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Silver nanoparticles mediated by *Callistemon citrinus* extracts and their antimalaria, antitrypanosoma and antibacterial efficacy

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ABSTRACT

In this report, three biosynthesized silver nanoparticles (AgNPs) obtained from the reduction of silver nitrate (AgNO₃) by the aqueous crude extracts of aerial parts of *Callistemon citrinus* plant were characterized by means of ultraviolet–visible spectroscopy (UV–vis), X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray (EDX), transmission electron microscopy (TEM) and Fourier transformed infrared (FTIR).

The XRD revealed that the AgNPs were crystalline in nature while the TEM showed that the shapes were spherical with an average size of 29 nm. The SEM and EDX demonstrated triangular shaped materials and that the AgNPs were made up of silver and oxygen only, absorption spectra confirm by UV–vis signifies the dispersed nature of the synthesized nanoparticles with absorption band observed at 280 nm for the leaf. FTIR had absorption bands at about 1700 cm⁻¹ in all spectra's establishing the C=O stretching owing to amide bond, another remarkable peak at 3400 cm⁻¹ was seen in the crude extract which was ascribed to the O–H stretching from water as a result of the aqueous nature of the plant extracts used. It is interesting to know that this peak was not seen in the AgNPs demonstrating the development of calcined AgNPs, in addition to this, peak at 420 cm⁻¹ was observed for all the three nanoparticles synthesized and this shows the successfully synthesis of the AgNPs. The antimicrobial activities of the of the AgNPs was also confirm via both gram positive and gram negative bacteria strains with a very significant inhibitory action, MIC values of 7.8125 mg/mL were documented for all the silver nanoparticles. Potent antiplasmodial activities with IC₅₀ ranging from 2.99–5.34 µg/mL were also recorded and a poor IC₅₀ of 107.30 µg/mL for antitrypanosoma activity of the leaf AgNPs was also documented.

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1. Introduction

The significant role of nanotechnology in present day research is gaining popularity among different researchers in the field of science; this has led to the growth of research in different areas such as drug design, environmental remediation, mechanics, cosmetics, medicine etc. Nanoparticles are simply inorganic or organic materials with size between 1 and 100 nm, their small size in relation to their large surface to volume ratio make them to be exceptionally significant. Several applications of nanoparticles in sensor technology, pharmaceuticals industry, processed food, optoelectronics, molecular biology, drug delivery

and biomedical system, biomimetic materials, production of polymeric membranes for filtrations, gas separation and waste treatment due to the large surface energy, extensive Plasmon excitation and specific electron structures induced by the efficient transition linking the molecular and metallic states have been documented [1–5].

Nanoparticles are synthesized through various methods like solvent dispersion, ionic gelation, super critical; fluid extraction, solid state reaction, chemical reactions, co-precipitation and polymerization technique. These procedures involve the use of chemicals that are costly, non-biodegradable and environmentally unfriendly. For this reason researchers have been busy seeking for alternative methods of synthesis using non-toxic and environmentally friendly biological methods and materials (green synthesis).

Biogenic mode of using different plant extracts and microorganisms through the route of green synthesis is useful due to its reduced environmental impact coupled with the generation of large quantity of nanoparticles that are not contaminated but are cost effective, simple

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energy conserving with well-defined size, morphology and compatible in the area of food and medical application [6,7].

Nanoparticles produced from green synthetic route are also found to have antioxidant properties [8], curative potential [9] and antimicrobial activity [10–15]. Different plants parts like seeds, flower, stem, fruits, skin or even their extracts are now used for nanoparticles production through green synthesis.

The function of these extracts or plant parts is to reduce and stabilize the nanoparticles [16]. This is brought about as a result of the diverse plant metabolites like amino acids, alkaloids, tannins, saponins, flavonoids, enzymes, vitamins and terpenoids embedded in the plant which are already established to possess therapeutic activity [17]. Plant extracts have the ability to reduce metal ions and this has brought about extensive concentration to green synthesis over the years [16,18–21] (Fig. 1).

Several application of synthesized nanoparticles obtained from different techniques have been found to have in-vitro diagnostic relevance [22–24]. It has been observed that silver nanoparticles exhibit antimicrobial properties against animal and human pathogens [25–27]. The efficacy of nanoparticles derived from silver metal against microbial and cancer ailment have been reported [28,29]. Nanoparticles are recognised to obstruct protein and DNA replication [30].

Numerous applications of metal nanoparticles in agriculture and crop production, antimicrobial food packaging, in wastewater effluent treatment etc. have all been documented [31–35]. Silver nanoparticles have been established to boost larvicidal activity against filariasis and malaria vector [36,37]. The antiplasmodial, anticancer and antifungal activities of silver nanoparticles have been documented [38–41]. The antimicrobial ability of silver nanoparticles relies on the size and environmental states like pH and ionic strength. The mechanism of antimicrobial potency of silver nanoparticles is through the slow discharge of toxic silver ions initiated by oxidation inside and outside the cell which tend to affect the membrane permeability of the microbial cells [42].

From a variety of metals exhibiting antimicrobial properties, silver has the most efficient antibacterial activity and has been found to be the least toxic to animal cells; this is why it is enormously used for therapeutic treatment. During World War 1 it was used to treat wounded soldiers in order to stall microbial growth [43], the therapeutic effectiveness have been well acknowledged for over 200 decades [44]. Silver is employed in nitrate form to bring about antimicrobial action but when added to plant extracts through green synthetic route, it amplifies the antimicrobial potency of the nanoparticles formed due to larger surface area brought about by the smaller size. A considerable disparity in the chemical component of plant extracts from the same species collected from different locations or environment may bring about diverse result in the application of silver nanoparticles.

In this study, we report on the synthesis, characterization and antimicrobial, antiplasmodial and antitrypanosomal properties of silver nanoparticles brought about by the reduction of AgNO_3 using plant parts (leaves, flower and seed) of *Callistemon citrinus*. To the best of our knowledge, no study has reported the antimicrobial, antiplasmodial and antitrypanosomal properties of the green synthesized AgNPs of *Callistemon citrinus* using its different plant parts.

2. Materials and methods

2.1. Materials

AgNO_3 was purchased from Merck, South Africa, Mueller-Hinton agar from Oxford.

Ltd. (Hampshire, England), dimethyl sulfoxide (DMSO) from Fluka Chemicals (Buchs, Switzerland). All other reagents used in this study were of analytical grade.

2.2. Organisms

Escherichia coli 0157:H7:ATCC 35150, *Vibrio alginolyticus* DSM 2171, *Salmonella typhi* ACC, *Staphylococcal enteritis* ACC, *Staphylococcus aureus* ACC, *Listeria ivanovii* ATCC 19119 and *Mycobacterium smegmatis* ATCC 19420.

2.3. Characterization

The synthesized materials were characterized with a number of techniques to ascertain the composition, structure and morphology of the materials. Bruker D8 advanced x-ray diffractometer (XRD) was used to determine the crystallinity and size of the materials. Perkin-Elmer Universal ATR 100 Fourier Transformed Infra-Red spectrophotometer (FT-IR) was employed to observe the vibrations of the samples while Scanning electron microscope (SEM) and Electron diffraction spectrophotometer (EDS) images were obtained using JOEL JSM-6390 LVSEM. SEM and EDS were used to ascertain the morphology and composition of the materials whereas Transmission electron microscope (TEM) images were recorded using JOEL 1210 transmission electron microscope at 100 kV accelerating voltage. This is required to have information on the shape and size of the synthesized materials. UV-visible spectra were obtained with Perkin-Elmer Universal absorption spectrophotometer.

2.4. Methods

2.4.1. Preparation of the plant extracts

The fresh plant part (leaves, flowers and seeds) of *Callistemon citrinus* were obtained from the University of Fort Hare vicinity and were air dried for about 27 days at ambient temperature, then grinded with a mechanical grinder (Polymix, PX-MFC 90D). About 50 g of the crude powdered samples were soaked in 400 mL distilled water and agitated on an orbital shaker for 24 h. The extracts were filtered with Whatman No.1 filter paper and the filtrates were then lyophilised into dry power, preserved with tightly stopped centrifuge tubes, refrigerated at 4 °C until needed for the silver nanoparticles synthesis.

2.4.2. Synthesis of nanomaterials

Exactly 12.5 mL each of the extracts (21.2 mg/mL) of the extracts was added to 90 mL of 1 mM solution of silver nitrate salt (AgNO_3). The mixtures in a conical flask was kept under continuous stirring condition (10 rpm) for about 5 h at room temperature with the reaction vessel covered with aluminium foil to avoid auto-reduction of the AgNO_3 due to photosensitivity. The initial colour when the flower, leaf and seed extracts were added to the aqueous AgNO_3 solution were ox-blood, pale cream and cream respectively. The appearance of

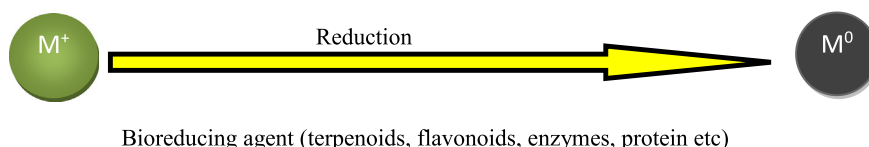


Fig. 1. Bioreduction mediated by plant metabolites.

darkish-brown, reddish-brown and deep-brown coloration confirmed the formation of the various nanoparticles.

2.4.3. *Plasmodium falciparum* culture and maintenance

The malaria parasites (*Plasmodium falciparum* strain 3D7) were preserved in RPMI 1640 medium containing 2 mM L-glutamine and 25 mM Hepes (Lonza). The medium was further supplemented with 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamycin and 2–4% hematocrit human red blood cells.

The parasites were cultured at 37 °C under an atmosphere of 5% CO₂, 5% O₂, and 90% N₂ in sealed T25 or T75 culture flasks. Parasitaemia (the concentration of parasites in the culture) was measured by light microscopy of Giemsa-stained thin blood smears.

2.4.4. Antiplasmodial activity

Parasite viability was measured using parasite lactate dehydrogenase (pLDH) activity according to the method described by Makler et al. [45]. Chloroquine (Sigma Aldrich) or artemisinin (Sigma Aldrich) was used as positive controls.

Screening of the samples against malaria parasites, which were added to the parasite cultures in 96-well plate and incubated for 48 h in a 37 °C CO₂ incubator was carried out at a concentration of 50 µg/mL. After the expiration of 48 h, the plate was removed from the incubator. Twenty micro litre of the culture was removed from each well and added to 125 µL of a mixture of Malstat and NBT/PES solutions in a fresh 96-well plate. These solutions were used to determine the activity of the parasite lactate dehydrogenase (pLDH) enzyme in the cultures. A purple product was formed when pLDH was present, and this product could be quantified in a 96-well plate reader at an absorbance of 620 nm (Abs₆₂₀). The Abs₆₂₀ reading in each well was thus a sign of the pLDH activity and number of parasites in that well.

2.4.5. Antitrypanosoma activity

The main cause of African sleeping sickness in human (human African trypanosomiasis and nagana (animal African trypanosomiasis) in cattle) is *Trypanosoma brucei* (*T.b.*) parasites. The subspecies accountable for Nagana (*T.b. brucei*) rarely affects humans and is commonly used for drug screening. To evaluate anti-trypanocidal activity, the synthesized nanoparticles were added to in vitro cultures of *T.b. brucei* in 96-well plate at a fixed concentration of 50 µg/mL. The mixture was incubated for about 48 h and the numbers of parasites that can survive the drug exposure was determined by adding a resazurin based reagent, this reagent was reduced to resorufin by living cells. Resorufin is a fluorophore (Excitation₅₆₀/Emission₅₉₀) and can thus be quantified in a multi-well fluorescence plate reader.

The results obtained were expressed as % parasite viability and this was achieved by comparing resorufin fluorescence in compound-treated wells relation to untreated controls. The test was carried out in duplicate wells, and standard deviation (SD) was also calculated. By and large, extracts that decreased parasite viability to <10–20% were considered for additional testing (e.g. dose-response and cytotoxicity assays). Pentamidine (an existing drug treatment for trypanosomiasis) was used as a positive control drug standard.

2.4.6. Single concentration screening

The single concentration approach was adopted where the compound of interest was added to the parasites and incubated for 48 h. This was a fast way to verify the anti-malarial activity (if any) of the compound of interest at a particular concentration, and mainly suitable when large numbers of samples were needed to be screened. As a rule of thumb, if a compound does not reduce parasite numbers by >80% at 10 µM (for pure compounds) or 50 µg/mL (for natural extracts), it is improbable to have a promising anti-malarial activity.

For each test sample concentration, the cell viability or percentage parasitemia was calculated. The test was carried out in a triplicate wells and standard deviation (SD) was derived. The % parasitemia of

the extracts were compared with any of these standard drugs: chloroquine (an anti-malarial drug) or emetine (which induced cell apoptosis) or pentamidine (an existing drug used in the treatment of trypanosomiasis) depending on the type of assay conducted.

2.4.7. Dose response

This assay was carried out to determine the IC₅₀ concentrations of the compounds (50% inhibitory concentration, or the concentration of the compound needed to kill 50% of the parasites in a culture). As a general rule, a compound with an IC₅₀ < 1 µM can be considered a promising anti-malarial, while an IC₅₀ < 0.1 µM is very good. A standard drug like Chloroquine or artemisinin with IC₅₀ values of approx. 0.02 µM is used for comparison. For natural extracts an IC₅₀ values ≤20 µg/mL are promising, and <1 µg/mL very good.

For each sample, percentage viability was acquire against Log (AgNPs extract concentration) and the IC₅₀ (50% inhibitory concentration) resolved from the resulting dose-response curve by non-linear regression using Prism 5 for Windows, Version 5.02 (Graph Pad Software, Inc.) program. Chloroquine, pentamidine or emetine was used as positive standards drugs based on the type of test carried out. Chloroquine, pentamidine and emetine gave IC₅₀ values in the range of 0.00001–100 µM. The samples were tested in concentration range of 250 to 0.11 µg/mL (3-fold-dilutions) for antitrypanosomal/antiplasmodial and from 125 to 0.057156 µg/mL (also in a 3-fold dilution series) for cytotoxic assays.

2.4.8. Cytotoxicity assay

The overt cytotoxicity of the synthesized nanoparticles was estimated against HeLa (human cervix adenocarcinoma) as described by Keusch et al. [46]. Stock solutions of the nanoparticles (20 mg/mL) were prepared in DMSO and later diluted with culture medium to 50 µg/mL, this was incubated in 96-well plate containing HeLa (human cervix adenocarcinoma) cells for about 48 h. The amounts of cells that were able to out-live exposure to the drugs were also established via resazurin based reagent and reading resorufin fluorescence in a multi-well plate reader.

The obtained result was articulated as %viability (the resorufin fluorescence in compound-treated wells compared to untreated controls). This test was carried out in duplicate wells and standard deviation (SD) was generated for the targeted compounds. The results for the cytotoxicity assay were also expressed as % cell viability (obtained from fluorescence reading in treated wells versus untreated control wells). Emetine (which induces cell apoptosis) was employed as a positive standard drug.

2.5. Statistical analysis

Analysis of data was done using origin software in the statistical computing system. This software put into consideration the adjustment of the regression coefficient square, R² (47). In addition, non-linear regression using Prism 5 for Windows, Version 5.02 (Graph Pad Software, Inc.) program was used to resolve IC₅₀ from the dose-response curve.

3. Results and discussion

3.1. Synthesis and characterization

The synthesized AgNPs obtained from the reduction of AgNO₃ by the plant parts extracts of *Callistemon citrinus* were characterized by a number of techniques prior to testing them for their antimicrobial, antiplasmodial and antitrypanosoma properties.

X-ray diffraction spectra of silver nanoparticles obtained from the reduction of AgNO₃ by aqueous extract of (B) leaf (C) flower and (C) seed parts of *Callistemon citrinus* is presented in Fig. 2. It can be observed that all synthesized materials are crystalline in nature upon the reduction of AgNO₃. Peaks observed at 2θ values of 28° and 34° for

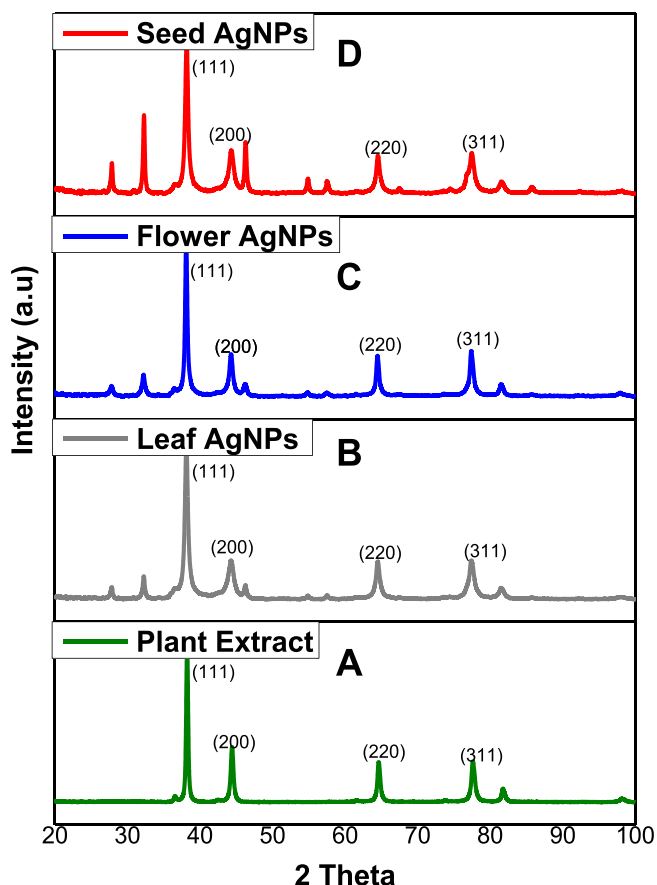


Fig. 2. X-ray Diffractogram of (A) *Callistemon citrinus* extract and AgNPs obtained (B) leaf (C) flower and (D) seed.

Fig. 2(B), (C) and (D) indicate the successful synthesis of AgNPs, these peaks are not observed in the diffraction pattern of Fig. 2A. Ojemaye et al. [47] reported that diffraction peaks at (111), (200), (220) and (311) are characteristic peaks of metal nanoparticles and these peaks are also observed in the diffractogram in Fig. 2(B), (C) and (D) confirming the successful synthesis of AgNPs. The crystallite size of all synthesized materials with (111) diffraction peak using Scherrer formula showed that all synthesized materials are in the size range of 25–32 nm.

Fig. 3 shows the FTIR spectra of (A) plant extract (B) Leaf-AgNPs (C) Flower-AgNPs and (D) Seed-AgNPs. Adsorption bands observed at 1700 cm^{-1} in all spectra are characteristic of the C=O stretching due to amide bond in the plant. A broad Peak observed at 3400 cm^{-1} in the spectra of plant extract (Fig. 3A) is attributed to O—H stretching from water since the plant extract is an aqueous one. This band is obviously missing from the spectra of Fig. 3B, C and D indicating the formation of calcined AgNPs. Also, stretching frequency observed at 420 cm^{-1} in Fig. 3B, C and D which is attributed to Ag—O band further confirm the successful synthesis of silver nanoparticles, this band is however not observed in the infrared spectra of the plant extract (Fig. 3A). Result similar to this was reported in recently published works [48]. The FTIR further confirms that some specific phytochemicals were accountable for the synthesis and stabilization of AgNPs as shown from the different peaks explained above.

Transmission electron microscope (TEM) was used to determine the shape, size and morphology of the synthesized materials (Fig. 4). From the micrographs, it can be observed that spherical shaped materials were synthesized with an average size of 29 nm. The average size obtained from the measurement using TEM complements the result

obtained from XRD therefore confirming that nanosized materials have been successfully synthesized. Worthy of note is that the plant part of *Callistemon citrinus* employed for the reduction of AgNO_3 did not influence the shape and size of the synthesized materials. This same observation was reported by Jyoti et al. [49].

Scanning electron microscope (SEM) and Electron diffraction spectrophotometer (EDS) were used to assess the morphology and composition of the materials obtained from the reduction of AgNO_3 by plant parts of *Callistemon citrinus* (Fig. 5). From the images, it can be seen that roughly triangular spherical shaped materials were synthesized; this morphology is characteristics of AgNPs [50]. Interestingly, EDS result show that the materials are only composed of silver and oxygen (from water molecule that might be left in the material after drying) with silver showing to possess about 89% of the overall composition of elements in the different materials.

Absorption spectra of the silver nanoparticles were measured using UV–visible spectrophotometer between 200 and 400 nm (Fig. 6). Broad peaks were observed around 235 nm for seed and flower mediated AgNPs indicating the dispersed nature of the materials synthesized using these plant parts. Absorption band observed at 280 nm for the leaf nanoparticles indicate the slow reduction rate of AgNO_3 using this plant part. Philip and Unni and Hyllested et al. [51,52] reported similar observation for the absorption maxima of AgNPs obtained from plants.

3.2. Antitrypanosoma activity

The antitrypanosoma activity of the three synthesized nanoparticles from *Callistemon citrinus* leaf, flower and seed were examined by *trypanosoma brucei* assay, it was found that their % viability at 50 $\mu\text{g/mL}$ were correspondingly $(19.740 \pm 4.09\%)$, $(35.043 \pm 0.76\%)$ and

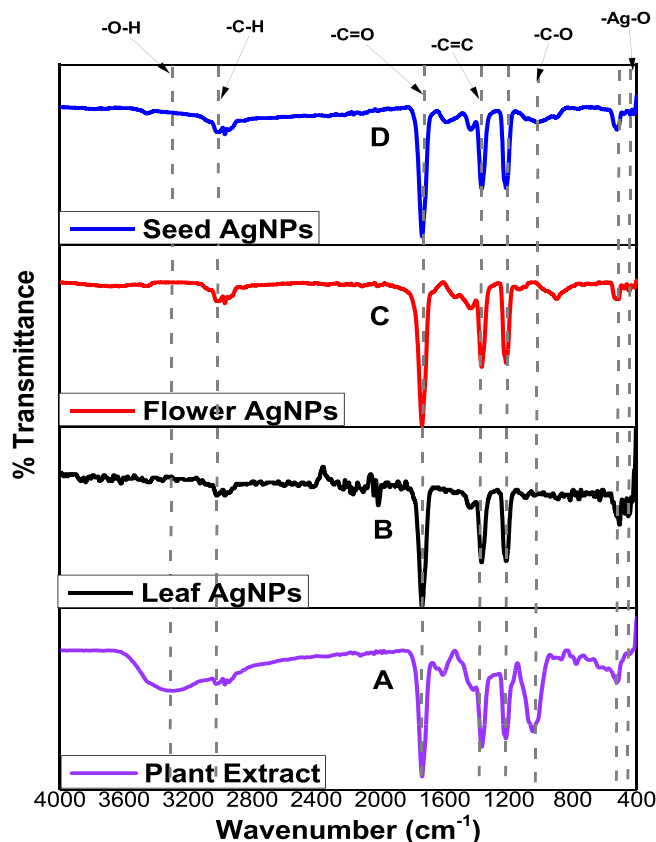


Fig. 3. FTIR spectrum of (A) *Callistemon citrinus* extract and AgNPs obtained (B) leaf (C) flower and (D) seed.

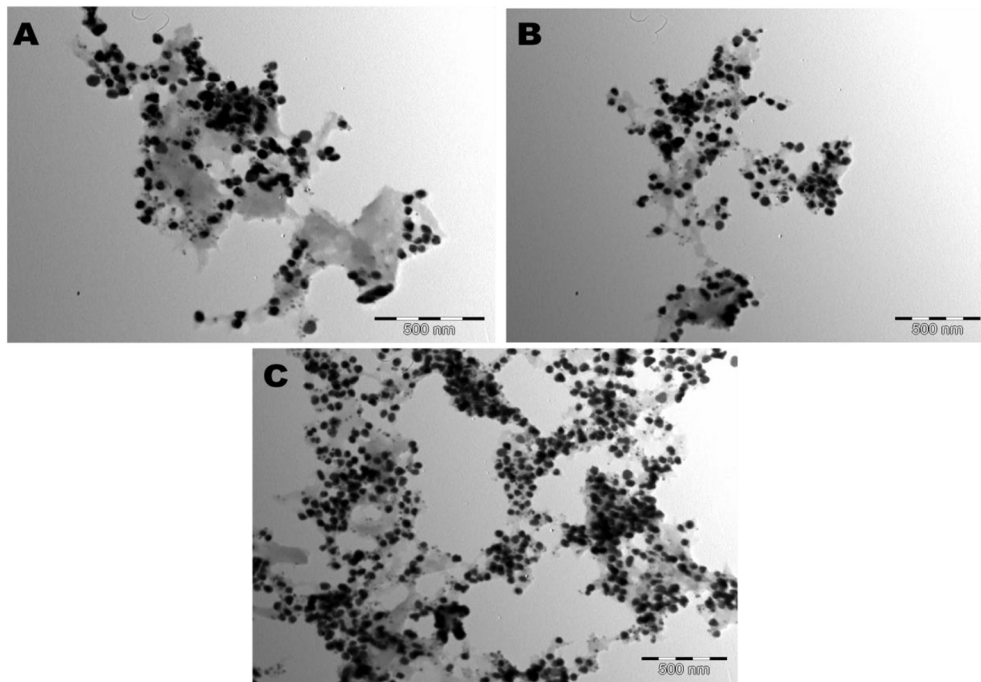


Fig. 4. TEM micrographs of *Callistemon citrinus* mediated AgNPs obtained (A) leaf (B) flower and (C) seed.

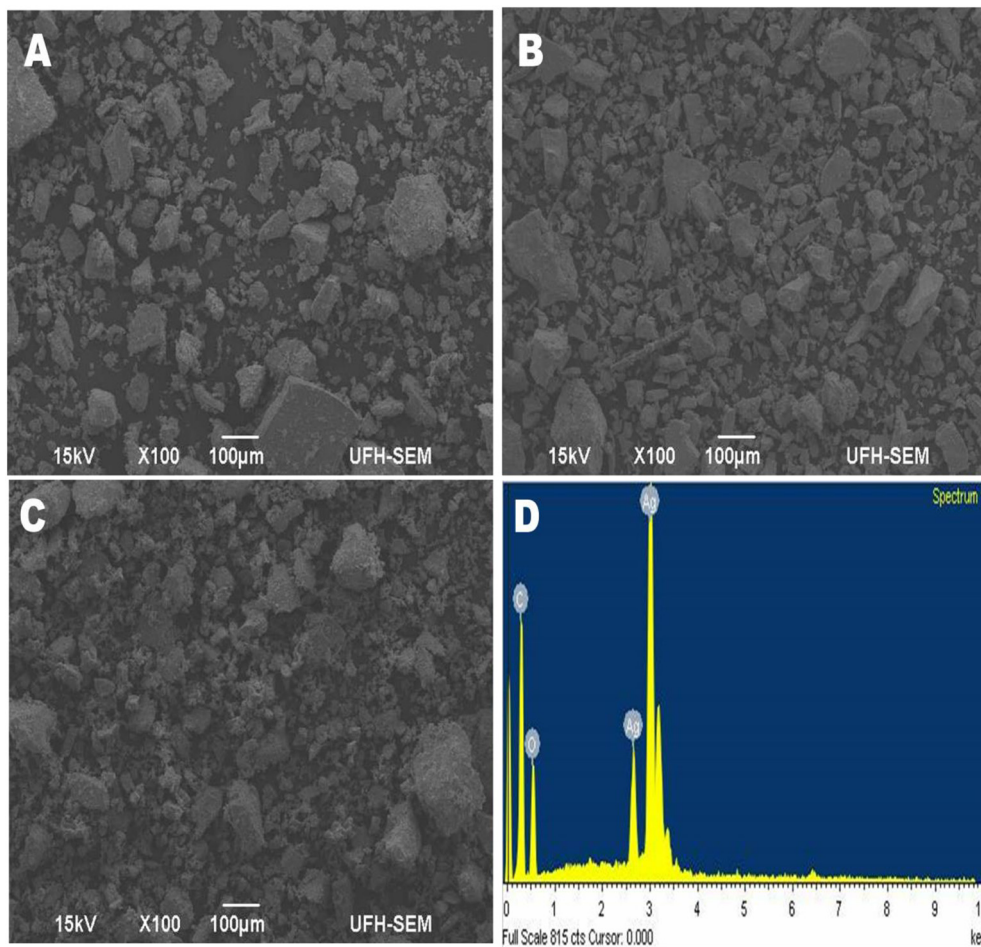


Fig. 5. SEM images of *Callistemon citrinus* mediated AgNPs obtained (A) leaf (B) flower (C) seed and (D) EDS of AgNPs.

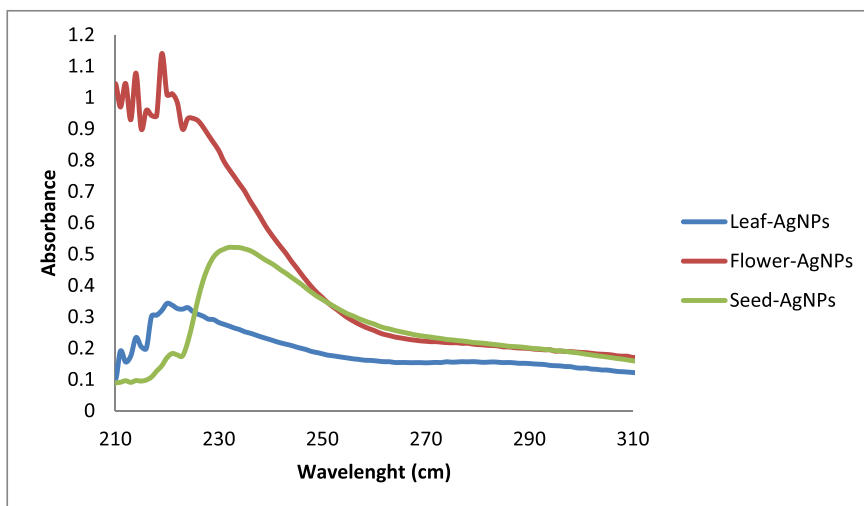


Fig. 6. Absorption spectra of AgNPs obtained with different plant parts.

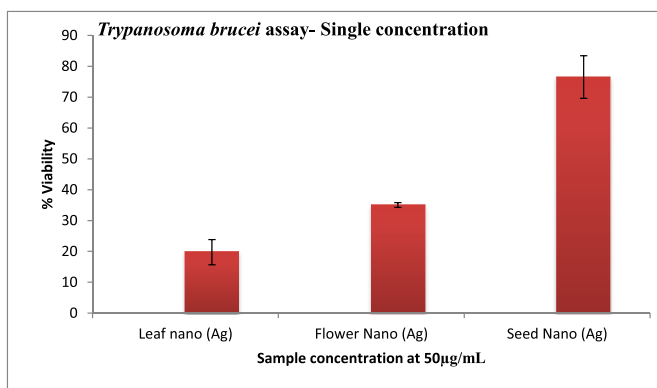


Fig. 7. Single concentration of trypanosome assay.

76.520 ± 6.90%) (Fig. 7). Only leaf nanoparticles brought about a significant decrease to 20% in trypanosome parasites at a concentration of 50 µg/mL, the other two synthesized nanoparticles (flower and seed) could not do so and were therefore considered inactive.

The IC₅₀ of the leaf nanoparticle obtained from the dose response curve was poorly active against trypanosomes at 107.30 µg/mL (Fig. 8). Interestingly, the crude extracts from the plant of study from where the Leaf, flower and seed AgNPs were synthesized from exhibited significant activities against *trypanosoma brucei* (*T.b.*) parasites with IC₅₀ of 11.06, 33.66 and 31.31 µg/mL respectively (Fig. 9).

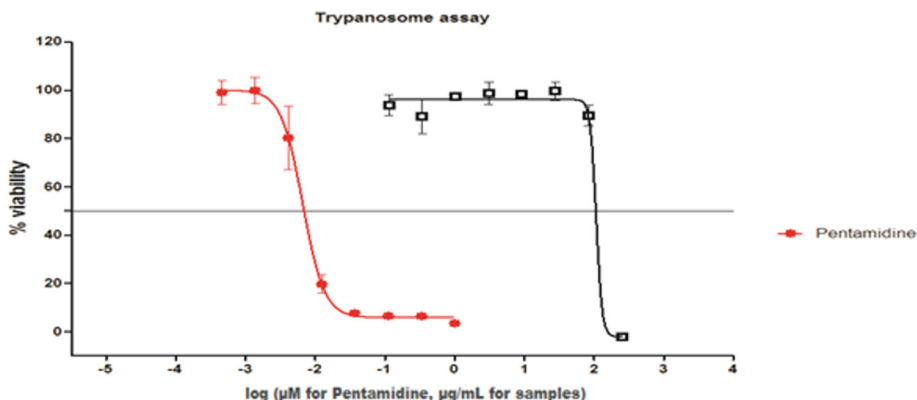


Fig. 8. Dose-response curve for trypanosome assay of Leaf AgNPs.

Bero et al. [53] recorded that IC₅₀ value of ≤20 µg/mL are regarded as good or very potent while IC₅₀ of between 20 and 60 µg/mL are considered as moderate but IC₅₀ > 100 µg/mL is termed not active. The antitrypanosoma action of methanolic extract of *Solanum schimperianum* from the Kingdom of Saudi Arabia gave an IC₅₀ of 0.61 µg/mL and another plant from the same region *C. tuberculata* showed an IC₅₀ of 0.5 µg/mL [54]. The presence of some secondary metabolites such as alkaloids, terpenoids, flavonoids, saponins, tannins, steroids and many others in plants may be responsible for their antitrypanosoma activities [55–57].

3.3. Antiplasmodial action

Three silver nanoparticles prepared from the leaf, flower and seed extracts of *Callistemon citrinus* were subjected to in-vitro screening of antiplasmodial activity against the malaria parasites (*Plasmodium falciparum* strain 3D7). The synthesized leaf nanoparticles obtained from *Callistemon citrinus* at a concentration of 50 µg/mL strongly decreased the viability of *Plasmodium falciparum*, while that of the synthesized flower also strongly decreased *Plasmodium falciparum* at the same concentration to (0.423 ± 1.125). In addition, the synthesized seed nanoparticle also showed strong % viability against same *Plasmodium falciparum* strain at (1.192 ± 4.38%).

Their percentage viabilities were then plotted against logarithm of synthesized nanoparticles concentration 250 to 0.11 µg/mL, 3-fold-dilutions and the IC₅₀ (50% inhibitory concentration) were obtained from the resulting dose-response curve by non-linear regression. For

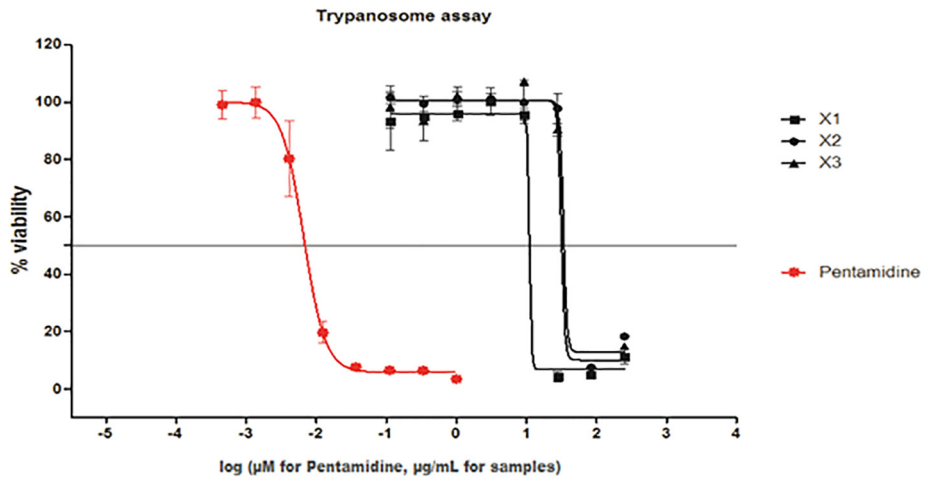


Fig. 9. Dose-response curve for trypanosome assay of crude seed, flower and leaf (X1, X2 & X3).

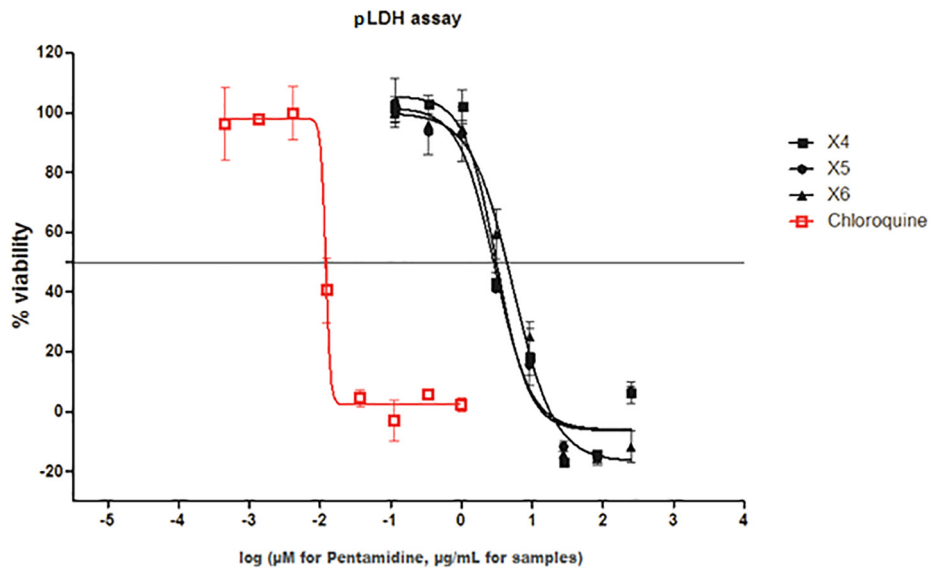


Fig. 10. Dose-response curve for pLDH assay. X4, X5 and X6 represents silver nanoparticles of leaf, flower and seed extracts.

comparative purposes, Chloroquine (an anti-malarial drug) was used as a comparative drug standard and IC₅₀ values yielded were in the range 0.01–0.05 μM.

The IC₅₀ for the leaf, flower and seed silver nanoparticles all showed strong antiplasmodial activity of (3.14, 2.99 and 5.34 μg/mL) respectively (Fig. 10). The flower AgNPs had the highest activity against

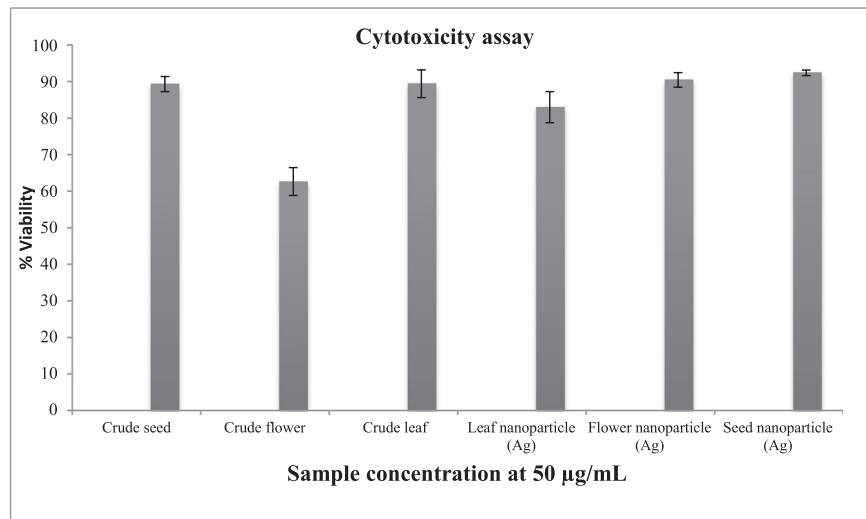


Fig. 11. Single assay concentration for cytotoxicity.

Plasmodium falciparum parasite followed by the leaf AgNPs and the seed AgNPs had the least. The crude extracts of the leaf, flower and seed from *Callistemon citrinus* plant were also tested against *Plasmodium falciparum* strain 3D7 parasite and were found inactive as they were not able to decrease the % viability of the *Plasmodium falciparum* parasite. For crude extracts, IC₅₀ values should definitely be below 100 µg/mL [58] although most promising antimalaria extracts displays IC₅₀ values under 10 µg/mL [59,60]. The three nanoparticles exhibited an IC₅₀ lesser than 10 µg/mL showing they are all promising candidates for antiplasmodial lead drug.

Some medicinal plants of South Africa origin like *Mimusops caffra*, *Hypoxis colchicifolia* and *M. obtusifolia* have been used by traditional healers in the Zulu communities to treat malaria, but among these three medicinal plants, it was established that *Hypoxis colchicifolia* did not exhibit any antiplasmodial action as asserted by the Zulu traditional healers [61]. Several medicinal plants from Mali and Sao-Tome like *Feretia apotantha*, *Securidaca longepedunculata*, *Guiera senegalensis*, and *Morinda citrifolia* have been recommended by traditional healers in these regions to combat against malaria parasite strains [62]. Perusal of literature on the antiplasmodial activity of both crude and silver nanoparticles obtained from *Callistemon citrinus* revealed to the best of our knowledge no report or documentation has been done up to date in south Africa.

3.4. Cytotoxicity activity

The leaf, flower and seed nanoparticles with the crude samples they were derived from were evaluated against HeLa (human cervix adenocarcinoma) cells and HEK 293 (human embryonic kidney) cells at a concentration of 50 µg/mL. It was revealed that the nanoparticles and the crude samples did not show any sign of cytotoxicity since the samples did not cause any significant cytotoxic effects at a concentration of 50 µg/mL (they did not reduced the viability of HeLa cells to below 50%) because their percentage cell viability were >70% (Fig. 11). Similar report on colloidal silver nanoparticle demonstrating low cytotoxicity toward goat blood RBC was documented by Santosh Kumar et al. [63]. This might be a hint of their safety as targeted drugs for mammalian organisms. The synthesized nanoparticles indicates a strong antiplasmodial and very poor antitrypanosomal activities devoid of toxicity on HeLa cells which strongly indicates that the effects on parasite cultures was not as a result of a general cytotoxic effect of the synthesized nanoparticles.

3.5. Antibacterial activity

Agar well diffusion method as described by Collins et al. [64] was used to test for the zone of inhibition of the nanoparticles material. Microorganisms were cultured and inoculated in Nutrient Broth (oxid), this was incubated for a day (24 h) at a temperature of 37 °C.

McFarland 0.5 turbidity standard was used to standardized the inoculums before inoculation to give a confluent growth. About 38 g of Mueller Hilton Agar (Oxoid) was dissolved in 1 litre of distilled water and the resultant mixture was autoclave for about 30 min at 15lbs and 121 °C, it was allowed to cool for about 1 h and about 15–20 mL were dispensed into sterilized petri dishes and permitted to solidify in the various petri dishes. In all the petri dishes containing Muller Hinton Agar, 6 mm diameter wells were made via an uncontaminated cork borer. The bacterial culture was adjusted to 0.5 Mc Farland turbidity standard and the test microbes (0.1 mL) were inoculated with a clean swab on the outer surface of the solid medium in the petri dishes. Into each of the wells was fed 62.5–15.625 mg/mL of the nanoparticles extracts obtained from the stock solution and labelled accordingly. The inoculated petri dishes were inverted and incubated at 37 °C for 24 h. After all night incubation, the diameter of each zone was measured and recorded. All tests were performed under hygienic conditions.

Table 1
Inhibition zone (mm) showing antibacterial activities of the nano-particles derived from *Callistemon citrinus* with the standard drug, ciprofloxacin against bacterial test organisms.

Microorganism Concentration	Positive control Ciprofloxacin (mg/mL)			Nano leaf (mg/mL)			Nano seed (mg/mL)			Nano flower (mg/mL)		
	62.5	31.25	15.625	62.5	31.25	15.625	62.5	31.25	15.625	62.5	31.25	15.625
Gram negative bacteria strains												
<i>Escherichia coli</i> 0157:H7-ATCC 35150	35.0 ± 4.0	30.0 ± 4.0	18.0 ± 0.9	18.0 ± 3.0	12.0 ± 3.0	7.0 ± 3.0	18.0 ± 3.0	15.0 ± 3.0	8.0 ± 3.0	25.0 ± 3.0	18.0 ± 3.0	10.0 ± 3.0
<i>Vibrio alginolyticus</i> DSM 2171	33.0 ± 2.0	25.0 ± 2.0	13.0 ± 0.4	13.0 ± 2.0	11.0 ± 5.0	9.0 ± 4.0	15.0 ± 2.0	12.0 ± 5.0	6.0 ± 4.0	15.0 ± 2.0	10.0 ± 5.0	8.0 ± 4.0
<i>Salmonella typhi</i> ACC	35.0 ± 1.0	32.0 ± 0.6	18.0 ± 3.0	13.0 ± 0.5	10.0 ± 1.0	8.0 ± 2.0	20.0 ± 0.5	18.0 ± 1.0	10.0 ± 2.0	15.0 ± 0.5	10.0 ± 1.0	8.0 ± 2.0
Gram positive bacteria strains												
<i>Staphylococcus aureus</i> ATCC	40.0 ± 5.0	32.0 ± 1.0	17.0 ± 0.2	20.0 ± 0.5	16.0 ± 0.1	8.0 ± 0.4	25.0 ± 0.5	23.0 ± 0.1	18.0 ± 0.4	22.0 ± 0.5	18.0 ± 0.1	15.0 ± 0.4
<i>Staphylococcus aureus</i> ACC	20.0 ± 2.0	15.0 ± 0.0	11.0 ± 2.0	10.0 ± 4.0	8.0 ± 1.0	7.0 ± 2.0	15.0 ± 4.0	15.0 ± 1.0	13.0 ± 2.0	10.0 ± 4.0	8.0 ± 1.0	8.0 ± 2.0
<i>Listeria ivanovii</i> ATCC 19119	35.0 ± 6.0	30.0 ± 0.2	22.0 ± 1.0	18.0 ± 2.0	12.0 ± 2.0	10.0 ± 1.0	18.0 ± 2.0	15.0 ± 2.0	13.0 ± 1.0	25.0 ± 2.0	20.0 ± 2.0	18.0 ± 1.0
<i>Mycobacterium smegmatis</i> ATCC 19420	35.0 ± 6.0	32.0 ± 0.0	24.0 ± 1.0	13.0 ± 0.4	10.0 ± 0.5	8.0 ± 1.0	15.0 ± 3.0	10.0 ± 0.1	8.0 ± 1.0	15.0 ± 0.5	15.0 ± 0.4	12.0 ± 0.5

Zone of inhibition (millimeter), ACC (AEMREG) culture collection, ATCC (American type collection centre), values are mean ± SD, n = 3.

Table 2

Minimum inhibitory concentration (MIC) values (mg/mL) for nanoparticles and standard drug.

Bacteria	Nanoleaf	Nanoseed	Nanoflower	Ciprofloxacin	DMSO
<i>Escherichia coli</i> 0157:H7:ATCC 35150	7.8125	7.8125	7.8125	15.625	0.5 mL VG
<i>Vibrio alginolyticus</i> DSM 2171	7.8125	7.8125	7.8125	15.625	0.5 mL VG
<i>Salmonella typhi</i> ACC	7.8125	7.8125	7.8125	7.8125	0.5 mL VG
<i>Staphylococcal enteritis</i> ACC	7.8125	7.8125	7.8125	15.625	0.5 mL VG
<i>Staphylococcus aureus</i> ACC	7.8125	15.625	7.8125	7.8125	0.5 mL VG
<i>Listeria ivanovii</i> ATCC 19119	7.8125	7.8125	7.8125	15.625	0.5 mL VG
<i>Mycobacterium smegmatis</i> ATCC 19420	7.8125	7.8125	7.8125	15.625	0.5 mL VG

VG = Visible growth.

3.5.1. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The micro-dilution procedure was adopted in order to evaluate the minimum inhibitory concentration (MIC) of the different samples. To achieve this 250 μ L of Mueller Hinton broth (MBH) was dispensed into each Eppendorf tubes. Concentrations ranging from (61.5–15.75) mg/mL of the samples were prepared from the stock solution of 125 mg/mL in DMSO by two fold serial dilution. Aliquot of 250 μ L from the highest concentration of the sample was added into the first tubes containing MHB to bring the final volume to 500 μ L. From this same tube 250 μ L of the mixture was removed and added to the second tube, the same thing was done for the third tube through a twofold serial dilution and the contents were carefully vortexed. Exactly 20 μ L from the inoculums' suspension of each bacterial strain (0.5 McFarland, $\sim 1 \times 10^8$ cfu/mL) was afterwards added and vortexed to allow sufficient mixing of the extract and broth. The positive and the negative control that were used are ciprofloxacin and DMSO respectively.

The test was carried out in duplicate and incubated at 37 °C for 24 h. The MIC values of the extracts were defined as the lowest concentration that showed no visible growth when compared with the control containing only MHB while the minimum bactericidal concentration (MBC) was ascertained by the pour plate method of all tube content without visible growth in the MIC method above onto fresh Mueller Hinton agar plates and the culture was then incubated for 24 h at 37 °C. The lowest concentration of extracts that did not show any colony growth on the solid medium after incubation period of 24 h was regarded as the MBC.

The synthesized nanoparticles from the aerial part of *Callistemon citrinus* demonstrated significant inhibitory activities (Table 1) against both gram positive and gram negative bacteria strains (*Escherichia coli* 0157:H7:ATCC 35150, *Vibrio alginolyticus* DSM 2171, *Salmonella typhi* ACC, *Staphylococcal enteritis* ACC, *Staphylococcus aureus* ACC, *Listeria ivanovii* ATCC 19119 and *Mycobacterium smegmatis* ATCC 19420). A similar report had been documented for the volatile oils of leaf and flower of *Callistemon citrinus*. While Khowdiary et al. also reported the moderate antibacterial activity of pure silver nanoparticles [65,66].

The nanoparticles synthesized from the flower of this plant exhibited the highest inhibitory activities (25.0 \pm 3.0 mg/mL & 25.0 \pm 2.0 mg/mL) against *Escherichia coli* 0157:H7:ATCC 35150 a gram negative bacteria and *Listeria ivanovii* ATCC 19119, a gram positive bacteria

strain at a concentration of 62.5 mg/mL among the nanoparticles. Seed and Flower nanoparticles displayed the same inhibitory activity of 15.0 \pm 2.0 mg/mL against *Vibrio alginolyticus* DSM 2171 at the highest concentration, while seed AgNPs demonstrated good activities of 20.0 \pm 0.5 mg/mL & 18.0 \pm 0.5 mg/mL against gram negative *Salmonella typhi* ACC at 62.5 mg/mL and 31.25 mg/mL, respectively. The lowest activities of 10.0 \pm 4.0 mg/mL were recorded for leaf and flower nanoparticle against gram negative *Staphylococcus aureus* ACC at 62.5 mg/mL. Same MIC values of 7.8125 mg/mL were recorded for all the bacterial strains (Table 2). Similarly 31.25 mg/mL of leaf AgNPs was able to kill (bactericidal) *Escherichia coli* 0157:H7:ATCC 35150 but twice the amount was needed by flower AgNPs to completely exterminate same *Escherichia coli* 0157:H7:ATCC, observation made here was that increase in concentration of the flower AgNPs brought about the bactericidal effect of *Escherichia coli* a Gram negative bacteria. This may be due to the fact that there is no established rule on Gram sensitivity as some researchers had documented that some Gram negative bacteria strains are more sensitive than the Gram positive ones depending on the extract used and their various constituents [67,68]. On the other hand the leaf AgNPs were bactericidal at a concentration of 31.25 mg/mL against *Staphylococcus aureus* ACC but the flower AgNPs were bacteriostatic against the same bacterial strain at the same concentration Table 3. The differences observed in the antimicrobial properties of the different synthesized AgNPs may be due to variation in the chemical constituents of the different plant parts coupled with some bioactive compounds such as alkaloids, tannins, terpenoids, ether and phenolic compounds like flavonoids, which are considered to be bacteriostatic and bactericidal as reported in our previous study [65]. Also variation in the bacteria strains used may contribute to the significant differences in the antibacterial properties since the outer membrane of Gram negative bacteria show great resistance and act as a relative impermeable barrier [69].

3.6. Conclusion

Observation made from this study is that the synthesized silver nanoparticles from the leaves, flowers and seed of *Callistemon citrinus* exhibited exceptional antiplasmodial and antibacterial activities devoid of any cytotoxic effect. The different results achieved from this work further confirms the use of this plant by traditional healers and established

Table 3

Minimum bactericidal concentration (MBC) values (mg/mL) for nanoparticles and standard drug.

Bacteria	Nanoparticle (Leaf)	Nanoparticle (Seed)	Nanoparticle (Flower)	Ciprofloxacin Positive control	DMSO Negative control
<i>Escherichia coli</i> 0157:H7:ATCC 35150	Bactericidal at 31.25 NG	Bactericidal at 31.25 NG	Bactericidal at 62.5 NG	Bactericidal at \leq 15.625 NG	0.5 mL VG
<i>Vibrio alginolyticus</i> DSM 2171	Bactericidal at 31.25 NG	Bactericidal at 31.25 NG	Bactericidal at 31.25 NG	Bactericidal at \leq 15.625 NG	0.5 mL VG
<i>Salmonella typhi</i> ACC	Bactericidal at 31.25 NG	Bactericidal at 31.25 NG	Bactericidal at 15.625 NG	Bactericidal at \leq 15.625 NG	0.5 mL VG
<i>Staphylococcal enteritis</i> ACC	Bactericidal at 62.5 NG	Bactericidal at 31.25 NG	Bactericidal at 15.625 NG	Bactericidal at \leq 15.625 NG	0.5 mL VG
<i>Staphylococcus aureus</i> ACC	Bactericidal at 31.25 NG	Bactericidal at 31.25 NG	Bactericidal at 31.25 NG	Bactericidal at \leq 15.625 NG	0.5 mL VG
<i>Listeria ivanovii</i> ATCC 19119	Bactericidal at 62.5 NG	Bacteriostatic at 15.75 VG	Bactericidal at 61.5 NG	Bactericidal at \leq 15.625 NG	0.5 mL VG
<i>Mycobacterium smegmatis</i> ATCC 19420	Bactericidal at 62.5 NG	Bactericidal at 62.5 NG	Bacteriostatic at 31.25 VG	Bactericidal at \leq 15.625 NG	0.5 mL VG

NG = No growth, VG = Visible growth.

that the AgNPs could be used as an excellent alternative to synthetic antiplasmodial and antimicrobial agent to combat malaria and infectious diseases from different bacterial strains.

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Competing interests

The author(s) assert that they have no opposing interests.

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