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Enzyme Inhibition and Antibiotics Properties of Six (6) Weeks Stable *Chrysophyllum albidum* leaf Silver Nano-particles.

Kingsley Ozioma Omeje[1]*, Florence N. Nworah[1], Benjamin O. Ezema[1] and Sabinus O. O. Eze[1]

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria

* Correspondence: kingsley.omeje@unn.edu.ng

ABSTRACT: Antibiotic resistance has posed a major public health challenge because of antibiotics misuse, overdose, underdose, ignorance on antibiotics usage and substandard production from the producers, thus the need for an alternative antibiotic agent production. Here, a commonly used antibiotic plant, *Chrysophyllum albidum* leave was used to produce silver nanoparticle (AgNPs) and characterized using XRD, FTIR, DSC and DLS. The characterization data showed the production of six (6) weeks stable AgNPs, with high antioxidant properties and amylase, glucosidase and cholinesterases inhibition properties. Similarly, the product exhibited stable antibiotics properties on *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* after six (6) weeks of storage at 4°C.

Keywords: Enzyme Inhibition; Antibiotics; medicinal plants; *Chrysophyllum albidum*; Silver Nano-particles; biotechnology

1. Introduction

The synthesis and characterization of Nanoparticles have been receiving great attention in recent times due to its wide array of applications in different areas of chemistry, medicine biology [1] and drug delivery [2]. The size of nanomaterials range is usually 1 to 100 nm [3].

Antibiotics are substances that inhibits the growth of microorganisms. Antibiotics resistance (AMR) is a major problem for public health especially now and has led to the prevention and treatment of different infections [4], with *S. aureus* and *K. pneumoniae* being the most prevalent pathogenic antibiotic-resistant organisms of global concern [4].

Chrysophyllum albidum is a perennial plant also known as African star apple, with pharmacological or medicinal potentials [5], treating stomach-ache and diarrhea [6] inhibit microbial growth of known wound contaminants [7, 8], malaria and yellow fever.

Some advantages associated with compounding medicinal plant into nanoparticle include increase in component concentration, solubility, and half-life, thus enhancing drug delivery to its target [2, 9]. Despite the advantages attributed to compounding medicinal plant into nanoparticle, there is dearth of literature on the silver nitrate nanoparticle of *C. albidum* which could increase the potency of this common and generally acclaimed antibiotic medicinal plant. One of the challenges affecting the efficacies of antibiotics could be the reduction of intended concentration due to the factors in the biological system, which could be overcome by compounding this medicinal plant into silver nanoparticles and increase its concentration at the target site, which forms the aim of this study. Here, the biological activities of *C. albidum* leaf silver nanoparticles (Ag-*C. albidum*-NPs) was reported.

2. Materials and Methods

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Reagents used include 1, 1- diphenyl-2-picryl hydrazyl (Sigma-Aldrich), $AgNO_3$, Acetylcholine iodide (Sigma-Aldrich), Butrylcholine iodide (Sigma-Aldrich) All the reagents used were of analytical grades.

1.1. Sample Collection and Preparation

The taxonomic classification of *Chrysophyllum albidum* was carried out by Mr. Felix Nwafor of Pharmacognosy Department, University of Nigeria, Nsukka as *Chrysophyllum albidum G. Don*. (*Sapotaceae)* with voucher number PCG/UNN/0359. The plant sample (Leaves) was dried by airdrying for 72hrs, ground and macerated in 1000 mL of 80 % ethanol for 72 hours. At the end of maceration, the mixture was filtered and concentrated for analysis.

1.2. Synthesis of silver nanoparticles

The synthesis of C. albidum silver nanoparticles Ag NPs was carried out as described by Osibe et al. [10] using aqueous *C. albidum* extract to an equal volume of 1 mM $AgNO_3$ and incubated at 25 °C for 72 h.

1.3. Characterization of nanoparticles

The thermostability of Ag-*C. albidum*-NPs was determined using differential scanning calorimetry (Shimadzu, Kyoto, Japan). Particle size was determined using dynamic light scattering (Malvern Zeta sizer, Worcestershire). X-ray diffraction and Phase identification of the formulated nanoparticle (Ag NPs) were recorded in the X-ray diffraction (Philips Diffractometer, The Netherlands). Functional groups of the synthesized Ag NPs were identified using Fourier transform infrared spectroscopy (Shimadzu, Kyoto, Japan).

1.4. In vitro Antioxidant Activity

1.4.1. 1, 1- diphenyl-2-picryl hydrazyl (DPPH) scavenging potential.

DPPH scavenging potential of Ag-*C. albidum*-NPs were assessed as described by Shen et al. [11].

DPPH radical scavenging (%)= $\frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100$

1.4.2. Determination of hydroxyl radical scavenging activity

The hydroxyl radical (-OH) scavenging activity was measured by the method of Jin *et al.* [12], and the absorbance was read at 536 nm. Scavenging activity (%) = (Abs. sample – Abs. blank)/(Abs0 – Abs. blank) x 100. Ascorbic acid was be used as reference standard.

1.4.3. Nitric oxide radical scavenging

The nitric oxide radical scavenging capacity of the fractions was measured by Griess reaction

Sangermeswaran et al. [13]. Ascorbic acid was be used as reference standard.

Percentage of inhibition = [(Ao– A1)/Ao] x 100.

1.4.4. Ferric cyanide (Fe^{3+}) reducing antioxidant power assay:

Reducing power of the extracts was measured by the direct reduction of $Fe^{3+}(CN-)6$ to $Fe^{2+}(CN-)6$ by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition of excess Fe^{3+} [14]. Finally, the reaction mixture absorbance was measured spectrophotometrically at 700 nm.

1.4.5. Acetylcholinesterase and Butrylcholinesterase inhibitory activity assay

The cholinesterases (AchE) and (BuchE) inhibition were studied using Ellman et al. [15]

. $AChE\ activity\ \% = \frac{Ao-A1}{Ao} \times 100$; $BChE\ activity\ \% = \frac{Ao-A1}{Ao} \times 100$;

1.4.6. α-amylase and α-glucosidase inhibition Assay

α-amylase activity inhibition assay was done as described by Gulati et al. [16] method. α-glucosidase activity inhibition assay was determined according to the procedure of Li et al. (2005).

Percentage (%) amylase and glucosidase inhibition = $\frac{Abs.Control - Abs.Sample}{Abs.Control} \times 100$

3. Results and Discussion

X-Ray Diffraction is an appropriate technique being used in determining the phases of materials (Ag-*C. albidum* nanoparticles): four intense diffraction peaks were apparent at 2hvalues of 20.56, 22.95, 28.56 and 29.33 corresponding to the Ag-planes respectively (Figure 1). The data generated is in tandem with the report of [17] and [10] with high intensity of the peaks corresponding to Ag.NPs [18].

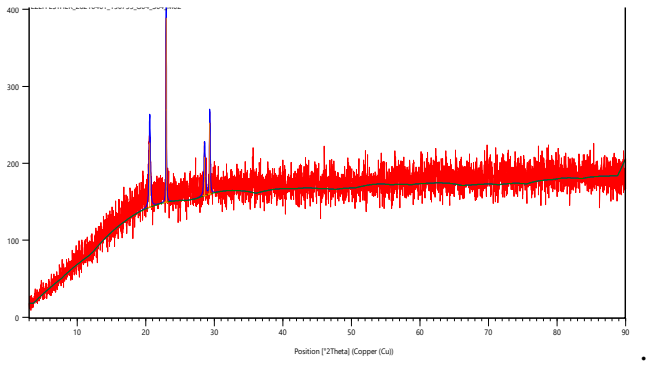
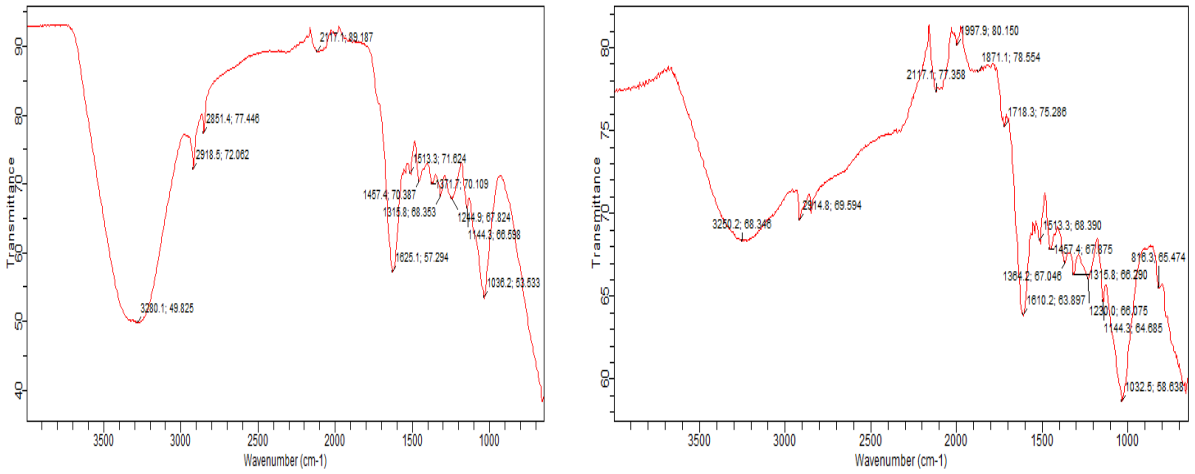


Figure 1. X-ray diffraction spectra of Ag-C. albidum-NPs synthesized from the leaf of *C. albidum.*

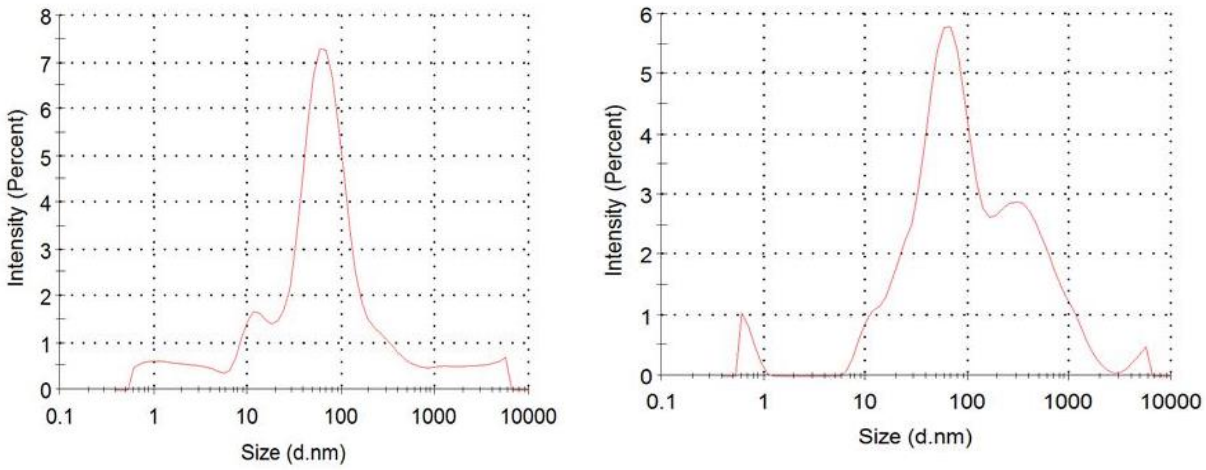
One of the tools used in analysing the functional groups present in a compound is Fourier transform infrared spectroscopy. Thus, the identification of the functional groups in both the aqueous extract of *C. albidum* and synthesized Ag *C. albidum* NPs as shown in Fig. 3a, b. The spectra of the *C. albidum* extract showed absorbance bands at 3280, 2918, 2851 and 2117. The broad band 3280 cm-^{1} that represents the stretching vibrations of N–H, 2918 and 2851cm^{1} bands corresponds to C–H stretching vibrations. Also, FT-IR spectra of the Ag-*C. albidum*-NPs shows absorbance band at 3250 cm, representing stretching vibrations of a N–H group, 2914 cm bands correspond to C–H stretching vibrations and 2117 cm represents N=N=N stretching. Furthermore, peaks at 1997, 1871 and 1718 cm represent amino acids, N-H^{+} charged amine and C=O stretching respectively. There was a shift in the absorbance from 3280 to 3250 cm1 with an increase in band intensity (Fig. 3b), suggesting a possible involvement of this functional group in the reduction of silver ions to nanoparticles. Several studies reported the use of FT-IR in evaluating the functional groups present in the plant material that aided in the biosynthesizes Ag-*C. albidum*-NPs and in identifying possible biomolecules responsible for some of the biological activities obtained [19, 20]. According to Tavan et al. [21], FTIR was used in identifying the functional groups of *P. frutescens* extract and Pf-AgNPs.



(**a**) (**b**)

Figure 3. (a) FT-IR Spectra of AgNPs,(b) FT-IR Spectra of Ag-*C. albidum*-NPs.

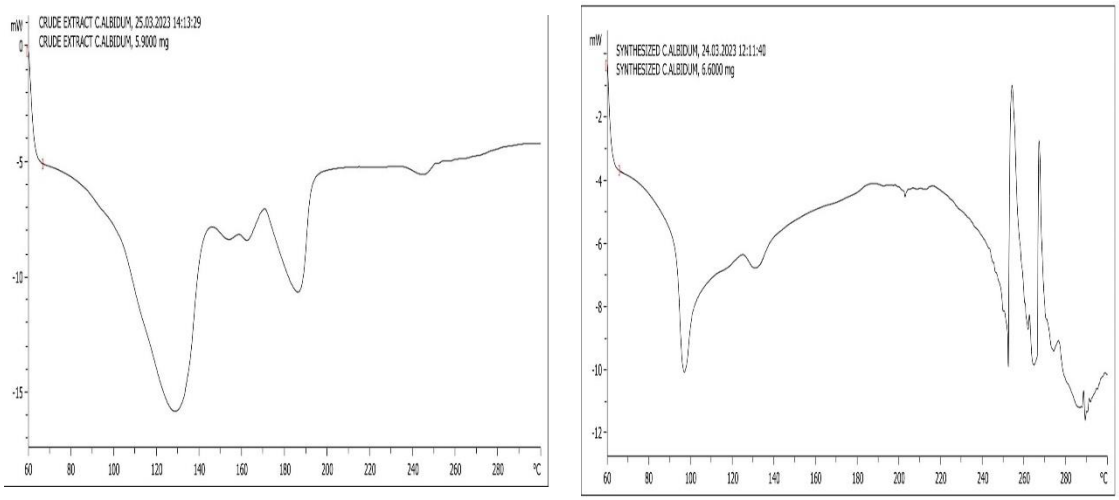
Dynamic light scattering (DLS) is used in measuring the size of the synthesized particle. The AgNPs gave peak diameter of 54.15 nm and Ag-*C. albidum*-NPs had modal peaks at 64.75 nm respectively as shown in Fig. 4a, b. DLS technique was described as a means of measuring the diameter of nanoparticles in suspension [21, 19]. They reported average diameter of 28.3 to 161 nm, with a Polydispersity Index of 0.72 [21]. The results of DLS agreed with reports of Omeje et al. [20] and Tavan et al. [21].



(**a**) (**b**)

Figure 4. (a) DLS of Spectra of AgNPs, (b) DLS Spectra Synthesized Ag-*C. albidum*-NPs.

The thermostability of the AgNPs and Ag-*C. albidum*-NPs was also evaluated using Differential scanning calorimetry (DSC). There was observation of that Ag-nanoparticle was releasing energy, with melting temperature of 129.8 and 188.6 °C, while Ag-C. albidum-NPs undergo exothermic reaction at 97.6 °C, with subsequent heating gave rise to endothermic activity at 257.4 and 270.1°C respectively (Fig. 5a, b).



(**a**) (**b**)

Figure 5. a): DSC thermogram of the AgNPs (b) DSC thermogram Ag-C. albidum-NPs.

There was *in vitro* inhibitory assay of α-amylase with both crude and Ag-*C. albidum*-NPs as shown in Fig. 6. The crude extract gave more than 50% inhibition of alpha amylase was obtained at high concentrations of 60 (53.22±1.5 %) and 80 mg/mL (56.62±0.4 %) respectively. Also, Ag-C. albidum-NPs gave 56.03±0.45 % inhibition of alpha amylase at 40 mg/ml and 53.24±0.06 % inhibition of alpha amylase at 80 mg/ml concentration of Ag-*C. albidum*-NPs (Fig. 6). At 20 and 100 mg/ml of both products, less than 50% inhibition of the enzyme respectively. The potential of crude and Ag-*C. albidum*-NPs to inhibit α-glucosidase was also studied and reported in Fig. 7. The rate of inhibition increased proportionally with increase in concentration of the extracts. At 60 mg/mL of the crude extract, more than 50% inhibition of the enzyme was reported, with a steady decline as the concentration increased (80 - 100 mg/mL). Similarly, highest percentage inhibition (47.22±0.4%) was obtained at 60mg/mL for Ag-*C. albidum*-NPs. The Ag-*C. albidum*-NPs could not achieve 50% inhibition of the enzyme at all concentrations studied (Fig. 7).

Also, the in vitro bioactivity of crude and Ag-NPs of *C. albidum* products were evaluated using acetylcholinesterase and butyrylcholinesterase. Various degrees of enzyme inhibition were obtained as shown in Figs. 8 and 9. The percentage inhibition of acetylcholinesterase of the crude leaf extract was 39.58±0.9, 55.15±0.03 and 79.82±0.83 % at 20, 40 and 60 mg/mL. Subsequent increase of concentration (80 - 100 mg/mL) gave AchE percentage inhibition of 66.56±0.22 and 63.32±0.70 respectively. Furthermore, the inhibitory capacity of the crude and Ag-*C. albidum*-NPs were evaluated on Butyrylcholinesterase, one of the enzymes responsible for stabilizing the nervous system and the results are shown in Fig. 9. There was >50% of butylrylcholinesterase activity inhibition at all the concentrations studied for both crude and NPs complex produced. Though, a concentration dependent increase in inhibition was observed from 20 – 60 mg/mL. High concentration (80-100 mg/mL) showed reduction in inhibition of the enzyme (Fig. 9). On the other hand, Ag-*C. albidum*-NPs gave consistent increase of inhibition with the concomitant increase in extract concentrations. According to Omeje et al. [23], the inhibition of some physiological enzymes involved in the aetiology of some chronic diseases (diabetes and Alzheimer's disease) are effective for their management.

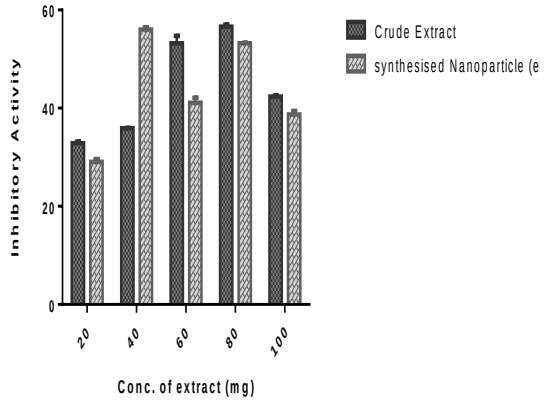


Figure 6. Amylase Inhibition by crude extract and Ag-*C. albidum*-NPs.

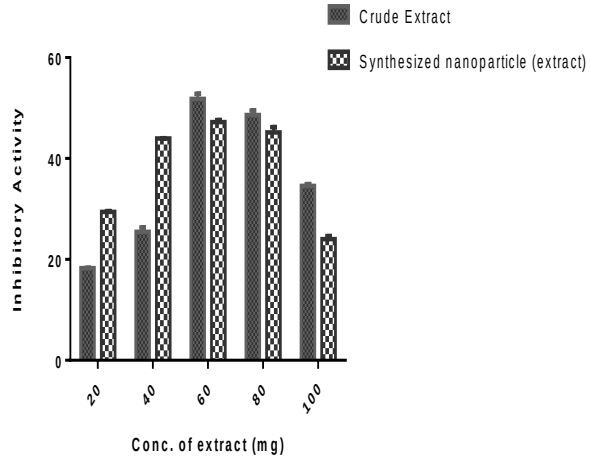


Figure 7. Glucosidase Inhibition by crude extract and Ag-*C. albidum*-NPs.

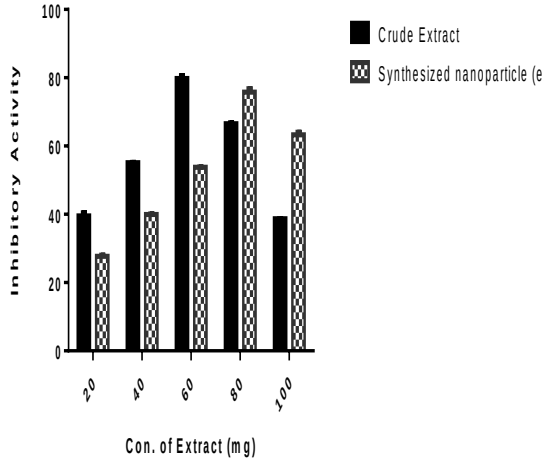


Figure 8. Inhibition of AchE by crude extract and Ag-C. albidum-NPs.

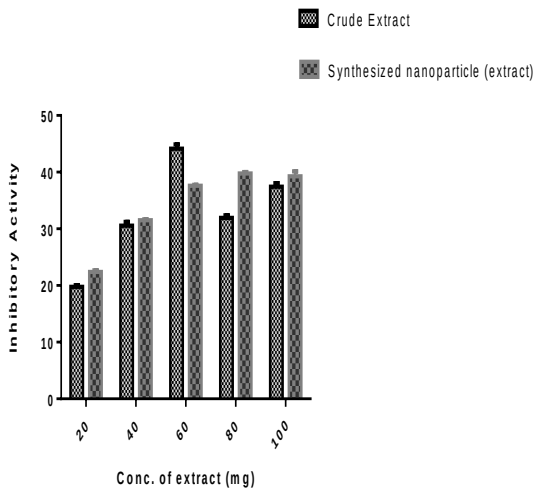


Figure 9. Inhibition of BuchE by crude extract and Ag-*C. albidum*-NPs.

Antibacterial potential of Ag-*C. albidum*-NPs were evaluated on some common bacteria pathogens such as *K. pnuemoniae, E. coli,* and *S. aureus* using graded concentrations of 6.5, 12.5, 25 and 50 mg and their zone of inhibitions reported. At 6.5 mg, there was 0.83 and 1.09 inhibition of *K. pnuemoniae* and *S. aureus* respectively. Subsequent increase of the extract concentrations to 12.5 mg gave 1.21, 3.06 and 3.27-mm zone of inhibition on *K. pneumonia*, *E. coli,* and *S. aureus* respectively. Also, a progressive increase in the zone of inhibition was obtained as the concentration of the extract increase from 25 to 50 mg/mL across all organisms studied as shown in Table 1. 50mg/ml gave the highest zone of inhibition among all organisms (*K. pneumonia*, *E. coli,* and *S. aureus*) studied as 9.88, 11.73 and 19.03 mm respectively. Though, significant difference in the zone of inhibitions was obtained for the extract when compared to a standard antibiotic (Streptomycin) (Table 1).

The power of medicinal material to scavenge free radicals in the cell is an essential pharmacological property. Thus, the *in vitro* ability of Ag-*C. albidum*-NPs to mop DPPH generated free radicals was evaluated. The radical scavenging potential of Ag-*C. albidum*-NPs increased concomitantly with increase in concentration (20-80mg/ml), except at 100mg when a significant decrease in the scavenging power was obtained. The report of this study is comparable to the study of NPs on *S. aureus*, and *E. coli* [27] and Pf-AgNPs was reported to exert antimicrobial power against *S. aureus, Candida albicans* and *E. coli* [21].

Table 1. Antibacterial Activity of Ag-*C. albidum*-NPs.

Conc. (mg)	*Klebsiella pneumoniae*	*Escherichia coli*	*Staphylococcus aureus*
6.5	0.83	-	1.09
12.5	1.21	3.06	3.27
25	6.92	19.41	21.75
50	9.88	11.73	19.03
Streptomycin	27.97	37.30	38.58

There was an increase in the scavenging potential of Ag-*C. albidum*-NPs and the standard antioxidant molecule (Vit. C.) at all concentrations studied (Table 2). Furthermore, the hydroxyl radical (OH*) scavenging ability of Ag-*C. albidum*-NPs was obtained to be increasing corresponding to increase in concentration, with 60 mg giving the highest desirable activity. Higher concentrations of 80 and 100 mg yielded lower inhibition, when compared to the standard (Vit. C) and 60 mg concentration. The inhibition of OH* by the compound was significantly low when compared to Ascorbic acid inhibition (81.53 %). Similarly, NO* scavenging power of Ag-*C. albidum*-NPs was studied at varying (20-100 mg/mL) concentrations respectively. None of the concentrations gave 50 % inhibition of

the radicals produced by nitroprusside as shown in Table 2. Though, 60 mg/mL also gave the highest inhibition of the radical (48.22±1.57 %). The varying concentrations scavenging power is significantly lower when compared to the 86.82±0.41 % inhibition obtained for Vit C. Table 2 shows the ferric reducing power of the Ag-*C. albidum*-NPs studied, a concentration dependent increase of electron transfer rate inhibition by the compound was obtained (20-60 mg), further increase in the concentration of the compound (80-100mg) led to decrease in the FRAP scavenging power of the compound (Table 2). Antioxidant's ability of medicinal plants are attributed plant rich in phytochemicals such as polyphenols and flavonoids [22]. Several plants have been reported to have radical scavenging powers [23, 21]. The radical scavenging power of NPs could be attributed to the presence of some compounds in *C. albidum* used for the synthesis. Similarly, Omeje et al. [23] also attributed antioxidant power of plant materials to the presence of tannins. This supports the earlier assertion of Deng et al. [24] on tannins and Patel et al. [25] on alkaloids, with flavonoids been described as a potent antioxidant agent [26].

Table 2. Antioxidant potentials of silver *C. albidum*-Nanoparticles.

Antioxidant Potentials	20 mg/mL	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL	Vit.C mg/mL)
DPPH (%)	20.11*a±0.01	30.10*b±1.00	42.71*c±1.99	42.06*c±2.60	39.88*c±0.10	73.20*±0.93
OH*Scavenging ability (%)	9.84*a±0.20	22.97*b±0.09	55.83*c±0.19	50.91*c±0.26	46.15*d±0.21	81.53*±0.86
NO*Scavenging ability (%)	19.93*a±1.30	27.08*b±0.77	48.22*c±1.57	47.80*c±0.90	43.83*c±0.11	86.82*±0.41
FRAP (%)	22.84*a±1.49	35.20*b±1.07	50.48*c±0.32	39.10*b±0.19	47.94*d±0.50	78.90*±0.88

Key: Data are triplicate results (mean ± standard error); data with (*) are statistically significant ($p < 0.0$5) to Vit. C (Standard); values with different lowercase are statistically significant ($p < 0.05$) across Concentrations.

Conclusively, green synthesis method was used to develop a commonly used antibiotic medicinal plant, (*Chrysophyllum albidum*) into silver nanoparticle (AgNPs) and characterized. From the results obtained in this study, *C. albidum*-Ag-NPs produced had good stability under ambient conditions for six (6) weeks, and the compound possessed free radical scavenging power, antibacterial properties and showed ability to inhibit alpha amylase, glucosidase, which have been implicated in diabetes aetiology. Also, *C. albidum*-Ag-NPs exhibited anti cholinesterases activities suggesting it could be appropriate in managing some neurological disease orders such as Alzheimer's disease. Overall, the study contributes to improving the concentration of active *C. albidum*-Ag-NPs with antibiotic, antioxidant and anticholinesterase medicinal plant that could be delivered to the site of action at higher concentration with prolonged shelf life of the material.

Authors contributions: Conceptualization, K.O.O., S.O.O.E., and F.N.N.; methodology, K.O.O., B.O.E. and F.N.N.; software, K.O.O., and B.O.E.; formal analysis, K.O.O. and B.O.E.; investigation, K.O.O., B.O.E., F.N.N. and S.O.O.E.; resources, K.O.O., B.O.E.; data curation, K.O.O., B.O.E., F.N.N. and S.O.O.E.; validation, K.O.O., B.O.E., F.N.N. and S.O.O.E.; visualization, K.O.O., B.O.E. and F.N.N.; supervision, K.O.O., B.O.E., F.N.N. and S.O.O.E; writing—original draft preparation, K.O.O., B.O.E., F.N.N. and S.O.O.E.; writing—review and editing, K.O.O., B.O.E., F.N.N. and S.O.O.E. All authors have read and agreed to the published version of the manuscript.

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