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Original Research Article

Biogenic Silver Nanoparticles from two Species of Malvaceae: Synthesis, Antimalarial, Antitrypanosomal, Antimicrobial Properties and their Potential towards HeLa Cell Line

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ABSTRACT

Both Gossypium barbadense (GB) and Gossypium hirsitum (GH) are members of the Malvaceae family and the cotton genus. In order to reduce silver nitrate, aqueous extracts of both plants were used to synthesize biogenic silver nanoparticles (AgNPs) (AgNO3). The techniques employed to describe them included X-ray diffraction, ultraviolet-visible spectroscopy, scanning electron microscopy, energy dispersive X-ray (EDX), transmission electron microscopy (TEM), and the fourier transformed infrared (FTIR) spectrophotometer. The AgNPs were found to be crystalline according to the XRD spectra, but TEM images showed that they were evenly distributed, free of aggregation, and in irregular shapes with an average size of 21 nm. Only silver, oxygen, and carbon were present in the nanoparticles, according to the SEM and EDX data. Both AgNPs have excellent antimalarial efficacy when tested *in vitro* with *Plasmodium falciparum*, with IC_{50} values of 1.2 and 0.96 g/mL, weak antitrypanosomal potentials, and a good track record of negligible cytotoxicity against the HeLa cell line. Both AgNPs exhibit potent antiplasmodial and antibacterial characteristics, making them intriguing candidates for use in nanomedicines and other contexts where related applications are needed. The aim of this study is to biogenically synthesize silver nanoparticles (AgNPs) using the aqueous leaf extracts of GB and GH, analyze their ability to target the HeLa cell line, and determine whether they have antimalarial and antitrypanosomal properties.

Keywords: *Gossypium barbadense* (GB) and *Gossypium hirsutum* (GH), AgNPs, cytotoxicity action, antimalarial activity, antitrypanosomal activity, IC_{50}.

Introduction

Natural products have an endless potential for new therapeutic leads due to their unparalleled chemical diversity. This chemical diversity has made medicinal plants a substantial source of biologically active compounds, and they have been extensively employed in the treatment of humans.[1,2] Nanoparticles (NPs) exhibit exceptional behaviour due to their small size and remarkably great ratio of surface region to volume; this distinctive capability makes them very useful in many areas of research like biosensors, optics, packaging, and drug delivery[3,4] and helps to determine some of the metal nanoparticles' properties such as their chemical, electrical, optical, physical, chemical, solubility, and stability.[5,6]

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Ionic coalition, solvent dispersion, fluid abstraction, compacted state reactions, chemical reactions, polymerization technique, and co-precipitation are all methods used to synthesize NPs. All the procedures highlighted above entail the use of chemicals like sodium borohydride (NaBH4), hexadecylamine (HDA), polyvinylpyrrolidine (PVP), and ethylene glycol (EG) as capping and reducing agents,[7,8,9] but these chemical solvents have been found to be expensive, non-biodegradable, and environmentally unsafe. To evade this difficulty, different approaches comprising the use of aquatic organaisms, microorganisms, and plants have been devised for the synthesis of nanoparticles using hypoallergenic and environmentally safe materials called "biogenic synthesis." The purpose of these plant extracts or microorganisms is to decrease and stabilize the nanoparticles.[10]This is achieved due to the different secondary metabolites embedded in plants, such as terpenes, amino acids, saponins, alkaloids, tannins, flavonoids, enzymes, and vitamins, which have already been recognized to possess medicinal activity.[11]Biogenic forms of various extracts from plants and microorganisms are now being used due to the production of large quantities of NPs that are eco-friendly, simple, non-adulterated, energy-conserving, well-distinct in shape and arrangement, and suitable for use in medicine and food applications.[12]These days, biosynthesis using plants is preferred over the use of microbes because of its simplicity and rapidity.[13] The whole aerial parts of plants, like the seeds, flowers, leaves, stem, root, fruits, or peels,[14,15,16]can be used to biosynthesize silver nanoparticles. The secondary metabolite in plant extracts is able

to bring about a reduction in metal ions; this has shifted the focus of a lot of researchers towards biogenic synthesis over the past year.[17] Parasitic diseases create serious health challenges for millions of people in the world due to serious limitations in their treatment and control methods.[18] Malaria is one of the parasitic diseases spread by the sting inflicted by Anopheles mosquitoes infested with Plasmodium species. There are generally four species of Plasmodium that infect humans: Plasmodium malariae, Plasmodium vivax, Plasmodium ovale, and Plasmodium falciparum, which is the major cause of morbidity and mortality. Malaria primarily affects poor people living in tropical and sub-tropical regions where the growth of these vectors and parasites is supported by temperature and rainfall.[19] The multiplication of *P. falciparum* and *Trypanosoma brucei* resistance to antiplasmodial and antitrypanosomal therapeutic drugs has contributed to the global reappearance of malaria and sleeping sickness, and these problems have catalyzed the search for new antimalarial and antitrypanosomal drugs.[20] Nanotechnology has shown significant progress and improvement in the management of parasitic infections,[20] this is achieved through the distinct characteristics of NPs like AuNPs, AgNPs, chitosan, and other metallic NPs. Gossypium barbadense (GB) and Gossypium hirsitum (GH) both belong to the cotton genus and the family Malvaceae; they are natural plants with fibers of immense economic importancec.[20] Both plants contain gossypol (1,1', 6,6', 7,7'-Hexahydroxy-5,5'-diisopropyl-3,3'-diméthyl-2,2'-binaphtalène-8,8'-dicarbaldéhyde), a yellow polyphenolic compound embedded in the pigment glands of the aerial parts of both plants (leaves, seeds, and root).[21] A recent study documented the antiplasmodial activities of ethanol and hexane aqueous extracts of *G. herbaceum* with IC_{50} values of 9.99 and 9.76 µg/mL respectively.[22] This compound has been examined by some researchers as human medicine, as male contraceptive and antihypertensive agents; in addition to these, it is also found to reduce the growth of both Trypanosoma cruzi, the causal agent of Chagas disease, and Entamoeba histolytica, responsible for amoebiasis.[23,24] It has also been discovered that these two plants contain cyclopropenoid fatty acids in the seed and tannins in the flower buds and leaves.[24] Both extracts from these plants have been documented to possess antihypotensive effects in rats, increase smooth muscle contraction in guinea pigs, and be used as alternative medicine for menstruation stimulants.[24] We find it interesting that there hasn't been any information reported on the biogenic synthesis of silver nanoparticles using Gossypium barbadense (GB) and Gossypium hirsutum (GH) extracts with their antiplasmodial, antitrypanocidal, and antimicrobial properties. In view of this, the authors present for the first time the biogenic synthesis of silver nanoparticles (AgNPs) obtained from the aqueous leaf extracts of two species (Gossypium barbadense GB and Gossypium hirsitum GH) of the Malvaceae family.

Materials and Methods

Reagents and Chemicals

Fluka Chemicals (Buchs, Switzerland) supplied dimethyl sulfoxide (DMSO), while South Africa (Merck) provided silver nitrate and Oxford Ltd, England provided Mueller-Hinton agar. The rest of the chemicals used in this research were of analytical grade.

Collection of Organisms

Clinically isolated microorganisms were employed in this experiment. Government Hospital Oyo in Nigeria graciously donated bacterial clinical isolates. The samples were delivered in petri dishes in complete anonymity, with no patient information. The working bacterial specimen was calibrated to the Mc Farland standard prior to the experiment (about 10^{4} CFU/mL). They include: *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, and Klebsiella pneumoniae*

Characterization

A D8 Bruker advanced x-ray diffractometer (XRD) was used to determine the crystalline structure and size of the biosynthesized materials. UV-visible spectra were obtained using a universal absorption spectrophotometer (Perkin-Elmer). Measurements were performed within the range of 400–800 nm with 1 s of initialization. The XRD arrays were recorded at a scanning rate of 4°/min using Cu K radiation (= 1.5406) operated at 40 KV and 40 mA. A Perkin-Elmer FTIR spectrometer with the all-purpose ATR sampling attachment was used to create the Fourier Transform Infrared (FTIR) spectra over a range of 400–4000 cm1. Using FTIR of the reduced AgNO3, the two biosynthesized AgNPs' surface chemistry was examined for the presence of functional groups. The two samples were placed on a copper grid covered in carbon, and TEM was used to examine the morphology and particle size of the materials, which was done on a JEM 2100. Additionally, SEM was used to observe the morphology of the biogenic NPs, which was accomplished on a JOEL JSM-6390 LV, and EDS, which took into account the elemental composition of the bio-synthesized NPs recorded using SEM sourced with an (JEOL).

Extracts preparation

On the 23rd of April, 2021, fresh leaves of *G. barbadense* and *G. hirsutum* were obtained from the grounds of the Emmanuel Alayande College of Education in Oyo, Nigeria.. Mr. Ayegba Sule, a taxonomist at the Department of Plant Science and Biotechnology, Kogi State University, Anyigba, Nigeria, identified and verified the plant materials. For record-keeping purposes, Voucher's specimen (Ogunmola GH and Ogunmola GB) numbers were given and the plants were deposited at the university herbarium. The samples were air-dried at ambient temperature for 2 weeks; they were pummelled with a power-driven grinder (Polymix, PX-MFC 90D); approximately 40 g of the grinded samples were immersed in 300 mL of distilled water distinctly followed by agitating them separately on an orbital mixer for 24 h, after which they were strained with a glass wool and the filtrates were lyophilised into dry powers. The powered were kept in a firmly stopped centrifuge tubes, refrigerated at 4 ^{o}C pending when it they were needed for the NPs synthesis.

Synthesis of Nanomaterials

Fifteen (15) mL of each extract (21.4 mg/mL) was combined with 95 mL of a 1 mM $AgNO_3$ salt solution. The two mixtures were continuously swirled at 12 rpm in a separate beaker for roughly 6 h without any heat, and the beakers were coated in aluminium foil to prevent auto-reduction of the silver nitrate solution caused by photosensitivity. The biogenesis of the various nanoparticles was validated by a colour change from greyish black to reddish brown and deep brown, respectively.[25] (Figure 1)

Plasmodium falciparum culture and maintenance

Plasmodium falciparum 3D7, a well-known malaria parasite, was kept alive in RPMI 1640 medium with 2 mM L-glutamine and 25 mM Hepes (Lonza). 5 percent Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 g/mL gentamycin, and 2–4 percent hematocrit human red blood cells were used to augment the medium. The parasites were cultured in sealed T25 or T75 culture flasks at 37°C in an environment of 5% CO_2, 5% O_2, and 90% N_2. Light microscopy of Giemsa-stained thin blood smears was used to calculate parasitaemia (parasite concentration in the culture) as reported by Larayetan *et al.*[25]

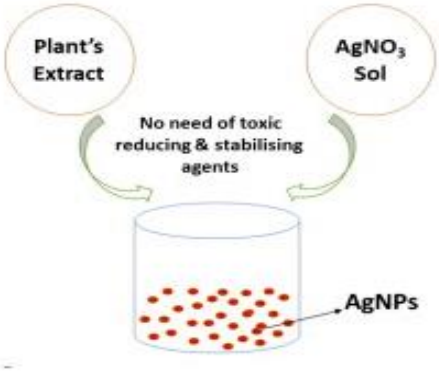

Figure 1: Green synthesis of silver nanoparticles

Single concentration screening
The extracts to be evaluated are introduced to the parasite at a single concentration and incubated for 48 hours; this is a useful method for validating the antiplasmodial efficacy (if present) of the tested extract at a molarity, and it is used when a large number of extracts need to be screened. Compounds should be able to reduce parasite numbers by more than 80% at a concentration of 10 M (for pure compounds) or 50 g/mL for natural extracts; if this is not reached by the tested extract or compound, it indicates that it is unlikely to have promising antimalarial activity. The cell viability is estimated from the concentration of the tested extracts or substance to produce percent parasitermia. To calculate the % parasitermia of the crude extract or compounds, the cell viability is determined from the concentration of the samples analyzed, and the results are compared to those of conventional drugs such as chloroquine (for antimalarial assays), emetine (for cytotoxicity assays), or pentamidine (an existing drug used to treat trypanosomiasis) for antitrypanosomal assays. The test is normally performed in triplicate wells and the standard deviation (SD) computed.
Dose response
This was done to confirm the IC_{50} concentration (The 50 % inhibitory concentration or the concentration of a substance capable of killing 50% of the parasites in the culture). This research was conducted according to Larayetan *et al.*[25] Any chemical with an IC_{50} less than or equal to (≤) 1 M is generally considered a potential antimalarial agent, whereas one with an IC_{50} of (≤) 0.1 M is considered an outstanding antimalarial agent. The extract is compared to conventional medications with IC_{50} values of around 0.02 M, such as chloroquine or artemisinin. Similarly, natural extracts with an IC_{50} of 20 µg/mL are effective, whereas those with an IC_{50} of 1 µg/mL are outstanding antimalarial agents. Plotting the percentage viability obtained from the single concentration assay against the Log (extract concentration) generated from the dose response curve by non-linear regression using the Prism 5 for Windows, Version 5.02 (Graph Pad Software, Inc.) program yields the 50% inhibitory concentration (IC_{50}) of each sample.

Antiplasmodial activity
The parasite viabilities of the GB and GH AgNPs extracts were assessed by parasite lactate dehydrogenase (pLDH) activity .through the method portrayed by Makler *et al.*[26] This was done by integrating both extracts (50 g/mL) separately with parasite cultures in a 96-well plate and incubating for 48 hours at 37 °C in a CO_2 incubator. A total of 20 microlitres (20 µL) of the above-mentioned mixture was removed from all well plates and introduced to 125 µL of NBT/PES solutions with Malstat in a different 96-well plate. The existence of (pLDH) activity was determined by reading the Abs_{620} in each plate, and the presence of (pLDH) activity is usually indicated by a purple colour. Sigma Aldrich provided chloroquine or artemisinin employed for the positive control.

Antitrypanosomal activity
The biosynthesized GB and GH AgNPs extracts were applied separately to *T. b brucei in vitro* cells on a 96-well plate at a fixed dose of 50 µg/mL to determine antitrypanocidal activity. For around 48 hours, the combination was incubated. The number of parasites that survived the drug exposure was determined by adding a resazurin-based reagent to the mixture, which was then converted to resorufin by the live cells. Since Resorufin is a fluorophore ($Excitation_{560}/Emission_{590}$), and can therefore be quantified in a multi-well fluorescence plate reader.[25]

Antibacterial assay
Antibacterial activity
Agar well diffusion technique was employed to determine the antibacterial activity of GB and GH AgNPs extracts Collin *et al.*[27] Our previous paper Larayetan *et al.*[25,28] explained this procedure. Briefly, the microbial cultures were inoculated into nutritional broth (Oxoid) and incubated for around 24 hours at 37 0.1 °C. With a clean cork borer, 6 mm diameter wells were equally created in the recently prepared and congealed Mueller Hilton agar (Oxoid) within the petri plates, and let to firm under hygienic conditions. The test microorganisms (0.1 mL) were inoculated using a germ-free swab on the exterior of the solid medium within each of the petri dishes after the bacterial culture was standardized to 0.5 McFarland turbidity standard. Various amounts of GB and GH AgNPs from the stock (3.125 to 100 mg mL^{-1}) were poured into each well and precisely labelled. The inoculated petri dishes were incubated for 24 hours at 37 °C. The entire petri dish was then examined for growth inhibition zones around each well, with the average diameter of these zones measured in centimetres.[25]

Minimum Inhibitory Concentration
The Agar dilution method was used to determine the minimum inhibitory concentration (MIC) of both GB and GH AgNPs against the tested bacteria; this approach was discussed in our earlier paper Larayetan *et al*.[25] In a nutshell, Mueller–Hinton agar was prepared by autoclaving and allowing to cool at 55°C. Each produced GB and GH AgNPs extract was diluted in a stock solution of dimethyl sulfoxide (DMSO). The agar medium was put into clean labelled Petri plates with the two biosynthesized AgNPs at concentrations of 100-6.25 mg/mL, and the mixture was softly stirred until the agar congealed. The test bacteria were inoculated into the labelled petri dishes with standardized inocula at (0.5 McFarland, 1 108 cfu/mL) and incubated at 37 °C for 24 hours under sanitary conditions The MIC was determined by measuring complete inhibition of growth at a specific concentration of GB and GH in duplicate for each treatment. Doxycycline was employed as a positive control in this study, whereas DMSO was used as a negative control.

Cytotoxicity assay
The GB and GH AgNPs biosynthesized were estimated against HeLa (human cervix adenocarcinoma) as depicted by Keusch *et al*.[29] HeLa cells were seeded onto clean 96-well plates and cultured for 24 hours in DMEM supplemented with 10% foetal bovine serum and penicillin/streptomycin/amphotericin B in a CO_2 incubator at 37 ºC. At a final concentration of 20 µg/mL, GB and GH AgNPs/crude extracts were distributed into the wells, followed by a day of incubation. A resazurin-based reagent from (Sigma Aldrich) was then added, and the combined mixture was incubated for another 3-5 hours. At the end of this time, the amount of cells able to survive drug exposure was estimated using a resazurin-based reagent and resorufin fluorescence was monitored in a multiwell plate reader at (Exc_{560}/Em_{590}) and expressed as percentage viability; this test was performed twice, with standard deviation (SD) calculated for both AgNPs and crude extracts.

Statistical analysis
The diverse data were analyzed using Origin software. This software takes into account the R^2 adjustment of the regression coefficient square. Non-linear regression was also used to examine the IC_{50} derived from the dose-response curve using the Prism 5 for Windows, Version 5.02 (Graph Pad Software, Inc) application.[25]

Results and Discussion

Biosynthesis and characterization
X-ray Diffraction Spectrophotometer
The x-ray diffraction spectra of bio-synthesized AgNPs as well as plant extract was carried out to collect information regarding the size of the particles synthesized and also the crystallinity of the materials,[30] Figure 2. The result obtained indicates that both AgNPs synthesized using GH and GB plant extracts were crystalline in nature. Furthermore, the size of the obtained nanoparticles determined using Scherrer formular by using the most intensed peak ($2\theta = 33^{o}$) shows that the crystallite size of the NPs are in the range 19 – 21 nm. This result compliments the result we obtained for the particles size of these materials when TEM was employed (Figure 2). Also, peaks obtained at (100, 110, 220 and 311) are typical of metal oxide nanoparticles (Figure 2), these peaks are very much missing in the spectra of the plant extract indicating the successful synthesis of AgNPs facilitated by GH and GB plant extracts. Figure 2: XRD analysis of plant extract including that of biogenic AgNPs from GH and GB.

Transmission Electron Microscopy (TEM)
Transmission Electron Microscopy (TEM) was engaged to verify the shape, size and morphology of GH and GB AgNPs as shown in Figure 3, it was established from the images of the TEM that the biosynthesized AgNPs were well dissipated and the micrographs of the biogenic

synthesis of the NPs of the two extracts GH and GB exhibited varying irregular shapes like spherical, triangular, pentagonal and prismatic patterns having an average size of 21 nm, this complement what was acquired in XRD analysis which further confirms that the nanoparticles were truly biosynthesized.

Scanning Electron Microscope (SEM)

The SEM images of GB and GH NPs prepared by biogenic reduction of AgNPs showed clusters of nanoparticles with different sizes Figure 4. It was revealed from the SEM images that there were irregularity in the shapes of GB and GH NPs, but major shapes that could be identified were cubic, triangular and spherical shapes.

Electron Diffraction Spectrophotometer (EDS)

Electron Diffraction Spectrophotometer (EDS) was done to ascertain the elemental composition of the nanocluster,[30] and the spectra revealed that AgNPs of GH and GB both exhibited strong silver (Ag) signal at 3 KeV Figure 5, although weaker peaks like carbon (C) and oxygen (O) elements were detectable in the EDS spectra at 0.4 and 0.6KeV respectively, these may have originated from the phyto-constituents of *G. hirsutum* (GH) and *G. barbadense* (GB) leaf extracts which are usually found attached to the surface of the synthesized AgNPs.

UV–vis Spectroscopy

This was used to ascertain the absorption spectra of the AgNPs which were found to be between 400-800 nm and similar to those reported in some literatures[25,30] Figure 6. The reaction between the two extracts of GH and GB leaves and $AgNO_3$ solution led to a change in colour from greyish black to dark brown, as an evidence of nanoparticles formation. The nucleation and onset growth started as early as 2 to 5 minutes in the Ag NPs. Broad peaks were seen about 468 nm and 472 nm for the GH and GB extracts respectively. The result of the UV-vis spectra signifies that the leaves of GH and GB brought about quick bio-reduction showing that the leaves of these plants are good reducing agents. This correlate the work of other researchers who have carried out similar research work using the leaf and seed extracts to bio-reduce silver nitrate solution (AgNPs).[25,30]

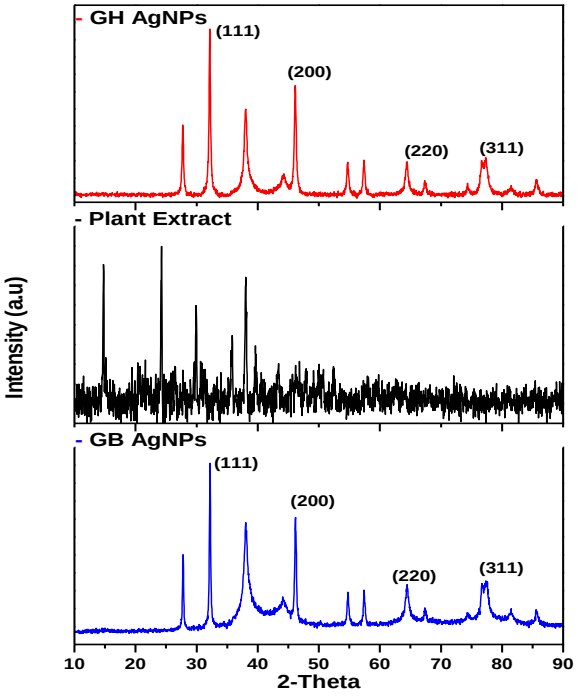

Figure 2: XRD analysis of plant extract including that of biogenic AgNPs from GH and GB.

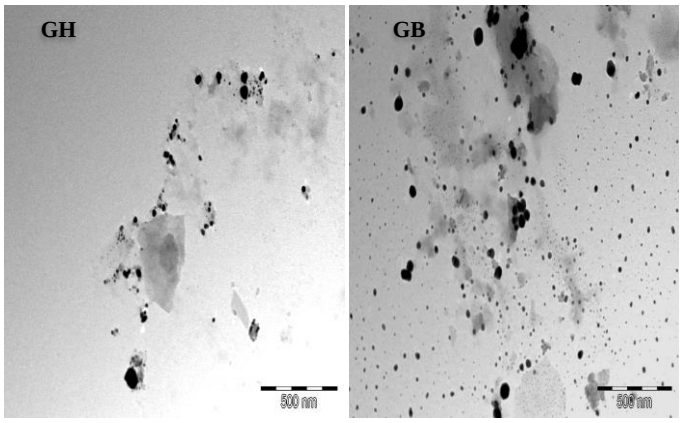

Figure 3: XRD patterns of AgNPs biosynthesized using GH and GB leaf extracts.

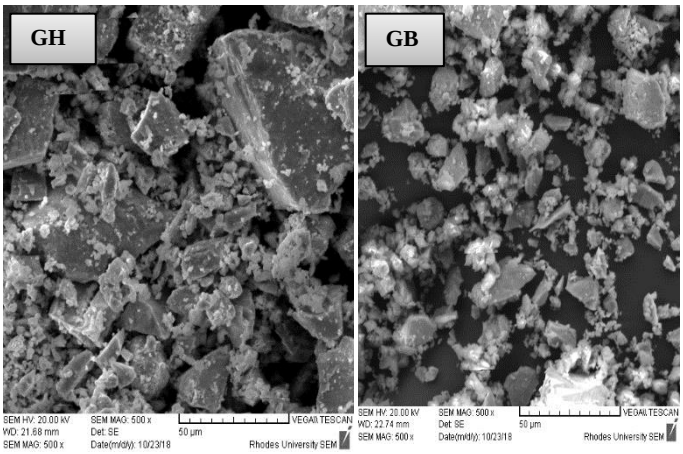

Figure 4: SEM images of GH-AgNPs and GB-AgNPs

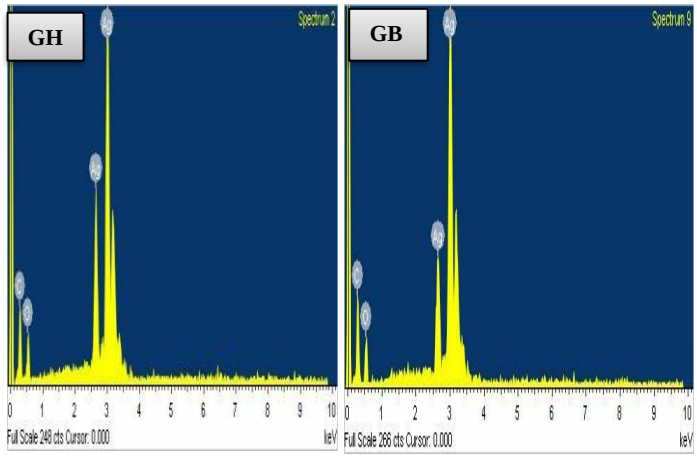

Figure 5: Scanning Electron Microscope Images of GH and GB-NPs.

Fourier Transform Infrared Spectroscopy (FTIR)

Figure 7 shows the Fourier Transform Infrared Spectroscopy (FTIR) of GH and GB NPs with plant extract. The presence of key functional groups in both the crude extract and the biosynthesized AgNPs confirmed the plant extracts dual action as capping and reducing agents, which was validated by FTIR analysis. In the FTIR examination, different vibrational bands such as 2868, 1597, 1403, 1279, and 1055 cm^{-1} were found. The broad and very intense peak of 3349 cm^{-1} may be as a result of the presence of hydroxyl functional groups in the phenolic and alcoholic compounds found in the bioactive constituents of the leaf extracts while the sharp peak at 1597 cm^{-1} may be credited to the presence of carbonyl compounds that participated in the bio-reduction

of Ag^1 ion to Ag^0. In addition to these, peaks between 1403 and 1279 cm^{-1} may be linked to C-N stretching mode in the aromatic amine groups.[25] The various vibrational bands as shown in the FTIR analysis revealed the presence of some important functional groups like alcohol, flavonoids, amine, amides, alcohols or phenols which might be accountable for the stabilization and bio-reduction of the AgNPs.[31] Worthy of note is the peak at about 500 cm^{-1} in the spectra of the GH and GB AgNPs attributed to the Ag-O band which is visibly absent in the spectra of the plant extract. This confirms the formation of bio-synthesized silver nanoparticles from GH and GB respectively.

Antiplasmodial action

Lately, parasitic infections have turn out to be a global health problem due to the resistant strains of protozoa[32] like *P. falciparum* and trypanosoma. The resurgence of resistance, especially in *P. falciparum*, has been the main contributor to the worldwide recurrence of malaria in the last 30 years or thereabout. Bearing in mind the side effects of currently available antiparasitic drugs and the harshness of parasitic diseases, it is essential to explore novel antiparasitic compounds with excellent activity, little toxicity, cheaper to get and have more potentials.

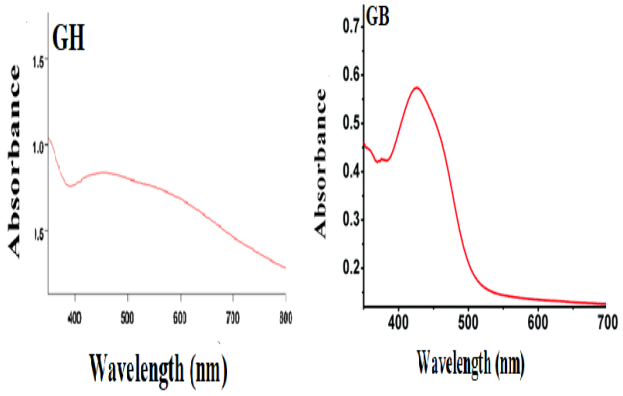

Figure 6: UV-visible spectra of bio-synthesized nanoparticles

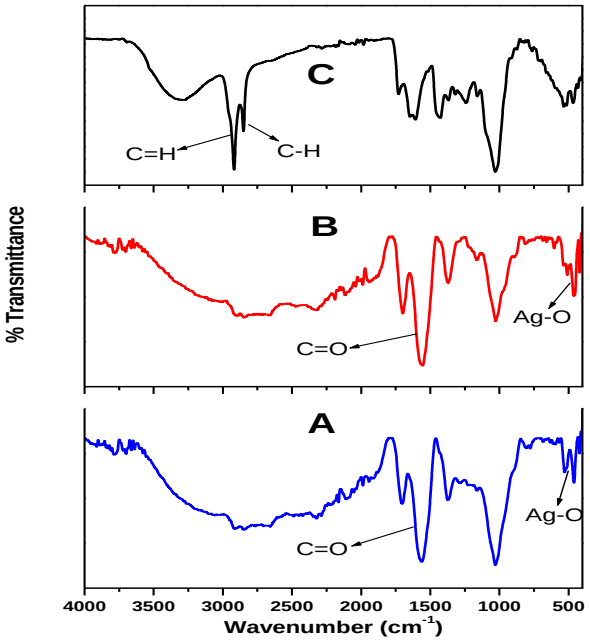

Figure 7: FTIR spectra of (A) GH-AgNPs (B) GB-AgNPs and (C) Plant extract.

The excellent behaviour exhibited by biosynthesized AgNPs is as a result of their small size and remarkably large ratio of surface area to volume; this unique potential makes them to be useful in many areas of research particularly in the area of nanomedicines. In this study the biosynthesis of GB and GH AgNPs from the aqueous leaf extract of two species of Malvaceae family were successfully carried out. The crude lyophilized aqueous extracts from *Gossypium Barbedense* (GB), *Gossypium hirsutum* (GH) and the silver nanoparticles (AgNPs) obtained from it were all screened for *in-vitro* antiplasmodial activity against *Plasmodium falciparum* strain 3D7 of malaria parasites, The extracts and AgNPs have % viability of (51.47 ± 1.38, 52.31 ± 0.87, 0.00 ± 1.81, 0.00 ± 0.99) respectively at a concentration of 20 µg/mL; any extract that has a promising antimalarial activity should produce a percentage (%) parasite viability less than 20 % (meaning more than 80 % of the parasites must have been killed).[33] Only the biosynthesized GB and GH extracts strongly reduced the viability of malaria parasites to less than 20 %. By graphing their percentage viabilities against the logarithm of the extract concentration (250 to 0.11 µg/mL, 3-fold dilution), the two AgNPs were put forward for pLDH IC50 screening. Non-linear regression was used to extract the 50% inhibitory concentrations (IC50) from the resulting dose-response curve. For comparison, chloroquine, a well-known anti-malarial medication with an IC50 of 0.01–0.05 µM, was employed as a positive reference. The IC_{50} of the GB and GH AgNPs were 1.2 and 0.96 µg/mL, respectively. Figure 8.

Bero *et al*.[34] documented that IC^{50} value of ≤ 20 µg/mL are considered as good or very effective while IC50 of between 20-60 µg/mL are regarded as fair but IC50> 100 µg/mL is termed inactive. GH AgNPs with IC_{50} of 0.96 µg/mL showed a better plasmodial activity than GB AgNPs with an IC50 of 1.2 µg/mL. Both AgNPs of GB and GH demonstrated they are guaranteed candidates for antiplasmodial lead drug. The results confirms that IC_{50} of the antiplasmodial activity of this present study inhibited the *P. falciparum* with IC_{50} of 0.96 µg/mL for *G. hirsitum* and 1.2 µg/mL for *G. barbadense*. Our present result demonstrates that biogenic AgNPs of *G. hirsitum* and *G. barbadense* would be good candidates for targeted antimalarial drugs against Chloroquine-sensitive *P. Falciparum.*

Antitrypanosomal activity

AgNPs from GB and GH, as well as their crude aqueous extracts, were evaluated against the trypanosome parasite, yielding percentage viability of (76.91 ± 11.91, 80.89 ± 0.60, 91.06 ± 1.90 and 86.50 ± 2.27 %) respectively. They were unable to reduce parasite viability to less than 50%, indicating that they were ineffective against trypanosome parasites and hence were not included for the *pLDH* IC50.

Antibacterial Potency and Minimum Inhibitory Concentration

The antibacterial activity of biogenic AgNPs against four Gram-negative (*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa, and Klebsiella pneumoniae*) and three Gram positive (*Streptococcus pyogenes, Streptococcus faecalis,* and *Staphylococcus aureus*) multidrug resistant bacteria is shown in (Figures 8 and 9). At a concentration of 100 mg/mL, GB AgNPs have a stronger inhibitory impact against Gram positive bacteria such as *Streptococcus pyogenes* (24.5 ± 2.1 mm), *Streptococcus faecalis* (24.0 ± 0.0 mm), and *Staphylococcus aureus* (20.0 ± 4.2 mm) than Gram negative bacteria, as shown in this study (Figure 9). Inhibitory activity of GH AgNPs against Gram positive bacteria such as *Streptococcus pyogenes*, *Streptococcus faecalis*, and *Staphylococcus aureus* follows a similar pattern. (22 ± 5.7, 23.0 ± 4.2 and 23.0 ± 7.1 mm) (Figure 10). According to this research, GB AgNPs had the greatest inhibitory effect against Streptococcus pyogenes (24.5 2.1). Figure 8. For Escherichia coli, *Pseudomonas aeruginosa, Proteus mirabilis,* and *Klebsiella pneumoniae*, the MICs of the GB and GH AgNPs are the same: 50.0 ± 0.0 mg/mL. *Streptococcus pyogenes* had the highest MIC value of 12.5 ± 0.0 mg/mL, followed by *Streptococcus faecalis* and *Staphylococcus aureus* (25.0 ± 0.0 mg/mL) achieved by GB and GH AgNPs, while the MICs of the positive control (Doxycycline) ranged from 6.25 – 12.5 mg/mL for all the bacteria tested. (Table 1)

The antibacterial activity of biosynthesized GB and GH AgNPs were found comparable to that of the positive standard drug Doxycycline.

This present study shows that Gram positive bacteria are more sensitive than Gram negative bacteria. The bactericidal effect of AgNPs against broad range of bacteria have been recorded in several studies.[25,33] The size of the NPs synthesized have been documented to influence their activity against bacteria, record showed that smaller sized NPs released more silver cations Ag^+ and are more effective in killing the bacteria compare to larger sized particles.[35,36]. The MICs of the biogenic AgNPs further confirms that GB and GH are biologically active against the tested bacteria as seen in (Table 1) The results displayed in this study implies that the biosynthesized AgNPs obtained from plant have certain constituents with antibacterial activity which could be used as novel antibacterial agents to combat common bacterial pathogens. Different fundamental mechanisms of how AgNPs work against microbes have been recorded. It is posited that the AgNPs bind themselves to the negatively charged exterior of the cell membrane and thus change the physical and chemical properties thereby knocking off-balance some vital functions like metabolic process, osmoregulation, electron transport and porosity of the cell,[37,38] in addition to this, the AgNPs can efficiently seep into the cell and intermingled with the DNA, protein and protein containing constituents because of their small particle size,[36] lastly the AgNPs has the capability to release Ag^+ capable of causing inequity thereby producing an augmented biocidal action in the cell.[39]

GB= Gossypium barbadense, GH= Gossypium hirsitum, DMSO= Dimethyl sulfoxide, n=2

Cytotoxicity activity

The crude aqueous extracts and the biosynthesized AgNPs from GB and GH were tested against HeLa cells at a concentration of 20 µg/mL. Since the extracts did not lower the viability of HeLa cells to less than 50%, the two biogenic NPs did not show any signs of cytotoxicity. Their respective percentage viabilities were (79.99 ±1.96, 76.26 ± 2.26, 104.54 ± 5.37, 97.35 ± 5.74 %) (Figure 11). The fact that none of these extracts are cytotoxic could indicate that they are safe to use as targeted medicines in mammals.

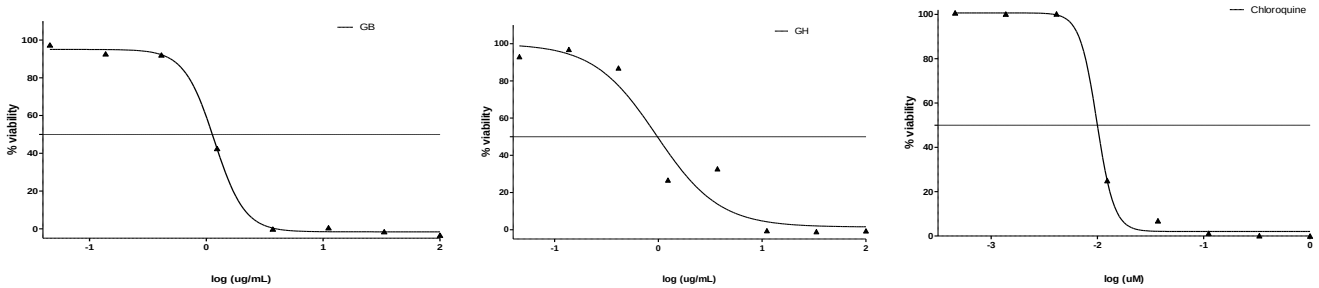

Figure 8: Dose-response curve for pLDH assay of GB, GH and Chloroquine

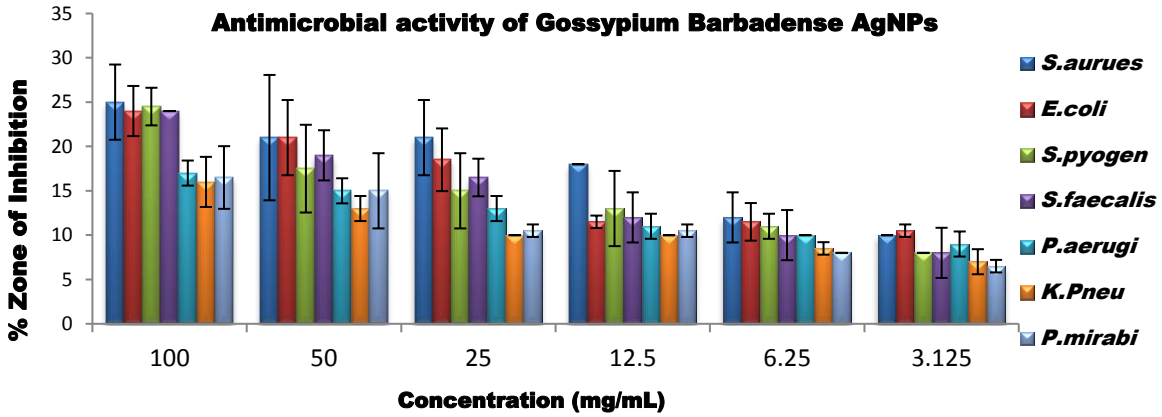

Figure 9: antibacterial activity of GB AgNPs

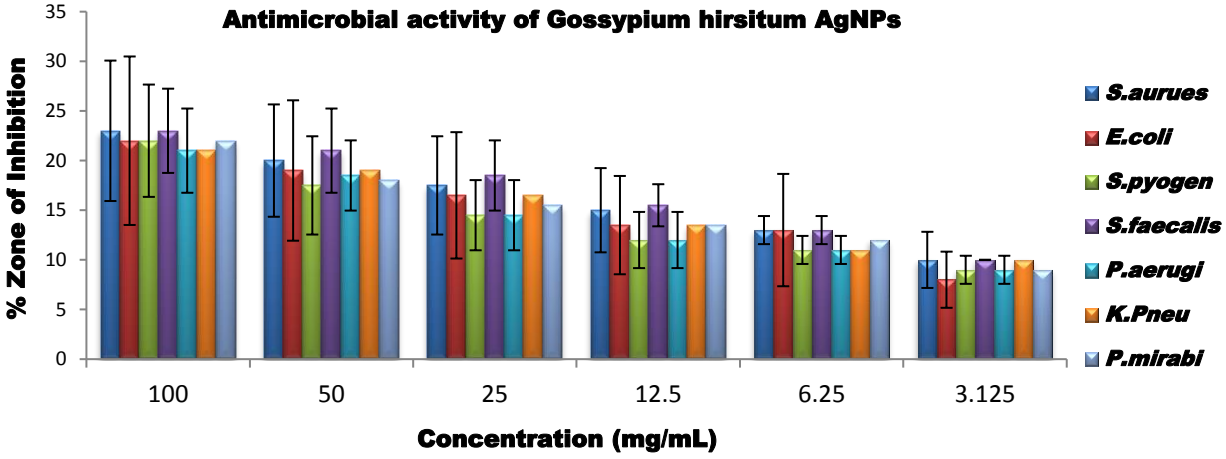

Figure 10: antibacterial activity of GH AgNPs

Table 1: Minimum Inhibitory concentration (MIC) values (mg/mL) for AgNPs of GB, GH and standard drug

Bacteria	GB-AgNPs	GH-AgNPs	Doxycycline Positive control	DMSO Negative control
Escherichia coli	50.0 ± 0.0	50.0 ± 0.0	6.25 ± 0.0	0.5 mL VG
Pseudomonas aeruginosa	50.0 ± 0.0	50.0 ± 0.0	6.25 ± 0.0	0.5 mL VG
Proteus mirabilis	50.0 ± 0.0	50.0 ± 0.0	6.25 ± 0.0	0.5 mL VG
Klebsiella pneumonia	12.5 ± 0.0	25.0 ± 0.0	6.25 ± 0.0	0.5 mL VG
Streptococcus pyogenes	25.0 ± 0.0	25.0 ± 0.0	6.25 ± 0.0	0.5 mL VG
Streptococcus faecalis	25.0 ± 0.0	25.0 ± 0.0	6.25 ± 0.0	0.5 mL VG
Staphylococcus aureus	25.0 ± 0.0	25.0 ± 0.0	6.25 ± 0.0	0.5 mL VG

GB= Gossypium barbadense, GH= Gossypium hirsitum, DMSO= Dimethyl sulfoxide, n=2

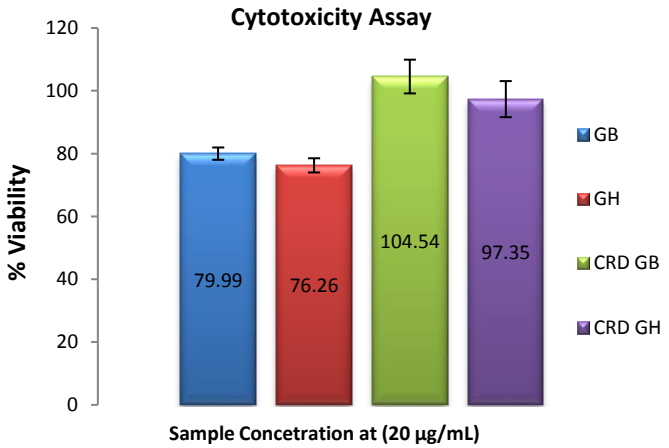

Figure 11: Cytotoxicity assays of crude GH & GB with GH & GB-AgNPs

Conclusion

The study revealed that the synthesis of AgNPs was successful. The biosynthesized AgNPs from GB and GH evaluated against HeLa cells did not show any sign of cytotoxicity because the extracts did not reduce the viability of HeLa cells to below 50%) Both AgNPs displayed potent antiplasmodial and antibacterial activities. Consequently, the biogenic AgNPs of GB and GH may possibly be promising candidates that could be employed in nanomedicines and other areas where such applications are needed, particularly with drug-resistant malaria agents. The non-cytotoxic of all these extracts may be a clue of their safety as targeted drugs for mammalian organisms.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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