

## **Green Synthesis, Characterization and *In-vitro* Antioxidant Property of Silver Nanoparticles Using the Aqueous Leaf Extract of *Justicia carnea***

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors GAA and JOI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IOS and MOO managed the analyses of the study. Authors SAA, SAJ, MDA, JDA and GAA managed the literature searches. All authors read and approved the final manuscript.

### **Article Information**

DOI: 10.9734/AJBGMB/2021/v7i330175

#### Editor(s):

(1) Dr. S. Prabhu, Sri Venkateswara College of Engineering, India.

#### Reviewers:

(1) Fateme Aboee, Islamic Azad University, Iran.

(2) Parhat Rozi, University of Chinese Academy of Science, China.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67240>

**Original Research Article**

**Received 02 February 2021**

**Accepted 08 April 2021**

**Published 13 April 2021**

### **ABSTRACT**

This study investigated the synthesis, characterization and *in vitro* antioxidant activity of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Justicia carnea*. The aqueous leaf extract of *J. carnea* was used as a potential reducing and capping agent. To identify the compounds responsible for the reduction of silver ions, the functional groups present in the plant extract were subjected to FTIR. The *in vitro* antioxidant activity of synthesized nanoparticles was evaluated in terms of ferric reducing antioxidant potential (FRAP), DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) free radicals scavenging assays. The surface plasmon resonance confirmed the formation of AgNPs with maximum absorbance at  $\lambda_{max} = 446$  nm. FTIR revealed the biological macromolecules of *J. carnea* leaf extract involved in the synthesis and stabilization of AgNPs. UV-Visible spectrophotometer showed absorbance peak in the range of 436-446 nm. The silver nanoparticles exhibited moderate antioxidant activities

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compared to standard antioxidants (ascorbic acid and BHT). These results confirmed this protocol as simple, eco-friendly, nontoxic and an alternative for conventional physical and chemical methods. It can be concluded that *J. carnea* leaf extract can be used effectively in the production of potential antioxidant AgNPs which could be useful in various bio-applications such as cosmetics, food and biomedical industry.

**Keywords:** *J. carnea*; reducing agent; capping agent; silver nanoparticles; nontoxic synthesis; FTIR; antioxidant.

## 1. INTRODUCTION

Nanoparticles (NPs) are a variety of particulate substances with a dimension less than 100 nm at least (1–100 nm) in the nanometer scale range or whose basic unit in the three-dimensional space is in this range. Depending on the overall shape, NPs can be 0D, 1D, 2D or 3D. The word 'nano' denotes one billionth of a meter or  $10^{-9}$  hence the scale in nanometers. Nanotechnology deals with the understanding and manipulation of matter in the order of nanometers to produce materials with entirely new properties and functions [1-4].

Synthesize of NPs can be either of these two strategies, one is "Top-down" and the other one is "Bottom-up approach. In the former, the bulk materials are gradually broken down by techniques like, ball milling, laser ablation etc. to nanosized materials, while the bottom-up approach build up atoms or molecules to nanometer range materials or structures. Bottom-up approach is often considered for chemical and biological synthesis of nanoparticles [2,5,6]. Characteristics of NPs such as their size, morphology and stability are determined by the method of preparation, nature of solvent, concentration, strength of reducing agent and temperature utilized during their synthesis [6].

In the synthesis of NPs, the size and shape of the desired nanoparticles could be obtained through physical (mechanical attrition) and chemical (sodium borohydride,  $\text{NaBH}_4$ ) methods in which chemical reductants and toxic chemicals are used as stabilizers during the synthesis. The use of these toxic chemicals on the surface of nanoparticles and non-polar solvents in the synthesis protocol decreases their biological and clinical applications [7]. Although, using chemical and physical methods may successfully yield pure and well-defined nanoparticles, but these methods are quite costly and potentially dangerous to the environment when not properly disposed [8,9].

With the growing demand for the development of clean, non-toxic, biocompatible and eco-friendly synthesis methods for synthesizing nanoparticles. The biological methods are now being considered to be safe, energy saving, cost-effective, sustainable and environmentally friendly synthesis processes [10,11]. Biological methods of synthesizing nanoparticles involve the use of microbes, plants and plant materials and it is one of the recent branches developing in nanotechnology, interconnecting nanotechnology and biotechnology, termed as "Nanobiotechnology" [2]. Hence, green synthesis is an alternative method for preparing nanoparticles with the use of plants and plant materials, bacteria and yeast [11]. Also, the elimination of hazardous chemicals such as hydrazine favors green synthesis as an eco-friendly one [5].

Reports from different researchers indicated silver nanoparticles (AgNPs) to be one of the promising nanoparticles due to its distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity [5,12]. Different plants have been used in the green synthesis of silver nanoparticles. Green synthesis of AgNPs from green leaves extract of *Calotropis procera* was reported by [9]. *Eugenia jambolana* leaf extract was used to synthesize AgNPs that indicated the presence of other secondary metabolites [13]. Silver nanoparticles were synthesized using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity was reported by [14]. AgNPs were synthesized using leaves of *Rhynchoetechum ellipticum*, and the results indicated the presence of polyphenols, flavonoids, alkaloids, terpenoids, carbohydrates, and steroids [15]. *Hesperidin* was used to form AgNPs of 20-40 nm [16]. Silver nanoparticles were synthesized from Mulberry leaves extract and it showed effective antibacterial activity toward *Staphylococcus aureus* and *Shigella* sp. [10] etc.

*Justicia carnea* belongs to the *Acanthaceae* family, it is an ornamental plant with about 250

genera and 2500 species [17]. Its common names include Brazilian plume flower, flamingo flower and Jacobinia [18,19]. Different shades of the plant occur during summer and it ranges from white, pink, red, rose, magenta, orange, purple to coral/apricot [18]. The flowers appear tube-like in shape and curves outward from the spike. The smaller varieties grow about 2 feet high while others may grow to 6 feet tall by 6 feet wide [18]. In Nigeria, the leaves of *J. carnea* is often concocted with edible vegetables to make soup, boiled separately in water to make tea or prepared by cooking with other medicinal plants for therapeutic purposes [20]. It is a medicinal plant used widely in Nigeria reported to have diverse functions, including blood-boosting potential [21].



Fig. 1. Pictorial view of *Justicia carnea* [18]

*J. carnea* has been reported to exhibit therapeutic properties such as hypoglycemic [21], antidiabetic [22], antioxidant [20,23], antiplasmodial [17], and anti-anemic and alleviation of lipid profile [24,25]. There is no documented scientific and experimental evidence on the use of *Justicea carnea* leaves in the synthesis of silver nanoparticles. This study investigated the synthesis, characterization and *in-vitro* antioxidant activity of AgNPs from the aqueous leaf extract of *Justicia carnea*.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

All the reagents purchased were of analytical grade and used without any further purification. Silver nitrate ( $\text{AgNO}_3$ ) was purchased in Lagos with a  $\geq 99.5\%$  purity. Distilled water and deionized water were used for preparing aqueous solutions all through the experiments

### 2.2 Collection of Plant Material

Fresh leaves of *Justicia carnea* were collected within the surroundings of Lagos State University, Ojo Lagos, Nigeria. The leaves were identified and authenticated by the Department of Botany, Lagos State University.

### 2.3 Sample Preparation and Extraction

Shown in Fig. 1 is the typical picture of *J. carnea* plant. The green synthesis of silver nanoparticles was prepared with slight modification following the method reported in the literature [10]. The harvested fresh leaves of *Justicia carnea* were detached from the stems, sorted, and washed with tap water at first, and then their surfaces were washed with distilled water until no impurities remained. The fresh leaves were cut into small pieces, and 60g was weighed and put into a 500ml beaker with 300ml of distilled water. The mixture was heated for 1 hour at  $60^\circ\text{C}$  while stirring occasionally until the color of the aqueous solution changes from watery to light yellow. Then the extract was allowed to cool at room temperature, filtered using the Whatman 42 filter paper and then centrifuged at 3000rpm for 15 minutes to remove the heavy biomaterials. The extract was stored in the refrigerator at  $4^\circ\text{C}$  for further use to synthesize Ag nanoparticles from  $\text{AgNO}_3$  precursor solution.

### 2.4 Synthesis of Silver Nanoparticles

100 mL ( $10^{-3}$  mM) aqueous solution of silver nitrate was prepared in Erlenmeyer flask, by weighing 0.17g of  $\text{AgNO}_3$  in 1L of distilled water. 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 beakers at room temperature (their notation shown in Table 1). To prepare 10 ml of 3 mM aqueous silver nitrate solution, 0.005g of  $\text{AgNO}_3$  was dissolved in 10 ml of deionized water. The solution was kept in dark chamber until solution colour changes to yellow to dark yellow. After, 15 minutes, 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 periodic sampling using the UV spectrophotometer.

### 2.5 Characterization of Silver Nanoparticles

A colour change from pale yellow to dark brown upon incubation due to surface plasma

resonance (SPR) vibration was observed indicating the formation of nanoparticles. The periodic scans of the optical absorbance between 200 nm and 800 nm with a Shimadzu UV-Visible spectrophotometer (UV-1800, Japan) were performed to investigate the reduction of silver ions by the aqueous leaf extract. Deionized water was used as reference for background correction of the experiments. A Fourier transform infrared spectrometer (FTIR) (Bruker Tensor 37 spectrometer) was used to obtain the infrared spectra of absorption and emission of the formed silver nanoparticles. FTIR spectra were recorded from wave number 600-4000  $\text{cm}^{-1}$  [9].

**Table 1. Notation of silver nanoparticles synthesized using *Justicia carnea* extract [26]**

Sample	Plant extract (ml)	AgNO <sub>3</sub> solution (ml)
Control	10	-
1	1	10
2	2	10
3	3	10
4	4	10
5	5	10

## 2.6 Antioxidant Assay

### 2.6.1 Ferric reducing antioxidant potential (FRAP)

Ferric reducing antioxidant potential (FRAP) of the extracts of *J. carnea* was measured according to the method reported by [27,28]. 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 used 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  $\mu\text{L}$  plant sample (0.1 mg/mL, in methanol) or standard was added. 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  $\mu\text{g}$  of ascorbic acid equivalents (AAE) per mL.

### 2.6.2 DPPH free radical scavenging activity

The DPPH free radical scavenging activity of aqueous extracts of *J. carnea* was assayed

according to the method reported by [29], with slight modification by [30]. The stock solution of the radical was prepared by dissolving 24 mg DPPH in 100 mL methanol, and was kept in a refrigerator until further use. The working solution of the radical was prepared by diluting the DPPH stock solution with methanol to obtain an absorbance of about 0.98 ( $\pm 0.02$ ) at 517 nm [31]. In a test tube, 3 mL DPPH working solution was mixed with 100  $\mu\text{L}$  plant extract (1 mg/mL) or the standard solution. The absorbance was measured at 517 nm for a period of 30 min. The percentage antioxidant or free radical scavenging activity was calculated using the formula below:

$$\% \text{Antioxidant activity} = [(Ac - As)/Ac] \times 100.$$

Where, Ac and As are the absorbance of control and sample, respectively. The control contained 100  $\mu\text{L}$  methanol in place of the plant sample.

### 2.6.3 ABTS<sup>•+</sup> radical scavenging activity

ABTS<sup>•+</sup> decolorization assay was measured using the method reported by (Re et al. 1999) with some modifications by Bursal. The working solution of ABTS<sup>•+</sup> radical was made by reacting ABTS (9.5 mL, 7 mM) with potassium persulfate (245  $\mu\text{L}$ , 100 mM), and raising the volume to 10 mL with distilled water. The solution was kept in the dark at room temperature for 18 hours, and then diluted with potassium phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 ( $\pm 0.02$ ) at 734 nm. Plant samples were prepared in methanol with dilutions 50–1250  $\mu\text{g}/\text{mL}$ . A sample (10  $\mu\text{L}$ ) was placed in a test tube and mixed thoroughly with 2.99 mL ABTS radical working solution. Absorbance of the resulting clear mixture was recorded at 734 nm. The percent antioxidant activity of the sample was determined using the following formula:

$$\% \text{Antioxidant activity} = [(Ac - As)/Ac] \times 100$$

Where Ac and As are the absorbances of the control and sample, respectively. The control was prepared by adding 10  $\mu\text{L}$  of methanol in place of the sample (Bursal et al., 2011).

### 2.6.4 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-values of less than 0.05.

### 3. RESULTS AND DISCUSSION

#### 3.1 Visual Observation and UV-Vis Spectroscopy

Silver nanoparticles exhibit a yellowish-brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Reduction of silver ions to silver nanoparticles could be followed by a color change and UV-Vis spectroscopy [12]. In this study, addition of plant extract of *J. carnea* into the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution to yellowish-to-yellowish brown as seen in (Fig. 2). Fig. 2. shows the photographs of sample solutions containing silver nitrate with aqueous leaf extract of *J. carnea* after 15 mins (left test tubes), and silver nitrate with aqueous leaf extract of *J. carnea* solutions after 3 hours on completion of the reaction (right test tubes). The appearance of a yellowish-brown color confirms the existence of silver nanoparticles in the solution (right test tubes). This result is similar to the report of [32] who synthesized silver nanoparticles from *Azadirachta indica* aqueous leaf extract. He and his team also reported a change in the colour of the solution to yellowish to reddish brown within reaction duration due to excitation of surface plasmon vibrations in silver nanoparticles.

Certain information about metal nanoparticles such as their shape, size and stability in aqueous suspension can be obtained using UV-Vis spectroscopy [32]. The recorded UV-vis spectra for silver colloid medium after 3 hours of moderate stirring at room temperature is presented in (Fig. 3). There have been reports of silver nanoparticles obtained through green synthesis having absorbance values in the visible range of 418 nm to 448 nm [10,12,32-34]. The absorption spectrum of silver nanoparticles spanned a wide range from 300nm to 700 nm with a prominent peak at 446 nm in this study. This peak indicates the formation of AgNPs because it is within the range of the surface plasmon resonance (SPR) for AgNPs. The absorbance value obtained in this study is similar to the work of [32] who obtained an absorbance value of 445 nm for the synthesis of AgNPs from *A. indica* aqueous leaf extract. The UV-vis spectra recorded, implied that a rapid bio-reduction of silver ions was achieved using *J. carnea* leaf extract as a reducing agent. The UV-vis spectrum and visual observation revealed that formation of silver nanoparticles occurred within 3 hours.

#### 3.2 FTIR Analysis

The aqueous leaf extract of *J. carnea* 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. 4 and Fig. 5) respectively.

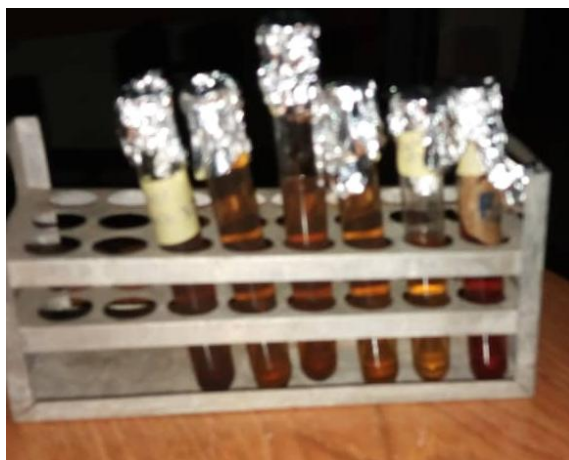


Fig. 2. Colour change observed in silver nitrate solution after 15 minutes (left) and 3 hours (right) of adding *J. carnea* aqueous leaf extract

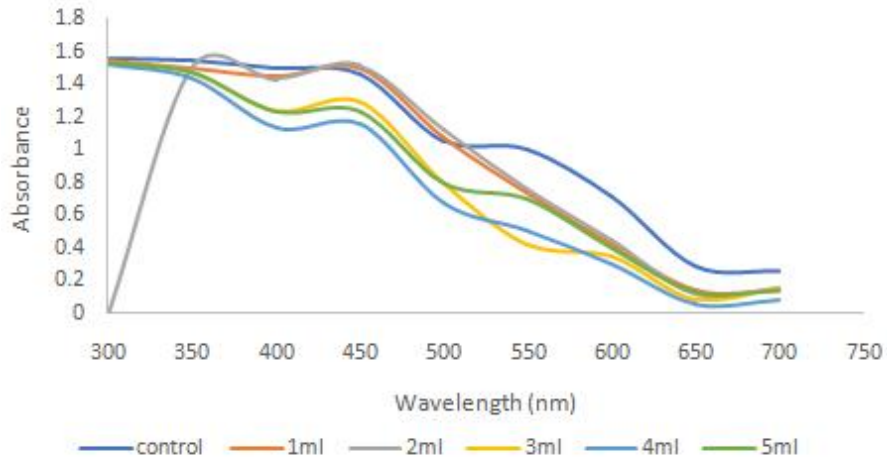


Fig. 3. UV-Vis absorption spectra of obtained silver nanoparticles after 3 hours of using *J. carnea* aqueous leaf extract as reduction agent.

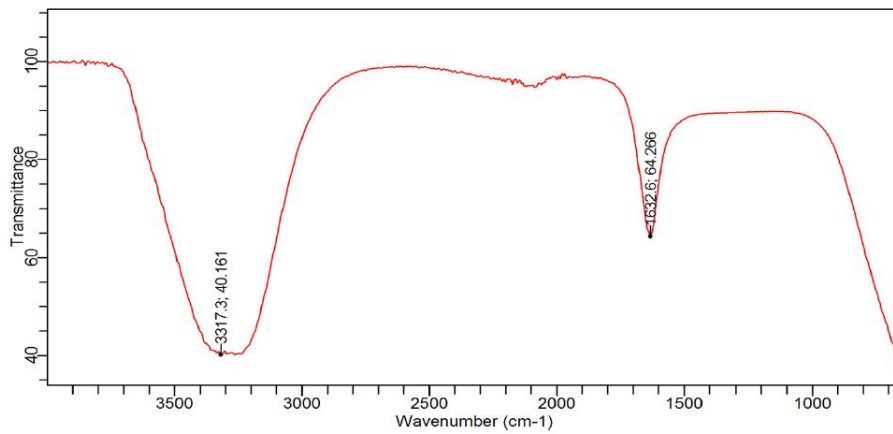


Fig. 4. FTIR Spectrum of *J. carnea* aqueous leaf extract

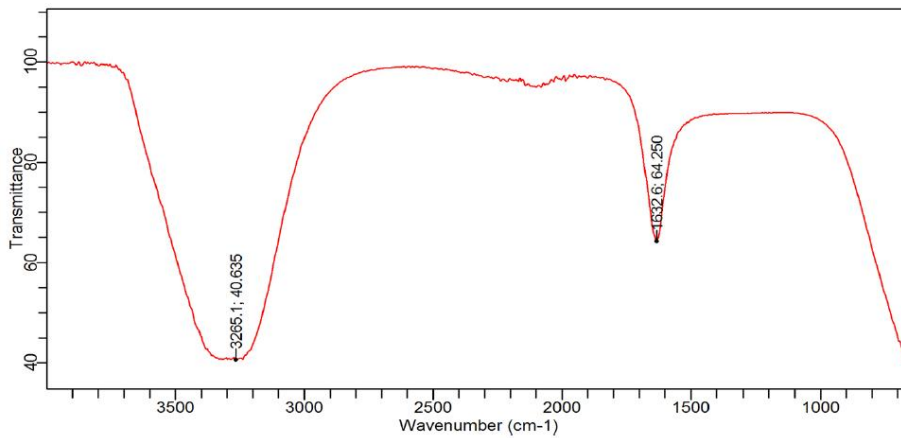


Fig 5. FTIR spectrum of AgNPs synthesized using *J. carnea* aqueous leaf extract

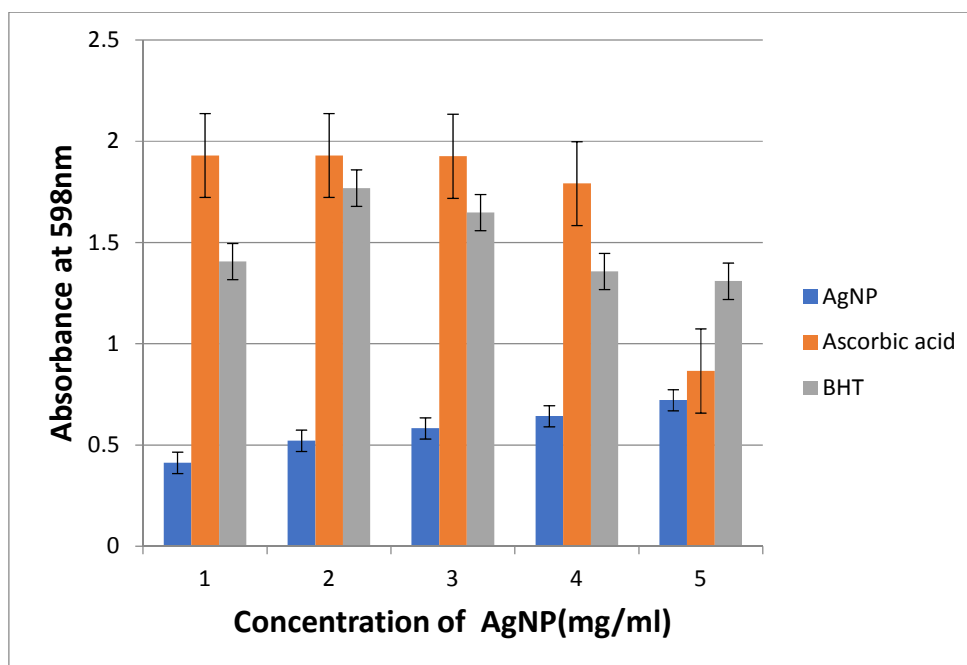
The interpretation of various functional groups at different bands of the FTIR spectrum for both (Figs. 4 and 5) is summarized in (Table 2). The bioactive constituents of *J. carnea* are flavonoids, phenols, ascorbic acid, with moderate amounts of lycopene and  $\beta$ -carotene [18,22]. The reduction and capping ability of *J. carnea* may be due to the presence of phenols in its aqueous leaf extract. Phenolics are strong antioxidants with high reducing capacity. Phenolic content in *J. carnea* leaf extract enabled the reduction of silver ions to nanoscale-sized silver particles due to the electron donating ability of the phenolic compounds [34]. From the FTIR results, it could be concluded that some of the bioorganic

compounds from *J. carnea* extract formed a strong coating/capping on the nanoparticles.

The proposed mechanism for the reduction of  $Ag^+$  by plants' phenolic compounds is explained by [34]. 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.

**Table 2. FTIR spectrum interpretation of various functional groups present in the leaf extract of *J. carnea***

S/No	Wavenumber (cm <sup>-1</sup> )	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
		Alkyne	C-H stretching
3	3317.3	Alcohol	O-H stretching
		Aliphatic primary amine	N-H stretching
		Aliphatic secondary amine	N-H stretching
		Alkyne	C-H stretching
		Alkyne	C-H stretching



**Fig. 6. A graph showing the FRAP of AgNPs from aqueous leaf extracts of *J. carnea* compared with that of standard antioxidants (Ascorbic acid and BHT).**

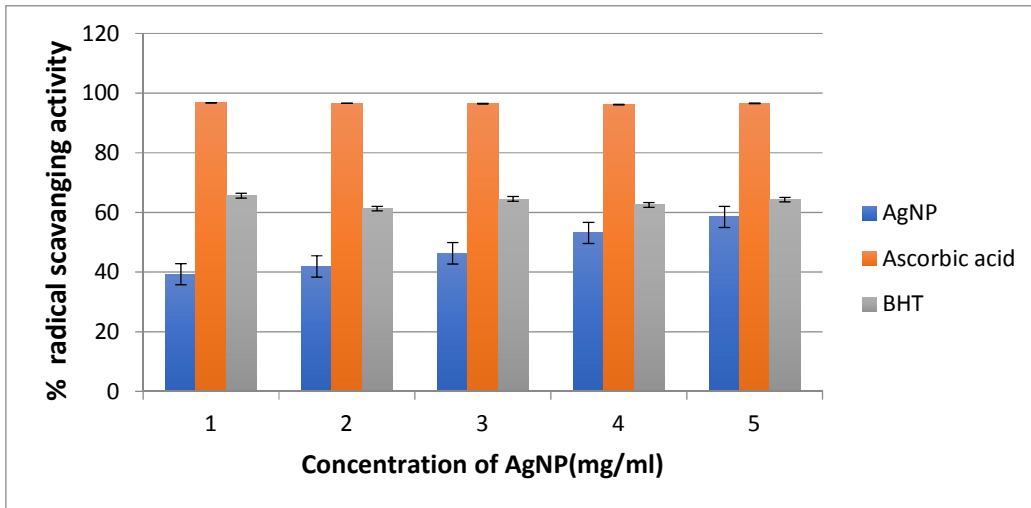


Fig. 7. DPPH radical scavenging activity (RSA) of AgNPs synthesized from *J. carnea* aqueous leaf extract compared with that of standard antioxidants (Ascorbic acid and BHT)

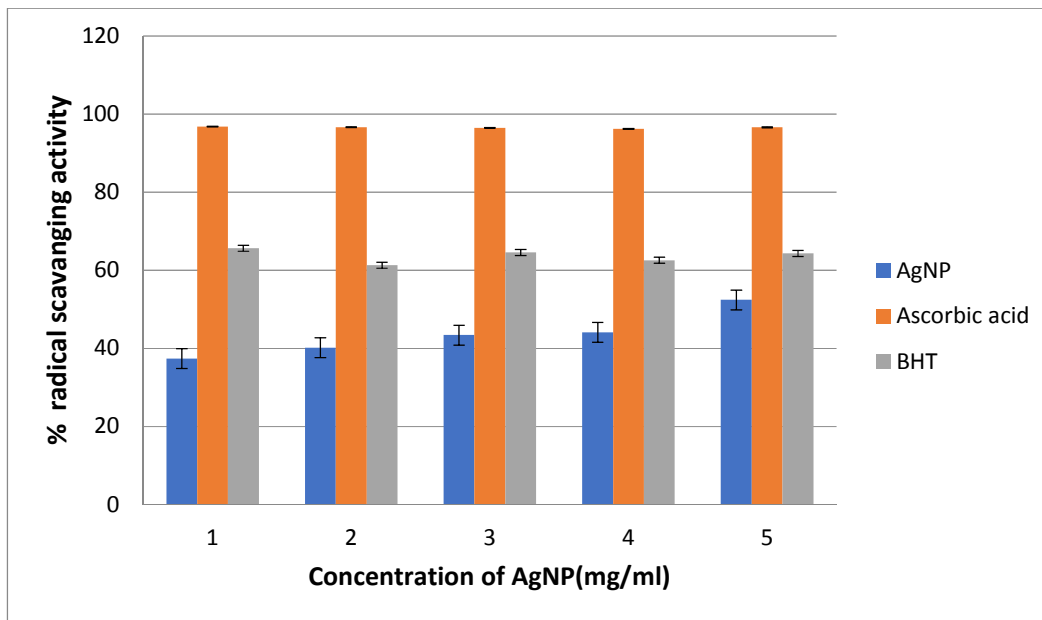


Fig. 8. ABTS<sup>+</sup> radical scavenging activity of AgNPs synthesized using *J. carnea* aqueous leaf extract compared with that of standard antioxidants (Ascorbic acid and BHT)

### 3.3 Antioxidant Assays

Silver nanoparticles are known to have antioxidant and antimicrobial properties [32,35]. In this study, we assessed the antioxidant potential of AgNPs synthesized from the aqueous extract of *J. carnea*. The results are presented in (Figs. 6,7,8) above. One similarity these antioxidant results have in common is that the antioxidant potential of the synthesized

AgNPs increased with increased concentration but none of them competed significantly with the standard antioxidants (Ascorbic acid and BHT) used during the assay.

The reducing potential of the synthesized AgNPs was evaluated by determining its ability to reduce Fe<sup>3+</sup> 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 [36]. The results for the ferric reducing activity of *J. carnea* 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 [22,23], where the aqueous extract of *J. carnea* 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  $Fe^{2+}$ .

The synthesized AgNPs and the standard antioxidants promoted inhibition of DPPH radical in (Fig. 7), 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 [23], where the standard antioxidant (BHA) exhibited a higher DPPH radical inhibition activity than that of the aqueous extracts of *J. carnea*.

The synthesized AgNPs exhibited a dose dependent ABTS radical scavenging property in (Fig. 8). Although, not as effective as that of the standard antioxidants. The antioxidant behavior of *J. carnea* AgNPs justifies their potential applications in the therapy of many diseases caused by inflammation and oxidative stress. The antioxidant mechanism behind the AgNPs of *J. carnea* could be that during the AgNPs synthesis, numerous *J. carnea* bioconstituents could be adsorbed onto the active surfaces of *J. carnea* AgNPs, which increases its surface area, and thereby interact and scavenge these free radicals efficiently [37]. 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 [22] present in the aqueous leaf extract.

#### 4. CONCLUSION

This study revealed that silver nanoparticles (AgNPs) can be obtained with a simplified method using the aqueous leaf extract of *Justicia carnea*. AgNPs synthesis using this plant extract

is simple, cost effective, precise, and eco-friendly. The presence of phenolic compounds in the aqueous leaf extract could be the reducing and capping agents for AgNPs synthesis, providing a safe, green process and avoiding input of any toxic chemicals. The synthesized AgNPs exhibited a moderate antioxidant activity which could be attributed to the presence of organic phytochemicals (flavonoids, phenols and carotenoids) in the aqueous leaf extract. This eco-friendly method could be a competitive alternative to the earlier known physical/chemical methods used for synthesis of silver nanoparticles. *J. carnea* leaf extract can be used effectively in the production of potential antioxidant AgNPs which could be useful in various bio-applications such as cosmetics, food, and biomedical industry.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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