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Silver nanoparticles (AgNPs) facilitated by plant parts of *Crataegus ambigua* Becker AK extracts and their antibacterial, antioxidant and antimalarial activities

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ABSTRACT

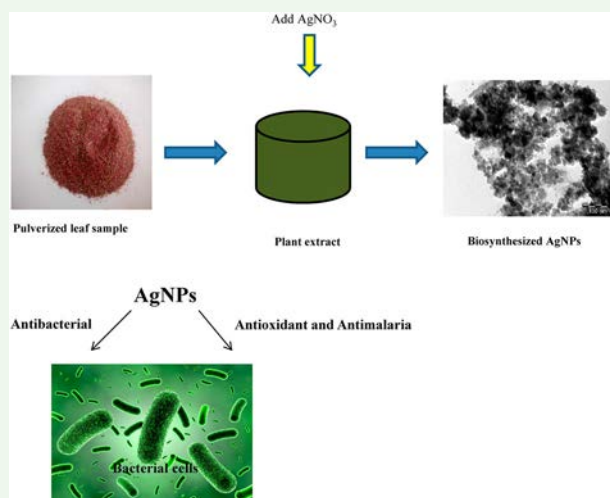
In this study, we aimed to synthesize, characterize, and investigate the efficacy of silver nanoparticles (AgNPs) derived from the fruit and leaf extracts of *C. ambigua* against free radical, bacterial strains and malarial parasites. Their antibacterial, antimalarial and antioxidant efficacy were examined by micro-dilution, parasite viability, and spectrophotometric techniques respectively. The result of characterization of the silver nanoparticles showed that biosynthesis of AgNPs was successful. FTIR analysis showed peak around 500 cm^{-1} ascribed to Ag-O peak for both nanoparticles. Also, spherically shaped materials from TEM images indicated that AgNPs were obtained. The AgNPs exhibited strong inhibitory activity on the seven test bacterial strains with minimum inhibitory concentration (MIC) ranging between $3.25\text{--}30.00\text{ mg mL}^{-1}$ and $15.00\text{--}30.00\text{ mg mL}^{-1}$ on the Gram-positive and Gram-negative bacterial strains respectively. The efficacy of AgNPs to reduce 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical to non-radical molecule (DPPH-H) was significant and the IC_{50} of AgNPs mediated by leaf extract was 0.21 and 0.80 mg mL^{-1} for the fruit silver nanoparticles. Similarly, the synthesized nanoparticles from the fruit and leaf parts of the plant showed excellent antimalarial properties with % inhibition of 100 at $20\text{ }\mu\text{g mL}^{-1}$ compared to the plant extract with lower % inhibition (48.5%). The AgNPs were observed to inhibit bacterial strains and have a very strong antioxidant and antimalarial activities indicating that plant parts of *C. ambigua* are very good precursors for non-artificial anti-microbial, antioxidant and antimalarial drugs.

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1. Introduction

Nanoparticle is a microscopic material that has at least one dimension below 100 nanometers in size. Owing to their significant thermal, chemical, optical, physical, and electrical properties; they are very useful in consumer goods,

pharmaceutical, chemical, environmental, energy, agricultural, and communication industries (1, 2). Nanoparticles (NPs) synthesis involved the use of different chemicals including ascorbate, sodium borohydride, elemental hydrogen, sodium citrate, Tollen's reagent, and ethylene

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glycol etc., which reduced metal ions in polar and non-polar solutions. These substances are very harmful and produced non-eco-friendly compounds (3). Studies have shown that plants facilitate better stable metal NPs, proven to be the greatest candidate for quick and large-scale production as compared to microbes and hazardous substances (4, 5). Recent studies have shown that production of metallic NPs via plant parts including the seed, leaf, and stem, is the most reproducible approach, cost effective, and simplest (5, 6). The preference for plant extract in NPs synthesis is due to presence of bioactive metabolites in most plants, including terpenes, alkaloids, protein and others, which serves as effective bioreducing compounds in synthesis of NPs. It has been suggested that metal nanoparticles mediated by plant's extract enhanced the bioactivity, broad spectrum properties and applications of NPs (3, 4). Biotechnology and pharmacological evaluation of many medicinal plant have been documented by several research scientists in the last two decades (7–9). Therefore, the use of phytochemicals in the preparation of NPs will builds a significant synergy between natural products and nanotechnology. Consequently, green nanotechnology which is performed devoid of significant environmental pollution will create novel sustainable, economically viable, eco-friendly and cutting edge technology.

Crataegus ambigua (hawthorn) of the family *Rosaceae* is a genus of small trees of over 200 species reported native to temperate region of South Africa (10). *Crataegus* species mostly grows between 6 and 14 m tall with thorny branches and small pome fruit. *Crataegus* thorns are small sharp-tipped about 1–3 cm long, arise either from the trunk or branches. In folk medicine, genus *Crataegus* is a curative for many ailments including cancers, diabetic, as well as central nervous, cardiovascular, and reproductive disorders (11). Studies have shown that plant leaf extracts function as anti-inflammatory, antibacterial, antidiabetic, anti-HIV, gastro, and cardio-protectives (12–18). Previous studies on genus *Crataegus* were principally on the flowers, berries and leaves of *C. azarolus*, *C. pinnatifida*, *C. aronica*, *C. monogyna*, *C. Mexicana* and *C. oxyacantha* species (10–12). Some of the reported isolated compounds from genus *Crataegus* are bioflavonoid, oligomeric, amines, procyanidins, polysaccharides, catecholamine, vitamin C, saponins, triterpenes, ursolic acid and purine derivatives (16–18). There is paucity of information on the nanoparticles and bioactive properties of *C. ambigua* as well as comparative investigation from the aqueous extracts of the leaf and fruit mediated NPs. Data from such study is not only important for broad knowledge of the plant's AgNPs but also its economic value. In this paper, we synthesized and characterized AgNPs facilitated by aqueous fruit and leaf extracts of *C. ambigua* and

investigated their antibacterial, antioxidant and antimalarial efficacies.

2. Materials and methodology

2.1. Chemicals

Chemicals and reagents employed in this study were of high purity and used as supplied by our vendors. Silver nitrate (AgNO_3), 97% (Merck Chemicals, South Africa). Mueller-Hinton agar (MHA) (Oxford Limited, Hampshire, England). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma – Aldrich, St Louis, USA) and dimethyl sulfoxide (DMSO), 98% (Fluka Chemicals, Buchs, Switzerland) were utilized in this study.

2.2. Fresh plant parts collection

Fresh leaves as well as the fruit of *Crataegus ambigua* were collected from the University of Fort Hare, Alice Campus locality and washed with deionized water (DW), air-dried at ambient atmospheric condition for 14 and 21 days respectively. Thereafter, they were separately ground to powder form with Polymix PX-MFC grinder. Forty-five grams (45 g) of each sample was macerated in 350 mL DW for 24 h. The extracts were filtered, freeze dried to dry powder and preserved at 4°C.

2.3. Silver nanoparticles (AgNPs) synthesis

AgNPs was synthesized by slowly adding 1 part of the aqueous extract of the different parts of the plant separately into 9 parts of 0.1 M AgNO_3 solution. The different mixtures were allowed to stir at room temperature until visible color change from light brown to black and precipitate were observed from the reacting solutions. The AgNPs solution was subjected to centrifugation with continuous washing with distilled water to obtain a pure material. This was followed by drying in an oven for 24 h. The AgNPs obtained was stored in an airtight sample bottle in a desiccator for further use (19).

2.4. Characterization

To ascertain the efficacy of the synthesized nanoparticles on microbial strains, materials/nanoparticles characterization was carried out with the aid of different instruments. Vibrational frequencies of the synthesized samples and plant extracts were obtained on Fourier transformed infra-red spectrophotometer (Perkin Elmer Universal ATR 100). Absorption spectra of the samples were recorded with UV-Visible absorption spectrophotometer (Perkin-Elmer Universal). TEM and SEM images

were obtained by using JOEL 1210 transmission electron microscope with accelerating voltage of 100 kV and JOEL JSM-6390 LVSEM respectively. The SEM instrument was also used to collect the electron diffraction spectrophotometer (EDS) images. In order to ascertain the crystallinity, phase and material size, the samples were subjected to X-ray diffractometer measurement using Bruker D8 X-ray diffractometer.

2.5. Bioactive assays

2.5.1. Antibacterial assay

The reference bacterial strains (BS) used for antibacterial assay were *Listeria ivanovii* (ATCC 19119), *Staphylococcus aureus* (NCIB 50080), *Enterobacter cloacae* (ATCC 13047), *Mycobacterium smegmatis* (ATCC 700084), *Streptococcus uberis* (ATCC 700407), and two (*Vibrio parahaemolyticus*, *Escherichia coli* 180) established multi-drug resistant bacteria (20–22). The BS were reported to be resistant to nalidixic, streptomycin, ampicillin, sulphamethoxazole as well as tetracycline (22). The agar diffusion technique as described by Collins et al. (23) and modified by Larayetan et al. (24) was adopted to examine the zone of inhibition of the bio-synthesized AgNPs. The cultured bacterial strains were inoculated in Nutrient Broth and the inoculums standardized (Mc Farland 0.5 $\sim 1 \times 10^8$ cfu.m⁻¹) before inoculation. Approximately thirty-eight (38 g) MHA was dissolved in 1 L of DW and autoclaved for 30 min at 121°C and 15 lbs. The MHA was left to cool, thereafter about 18–20 mL apportioned to sterilized petri dishes (PDs) and allowed to congeal. Afterwards, 6 mm diameter wells were bored into each MHA plate with a sterilized cork borer. Then, the bacterial culture (0.1 mL) adjusted to 0.5 Mc Farland turbidity was inoculated with a clean swab on the solid medium of the PDs. Subsequently, into each well was fed (60–7.5 mg mL⁻¹) of the AgNPs extract from the stock solution and they were labeled. Thereafter, the PDs were upturned and incubated at 37°C for 24 h. Thereafter, the zone of inhibition (diameter) of each was recorded. The test was carried out in parallel triplicate, under aseptic condition.

The minimum inhibitory concentration (MIC) of each biosynthesized AgNPs was evaluated by micro-dilution technique (25). Briefly, 250 μ L of Mueller Hilton Broth (MHB) was poured into each Eppendorf tube (EPT). Concentrations (in a two-fold serial dilution), ranging from 60 to 7.5 mg mL⁻¹ prepared in DMSO from the stock AgNPs extracts. From the highest concentration, an aliquot of 250 μ L was transferred to the first Eppendorf tube (EPT₁) containing the MHB, to bring the final volume to 500 μ L. Then, 250 μ L from the mixture of EPT₁ was removed and added to the second EPT₂. The two-fold serial dilution was carried out on third EPT₃ to final

EPT₄ and the EPTs contents were properly vortexed. Thereafter, 20 μ L from the inoculums' suspension of each BS was added and vortexed. DMSO and Ciprofloxacin were used as negative and positive control respectively, then incubated at 37°C for 24 h. The experiment was carried out in duplicate and the EPT nanoparticles extract having the lowest concentration without visible growth (WOG) was recorded as the MIC. Thereafter, pour plate method was used to determine the minimum bactericidal concentration (MBC) of all EPT content WOG in the MIC method above onto fresh nutrient agar plates and the culture incubated for 1 d at 37°C. Minimum bactericidal concentration (MBC) was the lowest concentration of AgNPs or extracts without any colony growth on the solid medium after the incubation period.

2.5.2. Antioxidant assays

Two different (2, 2-diphenyl-1-picrylhydrazyl [DPPH], and lipid peroxyl) radical scavenging assays were used to investigate the antioxidant activities of the *C. ambigua* extracts mediated AgNPs. In the DPPH assay, Liyana-Pathirana et al. (26) protocol with few changes (DMSO as diluent) was used. Concisely, DPPH (2.7 mM) in DMSO was made and 1 mL added to 1 mL of the AgNPs dissolved in DMSO (0.05–0.50 mg mL⁻¹), likewise for the reference drugs (RD). The reaction solutions were properly vortexed and incubated in the dark for 30 min at an ambient temperature. The absorbance of each mixture was then read at 517 nm against a reference blank containing DMSO. The assay was carried out in triplicate. The AgNPs efficacy to reduce DPPH• to non-radical molecule was calculated as an inhibitory percentage using the Equation (1):

$$\begin{aligned} &\% \text{ AgNPs or RD scavenging efficacy on DPPH}\bullet \\ &= \frac{(\text{Abs crl} - \text{Abs sap})}{(\text{Abs crl})} \times 100 \end{aligned} \quad (1)$$

Where Abs crl is the absorbance of the DPPH radical + DMSO; Abs sap is the absorbance of DPPH radical + AgNPs or reference drugs (RDs).

The synthesized AgNPs antioxidant efficacy against lipid peroxyl radical was evaluated applying the thiobarbituric acid technique as illustrated by Badmus *et al.* (16) with few modifications (egg yolk as lipid-rich source and DMSO as diluent). The AgNPs samples at increasing concentrations (0.05–0.50 mg mL⁻¹) in DMSO was added to 10% egg yolk homogenate (0.5 mL) and the reaction solution made up to 1 mL with DMSO. Thereafter, lipid peroxidation was stimulated by the addition of 0.05 mL of 0.07 M FeSO₄, then incubated at ambient temperature for 30 min. Subsequently, 1.50 mL of 10% acetic acid (pH

3.50) and 1.50 mL of 0.08% 2-thiobarturic acid in (1.10% sodium dodecyl sulphate and 20% trichloroacetic acid) were added and the mixture was vortexed and heated at 65°C for 60 min. Upon cooling, 0.50 mL of n-butanol was added to the reaction mixture and centrifuged for 10 min at 3000 rpm. The upper organic layer was then aspirated and the absorbance read at 532 nm. The percentage inhibition of lipid peroxy radical was calculated using the expression in equation A described above. The experiment was carried out in parallel triplicate and the average % inhibition of the radical evaluated.

2.5.3. Antimalarial assay

For *in-vitro* antimalarial assay, *P. falciparum* parasites maintained at 37 °C in human erythrocytes (O+/A+) was suspended in a mixture of [25 mM HEPES (Sigma-Aldrich), D-glucose (20 mM) (Sigma-Aldrich), hypoxanthine (200 µM) (Sigma-Aldrich), sodium bicarbonate (0.2%), Gentamicin (24 µg mL⁻¹) (Sigma-Aldrich) and AlbuMAX II (0.5%)] making up the RPMI 1640 medium (Sigma-Aldrich) in a gaseous environment of 5% O₂, 90% N₂ and 5% CO₂ as described in previous studies (27, 28). *In vitro* ring-stage intra-erythrocyte cultures of *P. falciparum* parasite, in a mixture of 200 µL at 1% haematocrit, 1% parasitaemia (NF54) were treated with the nanomaterials and plant extracts. A positive control, 1 µM Chloroquine diphosphate and a negative control, complete RPMI media served as the controls for this assay and grown for 4 days at 37°C under the gaseous environment in 96-well plates. After the 4 days growth period, *P. falciparum* parasite cultures (100 µL each) were mixed with SYBR Green I lysis buffer (20 mM Tris, 5 mM EDTA; 0.08% (v/v) Triton X-100; 0.008% (w/v) saponin; 0.2 µL mL⁻¹ 10 000x SYBR Green I, Invitrogen; pH 7.5). The samples were incubated for 60 min at 20° C, then fluorescence was determined using a GloMax®-Explorer Detection System with Instinct® Software (Promega, emission at 538 nm and excitation at 485 nm). The “background” fluorescence was subtracted from the total fluorescence measured for each sample to provide a measure of parasite proliferation.

Data obtained for all biological assays were analysed on Excel, and graphs determined using GraphPad 7 and experiments were performed in triplicate for three biological repeats (*n* = 1).

3. Results and discussion

3.1. Characterization of the synthesized materials

UV-Visible spectra of silver nanoparticles synthesized from the plant parts of *Crataegus ambigua* Becker AK

were obtained by scanning the aqueous solutions of the samples through UV-Visible spectrophotometer from 690 to 190 nm. The spectra obtained (Figure 1), showed a sharp peak at about 260 and 290 nm for both leaf and fruit AgNPs. These peaks are assigned to the absorption bands of metal nanoparticles indicating steady and gradual reduction of silver nitrate by the plant part extracts. Results similar to our observation in this study on the absorption spectra of AgNPs synthesized via green route were also published by Philip and Unni and Hillesden *et al.* (20, 21).

SEM and EDS images of the bio-synthesized AgNPs and leaf plant extract were collected to unravel the surface morphology and elemental composition of the synthesized materials respectively (Figure 2). The morphology of the nanoparticles was observed to be non-uniform spherically shaped according to the image captured from the SEM micrograph (Figure 2(a,b)). Although, few aggregations of the particles were observed and specifically more in the image of AgNPs mediated by fruit plant part (Figure 2(b)) than in the AgNPs from leaf plant part (Figure 2(a)) which can be ascribed to the uneven dispersion of the plant extract in solution, the particles obtained were still well dispersed. The SEM image (Figure 2(c)) of the plant extract showed a flower like material which was different from what we obtained for the nanoparticles indicating the successful formation of AgNPs. EDS image showed that pure AgNPs were obtained after the bio-reduction of AgNO₃ salt. This result is similar to the observations previously published by Carmona *et al.* (22).

TEM images of green synthesized AgNPs from the different plant parts of *Crataegus ambigua* Becker AK are presented in Figure 3. The recording of the images of these samples by TEM is important to determine the shape and size of the nanoparticles synthesized. From the micrograph, it can be noticed that the nanoparticles

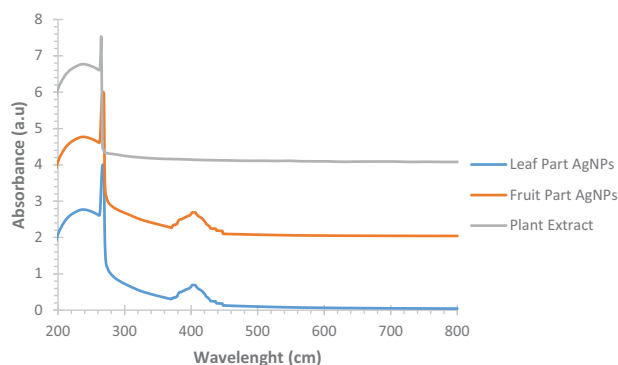


Figure 1. UV-Visible spectra of synthesized AgNPs and plant extract.

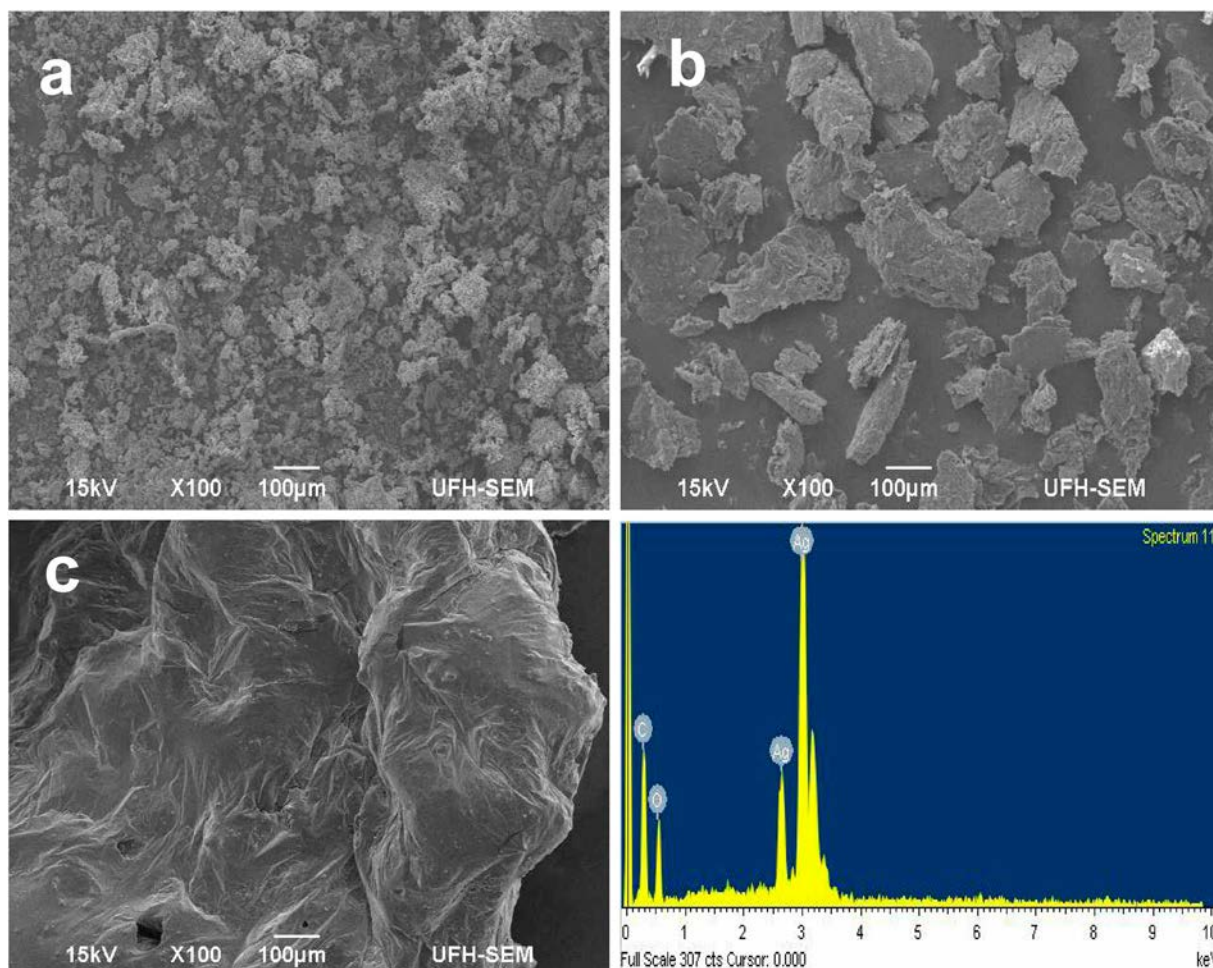


Figure 2. Scanning electron micrograph of AgNPs obtained from (a) leaf and (b) fruit parts of *Crataegus ambigua* Becker AK plant (c) plant extract and EDS micrograph of AgNPs.

are spherical in shape with average size of 32 nm which is in agreement with the result obtained for the size of these particles using XRD (Figure 4). Also, worthy of mention is that the plant part type used for the synthesis of these nanoparticles did not influence the size and shape of the synthesized nanoparticles. Similar result was reported in previously published works on AgNPs synthesized using plant parts (24, 29, 30).

The successful synthesis of AgNPs by the bio-reduction of AgNO_3 using the fruit and leaf extracts of *C. ambigua* was confirmed using XRD (Figure 4). The analysis done on these nanoparticles using XRD (from 2θ values between 10° and 90°) revealed that diffraction peaks at 2θ values of 33.5° , 44° , 65° and 77.4° for both fruit and leaf biosynthesized nanoparticles showing that the materials are crystalline in nature and characteristics of AgNPs. In addition, diffraction peaks at 2θ values of 28° , 32.6° , 47° , 55° and 57° could be ascribed to the presence of silver chloride (AgCl) resulting from the reaction of between Ag^+ from silver

nitrate and chloride ion (Cl^-) from the phytochemicals in the plant extract (30). Although, we observed stronger intensity in the peaks of the fruit biosynthesized AgNPs in the diffractogram (Figure 4) as compared to that of the leaf biosynthesized AgNPs, the sizes of the materials were around 30 nm using 2θ value 33.5° in the Scherrer formula (Nuffield). Similar results like the one we obtained from this study for XRD was published by Okaiyeto et al. (30).

For the vibrational frequencies information of the synthesized materials, their FT-IR spectra were recorded from 400 to 4000 cm^{-1} using ATR Perkin Elmer FT-IR spectrophotometer. The vibrational frequency observed at about 500 cm^{-1} for both materials is assigned to the Ag-O vibrational band which is characteristic of metal nanoparticles (Figure 5). Also, bands observed at about 1100 and 1650 cm^{-1} is assigned to the stretching band of C-O and C=O respectively probably from the different plant part extracts. The band at about 3500 cm^{-1} is as a result of the presence of water

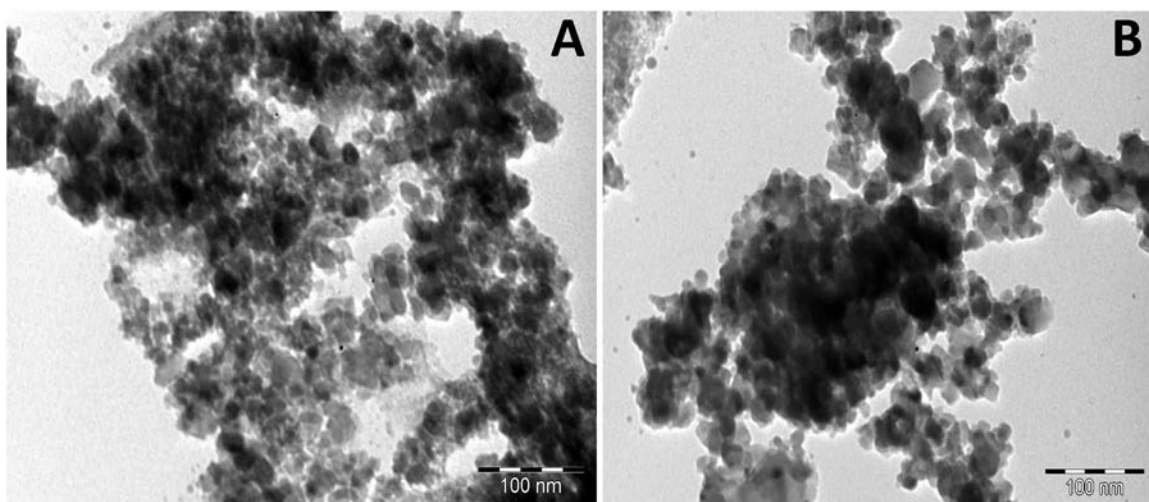


Figure 3. TEM images of AgNPs obtained from (A) leaf and (B) fruit parts of *Crataegus ambigua* Becker AK plant.

molecules from the washing of the materials after synthesis, this could be attributed to the incomplete drying of the particles.

3.2. Antibacterial activity

The silver nanoparticles mediated by the leaf and fruit extracts of *Crataegus ambigua* exhibited strong antibacterial activities against the seven test bacterial strains

(Table 1). In our previous report, similar antibacterial effects were recorded for essential oil of the same plant parts (25) while Larayetan et al. (24) documented significant antibacterial efficacy of *Callistemon citrus* extracts facilitated silver nanoparticles.

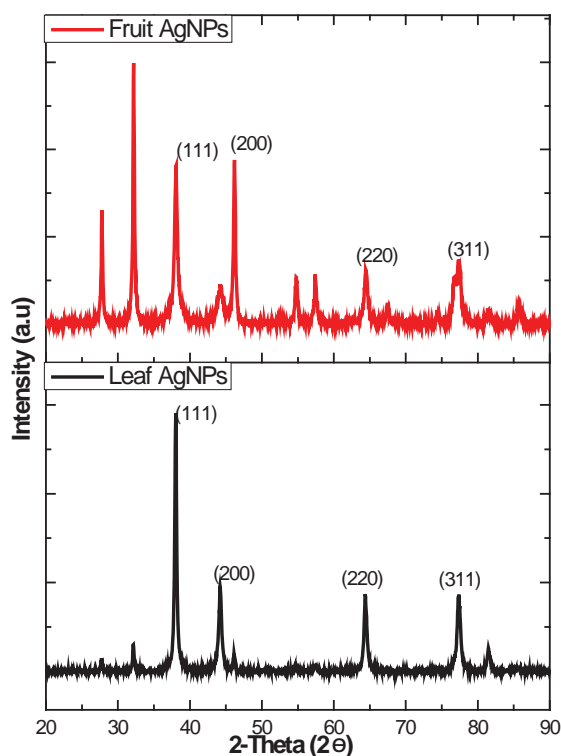


Figure 4. XRD spectra of AgNPs from fruit and leaf parts of *C. ambigua*.

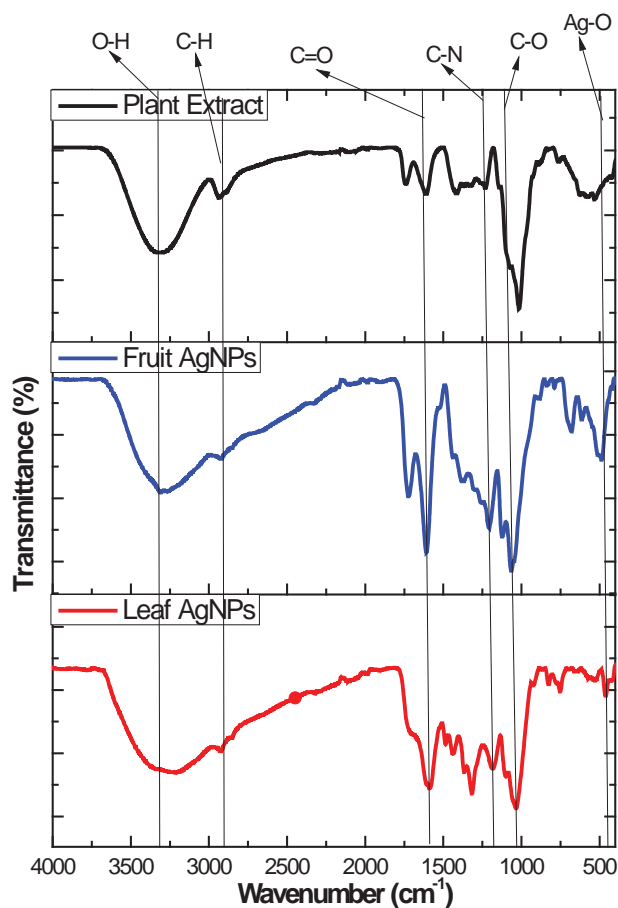


Figure 5. FTIR spectra of AgNPs from *C. ambigua* and its extract.

Table 1. Zones of inhibition (mm) of silver nanoparticles mediated by *C. ambigua* extracts and positive drug on bacterial strains.

| Test bacterial strains | AgNPs leaf (mg. mL ⁻¹) | | | | AgNPs Fruit (mg. mL ⁻¹) | | | | Ciprofloxacin ^a (mg. mL ⁻¹) | | | |
|------------------------------------|------------------------------------|-------------|------------|-----------|-------------------------------------|------------|------------|----------|--|------------|------------|-----------|
| | 60.00 | 30.00 | 15.00 | 7.500 | 60.00 | 30.00 | 15.00 | 7.50 | 60.00 | 30.00 | 15.00 | 7.50 |
| <i>L. ivanovii</i> ATCC19119 | 25.00 ±0.30 | 15.00 ±0.01 | 7.50±0.20 | 4.50±0.10 | 11.50±0.03 | 8.00±0.00 | 5.00±0.10 | Ngb | 32.00±0.02 | 17.50±0.10 | 10.50±0.00 | 6.00±0.30 |
| <i>S. aureus</i> NCIB50080 | 24.00±0.01 | 13.50±0.20 | 7.00±0.01 | Ngb | 12.40±0.02 | 6.00±0.30 | N/D | Ngb | 29.00±0.10 | 14.00±0.30 | 9.50±0.20 | 7.00±0.10 |
| <i>M. smegmatis</i> ATCC700084 | 25.00±0.02 | 15.50±0.03 | 10.00±0.01 | 8.00±0.02 | 9.50±0.30 | 6.50±0.01 | 5.00±0.30 | Ngb | 30.00±0.30 | 19.5± 0.03 | 8.00±0.02 | 5.80±0.00 |
| <i>V. parahaemolyticus</i> * | 14.00±0.20 | 9.40±0.03 | 7.50±0.20 | Ngb | 11.40 ±0.10 | 9.80±0.03 | 7.50±0.00 | 4.5±0.20 | 31.50±0.20 | 15.5±0.02 | 8.50±0.11 | 5.50±0.30 |
| <i>E. cloacae</i> | 11.40±0.03 | 9.40±0.20 | 8.50±0.10 | 4.60±0.30 | 8.00±0.20 | 5.60±0.20 | Ngb | Ngb | 34.00±0.03 | 19.0± 0.10 | 11.50±0.20 | 6.50±0.01 |
| ATCC13047 | 8.5±0.02 | 6.00±0.01 | 5.6±0.30 | Ngb | 7.50± 0.10 | 5.80 ±0.02 | 4.00± 0.04 | Ngb | 28.40±0.30 | 13.5±0.20 | 8.00±0.01 | 5.50±0.20 |
| <i>E. coli</i> 180 * | | | | | | | | | | | | |
| ATCC700728 | 9.20±0.30 | 7.50 ±0.20 | 5.40±0.20 | Ngb | 6.50±0.03 | 5.30±0.10 | 4.50±0.02 | Ngb | 32.00±0.10 | 16.40±0.03 | 9.5±0.03 | 7.00±0.03 |
| <i>Str. Ueberis</i> ATCC 700407 | | | | | | | | | | | | |

Note: ATCC: American type collection center. * Multi-drug resistance bacterial strain from our (AEMREG) stock culture. ^a positive control, Ngb: negligible (<4.0 mm).

The silver nanoparticles (AgNPs) synthesized from the plant's leaf extract displayed significant higher zone of inhibitions ranging from 11–25, 9.5–15 and 7.5–10 mm at 60, 30.00, and 15 mg mL⁻¹ respectively against *L. ivanovii*, *S. aureus* and *M. smegmatis* when compared to fruit AgNPs at same concentrations (Table 1). The anti-bacterial activities of *C. ambigua* extracts mediated silver nanoparticles against *S. aureus*, *L. ivanovii* and *M. smegmatis* in this study are similar to AgNPs from *Calistemon citrus* reports by Larayetan et al. (24). However, our synthesized AgNPs exhibited superior inhibitory activities against these three gram-positive bacterial strains. The leaf silver nanoparticles had the lowest MIC value against *M. smegmatis* at 3.25 mg mL⁻¹ (Table 2). In contrast, the MIC values for fruit AgNPs ranges from 15 to 30.00 mg mL⁻¹ against the seven test bacterial strains.

The leaf, fruit and Ciprofloxacin (positive control) had same MIC value (15.00 mg mL⁻¹) against *S. aureus*, however the leaf AgNPs and Ciprofloxacin exhibited bactericidal effect at 30.00 and 15.00 mg mL⁻¹ respectively, while the fruit nanoparticles was bacteriostatic at 30.00 mg mL⁻¹ (Table 2). Interestingly, the leaf silver nanoparticles and Ciprofloxacin demonstrated bactericidal effect against *M. smegmatis* and *E. cloacae* at 7.5 and 15.0 mg mL⁻¹ respectively. In this present study, the leaf, fruit nanoparticles and Ciprofloxacin exhibited higher efficacy against gram-positive than gram-negative bacterial strains except on *Str.uberis*. Previous studies have shown that due to the outward complex membrane of gram-negative bacterial, consisting of hydrophilic lipopolysaccharide (31), creates a barricade toward hydrophobic chemicals, affording gram-negative bacteria with more tolerance toward hydrophobic anti-bacterial chemicals like those present in plant extracts (6, 7). Gram- negative bacterial resistance against phyto-chemical derivatives has also been suggested to be the existence of multi-drug resistant sites that stimulates the production of amphipathic toxins (32–34).

3.3. Antioxidant activity

Antioxidant activities of leaf and fruit mediated nanoparticles were investigated using DPPH (DPPH•) and Lipid peroxyl radical (LP•) radical scavenging tests. The scavenging effects on the two different radicals by two AgNPs and the RDs were concentration dependant (Figures 6 and 7). In the DPPH assay (Figure 6), the antioxidant effect of the silver nanoparticles was superior to the two RDs used at increasing concentrations (0.05–0.50 mg mL⁻¹). To ascertain the antioxidant efficacy against a specific radical, the LP• quantitative test showed that the leaf and fruit AgNPs exhibited

Table 2. Antibacterial activities of silver nanoparticles mediated by *C. ambigua* extracts and positive drug.

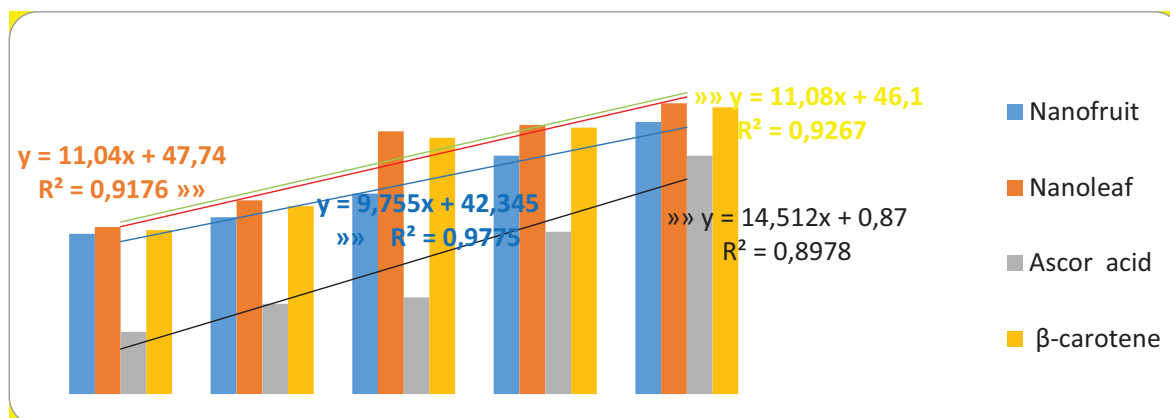
| Bacterial strains | AgNPs of <i>C. ambigua</i> extracts | | | | Controls | | Distilled H ₂ O _b (0.5mL) |
|-----------------------------------|-------------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|--|
| | nanoleaf | | nanofruit | | Ciprofloxacin ^a | | |
| | MIC (mg.mL ⁻¹) | MBC (mg.mL ⁻¹) | MIC (mg.mL ⁻¹) | MBC (mg.mL ⁻¹) | MIC (mg.mL ⁻¹) | MBC (mg.mL ⁻¹) | |
| <i>L.ivanovii</i> ATCC19119 | 7.50 | WOVG at 15.0 bactericidal | 15.00 | WOVG at 30.0 bactericidal | 7.50 | WOVG at 7.50 bactericidal | WG |
| <i>S. aureus</i> NCIB50080 | 15.00 | WOVG at 30.0 bactericidal | 15.00 | WG at 30.0 bacteriostatic | 15.00 | WOVG at 15.00 bactericidal | WG |
| <i>M. smegmatis</i> ATCC700084 | 3.25 | WOVG at 7.50 bactericidal | 30.00 | WOVG at 30 bactericidal | 3.25 | WOVG at 7.50 bactericidal | WG |
| <i>V. parahaemolyticus</i> * | 15.00 | WOVG at 30.0 bactericidal | 30.00 | WG at 30.0 bacteriostatic | 15.00 | WOVG at 15.0 bactericidal | WG |
| <i>E. cloacae</i> ATCC13047 | 15.00 | WOVG at 15.0 bactericidal | 30.00 | WOVG at 30 bactericidal | 15.00 | WOVG at 15.0 bactericidal | WG |
| <i>E. coli</i> 180 ATCC700728 | 30.00 | WG at 30.0 bacteriostatic | 30.00 | WG at 30.0 bacteriostatic | 30.00 | WOVG at 30.0 bactericidal | WG |
| <i>Str.Uberis</i> ATCC700407 | 15.00 | WOVG at 30.0 bactericidal | 15.00 | WG at 30.0 bacteriostatic | 7.50 | WOVG at 7.50 bactericidal | WG |

Note: MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; WOVG: without visible growth; WG: with visible growth, a: positive control drug, b: negative control.

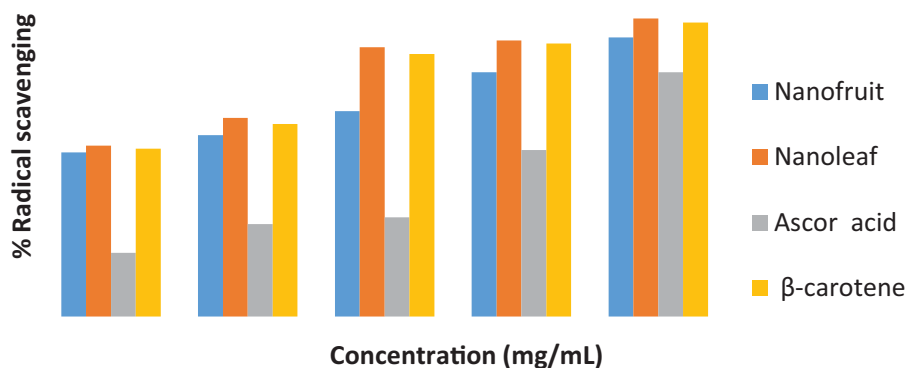
significant radical scavenging effect, acting as good electron donors in the vitro experiment (Figure 7). Standard curves produced (Figures 6 and 7) from % inhibitions against concentrations (Tables 3 and 4) on the AgNPs

and RDs were both linear. Linear regression equation generated (Figures 6 and 7) from each silver nanoparticles and RDs was used to calculate IC₅₀ value. The regression equations generated (Figure 6) from DPPH

DPPH



$p < 0.05$

**Figure 6.** Antioxidant activities of silver nanoparticles and reference drugs on DPPH radical.

Lipid peroxidation

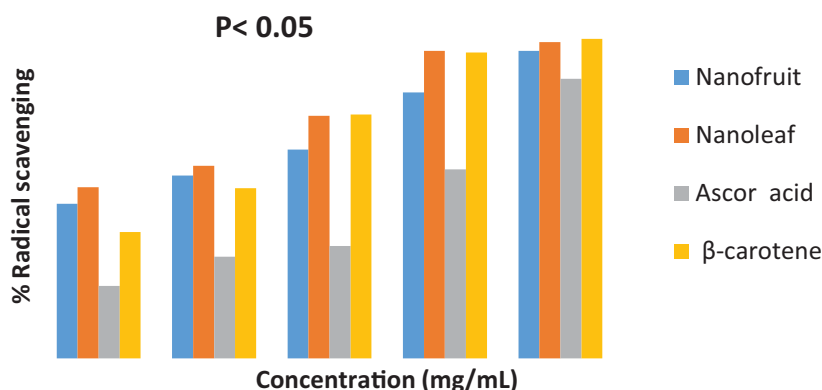
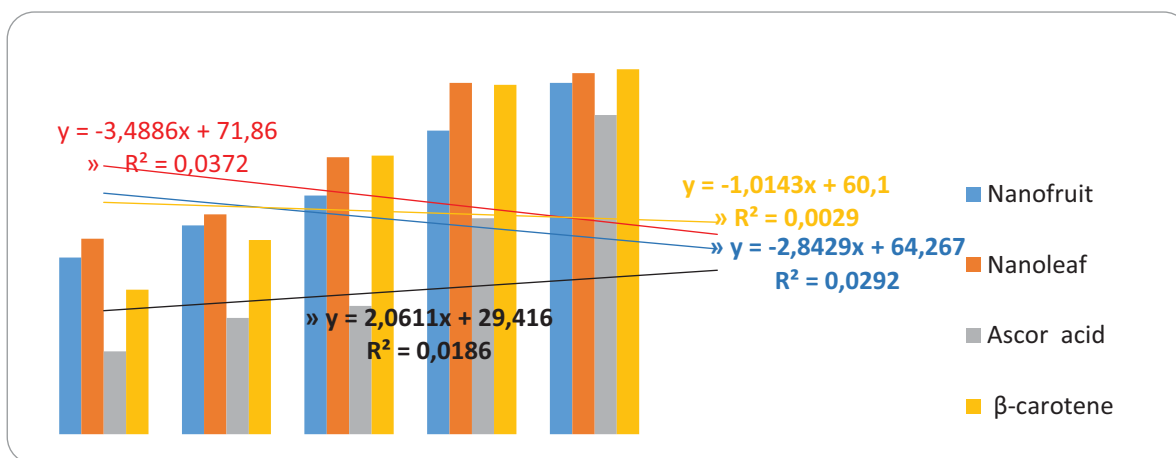


Figure 7. Antioxidant activities of silver nanoparticles and reference drugs on lipid peroxyl radical.

data: for the fruit silver nanoparticles (nanofruit), $y = 9.755x + 42.345$; $R^2 = 0.9775$: thus x (IC_{50} for nanofruit) $= 50 - 42.345/9.755 = 0.78 \text{ mg mL}^{-1}$.

For the leaf silver nanoparticles (nanoleaf), $y = 11.04x + 47.74$; $R^2 = 0.9176$: hence x (IC_{50} for nanoleaf) $= 50 - 47.74/11.04 = 0.20 \text{ mg mL}^{-1}$. Similarly, the IC_{50} values for the two RDs (ascorbic acid 3.39 mg mL^{-1} and β-carotene 0.35 mg mL^{-1}) were calculated from each regression equation (Figure 6). From the lipid

peroxyl data and regression equations (Table 4 and Figure 7), the two AgNPs reduced the LP• to a non-radical molecule (LPH), attaining 50% decrease with an IC_{50} value of 1.65 mg mL^{-1} for the nanofruit, 1.17 mg mL^{-1} for the nanoleaf. Both demonstrated superior antioxidant efficacy against lipid peroxidation when compared to the IC_{50} value of 3.41 mg mL^{-1} obtained for ascorbic acid and 1.81 mg mL^{-1} for β-carotene.

Table 3. Silver nanoparticles and Reference drugs effects against DPPH radical.

| Conc. mg.mL^{-1} | % Nanofruit | Inhibition ^a Nanoleaf | Ascorbic acid | β-carotene |
|------------------------------|----------------|-------------------------------------|---------------|------------|
| 0.05 | 54.70 | 57.00 | 21.23 | 56.00 |
| 0.10 | 60.45 | 66.20 | 30.80 | 64.20 |
| 0.20 | 68.50 | 89.80 | 33.07 | 87.50 |
| 0.40 | 81.40 | 92.00 | 55.48 | 91.00 |
| 0.50 | 93.00 | 99.30 | 81.45 | 98.00 |

^a% inhibitions are average of three parallel LP experiment on AgNPs and reference drugs.

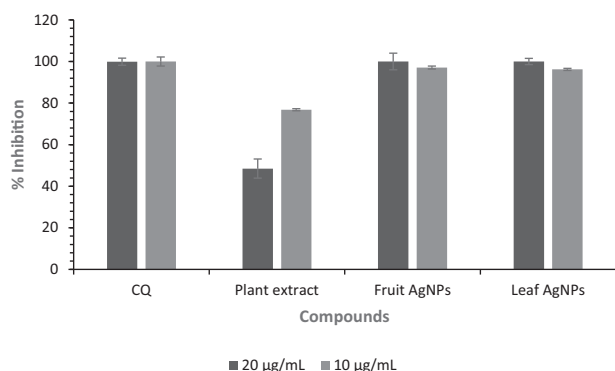
Table 4. Silver nanoparticles and Reference drugs effects against lipid peroxyl radical.

| Conc. mg.mL^{-1} | % Nanofruit | Inhibition ^a Nanoleaf | Ascorbic acid | β-carotene |
|------------------------------|----------------|-------------------------------------|---------------|------------|
| 0.05 | 45.00 | 49.8 | 21.10 | 36.80 |
| 0.10 | 53.20 | 56.00 | 29.60 | 49.50 |
| 0.20 | 60.80 | 70.6 | 32.70 | 71.00 |
| 0.40 | 77.40 | 89.50 | 55.00 | 89.00 |
| 0.50 | 89.50 | 92.00 | 81.38 | 93.00 |

^a% inhibitions are average of three parallel LP experiment on AgNPs and reference drugs.

Table 5. % inhibition of asexual parasite of chloroquine reference standard.

| CQ | Conc. (μM) | Asexual inhibition (%) |
|----|-------------------------|------------------------|
| | 1 | >99% |
| | 5 | >99% |

**Figure 8.** % inhibition of asexual parasites for reference standard, plant synthesized nanomaterials and plant extract concentrations at 20 and 10 $\mu\text{g/mL}$.

3.4. Dual point antimalarial analysis

Reference compound used for this assay was Chloroquine (CQ), it typically produced the average % inhibition of asexual parasite proliferation at 1 and 5 μM as presented in Table 5.

The nanoparticles and plant extract of *C. ambigua* were screened for *in vitro* asexual activity using the SYBR Green assay on the NF54 strain of *P. falciparum* parasites. Each material was evaluated at concentrations of 20 and 10 $\mu\text{g mL}^{-1}$. Figure 8 indicates the percentage inhibition obtained against asexual parasites for nanomaterials and plant extract concentrations at 20 and 10 $\mu\text{g mL}^{-1}$ for each series, actual values are provided in Table 6.

From Table 6, we observed that nanomaterials synthesized from the fruit and leaf parts of *C. ambigua* plant showed good activity with % inhibition of 100 at 20 $\mu\text{g mL}^{-1}$ and 97.1 and 96.2 at 10 $\mu\text{g mL}^{-1}$ respectively. This is contrary to the result obtained for the plant extract in which moderate % inhibition (48.5) was observed. The data showed good reliability and reproducibility with a Z-factors = 0.91 and %CV = 2.15. This result is consistent with previously published data (35).

4. Conclusion

This study investigated the potency of silver nanoparticles biosynthesized from the fruit and leaf extracts of *C. ambigua* against some bacterial, antimalarial strains and its scavenging properties. The result of characterization revealed that the silver nanoparticles were synthesized and possessed non-uniform spherical shapes

Table 6. In vitro activity of nanomaterials and plant extract against asexual *P. falciparum* parasites, obtained at concentrations of 20 and 10 $\mu\text{g mL}^{-1}$.

| Compound name | Dual Screens | | | |
|---------------|-------------------------------|------|--------------------------|------|
| | Asexual parasites, SYBR Green | | | |
| | 20 $\mu\text{g mL}^{-1}$ | | 10 $\mu\text{g mL}^{-1}$ | |
| | % Inhibition | | % Inhibition | |
| | Ave | SD | Ave | SD |
| CQ* | 99.9 | 1.71 | 99.99 | 2.18 |
| Plant extract | 48.5 | 4.6 | 76.8 | 0.5 |
| Fruit AgNPs | 100.0 | 4.0 | 97.1 | 0.7 |
| Leaf AgNPs | 100.0 | 1.5 | 96.2 | 0.5 |

*CQ = was used as a control compound, ($n = 1$, three biological assays with technical triplicates).

with an average size of about 30 nm as observed from SEM and TEM analysis respectively. The AgNPs were observed to inhibit bacterial strains and possess very strong antioxidant and antimalarial activities indicating that plant parts of *C. ambigua* are very good non-artificial anti-microbial, antimalarial and antioxidant drugs.

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Disclosure Statement

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