



Green Synthesis, Antioxidant and Antimicrobial Activity of Silver Nanoparticles Using *Gnetum africanum* Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The synthesis and application of nanoparticles is an important area of research that is gaining attention recently. In this recent project, we report the synthesis of silver nanoparticles, AgNP using aqueous solution of silver nitrate and *Gnetum africanum* leaf extract (reducing agent). The synthesis of AgNP was achieved by mixing aqueous solution of silver nitrate (70ml, 15.75mM) with a solution of *Gnetum africanum* leaf extract (100 ml) in a reaction flask and allowed to stand for 24 hours in a dark cupboard. A color change from light brown to yellowish brown was observed which indicated that synthesis of silver nanoparticles took place. The presence of AgNP was ascertained using UV-vis spectra analysis and absorption at 442 nm showed the presence of AgNP. The antioxidant assay of both the synthesized AgNP and the leaf extract was determined using DPPH. Antimicrobial activity was conducted using three different organisms which were *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* respectively. The antioxidant results using DPPH scavenging ability of AgNP showed that at concentrations of 2mg/ml, 1mg/ml and 0.1mg/ml, the percentage inhibition of DPPH by AgNP was 61.69, 53.06 and 38.31 respectively and that of *Gnetum africanum* leaf extract was 81.32, 78.49, and 58.29 respectively at the same concentrations using

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Ascorbic acid as a standard. The antimicrobial activity of both the synthesized AgNps and *Gnetum Africanum Leaf* extract using one gram positive bacteria (*Staphylococcus aureus*) and two gram negative bacteria (*Escherichia coli* and *Pseudomonas*) revealed that the synthesized AgNps showed lesser activity than *Gnetum africanum* leaf extract for both the gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas*) and (*Escherichia coli*). From the above findings, it can be observed that *Gnetum Africanum Leaf* extract reduced Ag^+ to Ag^0 and also both the synthesized AgNps and the *Gnetum Africanum Leaf* extract showed reasonable antioxidant activity against DPPH and antimicrobial activity against the tested microorganisms. This implied that both samples have medicinal values.

Keywords: *Gnetum africanum*; silver nanoparticles(AgNps); 2,2-diphenyl-1-picrylhydrazyl(DPPH); *Staphylococcus aureus*; *Escherichie coli* and *Pseudomonas*; antioxidant assay; antimicrobial activity.

1. INTRODUCTION

With antimicrobial resistance increasingly becoming a global concern, scientists have expanded the search for potentially new and safe antimicrobial agents to plants. There has been a steady increase in the use of plant remedies worldwide, including the search for new plant compounds that present potentials as sources of useful drugs for the treatment of diseases [1].

The *Gnetum africanum* plant popularly known as Afang and *Gnetum africanum* in Nigeria and Cameroon, is a member of Gnetaceae family, which has been used widely in treatment and curing of so many ailments and as food. It has been regarded as a medicinal plant, due to its natural content of active ingredients, with anti-inflammatory and anti-carcinogenic properties and hence has, by virtue of its physico-chemical composition, tremendous health benefits [2].

The study of Nanoparticles has become fascinating because of their outstanding compositions in their size and shape, optically dependent, electrical, and magnetic properties. Also, is its importance in different areas of science such as chemical sciences, medical, environmental, physical sciences as well as engineering, pharmacological fields etc [3]. The use of AgNPs as antibacterial agents has a long history in the health industry [4]. The anti-pathogenic mechanisms of AgNPs although not fully understood have been explained to be by microbial damage, microbial sub-cellular structure damage, adhesion to microbial cells, ROS and free radical generation, microbial wall piercing and penetration inside cells and modulation and modification of microbial signal-transduction pathways [5].

In the Green synthesis of AgNPs, who synthesized silver nitrate with neem leaf extract,

discovered the color change of the synthesized solution after the addition of the silver nitrate solution to the neem leaf extract, and observed the color change to brownish gray, which indicates the formation of AgNPs and reduction of Ag^+ to Ag^0 [6].

The objective of the study was to synthesize silver nanoparticles using *Gnetum Africanum*, leaf extracts and examine the antioxidant and antimicrobial activity of the synthesized silver nanoparticles.

2. MATERIALS AND METHODS

2.1 *Gnetum africanum* Leaf (Sample) Collection

The fresh leaf sample was collected from Thinkers corner, Enugu State and was identified as *Gnetum Africanum* By a botanist.

2.2 Preparation of the Leaf Sample

The fresh leaves were washed thoroughly using distilled water and were air-dried for 2weeks. After drying, they were powdered using an electric blender. 90g of this powdered sample was weighed and mixed with 700ml of distilled water in a measuring cylinder. The mixture was heated using a Bunsen burner for 1hour 20 minutes in a fume hood, after which it was allowed to cool for 30 minutes. The mixture was filtered into a beaker, first using a muslin cloth, the filtrate of which was then filtered using a filter paper.

2.2.1 Synthesis of silver nanoparticles (AgNPs)

The 15.75mM silver nitrate ($AgNO_3$) solution was dissolved in 100ml of distilled water in the absence of light to avoid the decomposition of

silver nitrate by light. 100 ml of the *Gnetum africanum* leaf extracts was measured into a conical flask. 70 ml of the above silver nitrate solution was mixed with 100 ml extracts solution. It was then placed in a dark cupboard for 24 hours for the synthesis of AgNPs to take place. It was then removed after 24 hours and concentrated using a water bath to obtain a semi-solid product.

2.3 Antioxidant Assay

Antioxidant activity of both the synthesized AgNPs of the leaf extract of *Gnetum africanum* with AgNO₃ and the unsynthesized extract were determined by 2,2 diphenyl-1-picrylhydrazyl (DPPH) assay.

Stock solution of 0.07mmol/L of DPPH was prepared with methanol. 1.0ml of various concentrations (0.1, 0.5 and 1mg/ml) of the synthesized AgNPs of both the Synthesized and non-synthesized sample extract, were added to 6 different test tubes 3 for each. 1.0ml of the prepared solution of DPPH was added to each of the test tubes containing the sample. The mixture was shaken gently and placed in a dark room for 30minutes at room temperature. After 30minutes, it was removed from the dark room and the absorbance recorded at 517 nm against methanol control. Methanol was used as control and ascorbic acid was used as standard. % for DPPH radical scavenging activity was calculated with the formula:

$$\% \text{ DPPH radical scavenging} = \frac{A_0 - A_1}{A_0} \times 100$$

Where % inhibition - %DPPH radical scavenging

A₀ = control absorbance

A₁ = sample absorbance. [7].

2.4 Antimicrobial Activity Determination

The antimicrobial activity of the synthesized AgNPs and unsynthesized leaf extract of *Gnetum africanum* was tested against three different bacteria. The organisms are *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative) and *Pseudomonas* (gram negative). Preparation of both the nutrient broth and media (the nutrient agar) was carried out according to the manufacturing guide.

A syringe was used to measure out 3ml of dimethyl sulfoxide (DMSO) used to dissolve the

extract. A cock borer was then used to make three holes on each of the culture plates. 0.5ml of the dissolved extract was introduced carefully so as not to mistakenly touch the media, into the holes of the respective bacteria as labeled, one of each hole on the media was used as a control. It was incubated for 24 hours at 37 °C.

The antimicrobial activity was determined using the zone of inhibition (mm) of the bacteria with ciprofloxacin and chloramphenicol used as standards.

3. RESULTS

3.1 Silver Reduction

According to (Kan-Senet *al*, 2003) explanation of synthesizes leaf extract, a yellowish-brown color change was observed after the aqueous *Gnetum africanum* leaf extract was added to the silver nitrate solution, which indicates the reduction of silver ion to silver nanoparticles. It was then preserved in a dark cupboard for 24hr. After 24hr the color further changed to deep brown which indicates that synthesis has occurred completely [8].

3.2 Antimicrobial Activity

Antimicrobial activity of synthesized AgNPs and the leaf extract of *Gnetum africanum* showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas*. The synthesized AgNPs showed the zone of inhibition while in the leaf extract of *Telfairia Occidentalis* Only *Escherichia coli* showed a zone of inhibition against the gram negative organisms used. Ciprofloxacin and Chloramphenicol were used as standard drugs for gram positive and gram negative organisms respectively [9].

3.3 Antioxidant Assay

3.3.1 DPPH scavenging property of synthesized AgNPs of *Gnetum africanum*

The antioxidant activity that was carried out for synthesized *Gnetum africanum* using silver nitrate and unsynthesized *Gnetum africanum* Leaf extract was determined by 2, 2diphenyl-1-1-picrylhydrazyl (DPPH) assay. The graph of % radical scavenging activity is plotted against the three concentrations and was evaluated with ascorbic acid as the standard antioxidants. Methanol was used as the control, which has

absorbance of 0.322%. DPPH radical scavenging activity was calculated with this formula [10].

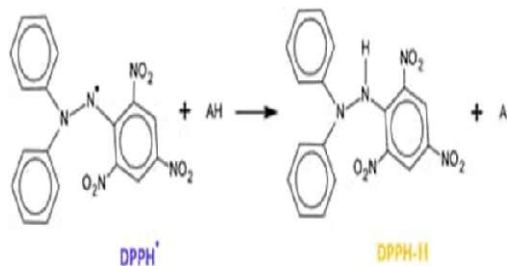


Fig. 1. The structure DPPH activity with antioxidants [11]

4. DISCUSSION

The research findings showed that *Gnetum africanum* leaf extract reduced Ag^+ to AgNPs after addition of aqueous *Gnetum africanum* leaf extract and it was confirmed after a color change of yellowish-brown was observed. After 24 hours of being stored in a dark cupboard, the color changed from yellow-brown to deep brown. The brown color was as a result of the surface Plasmon resonance which is a very important property of nanoparticles.

The synthesized silver nanoparticles and *Gnetum africanum* leaf extract were tested against gram positive and gram negative microorganisms, in which the synthesized AgNPs and extract of *Gnetum Africanum* showed maximum inhibition on the gram positive (*Staphylococcus aureus*) microorganisms and minimum inhibition on the gram negative microorganism (*E. coli* and *Pseudomonas*). The extract of *Gnetum Africanum* showed higher activity in the gram positive organism (*S. aureus*) and in one of the gram negative organism (*Pseudomonas*) and low activity in one of the gram negative organisms (*E. coli*), then the synthesized AgNPs of *Gnetum Africanum* showed higher activity in the gram positive organism (*S. aureus*) and in one of the gram negative organism (*E. coli*). Then it showed lower activity in one of the gram negative organisms (*Pseudomonas*). This means that both the synthesized AgNPs and the extract of *Gnetum Africanum* has more inhibitory activity on the gram positive organism than on the gram negative organism. This means that the *Gnetum Africanum* leaf extract has more activity on both the gram positive and gram negative organisms. This means that the *Gnetum africanum* leaf

extract can penetrate the high resistance cell wall of gram positive and gram negative organisms.

The antioxidant activity examined against DPPH was observed to have reasonable scavenging property for both the synthesized and *Gnetum africanum* leaf extract, where the absorbance of the synthesized, *Gnetum africanum* and ascorbic acid are shown in the table in the previous chapter and the ascorbic acid was used as the standard. The antioxidant activity was observed to be high in the synthesized *Gnetum africanum* leaf extract at the concentration 0.1mg/ml, when compared with that of the ascorbic acid, and for the *Gnetum africanum* leaf extract was observed to be more active at the concentration of 0.1mg/ml which is a high concentration, when compared with the ascorbic acid. This result implies that for the synthesized AgNPs *Gnetum africanum* leaf extract to be effective in drug formulation the concentration of the nanoparticles should not be so high and for the extract of *Gnetum africanum* nanoparticles to be effective it should be at the same concentration or higher.

DPPH is an antioxidant assay based on electron transfer, produces violet solution which when immersed in ethanol, the free radical which is stable at room temperature, is reduced to colorless in the presence of an antioxidant molecule. Antioxidant is a general term for any compound or molecule that can inhibit the activity of free radicals. Antioxidant is a general term for any compound or molecule that can inhibit the activity of free radicals. These free radicals lack completion of electrons thereby attacking a molecule to steal its electron from the molecule and in the process damage the molecule. Antioxidants neutralize the free radical by giving off its own electron, antioxidants acts as a natural or total switch off for the free radicals. The use of DPPH assay provides a simple and well modified way to examine antioxidants by spectrophotometry. Antioxidants neutralize the free radical by giving off its own electron, therefore it means that the antioxidants acts as a natural or total switch off for the free radicals. The body or the cell generates free radicals from some environmental factors such as smoking of tobacco and cigarettes, excessive alcohol intake, air pollution, high intake of polyunsaturated fatty acids, ultraviolet rays etc. These environmental factors generate the R.O.S (reactive oxygen species). These R.O.S are a group of free radical

Table 1. Antimicrobial activity of synthesized nanoparticle on gram positive and gram negative

Microorganisms	Zone of inhibition synthesized AgNP	Zone of inhibition of <i>Gnetum africanum</i>	Zone of inhibition of standard drug (mm). Ciprofloxacin	Zone of inhibition of standard drug (mm) Chloramphenicol (10mm)
<i>Staphylococcus aureus</i> .(N ⁺)	20mm	35mm	30	--
<i>Escherichia coli</i> (N ⁻)	15mm	30mm	30	--
<i>Pseudomonas</i> (N ⁻)	15mm	30mm	-	20

Table 2. % DPPH radical scavenging

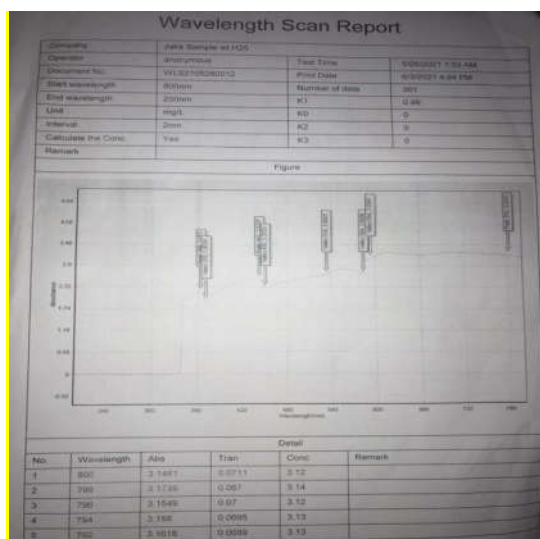
S/N	Sample	Concentration (mg/ml)	Absorbance (nm)			% inhibition of AgNP against DPPH
			A	B	Average	
1	<i>Gnetum africanum</i> leaf extract	2	0.118	0.119	0.178	81.32
2	<i>Gnetum Africanum</i> leaf extract	1	0.137	0.138	0.138	78.49
3	<i>Gnetum Africanum</i> leaf extract	0.1	0.269	0.266	0.268	58.29
1	Synthesized AgNps	2	0.243	0.244	0.244	61.69
2	Synthesized AgNps	1	0.299	0.298	0.299	53.06
3	Synthesized AgNps	0.1	0.294	0.292	0.293	38.31

Table 3. Comparison of synthesized Nanoparticles, Extracts of *Gnetum africanum* and Ascorbic Acid

Concentration	Synthesized <i>Gnetum africanum</i> leaf extract % inhibition of DPPH	<i>Gnetum Africanum</i> leaf extract % inhibition of DPPH	Ascorbic acid % inhibition of DPPH
2	61.69	81.32	96.40
1	53.06	78.49	93.33
0.1	38.31	58.29	96.40



Aqueous solution of *Gnetum africanum* leaf



UV-Visible result for Synthesized AgNPs

active molecules that are generated from oxygen. When R.O.S are introduced in the system, plants that scavenge the R.O.S contain a high complex antioxidant defense system, which scavenges low generated toxic oxygen metabolites by raising the level of endogenous antioxidant defense ability. So therefore constant taking of some natural antioxidants leads to some beneficial health aspects. E.g heart disease, improving weak vision etc.

5. CONCLUSION

The emergence of AgNPs synthesis by biological methods has been a significant development in the field of nanoparticles. The interest for this type of synthesis has increased rapidly because these methods do not involve the use of

hazardous or toxic chemicals. The use of plants, easily accessible factors, has led to the production of AgNPs through a simple, fast, cost effective and eco-friendly process. The synthesis of nanoparticles was attributed to the abundance of biomolecules in plant extract. The sample shown in the experiment was able to reduce silver ions to silver nanoparticles and exhibit great antibacterial and antioxidant properties. Silver nanoparticles synthesized from *G.africanum* can be incorporated into nanoparticles and applied as antibacterial agents and antioxidants [12].

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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