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Formulation of silver nanoparticles from the leaves extract of *Vernonia amygdalina*

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ABSTRACT

Background: Nanoparticles are small particles that range in size from 1 to 100 nm. *Vernonia amygdalina* belongs to the family of Asteraceae and it has been used traditionally to treat malaria, typhoid fever, microbial infections, diabetes mellitus, infertility and gastrointestinal disorders. The aim of this research was to synthesize silver nanoparticles (AgNPs) from the leaves extract of *Vernonia amygdalina* and to evaluate the antioxidant and antibacterial effects of the nanoparticles.

Method: The silver nanoparticles were synthesized using green method and the particles were evaluated using scanning electron microscope (SEM) and X-ray diffraction (XRD). The free radical scavenging effects of the AgNPs and extract were analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The antibacterial effect of the leaves extract and the AgNPs were determined by disk diffusion method on *Escherichia coli and Staphylococcus aureus*.

Results: SEM analysis of the formulated nanoparticles revealed a poly-dispersed spherical shaped nanoparticle with particle size of 70-90 nm. The DPPH assay revealed IC_{50} values of 2.80, 2.10 and 5.20 mg/mL for the leaves extract, silver nanoparticles and ascorbic acid respectively. The X-ray diffraction of the nanoparticles revealed a crystalline and polymorphic molecular structure and also confirmed that they are composed of biomolecular compounds which were responsible for the reduction of silver ions.

Conclusion: Silver nanoparticles were formulated using *Vernonia amygdalina* leaves extract. The formulated nanoparticles possessed significant antibacterial and antioxidant effects and also have potential pharmaceutical applications.

1. Introduction

Nanoparticles are very small materials that range in size from 1 to 100 nm. They have an increased surface area (1000 g/m^2) and unique electronic, mechanical, magnetic, photonic, physical and chemical properties[1]. They have a wide application from gene to drug therapy. They can be divided into many categories based on their features, forms, and sizes. Their optical qualities are affected by their size, which results in variable colours due to absorption in the visible region. Their distinctive size, shape, and structure influence their reactivity, toughness and conductivity. They have a variety of applications such as energy-based research, catalysis, tumor imaging, medical and pharmaceutical applications[2]. Nanoparticles can also act as a carrier for anticancer agents for targeted delivery of drugs to tumours. They also have the ability to hold thousands of molecules of a drug and overcome the challenges of solubility, stability and resistance in drug delivery. Nanotechnology is being used by medical researchers to develop more effective and efficient means of delivering

drugs to specific tissues, such as cancer chemotherapy[1].

Vernonia amygdalina (VA) belongs to the family, Asteraceae and it is a tropical African shrub or small tree that grows in abundance. They are also widely found in Asia, where they can be found in natural vegetations and commercial plantations[3]. It is commonly known in Africa as 'African bitter leaf,' 'Ewuro' in Yoruba, 'Onugbu' in Igbo and 'Oriwo' in Edo. The leaves are green in colour and have a distinct odour and bitter taste[4]. After washing and boiling to remove the bitter taste, the leaves of VA are used as soup seasonings[5]. It is used to make the popular Nigerian bitter leaf soup "Onugbo," as well as a spice in the Cameroonian dish "Ndole"[6]. The herb is extracted into a tonic and consumed for therapeutic purposes in various parts of Africa, such as Nigeria[7]. *Vernonia amygdalina* has also been used ethnomedically to treat diseases such as malaria, infertility, diabetes, veneral infections and gastrointestinal disorders[8,9,10]. *Vernonia amygdalina* extracts have been shown to possess antihelmintic, antimalarial, antitumorigenic, bacteriostatic and bactericidal activities against certain bacteria[11,12]. Nwajo[13] reported that the leaves extract possessed hypoglycaemic and hypolipidemic effect *in vivo*. Traditional healers also prescribe the aqueous extract for the treatment of a variety of ailments, including emesis, nausea, diabetes, loss of appetite, diarrhoea, gastrointestinal tract disorders, sexually transmitted illnesses and diabetes mellitus[14,15]. Nzekekwu and Abosede[16] had previously reported the green synthesis and characterization of silver nanoparticles using leaves extract of *Azadirachta indica* and *Vernonia amygdalina.* Similarly, Narayanaswamy *et al.,*[17] also formulated silver nanoparticles using leaves extracts of *Clitoria ternatea* and *Solanum nigrum* and studied the antibacterial effect against common nosocomial pathogens. Hence, the aim of this study was to formulate silver nanoparticles from *Vernonia amygdalina* leaves extract as an acceptable dosage form to mask the bitter and obnoxious taste as well as to evaluate the antioxidant and antibacterial effects.

2.0 Materials and Methods

2.1 Materials

Silver nitrate (BDH Company Limited, UK), 2,2-diphenyl-1-picrylhydrazyl (Sigma Aldrich, Germany), Gentamicin (Ranbaxy, India). The microorganisms used in the study were laboratory strains. Fresh *Vernonia amygdalina* leaves were collected from the botanical garden of the University of Benin, Benin-City, Nigeria.

2.2 Methods

2.2.1 Identification and extraction of *Vernonia amygdalina* leaves

Vernonia amygdalina leaves were collected from the botanical garden of the University of Benin, Ugbowo, Benin-City, Nigeria (Latitude: 6.3931°N; Longitude: 5.6195°E) in June, 2022. The collection was done during the rainy season in the morning as previous studies have shown that all the secondary metabolites in the leaves will remain intact when collected in the early hours of the morning[16]. The specimen was prepared and deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin-City, Nigeria with voucher specimen number of F904 for reference purposes. The leaves were air-dried for 14 days and then size reduced with wooden mortar and pestle.

2.2.2 Preparation of plants aqueous extract

20g of dried *Vernonia amygdalina* leaves extract was weighed into a 1 L beaker and 800 mL of deionized water was added and then stirred for 30 min. The mixture was allowed to settle for 6 h at room temperature and the aqueous extract was filtered using Whatman no. 1 filter paper into a clean conical flask.

2.2.3 Green synthesis of silver nanoparticles from *Vernonia amygdalina* leaves extract

The silver nanoparticles were prepared by reacting 0.1 M aqueous silver nitrate ($AgNO_3$) solution with the *Vernonia amygdalina* leaves extract in ratio 9:1 with constant agitation using a magnetic stirrer at 300 rpm for 10 h at 25°C. On addition of $AgNO_3$ to the extract, colour change was noticed after 5 min from yellow to brown. A change in colour of the solution from yellow to dark brown shows the bio-reduction of silver ion (Ag^+) to silver nanoparticles (AgNPs). The solution was then kept for 48 h in the dark at 25°C and was then centrifuged at 6000 rpm for 20 min and the supernatant layer was separated from the residue which was transferred to a clean watch glass and dried in an oven at 50°C for 24 h to obtain dry powder particles[18].

2.2.4 UV/Visible Analysis

Validation of the extract was done using a UV/Visible spectrophotometer (PG instrument, Model T70, USA). A serial dilution of each of the aqueous extracts was prepared and the wavelength of maximum absorbance was obtained by scanning over a wavelength range of 800 to 200 nm.

2.2.5 Fourier Transform Infra-Red Spectroscopy (FTIR)

The chemical functional groups of the formulated nanoparticles were determined using Fourier transform infrared (FTIR) spectrophotometer (Shimadzu, Japan) and was scanned between 4000-1000 cm^{-1}.

2.2.6 Scanning Electron Microscopy

The morphology of the nanoparticles was investigated using scanning electron microscope (Quanta 200 FEG model). The sample was put on a sample holder and gold coated before the microscopy was done[2].

2.2.7 X-ray Diffraction (XRD) Analysis

XRD analysis of the formulated silver nanoparticles was done using Rikagu generator (XRD Rikagu Rint 2000, Japan) at a voltage of 25kV, 20mA current intensity, 2Θ angle and 3°min^{-1} in the range of 4-50°.

2.2.8 Evaluation of antioxidant activity of the extract using DPPH

The antioxidant activity of the leaves extract and the nanoparticle were investigated according to previous method used by Pavithra and Sasikumar[19] with some modifications. The extract and the nanoparticle (0.1 g) each were used to prepare a 10 mg/mL stock solution. Serial dilutions were done to obtain 5, 2.5, 1.25 and 0.25 mg/mL solutions. The DPPH (0.8 mM) was prepared in methanol and then added to the test tubes containing the samples and was incubated for 30 min in the dark. The absorbance of each samples was measured at 480 nm using UV-spectrophotometer. The percentage inhibition was calculated using equation 1.

$$\text{Percentage inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}$$

- - -Equation 1

2.2.9 Antimicrobial activity

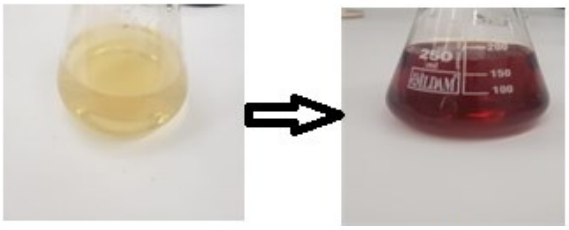
Antibacterial activities were determined by agar difference method[20]. *E. coli* and *S. aureus* plates were incubated at 37°C. Nanoparticles equivalent to 25 mg, 50 mg and 375 mg of the plant extract were soaked in 5 mL of dissolution medium for 12 h to give 5 mg/mL, 10 mg/mL and 75 mg/mL concentrations respectively. Released extracts were collected and placed in the bacterial media. Gentamicin was used as the standard. It was then cultured at 37°C for 24 h after which clear zones of inhibition were measured using a transparent rule.

2.2.10 Data analysis

All tests were done in triplicate, and the mean and standard deviations (SD) were determined. The results were expressed as mean±SD.

3. Results

The aqueous extract of *Vernonia amygdalina* leaves was used for the green synthesis of silver nanoparticles. The silver nanoparticles (AgNPs) appear brownish in colour in aqueous medium as a result of surface plasmon vibrations[16]. As the extract was added to aqueous silver nitrate solution, the colour of the extracts changed from yellow to brown indicating silver nanoparticles formation (Figure 1).



A B

Figure 1: Formulation of silver nanoparticles and sequence of colour change with time (A) Just after mixing the silver nitrate solution with the extract and (B) After 6 h of mixing the silver nitrate solution with the extract.

3.1 UV-Visible Spectra Analysis

Silver nanoparticles formulated from *V. amygdalina* leaves extract demonstrated maximum absorbance at 480 nm after 30 min of incubation. Surface plasmon resonance of AgNPs generated by reduction of aqueous Ag^{+} was responsible for the absorption bands[16] in the visible spectra.

3.2 FTIR Spectroscopy

The functional groups responsible for stabilizing and capping the *Vernonia amygdalina* silver nanoparticles were also investigated using FT-IR in the range of 4,000-1000 cm^{-1}. Figures 2 and 3 reveal a weakly defined peak at 1,280 cm^{-1} attributed to a carboxy (–C = O) stretching, and a broad band between 3,500 and 2,900 cm^{-1} attributed to bounded hydroxyl (–OH) or amine group (–NH) and aliphatic (–CH). Carboxyl (–C = O) and methyl group ($–CH_3$) stretching vibrations were also assigned to the peak centered at 1,414 cm^{-1}.

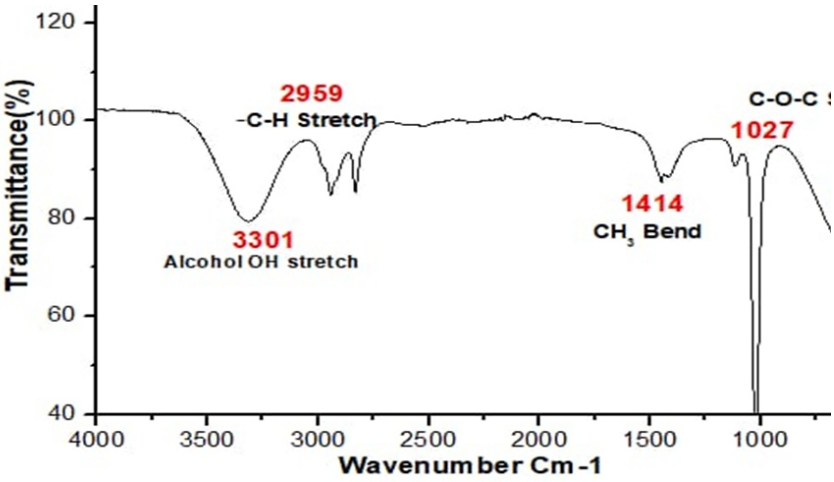


Figure 2: FTIR spectrum of *Vernonia amygdalina* leaves extract

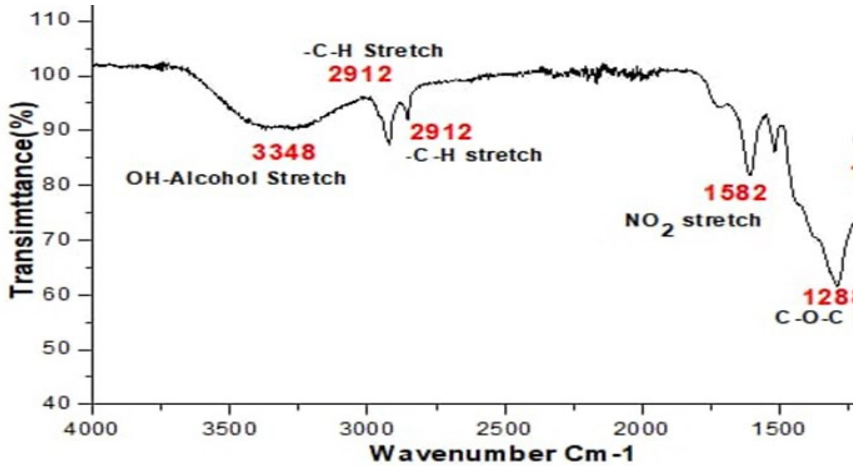


Figure 3: FTIR spectrum of *Vernonia amygdalina* silver nanoparticles

3.3 Scanning Electron Microscopy (SEM)

SEM is used to analyze the shape of the formulated nanoparticles as well as their structural composition. The synthesized *Vernonia amygdalina* nanoparticles had a disc shaped with a spherical dimension as a bright particle pattern was clearly observed (Figure 4).

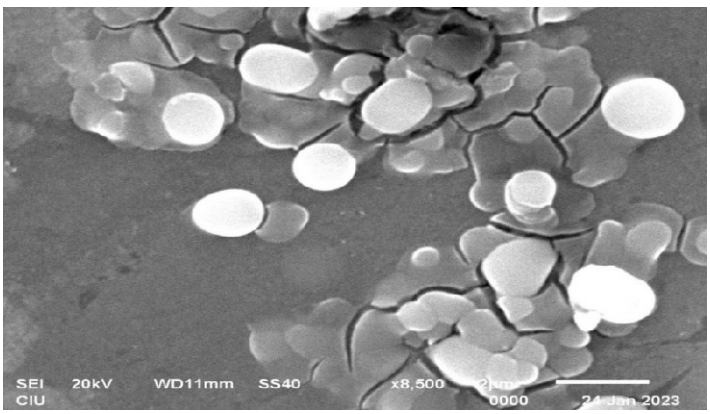


Figure 4: SEM image of the formulated *Vernonia amygdalina* nanoparticles

3.4 XRD Analysis

XRD analysis was done to elucidate the crystalline and polymorphic structure of the formulated AgNPs. From the Bragg's reflection peaks shown in the XRD pattern, it was possible to determine the crystallinity of AgNPs which showed the largest intensity peak at 1300 plane, along with smaller intensity peaks at 110, 281, 300, 307, 485, and 503 (Figure 5).

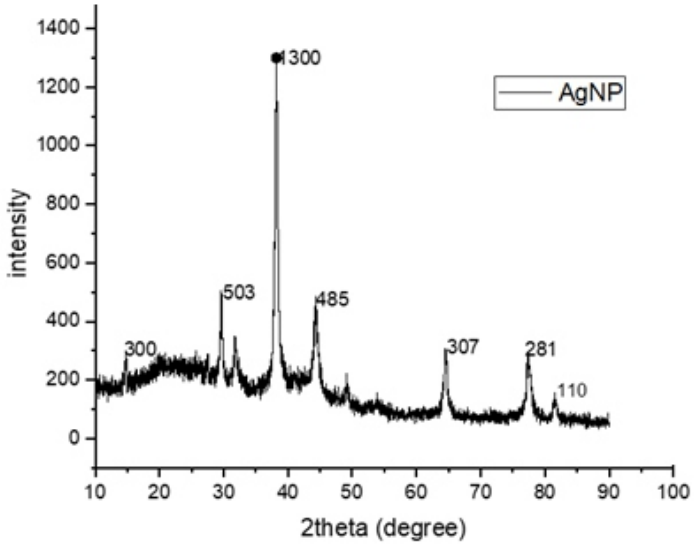


Figure 5: XRD spectrum of the formulated silver nanoparticles

3.5 Antioxidant activity

DPPH scavenging activity of *Vernonia amygdalina* leaves extract and silver nanoparticles

The quantitative antioxidant assay results were expressed as IC_{50} and percentage inhibition (Table 1 and Figure 6). The result revealed that the extract had higher activity than the silver nanoparticles at all concentrations. Although, the ascorbic acid showed higher activity than both the leaves extract and silver nanoparticles. On the basis of the percentage inhibition, the antioxidant effects demonstrated by all the samples were concentration dependent. From the graph of the percentage inhibition against the concentration, the IC_{50} of the leaves extract, silver nanoparticles and ascorbic acid were 2.80 mg/mL, 2.10 mg/mL and 5.20 mg/mL respectively.

Table 1: Percentage inhibition of extract, silver nanoparticles and ascorbic acid against DPPH

Conc. (mg/mL)	Silver nanoparticles (%)	Extract (%)	Ascorbic (standard) (%)
10	18.15	23.45	35.27
5	29.24	35.25	56.19
2.5	48.13	55.16	72.13
1.25	64.56	75.24	84.26
0.625	81.27	85.21	91.68

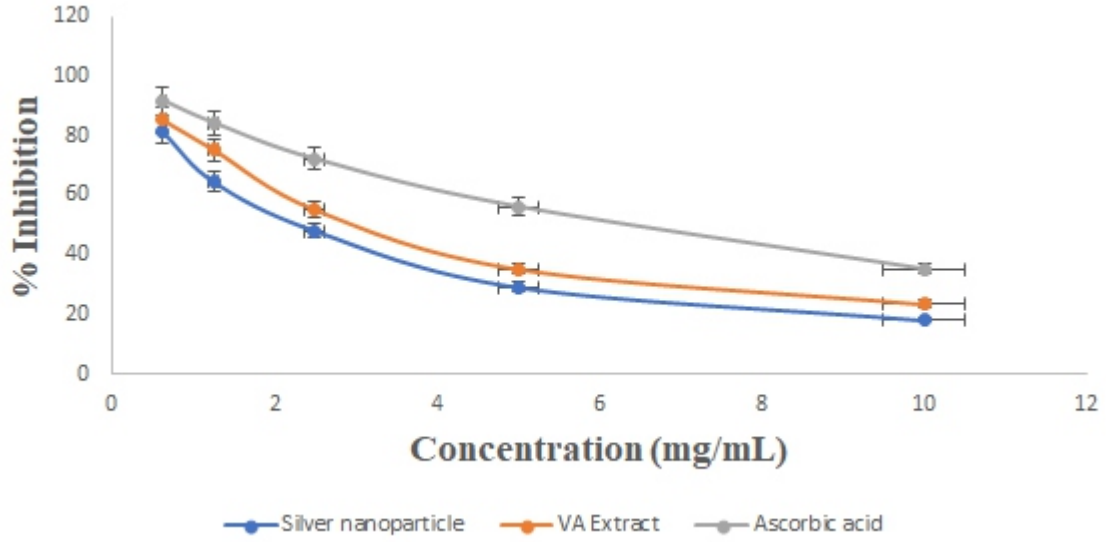


Figure 6: Percentage inhibition of VA extract, silver nanoparticle and ascorbic acid on DPPH

3.6 Antimicrobial activity of *Vernonia amygdalina* nanoparticles

The *Vernonia amygdalina* leaves extract and nanoparticles showed comparable antimicrobial activity relative to the positive control (gentamicin) and the zone of inhibition is presented in Table 2. The results showed that nanoparticles demonstrated significant antibacterial activity against all test bacteria, with the highest activity demonstrated against *S. aureus*. The zone of inhibition of growth of all the bacteria used in the study ranged 5.64-7.43 mm for the aqueous leaves extract and 8.46-9.85 mm for the formulated silver nanoparticles.

Table 2: Antimicrobial activity of *Vernonia amygdalina* leaves extract and nanoparticles

Organisms	*V. amygdalina*	Nanoparticles	Gentamicin	$AgNO_3$	Distilled water
S. aureus	7.43±0. 08	9.85 ±0.02	18.42 ±0.02	8.52 ±0.01	0
E. coli	5.64 ±0.10	8.46 ±0.01	12.41 ±0.11	6.52 ±0.01	0

Results are expressed as Mean ±SD of three replicate readings.

4. Discussion

Silver nanoparticles were synthesized by bioreduction of silver nitrate solution using *Vernonia amygdalina* leaves extract and there was a colour change from yellow to brown confirming the biosynthesis of the nanoparticles. Similar colour change has been previously reported by Arya *et al.,*[21] which confirmed the completion of reaction between the leaves extract and $AgNO_3$. FTIR analysis of the synthesized *Vernonia amygdalina* silver nanoparticles showed that the hydroxy, methyl, carboxyl functional groups present in the aqueous leaves extracts of *Vernonia amygdalina* were responsible for the reduction Ag^+ to Ag^0 and the synthesis as well as the stabilization of the formulated VA silver nanoparticles[16]. SEM analysis revealed a poly-dispersed spherical shaped nanoparticle with a particle size of 70-90 nm. Findings from previous studies observed a similar result in the shape as well as morphology of the synthesized AgNPs[16]. The X-ray diffraction analysis of the nanoparticles revealed a crystalline and polymorphic molecular structure and also confirmed that they are composed of biomolecular compounds which were responsible for the reduction of silver ions. The majority of the diffraction peaks were recorded between an angle of 15° and 90°. The peaks' widening suggests that AgNPs was formed at the nanoscale scale. The Bragg's reflection peaks for AgNPs were found at 2 Θ 83°, 79°, 10°, 65°, 45°, 29°, and 39° which correspond to 110, 281, 300, 307, 485, 503 and 1300 lattice planes respectively. Similar results were

obtained by Nzekwe and Abosede[16].

The percentage inhibitions and IC_{50} values were used to express the free radical scavenging activity of the extract and silver nanoparticles which were then compared with ascorbic acid (standard). From the results, the percentage inhibition increases as the concentration decreases. The lower the IC_{50}, the higher the activity. The antioxidant effect demonstrated by the extract may be due to the presence of phenolic compounds which are more in the extract compared to the nanoparticles. The extract and the formulated nanoparticles had a good antioxidant and DPPH scavenging activity when compared with ascorbic acid. Findings from the study showed that VA leaves extract and nanoparticles were able to scavenge DPPH in a concentration-dependent manner and ascorbic acid being the reference antioxidant had a better scavenging activity than the extract and the silver nanoparticles[22]. The VA extract and the silver nanoparticles demonstrated significant inhibitory activity against *S. aureus* and *E. coli*. The findings from this study is similar to previous study done by Farombi and Owoeye[15], who reported that silver nanoparticles of *Vernonia amygdalina* possess significant antioxidant and chemopreventive properties which may be due to the presence of flavonoids, tannins and saponins in the extract[23,24].

5. Conclusion

Silver nanoparticles were synthesized using *Vernonia amygdalina* leaves extract and the formulated nanoparticles contained some secondary metabolites such as saponins, flavonoids, tannins and phenols which were responsible for the antibacterial and antioxidant activities[15].

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

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