Characterization and antioxidant potential of silver nanoparticles (AgNPs) synthesized using the aqueous leaf extract of *Calotropisprocera*: An *In vitro* study

ABSTRACT

Plant mediated synthesis of nanoparticles have been considered as green route and a reliable technique for the synthesis of nanoparticles due to its eco-friendly approach. This research evaluated the characterization and antioxidant potential of silver nanoparticles (AgNPs) synthesized using the aqueous leaf extract of *Calotropisprocera.* The silver nanoparticles were synthesized using different concentrations of the extract ranging from (1-5mg/ml) with 10ml silver nitrate solution. The synthesis of silver nanoparticles was confirmed by UV-Vis spectrophotometer. The silver nanoparticles was characterized by Fourier Transform Infra-Red spectroscopy. The antioxidant activity of the synthesized silver nanoparticles was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Potential (FRAP), Reducing power and ABTS Assays. FTIR revealed the biological macromolecules of *Calotropisprocera* aqueous leaf extract involved in the synthesis and stabilization of AgNPs. UV-Visible spectrophotometer showed absorbance peak in the range of 436-446 nm. The synthesized AgNPs significantly exhibited increased free radical scavenging activity although lesser when compared with vitamin C and Butylated Hydroxyl Toluene (BHT) which were used as standards. In conclusion, the synthesized silver nanoparticles using *Calotropisprocera* aqueous extract possess antioxidant activity due to the presence of bioactive molecules on the surface of the silver nanoparticles which could be useful in various bio-applications such as cosmetics, food, and biomedical industry.

Keywords: *Calotropisprocera*, Silver nanoparticles, Antioxidant, FTIR

INTRODUCTION

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology, that deals with the synthesis, manipulation and use of particles ranging in size 1 to 100 nm(Kannan et al., 2010). Such particles are called nanoparticles(NP) their unique

physical, chemical and biological properties could be attributed to their small sizes and large surface area(Nanda and Saravanan, 2009). The synthesis of noble metal nanoparticles attracts an increasing interest due to their new and different characteristics as compared with those of macroscopic phase, that allow attractive applications in various fields such as antimicrobials(Priyadarshini et al., 2013), medicine, biotechnology, microelectronics, catalysis, information storage and energy conversion(Yeo et al., 2003). Various literatures describe many ways to synthesize nanoparticles which include physical, chemical, and biological method.Thephysical and chemical methods used for the synthesis of nanoparticles are not only energy consuming but also non eco-friendly due to the use of hazardous solvents and stringent techniques(Guozhong, 2004).

Calotropisprocera(family: Asclepiadaceae) (*C. Procera*) commonly known as milkweed or giant weed is a cultivable wild xerophytic shrub with important medicinal properties , it is found across Africa, Asia and South America, it is commonly grown in wastelands and roadside area(Iqbal et al., 2005). There is a number of species of *Calotropis*but most commonly available species include *C. sussuela,C. gigantean (Linn),* and *C. Procera*(Lorenzi and Matos, 2002).*C. Procera* can withstand drought, salt tolerant and it disperse seeds through wind and animals. It was identified as a weed along roadsides and overgrazed native pastures. It produces milky white latex which possess various curative properties(Magalhães et al., 2010).The medicinal potential of *Calotropisprocera* has been known to traditional systems of medicine for a while now with its leaves being widely used. The use of the plants, plant extracts, and pure compounds isolated from natural sources has always provided a foundation for modern pharmaceutical compounds. *Calotropisprocera* is a well-known plant and has been traditionally used for diarrhoea, stomatic, sinus fistula, and skin disease, and the leaf part is used to treat jaundice (Yogesh et al., 2010).

Owing to the growing need to reduce or eliminate the use of environmental-toxic substances, as the biogenic principles describe, the synthesis of nanoparticles using biological entities has received increasing attention in the last decade(Anastas and Kirchhoff, 2002).The biosynthetic procedures involve using microorganisms and plants . Among biological methods, the use of plant extract is the best, eco-friendly, cheaper and relatively fast as compared to microbe assisted synthesis(Mittal et al., 2013). Plant extracts have been found to be useful in the synthesis of metal nanoparticles as they possess phyto-components like polyphenols, alkaloids, flavonoids, fatty acids and proteins which act as reducing and capping agents(Saxena et al., 2012).The synthesis of noble metal nanoparticleshave attracted enormous attention due to their new and unique characteristics and their wide range of applications in various fields such as medicine, biotechnology, optics, catalysis (Gopinath et al., 2012). Among Noble metal Nanoparticles, Silver Nanoparticles (Ag NPs) are known for their versatile applications in biomedical field like antioxidant, antibacterial, anticancer activities(Percival et al., 2007).

The medicinal features of plants have been investigated, in view of new scientific advancements, all over the world due to their active pharmacological activities and economic feasibility. Antioxidant compounds possess anti-bacterial, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-inflammatory or antiviral activities to a greater or lesser extent(Pattnaik et al., 2017). Antioxidant compounds are found to be beneficial in management of diabetes and other disorders so the attention has been focused to natural products with antioxidant activities.The aimof this study was to synthesize and characterize

silver Nanoparticles using *Calotropisprocera*aqueous extract and screening of its Antioxidant potential.

METHODS

COLLECTIONS OF PLANTS MATERIAL.

The leaves of *Calotropisprocera* were collected from an area around Lasu main campus ojo, Lagos State; the plant wasauthenticated by the Department of Botany Lagos State University.

PREPARATION OF PLANTS EXTRACT

Fresh and healthy leaves of *Calotropisprocera* were collected, washed thoroughly with tap water first and then with distilled water until no impurities remained. Then the fresh leaves were incised into small pieces. 60g was weighed and transferred intoa beaker containing 300mL of deionized water .The mixture was heated for 1hr at 60^{o}C while stirring occasionally and then allow to coolat room temperature .The mixture was filtered using the Whatman filter paper and then centrifuged at 3000rpm for 15mins.The extract was stored in the refrigerator at 4^{o}C for further use to synthesize silver (Ag) nanoparticles (Awwad and Salem 2012).

SYNTHESIS OF SILVER NANOPARTICLES

100mL (0.001M) aqueous solution of silver nitrate was prepared in Erlenmeyer flask. Then 1.0, 2.0, 3.0, 4.0 and 5.0 mL of leaf extract were added separately to 10mL aqueous silver nitrate solution kept in separate test tubes at room temperature. The solution was kept in dark chamber until solution colour changes to yellow to dark yellow. After, 15 min, the solution turns yellow to yellow-red or dark brown indicating the formation of silver nanoparticles. The bio-reduction of silver ions was monitored by checking the absorbance of the samples between 300 and 700nm with a UV-vis spectrophotometer.

ANTIOXIDANT EVALUATION.

DPPH RADICAL SCAVENGING ACTIVITY.

The Antioxidant potential of the synthesized silver nanoparticles was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl(DPPH) free radical. DPPH (24 mg) was dissolved in 100 ml methanol (stock solution). In a test tube, 3 mL DPPH solution was mixed with 100 µL nanoparticle solution, different concentrations of the nanoparticles solution was used (1–5mg/ml) . The absorbance was measured at 517 nm for a period of 30 min. The percent antioxidant or radical scavenging activity was calculated using the following formula: %Antioxidant activity = [(Ac − As)/Ac] × 100 where, Ac and As are the absorbance of control and sample, respectively. The control contained 100 µL silver nitrate in place of the nanoparticles solution(Shah et al., 2013).

FERRIC REDUCING ANTIOXIDANT POTENTIAL (FRAP)

FRAP reagent was prepared by mixing in 25 mL acetate buffer (30 mM; pH 3.6), 2.5 mL TPTZ solution (10 mM) and 2.5 mL ferric chloride solution (20 mM). The mixture was incubated for 15 min at 37 °C before use. Ascorbic acid (vitamin C) was employed as a standard in this assay, and its calibration curve was obtained by using its concentrations ranging from 50 mg/L to 500 mg/L in water. To 2.85 mL FRAP reagent in a test tube, 150 µL nanoparticles solution was added, different concentrations of nanoparticles solution(1-

5mg/mL) were used. The mixture was incubated for 30 min in the dark, and its absorbance was measured at 593 nm. The blank contained an equal volume of methanol instead of the plant sample. The results were reported as µg of ascorbic acid equivalents (AAE) per mL (Huang et al., 2005).

ABTS$^{\bullet+}$ DECOLORIZATION ASSAY

The working solution of ABTS•+ radical was made by reacting ABTS (9.5 mL, 7 mM) with potassium persulfate (245 µL, 100 mM), and raising the volume to 10 mL with distilled water. The solution was kept in the dark at room temperature for 18 h, and then diluted with potassium phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 (±0.02) at 734 nm(Bursal and Gülçin, 2011). A sample (10 µL) was placed in a test tube and mixed thoroughly with 2.99 mL ABTS radical working solution, different concentrations of nanoparticles solutions(1-5mg/ml) were used. Absorbance of the resulting clear mixture was recorded at 734 nm. The percent antioxidant activity of the sample was determined using the following formula:
%Antioxidant activity = [(Ac − As)/Ac] × 100 where Ac and As are the absorbance of the control and sample, respectively. The control was prepared by adding 10 µL of silver nitrate solution in place of the sample.

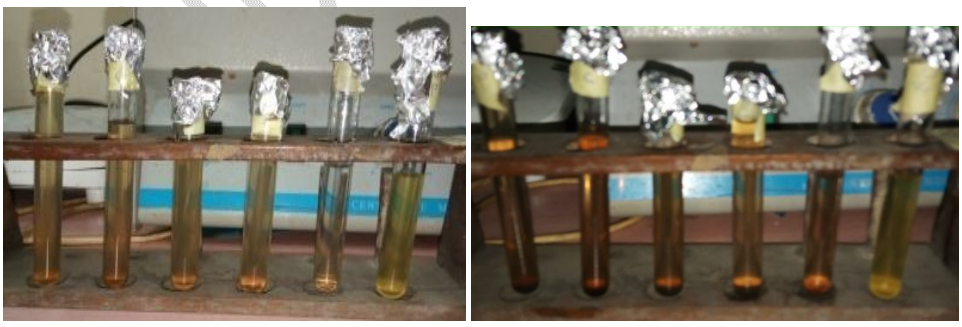
STATISTICAL ANALYSIS

Data obtained were subjected to statistical analysis. Spectra data were plotted using OriginPro v.9.1 software while GraphPad Prism v6 and MS Excel were used for other plots. For comparisons between samples, data was analyzed by one way ANOVA and Tukey's multiple comparison test was employed to test for significant differences. The results were considered significant at $p<0.05$.

RESULTS

VISUAL OBSERVATIONS

This results shows the synthesis of silver nanoparticles using *Calotropisprocera*extract, the synthesis was confirmed using the UV-vis spectrophotometer and was characterized using FTIR.



(a) (b)

Figure 1: Colour change observed after (a) 15 to 30mins of synthesis (b) 3hrs of the synthesis

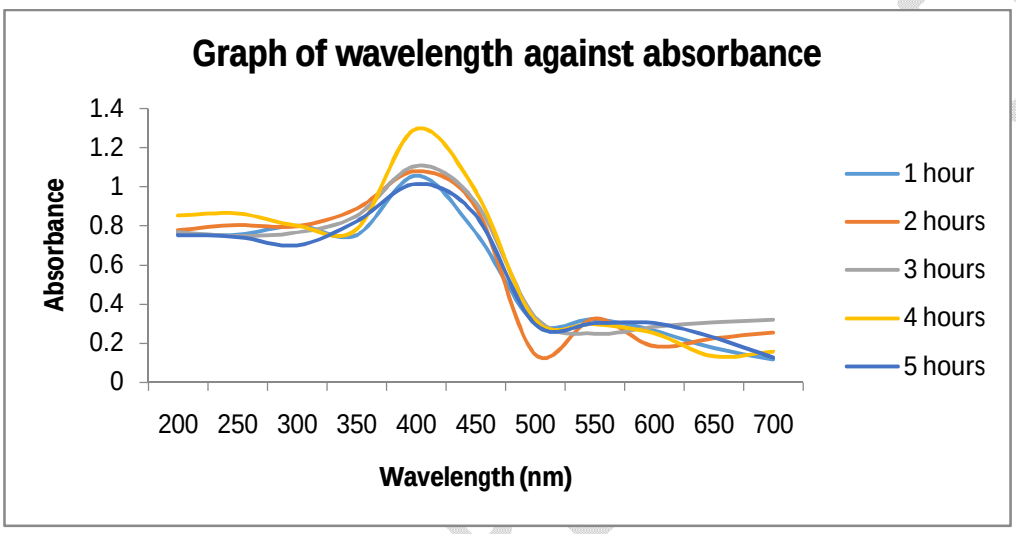
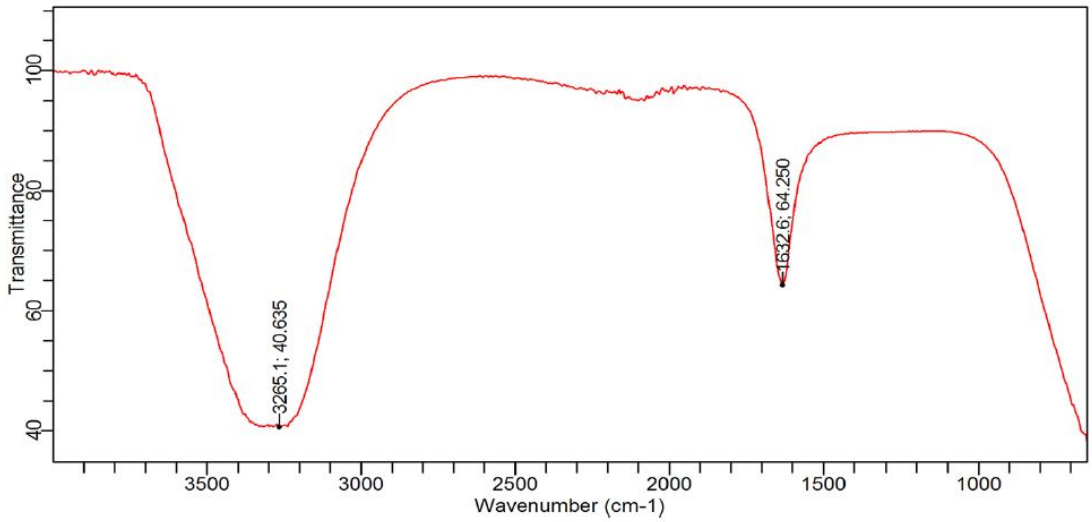


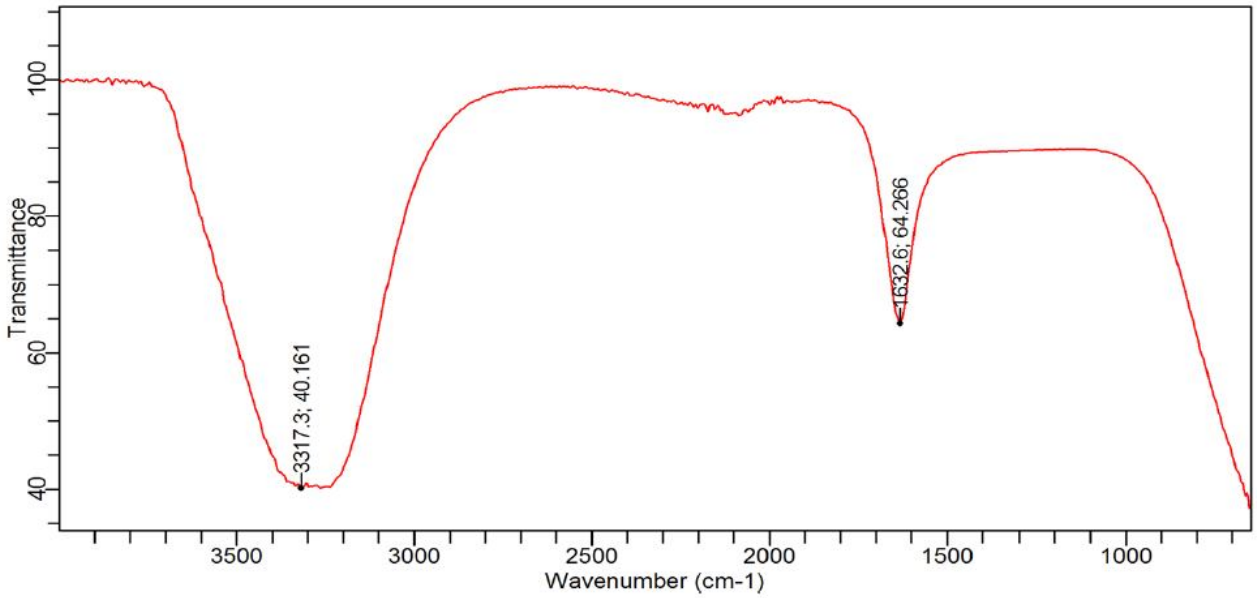
Figure 2: UV-Vis absorption spectra of obtained silver nanoparticles at different time intervals using *Calotropisprocera* aqueous leaf extract as reduction agent.

FTIR analysis

The aqueous leaf extract of *Calotropisprocera*played a dual role as a reducing and capping agent in the synthesis of AgNPs. This was due to the presence of some functional groups which was confirmed by the FTIR analysis of both the aqueous leaf extract and that of the synthesized silver nanoparticles in (Fig. 3 a and Fig 3b) respectively.



(a)



(b)

Fig 3: Fourier transform infrared (FTIR) spectra of (a) *Calotropisprocera* extract (b) AgNP synthesized using *Calotropisprocera* extract.

The interpretation of various functional groups at different bands of the FTIR spectrum for both (Fig.3a &b) is summarized in (Table 1) below. The bioactive constituents of *Calotropisprocera*are flavonoids, phenols, ascorbic acid, with moderate amounts of lycopene and β-carotene(Naser et al., 2019). The reduction and capping ability of

*Calotropisprocera*may be due to the presence of phenols in its aqueous leaf extract. Phenolics are strong antioxidants with high reducing capacity. Phenolic content in *Calotropisprocera*leaf extract enabled the reduction of silver ions to nanoscale-sized silver particles due to the electron donating ability of the phenolic compounds (David et al., 2014). From the FTIR results, it could be concluded that some of the bioorganic compounds from *Calotropisprocera*extract formed a strong coating/capping on the nanoparticles.

Table 1. FTIR spectrum interpretation of various functional groups present in the leaf extract of *Calotropisprocera*

S/No	Wavenumber (cm^{-1})	Compound class	Groups
1	3265.1	Alcohol	O-H stretching
		Carboxylic acid	O-H stretching
2	1632.6	Alkene	C=C stretching
		Conjugated alkene	C=C stretching
		Amine	N-H bending
		Cyclic alkene	C=C stretching
3	3317.3	Alcohol	O-H stretching
		Aliphatic primary amine	N-H stretching
		Aliphatic secondary amine	N-H stretching
		Alkyne	C-H stretching

The proposed mechanism for the reduction of Ag^+ by plants' phenolic compounds is explained by (David et al., 2014). Briefly, Ag^+ ions can form intermediate complexes with phenolic OH groups present in phenols (e.g., gallic acid) which subsequently undergo oxidation to quinone form with consequent reduction of Ag^+ to AgNPs. Also, the quinoid compound produced due to the oxidation of the phenol group in phenolics can be adsorbed on the surface of nanoparticles, accounting for their suspension stabilization.

Antioxidant assay

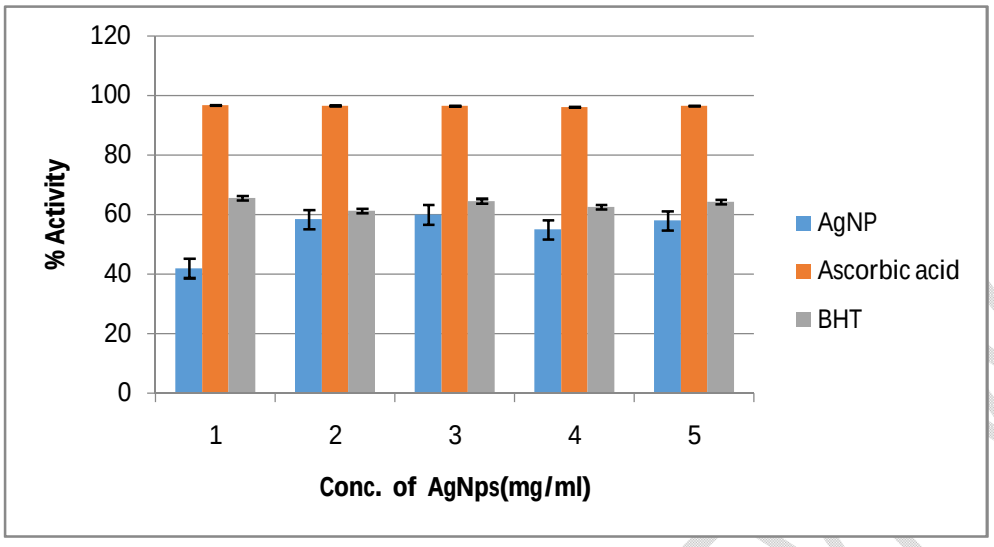


Figure 4: DPPH radical scavenging activity of AgNPs synthesized by *Calotropisprocera* extract

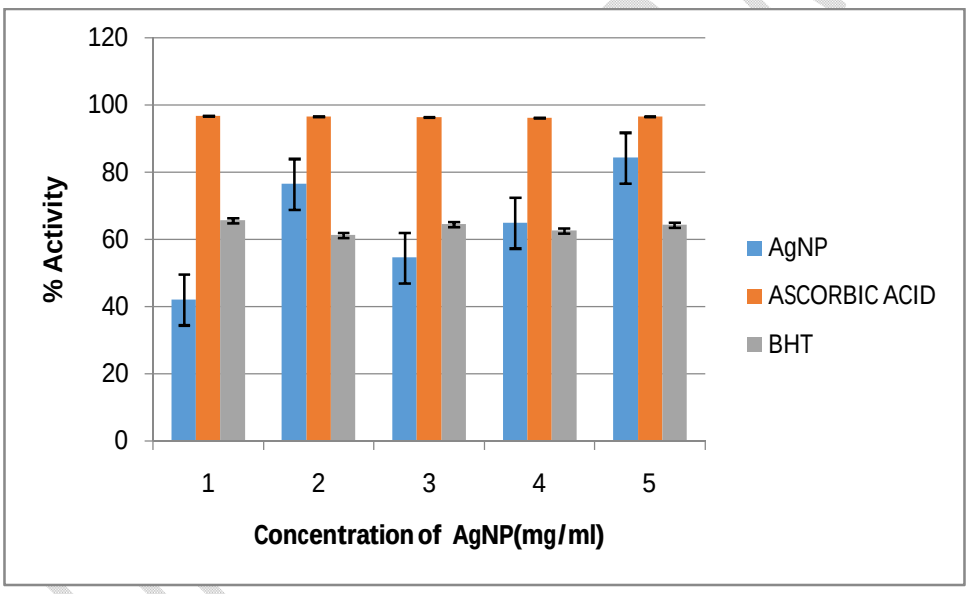


Figure 5: ABTS•+ radical scavenging activity of AgNPs synthesized using *Calotropisprocera* extract compared against standards (BHT and Ascorbic acid).

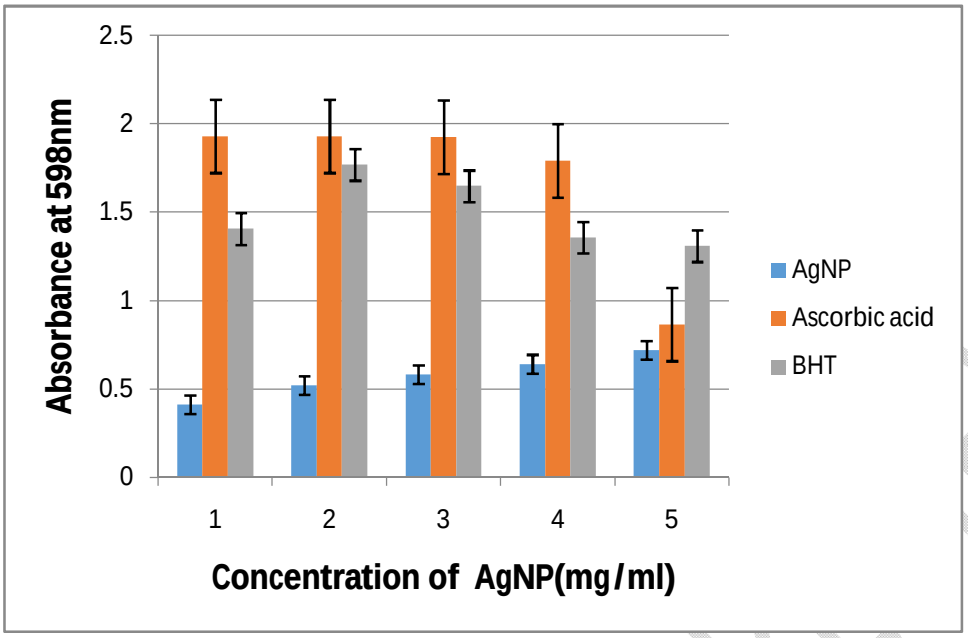


Figure 6: Ferric reducing antioxidant potential (FRAP) values of different concentration of silver nanoparticles compared against standards (BHT and Ascorbic acid).

DISCUSSION

The synthesis of nanoparticles was initiated once the plant extract of *Calotropisprocera*was introduced into 0.001M aqueous AgNO3 solution. Silver nanoparticles were synthesized at different concentrations of leaf extract (1, 2, 3, 4 and 5 mg/ml). The colour of the reaction mixture changed from pale-yellow then light brown to dark brown exponentially with reaction-time as aggregation proceeds which indicated the formation of silver nanoparticles (this was also confirmed using UV spectrophotometer. Appearance of different colours at different time intervals could indicate that the morphology (shape, size and the size distribution) of silver nanoparticles alters with the reaction time.

Ag nanoparticles synthesized in each extract solution was confirmed using UV-Vis spectroscopy method. This was done to determine the characteristics of the peak spectrum of the Ag nanoparticle wavelength prepared for each different concentrations of plant extract. The characteristics of Ag nanoparticles normally appear at a wavelength interval of 400–500 nm. UV-Vis spectra of Ag nanoparticles synthesized using the *Calotropisprocera* aqueous extract showed a maximum absorbance between 400-500nm, with 2mg/ml having the highest absorption maximum at 450nm. This shows that the Ag nanoparticles have formed in the extract, where the Ag^{+} has been reduced to Ag^{0}. Proteins and all secondary metabolites of extract play a critical role in both the reducing and capping mechanism for nanoparticle formation(Marslin et al., 2018).

Fourier transform infrared spectroscopy (FTIR) analysis was further used to identify the biomolecules from the plant extract responsible for the reduction of silver ions and for the efficient stabilization of the silver nanoparticles(David et al., 2014). FTIR analysis confirmed the dual role of the Plant extract as reducing and capping agent. The FTIR spectra of the plant extract and the synthesized AgNPhad similar absorption spectra, the bands appeared at 3321.1 and 1636.3 which indicates the presence of O-H and C=C stretching vibration respectively .

The synthesized AgNPs and the standard antioxidants promoted inhibition of DPPH radical in (Fig. 4), although the synthesized AgNPs exhibited its antioxidant activity with increasing concentrations. However, the percentage inhibition of the DPPH radical by the standards (Ascorbic acid and BHT) were higher than that of the synthesized AgNPs. This result is similar to the work of (Kelechi et al., 2019), where the standard antioxidant (BHA) exhibited a higher DPPH radical inhibition activity than that of the aqueous extracts of*Calotropisprocera.*

The synthesized AgNPs exhibited a dose dependent ABTS radical scavenging property in (Fig. 5). Although, not as effective as that of the standard antioxidants. The antioxidant behavior of *Calotropisprocera*AgNPs justifies their potential applications in the therapy of many diseases caused by inflammation and oxidative stress. The antioxidant mechanism behind the AgNPs of *Calotropisprocera*could be that during the AgNPs synthesis, numerous *Calotropisprocera*bioconstituents could be adsorbed onto the active surfaces of *Calotropisprocera*AgNPs, which increases its surface area, and thereby interact and scavenge these free radicals efficiently (Saratale et al., 2017). It could be presumed that the synthesized AgNPs has antioxidant potential. The antioxidant potential could be due to the presence of flavonoids, phenols, and carotenoids which are known antioxidants(Chidi et al., 2020) present in the aqueous leave extract.

The reducing potential of the synthesized AgNPs was evaluated by determining its ability to reduce Fe^{3+} ion because the reducing power is a measure of the electron-donating capacity of its bioactive compounds and may serve as a significant indicator of its antioxidant activity(Elekofehinti et al., 2013). The results for the ferric reducing activity of *Calotropisprocera*leaf compared to Ascorbic acid and BHT used as standards are reported in (Fig. 6). The synthesized AgNPs exhibited a dose-dependent reducing power potential which shows that they are capable of donating an electron and this increased with an increase in concentration when compared to the standards, although not as effective as the standards. This result contradicts the report made earlier by (Kelechi et al., 2019, Chidi et al., 2020), where the aqueous extract of *Calotropisprocera*exhibited a higher ferric reducing potential compared to the standard antioxidant (BHA). This result suggests the presence of reductants in the plant extracts. The increase is as a result of the reduction of Fe^{3+} to Fe^{2+}.

CONCLUSION

In conclusion, the effectiveness of using *Calotropisprocera*extract as a good source for the synthesis of AgNPs. Based on the obtained data, the phytosynthesizedAgNPs displays antioxidant activity *in vitro*. Further studies are needed to confirm these properties of the synthesized AgNPs using *Calotropisprocera*extract *in vivo*

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