eppendorf tube, and allowed to stand at 37 C in the incubator for 30 min. After the formation of clot, the tube was re-weighed and the weight of blood clot was obtained (X) by subtracting the initial weight of the tube. The blood was then treated with 100 microliter of the various AgNPs, AgNO3, and extracts. The tubes were further incubated at 37 C for 90 min, and then checked for thrombolysis. All the tubes were inverted, to allow lyzed clot to separate from clot, and then released. The final weight of the tube with the remaining blood clot was taken, from which the initial weight of tube was subtracted to obtain the weight of non-lyzed blood clot (Y). The thrombolytic activity was calculated thus:

$$\text{Thrombolytic activity} = \frac{X-Y \times 100}{X}$$

Furthermore, microscopic examination of the lyzed blood clot was undertaken as previously described by Lateef *et al*. (2017).

4.0 RESULTS

4.1 Silver Nanoparticles Synthesis

The result for the silver nanoparticles synthesis are presented in Plate 1-3.

The formation of AgNPs began just after mixing the flower extract into the $AgNO^3$ solution (1 mM and 5mM). After the addition of extracts, the $AgNO^3$ began changing its color from colorless to yellowish brown in approximately 20 minutes, and, finally, the solution turned from a light brown to dark brown color in approximately 40–45 minutes at room temperature. A dark brown color appeared within 1 hour at a high temperature (60 ^{o} C). Color changes were more intense at the higher temperature than at room temperature

4.2 UV–Visible Spectroscopy Analysis

The UV–Vis Spectrum result are presented in Figure 1-5.

A UV–visible spectral analysis of the mixed solution (peel extract and $AgNO^3$) was also performed to confirm the formation of AgNPs. The wavelength for the maximum absorbance of $AgNO^3$1 was 300nm. The wavelength for the maximum absorbance of $AgNO^3$2 was 350nm-400nm. The wavelength for the maximum absorbance of the plant extract was 350nm-400nm. The wavelength for the maximum absorbance of AgNPs1 was 350nm-450nm. The wavelength for the maximum absorbance of AgNPs2 was 350nm-450nm.

4.3 Fourier Transform Infrared (FTIR)

The FITR spectra of peel extract of *Blighia sapida* are presented in Figure 6-8.

The FT-IR spectrum of AgNPs synthesized and plant extract at 25 ◦C is presented in Figure 6-8. Various peaks of FT-IR represent different functional groups. The FT-IR spectra had major vibration modes at 2000, 1311.87, 1166.23, 1118.73, 1055.41 and 982.59 for AgNPs1. The FT-IR spectra had major vibration modes at 2000, 1378.36, 1232.72, and 1087.07 for AgNPs2. The FT-

IR spectra had major vibration modes at 2000, 1908.71, 1172.36, and 1049.078 for the plant extract. All of these spectra signified different functional groups. The results of the FT-IR analysis suggested the existence of different groups of various secondary metabolites

4.3 Antibacterial Activity of *Blighia sapida* Silver Nanoparticles (BS-AgNPs)

The antibacterial activity of BS-AgNPs are presented in Table 1 and Plate 4.

The antibacterial activity of BS-AgNPs was tested against six isolates including *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis* with concentrations of 20, 40, 60, 80, and 100 mg/mL (1mM, 5mM). Maximum inhibition was observed against *Staphylococcus aureus* (11mm, 29mm) while it was not effective against *Proteus mirabillis*

4.4 Antifungal Activity of *Blighia sapida* Silver Nanoparticles (BS-AgNPs)

The anti-fungal activity of BS-AgNPs are presented in Table 2 and Plate 5.

The anti-fungal activity of BS-AgNPs was tested against three fungal isolates including *Aspergillus niger* and *Aspergillus flavus* with concentrations of 150, 100, and 50 microgram/milliliter (1mM, 5mM). Maximum inhibition (43mm) was observed against *Aspergillus niger* at concentrations of 100 mg/mL in 1mM. Maximum inhibition (31mm) was observed against *Aspergillus flavus* at concentrations of 100 mg/mL in 1mM. The AgNPs (1mM, 5mM) was also tested against *Candida* at concentration of 20, 40, 60, 80, and 100 mg/mL.

4.4 Anticoagulant and Thrombolytic Activity of AgNPs

The anticoagulant result of AgNPs are presented in Plate 6.

It was observed from plate 5 that the control blood without EDTA and AgNPs was clotted the one blood with EDTA and AgNPs prevented coagulation (Plate 5). The same result was also observed in the photomicrograph of the blood with or without EDTA and AgNPs (Plate 6, 7 and 8).

The thrombolytic result of AgNPs are presented in Plate 7.

The AgNPs dissolved the preformed blood clot on slides almost instantaneously with very high thrombolytic activity, whereas the negative controls of AgNO3- treated and nest extract-treated blood clots did not lead to lysis of the blood clot. The initial weight of 1mM is 1.05 and final weight after incubation is 1.53. The initial weight of 5mM is 1.08 and final weight after incubation is 1.56

$$\text{Thrombolytic Activity} = \frac{X-Y}{X} \times 100$$

$$\text{For 1mM} = \frac{1.53-1.05}{1.53} \times 100$$

$$= 31.3\%$$

$$\text{For 5mM} = \frac{1.56-1.08}{1.56} \times 100$$

$$= 30.76\%$$

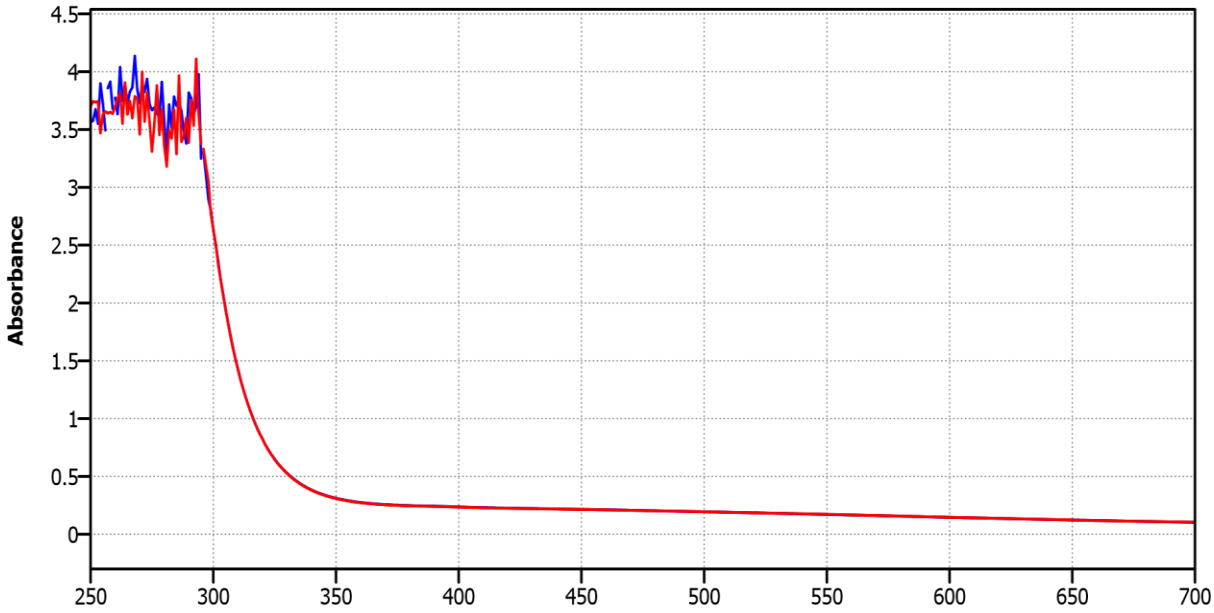


Figure 1. UV-Vis Spectrum of $AgNO^3$

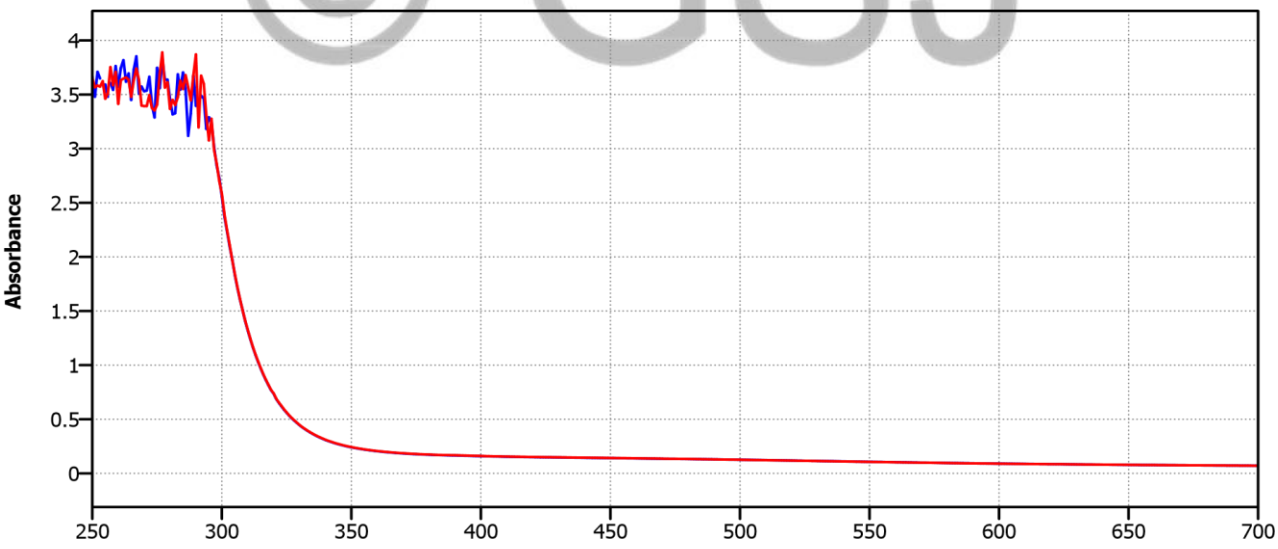


Figure 2. UV-Vis Spectrum of $AgNO^3$

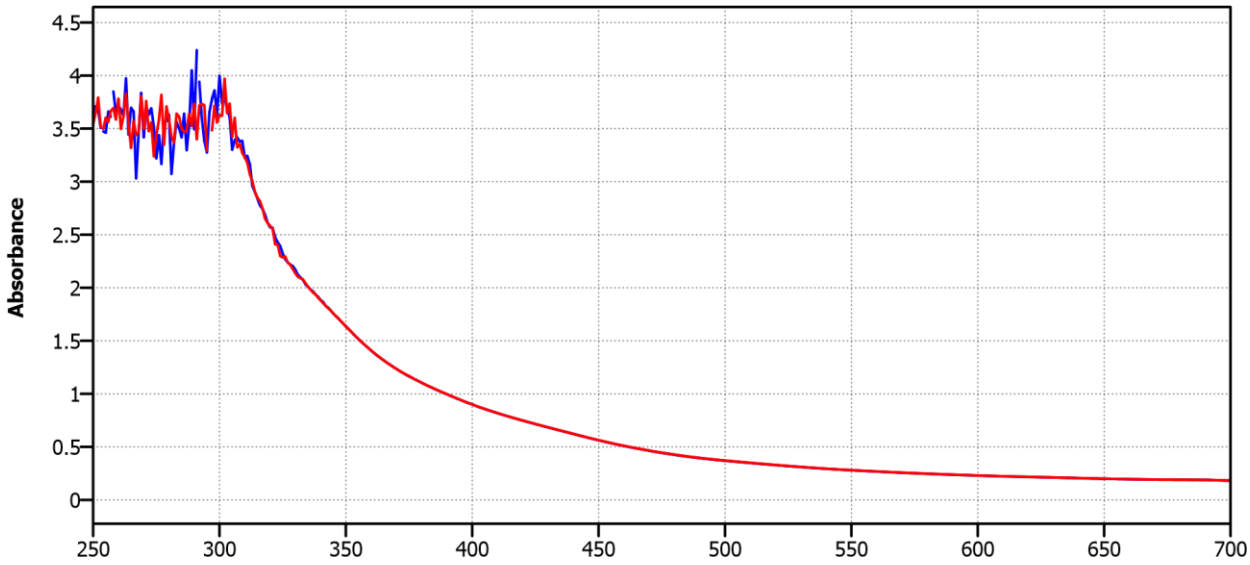


Figure 3. UV-Vis Spectrum of the Plant Extract

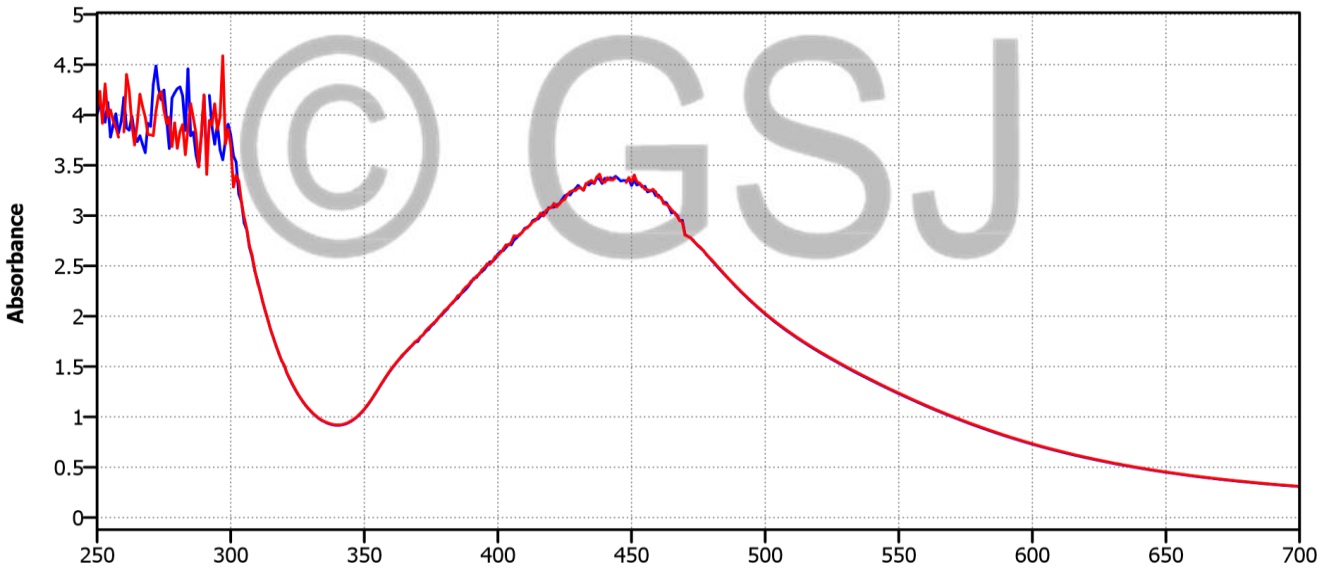


Figure 4. UV-Vis Spectrum of the AgNPs

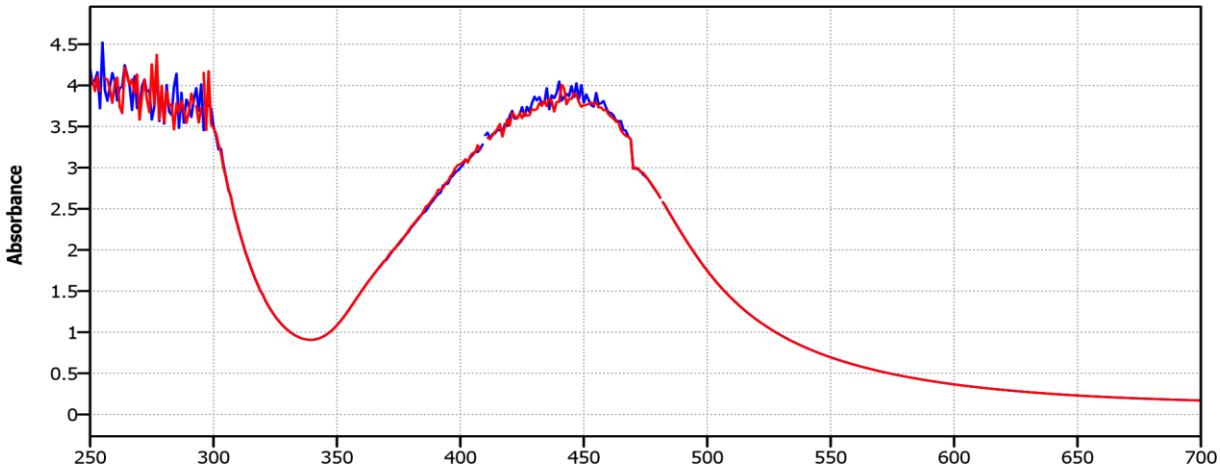


Figure 5. UV-Vis Spectrum of the AgNP

Figure 6. FITR Analysis of AgNPs 1

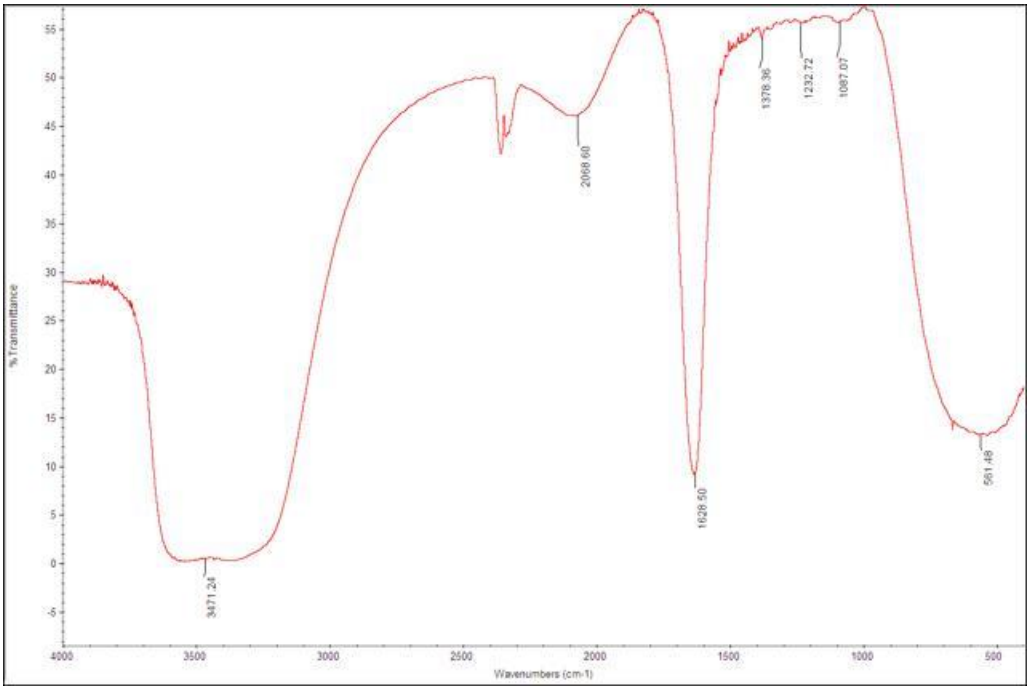


Figure 7. FITR Analysis of AgNPs 2

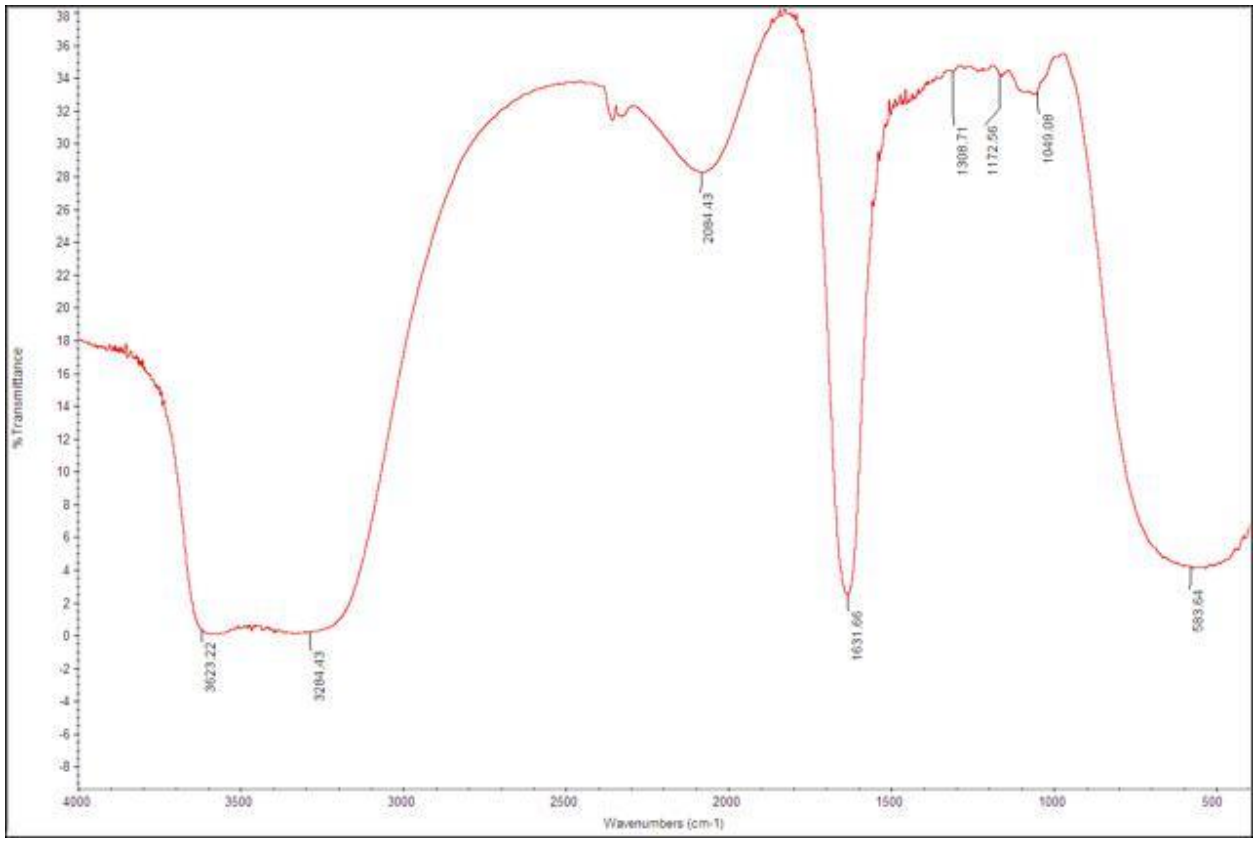


Figure 8. FITR Analysis of the Plant Extract

PEEL EXTRACT CONCENTRATION (mg/ml)	DIAMETER ZONES OF INHIBITION (mm)													
	E.coli		KP		KO		SAL		SA		PA		PM	
	1mM	5mM	1mM	5mM	1mM	5mM	1mM	5mM	1mM	5mM	1mM	5mM	1mM	5mM
20	0	9	0	11	0	0	0	11	0	14	0	12	0	0
40	0	11	0	12	0	11	0	13	0	18	0	13	0	0
60	0	13	10	0	0	11	9	13	9	19	11	12	0	0
80	0	12	12	12	0	10	11	13	1	25	13	14	0	0
100	0	12	13	15	0	13	12	14	11	27	14	17	0	0

Table 1. Antibacterial Assay of Test Organisms to the Aqueous Peel Extract of *Blighia sapida* at Different Concentrations using Disc and Agar Well Method

Keywords: 0=resistant, E.coli= *Escherichia coli,* KP= *Klebsiella pneumoniae,* KO= *Klebsiella oxytoca,* SAL= *Salmonella,* SA= *Staphylococcus aureus,* PA= *Pseudomonas aeruginosa,* PM= *Proteus mirabilis*

Table 2. Anti-fungal Assay of Test Organisms to the Aqueous Peel Extract of *Blighia sapida* at Different Concentrations using Poisoning Method

			Growth Inhibition (%)		
S/N	**Isolates**	**Control**	**150(mm)**	**100(mm)**	**50(mm)**
1.	*Aspergillus niger* (1mM)	49	(30, 40)	(32, 33)	(37, 50)
	(5mM)	59	(5, 90)	(13.5, 73)	(18, 64)
2.	*Aspergillus flavus* (1mM)	33	(9, 72.7)	(37, 18.1)	(43, 27)
	(5mM)	35	(7, 78.5)	(13, 62.8)	(39, 8.57)

Table 2.1 *Candida* Zone of Inhibition

	Zone of Inhibition				
Peel Extract Concentration	**20**	**40**	**60**	**80**	**100**
1mm	0	0	13.5	17.5	15.5
5mm	0	0	13.5	17.5	16.5

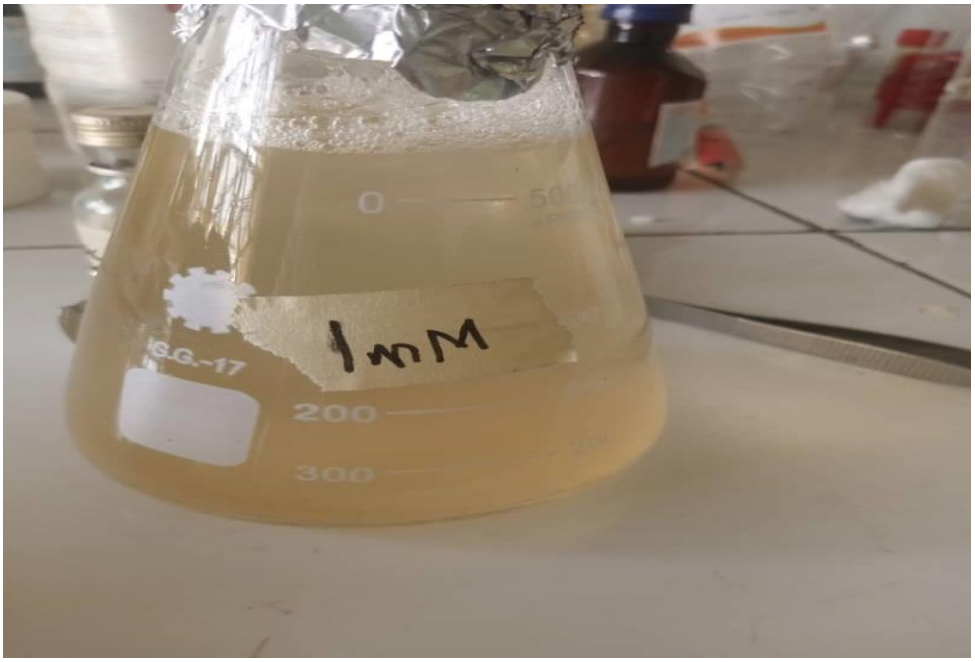


Plate 1. Biosynthesized Silver Nanoparticles at 25 ℃ (1mM)

Plate 2. Biosynthesized Silver Nanoparticles at 25 ℃ (5mM)

Plate 3. Biosynthesized Silver Nanoparticles at 60 ℃ (1mM, 5mM)

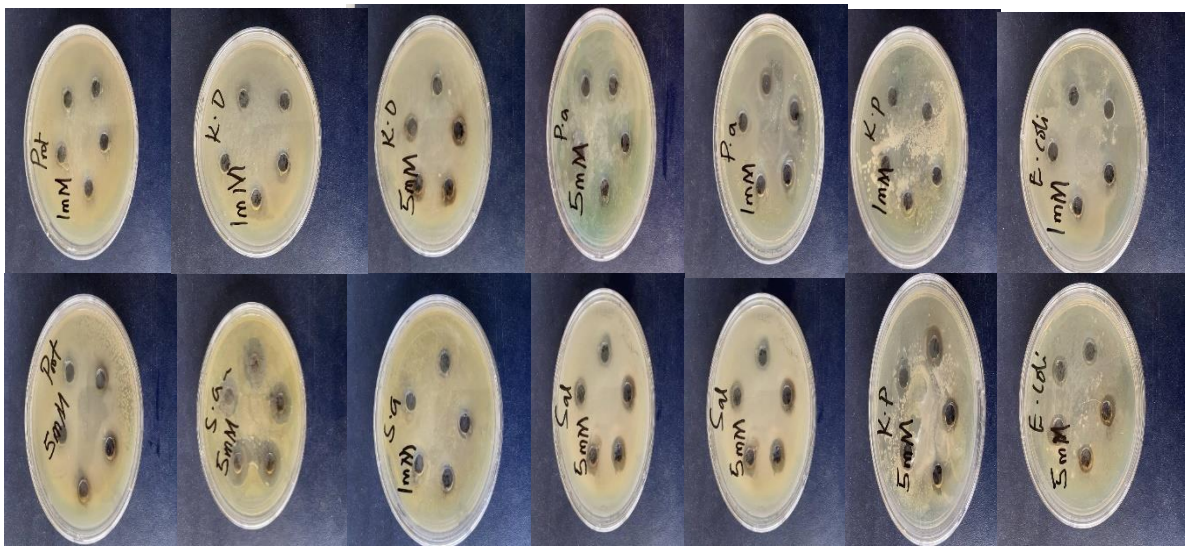
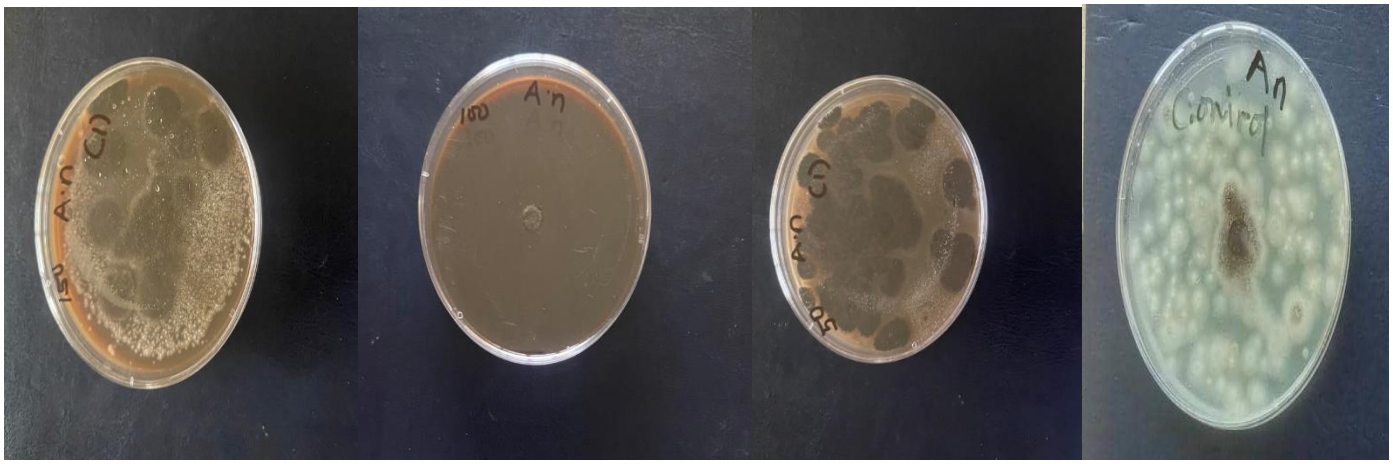


Plate 4. Antibacterial Activity of *Blighia sapida* Silver Nanoparticles (BS-AgNPs)

Silver Antifungal Activity

150 mg/mL 100 mg/mL 50 mg/mL Control

Aspergillus niger



Aspergillus flavus

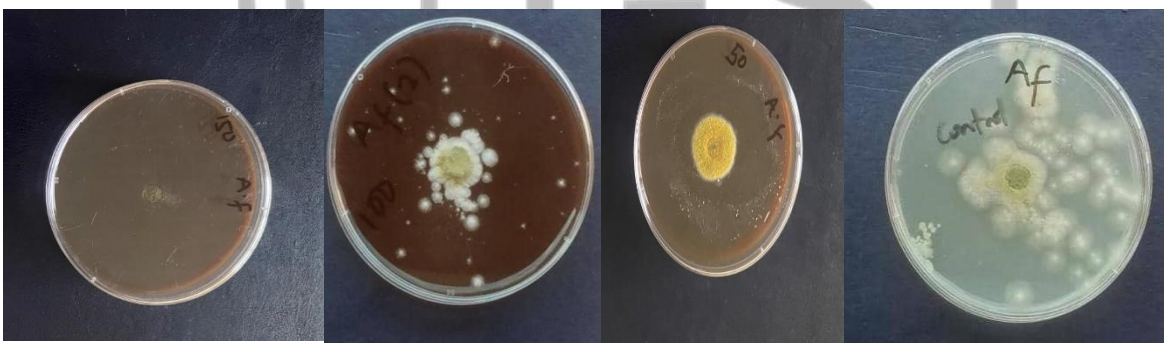


Plate 5. Antifungal Activity of *Blighia sapida* Silver Nanoparticles (BS-AgNPs)

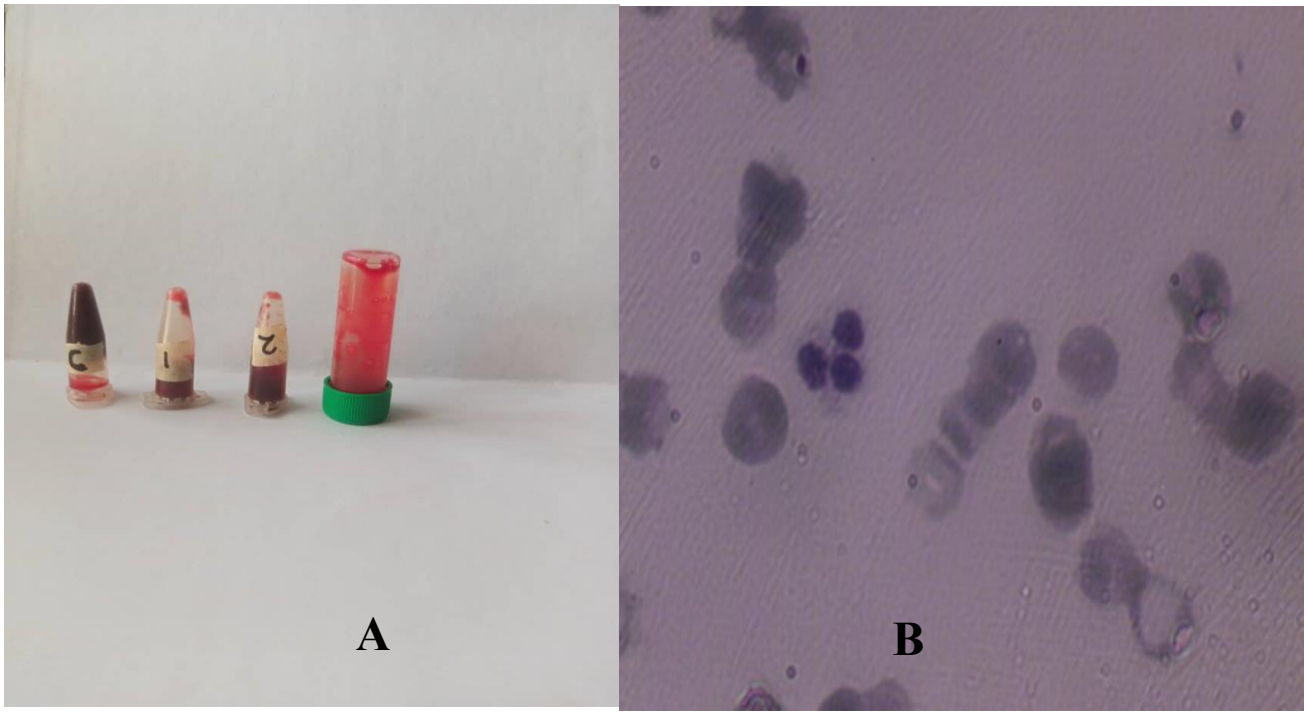


Plate 6. (A) Anticoagulant activity of Biosynthesized AgNPs; (B) Microscopic View of Biosynthesized AgNPs

Plate 7. Thrombolytic Activity Biosynthesized AgNPs

CHAPTER FIVE

5.0 Discussion

Blighia sapida have some alkaloidal content, and alkaloids are very useful defense systems for plants (Odeniyi *et al.,* 2020). They protect the plant against herbivores and pathogens. Hence, it can be said that *Blighia sapida* have anti-inflammatory, antioxidative, anticarcinogenic, anti-allergic, immunomodulatory, antifungal, antibacterial, and protective functions (Odeniyi *et al*., 2020). This study was carried out to biogenic synthesis of silver nanoparticles using the peel extract of *Blighia sapida* and evaluation of the nanoparticles for antimicrobial, anticoagulant and thrombolytic activities.

Color changes in the reaction mixture ($AgNO^3$ Solution + Peel Extract of *Blighia sapida*) confirmed the synthesis of silver nanoparticles. The color of the reaction mixture solution changed from colorless to yellowish, then to yellowish brown at 1mM and finally to dark brown at 5mM. The color change at 1mM was similar to the studies of Singh *et al.* (2021) who observed yellowish brown color at 1mM during synthesis of Silver nanoparticles. The color change at 5mM was similar to the studies of Singh *et al*. (2021) who observed yellowish brown color at 5mM during synthesis of Silver nanoparticles Anandalakshmi *et al.,* (2016). The color changes in the solution showed the presence of AgNPs due to the excitation of surface plasmon vibrations. The synthesis of AgNPs at room temperature takes more time when compared to the 60 ◦C, which may be due to the high temperature that speeds up the process. This findings of this study agrees with the study of Shah *et al*. (2021) and Singh *et al.* (2021).

UV–Vis spectroscopy is the most important technique and the simplest way to confirm the formation of nanoparticles. The absorbance spectrum of the colloidal sample was obtained in the

range of 300–450 nm, using a UV–Vis spectrometer Shimadzu-UV 1800 with distilled water as a reference. Metal nanoparticles have free electrons, which yield a surface plasmon resonance (SPR) absorption band, due to the mutual vibration of electrons of metal nanoparticles in resonance with light wave. The appearances of the peaks show the characteristics of surface plasmon resonance of silver nanoparticles. The metal nanoparticles, such as silver, have free electrons, which contribute to the surface plasmon reasonance (SPR) absorption band. The UV–Vis spectrum shows the important role of $AgNO_3$ and the presence of ingredients in the leaves for the formation of silver nanoparticles (Anandalakshmi *et al.,* 2016). A higher absorbance peak (450 nm) was obtained from AgNPs formed at 60 ^{0}C, which showed that a higher number of nanoparticles were synthesized at 60 ^{0}C. This agrees with the studies of Jan *et al*. (2020).

FT-IR studies of AgNPs were carried out to recognize the possible biomolecules responsible for the reduction of the Ag+ ions and the capping of the bioreduced AgNPs biosynthesized by *Blighia sapida* peel extracts. Previous studies have revealed that carbonyl, amide, and amino groups show a tendency to bond with metal particles. This helps to form a layer on the metallic nanoparticles, which ensures their stabilization and agglomeration. The amide and other functional groups in the extract can probably influence the interaction of AgNPs with peptides or carbohydrates, thus stabilizing them. The smaller size and crystal structure of AgNPs have excellent antimicrobial potential (Nadaf and Kanase, 2019). Biosynthesized AgNPs have shown absorption peaks in regions that are already related to the presence of polyphenols capped by AgNPs. The FT-IR spectrum band at 982.59, and 1055.41 cm^{-1} represents the vibration of C=C alkenes and the presence of the methoxy group ($–OCH_3$). The band at 1166.23, 1118.73 cm^{-1} was assigned for the C–N (amines) stretch vibration of the proteins. The band at 1378.36 cm^{-1} represents the N=O symmetry stretching, which is typical of the nitro compound. The band at

1908.71 cm^{-1} relates to C–N and C–C stretching, indicating the presence of proteins. The spectrum peaks of 2000 proved the presence of alkynes N=C=O and O=C=O, respectively. The findings of this studies were similar to those in previous reports Singh *et al.* (2021); and Joshi *et al*. (2018). The results of the FT-IR study proved the presence of various phytochemical groups in *Blighia sapida* peel extracts. The presence of various characteristic functional groups may be responsible for the medicinal properties of *Blighia sapida* peel extracts (Singh and Sharma, 2019).

The biosynthesized silver nanoparticle contained silver, which boosted the antimicrobial effects of the nanoparticle, as silver is found to have antimicrobial properties (Adebayo *et al.*, 2020). Odeniyi *et al.* (2020) found out that *Blighia sapida* contain phytochemicals such as tannins, flavonoids and saponins. Tannins inhibit extracellular microbial enzymes, reduce bioavailable iron, and form hydrogen bonds, specific interactions with proteins such as enzymes or cell envelopes, and complex formulations with polysaccharides. Tannins have been found to have antimicrobial activity against fungi, bacteria and yeast. Flavonoids exhibit a wide range of activity, ranging from antimicrobial to anti-inflammatory, analgesic, anti-allergic, and antioxidant effects. They help reduce the risk of cancer and prevent menopausal symptoms (Mebude *et al.,* 2017). Their antibacterial effects are thought to come from their ability to form complexes with bacterial cell walls and extracellular and soluble proteins (Mebude *et al.,* 2017). The antibacterial activities of AgNPs have been linked to the interaction of AgNPs with sulfur and phosphorus containing constituents of the bacterial cell to initiate cell killing by attacking the respiratory chain and cell division (Lateef *et al*., 2017). It has been suggested by earlier reports that depending upon the damage of the bacterial cell wall, Ag NPs can penetrate the cell wall. The Ag NPs could then affect the functions of important biomolecules, such as DNA, proteins, lipids, and respiratory enzymes that cause oxidative stress by liberating ROS and damage of nucleic acids and proteins, which may

lead to the death of the bacterial cell Singh *et al.*, (2020). AgNPs was effective against selected drug resistant bacteria. The results in this study suggested that the antibacterial activity of AgNPs could be caused by the destruction of the microbial cell membrane which indicate the potency of AgNPs synthesized in this study as potent anti-bacterial agent.

Nanoparticles have the capability to disrupt both fungal cell walls and membranes thereby leading to leakage of intracellular constituents that may herald death. They can also ensure fungal death through the generation of reactive oxygen species and hydroxyl radicals (Lateef *et al*., 2017). The AgNPs completely inhibited the growth of *Aspergillus niger* and *Aspergillus flavus* at tested concentrations of 50, 100 and 150 microgram/milliliter which is line with the report of Lateef *et al*. (2017) indicating the potency of AgNPs synthesized in this study as potent anti-fungal agent (Sumaira *et al*., 2017). The AgNPs (1mM, 5mM) was also tested against *Candida* at concentration of 20, 40, 60, 80, and 100 mg/mL. These observations are in contrast to the profuse growth that were obtained on the control plates. The antifungal activities agree with previously published results (Lateef *et al*., 2016; and Ojo *et al.*, 2016).

The AgNPs showed potent blood anticoagulation and thrombolytic activities from the slide and microscopic observations. The AgNPs prevented formation of blood clot when used as anticoagulation agent, which compared favorably with the positive control using EDTA The blood coagulation system is important to maintain steady blood flow, forestall bleeding and assists the innate immune system to prevent the spread of infectious agents. This is not without a disadvantage, as the formation of blood clot arising from infection can damage tissues and leads to organ failure, often associated with cardiovascular disorders, autoimmune reactions, allergic responses, injuries, and emergence of cancer. While blood clotting is necessary to curb excessive bleeding, its timely dissolution is equally important to prevent thrombosis and maintain

homeostasis. The timely and efficient dissolution of blood clot are key factors in achieving desirable outcome in patients with ischemia (inadequate supply of blood to organs), thereby necessitating optimization of the treatment regime using nanotechnology (Lateef *et al*., 2017).

Thrombolysis of 31.3% and 30.76% was obtained 1mM and 5mM AgNPs respectively. The reduced thrombolytic activity of 1mM BS-AgNPs compared to 5mM BS-gNPs may be attributed low fibrinolytic activity, which is a probable mechanism in the dissolution of blood clot. The results herein support earlier evidences of thrombolytic actions of AgNPs (Lateef *et al*., 2016). The differences observed in the activities can be related to the attributes of the particles; particularly size and morphology as well as the nature and distribution of the capping molecules. It is evident that extracts alone can impact some degree of thrombolysis as seen in this study and in the works of Lateef *et al.* (2016). The microscopic examination revealed that the red blood cells were free from the fibrin network and also retained the biconcave disc nature. While blood clotting is necessary to curb excessive bleeding, its timely dissolution is equally important to prevent thrombosis and maintain homeostasis (Devi *et al.*, 2016). The timely and efficient dissolution of blood clot are key factors in achieving desirable outcome in patients with ischemia (inadequate supply of blood to organs), thereby necessitating optimization of the treatment regimes using nanotechnology. The traditional antithrombotic treatments, such as streptokinase, have such limitations in their suitability for application, including short half-life, neutralization of the foreign agents by antibodies, and danger of excessive bleeding (Devi *et al.*, 2016).

5.1 Conclusion

This study demonstrated the eco-friendly synthesis of AgNPs using the peel extract of *Blighia sapida*. The formation of AgNPs was confirmed through UV-Vis spectrum analysis and FTIR analysis. The bio-synthetized AgNPs displayed remarkable antimicrobial activities against multi-drug resistant bacteria and fungi. In addition, potent blood anticoagulation and thrombolytic activities were obtained for the AgNPs. Thus, they could be of great importance as microbial growth inhibitors, making them useful in antimicrobial control systems and medical devices.

5.2 Recommendation

It is therefore recommended that further investigations for clinical applications such as drug delivery and anticancer activities of these agents be assessed.

5.3 Future Contribution

An extensive research is required, especially in in vivo conditions, in order to find out the accurate dose and toxicity assessment of AgNPs before clinical practice of these nanoparticles. Moreover, future work may also be accompanied with the identification of end-capping phytocompounds responsible for these biological activities.

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