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Biosynthesis of Eco-Friendly Silver Nano-Particles using Dry Fluted Pumpkin *(TelfairiaOccidentalis)* Leave Extractas Reducing Agent

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ABSTRACT

The Biosynthesis of Eco-Friendly Silver Nano-Particles using Dry Fluted Pumpkin (*TelfairiaOccidentalis*) Leave Extract as Reducing Agent was carried out. The research used the extract which was prepared from dry fluted pumpkin leaves to reduce 0.01 M silver nitrate solution without the addition of any capping agent. During the biosynthesis process qualitative measures like 15 minutes interval observation for 1 hr., observation of precipitates formation, and laser light pointer were taken to establish the formation of AgNPs in the solution. The AgNPs for characterization were produced by allowing the biosynthesis solution to remain in the cardboard for 24 hours before filtering and drying the residue in the oven at 110°C. The residue which was the AgNPs was characterized using ED-XRF and SEM. The NPs produced were of average size of 30 nm and angular and cubicle in shape. The chemical composition of the NPs was 71.8% Ag_2O, 25% SiO_2, 2.18% Cl and other trace compounds. The NPs; given the eco-friendly method of their biosynthesis can be further purified to be used on wound bandage.

Keywords: *Reducing agent, Telfairiaoccidentalis, Nano-particles, Wound, Purity, Extract*

1. Introduction

Manigandan*et al.*, (2017), posited that ‘‘ the unique physicochemical properties of nanomaterials are attractive for use in a variety of technologies due to the factors such as conductivity, magnetic property, and optical sensitivity by the characteristics such as small size, shape, surface structure and chemical composition. Modifying the properties of nanoscale materials generally involves control over the physicochemical features of the material. Noble metal nanostructures have created attention due to their extensive applicability in various domains. The advent of nanomaterials and their production sharpened man's precision in production especially the concept of top-down technique, from bulk to small and even smaller dimensions up to manufacturing molecule-to-molecule or even atom-to-atom and the bottom-up technique (Katsnelson, 2007; Preetha, and Rani, 2012; Obikwelu, 2012).

Silver (Ag) is a soft, translucent gray transition metal with an atomic number of 47. This element has the highest thermal and electrical conductivity of all the metallic elements found so far on Earth. The electronic configuration of Silver is $[Kr]4d^{10}5s^{1}$. It can be found in pure form or alloyed with other elements. It is considered a precious metal and is used for coins and bullions as part of monetary systems; it can be found in jewelry and ornaments. The photographic film industry has relied on the photochemical reactivity of silver and silver halide nanoclusters for many years to generate photographs (Poinern, 2015). Because of its close association with human history in several applications, silver has also been tested and used in dentistry in the form of amalgams with mercury for fillings to repair tooth decay. In addition, silver compounds have long been known for their antimicrobial activity. With the advent of a plentiful supply of antibiotics after World War II, there has been a major shift away from metal ions and their antimicrobial effects (oligodynamic effects) until recently. As microbes can evolve, resistant bacterial strains, for example, are capable of handling the latest most potent generations of antibiotics, and coupled with their ability to pass on this resistance to others. It makes them formidable pathogens. Thus, there has been resurgence in the manufacture of nano-silver not only as an antibacterial, but also for potential application against fungi, viruses, and other pathogenic species. There is currently a drive to make nano-silver and apply it as coating for various medical components to stop infections. For instance silvernanoparticles sprayed bandages can be used to tie wounds to avoid bacterial infections. Scientist Robert Burrell created a process to manufacture antibacterial bandages using nanoparticles of silver. Silver ions block microbes' cellular respiration. In other words, silver smothers harmful cells killing them (Kuldeep*et al.,* 2012;Biswas, *et al*., 2015; Shreya, *et al*., 2015; Benakashani, *et al.,* 2016; Bansal, *et al.,* 2017).

Here comes the issue of method of preparation; ecofriendly and harmless substances as reducing agents and capping chemicals are preferred. Any method therefore that will promote safer and purer form of silvernanoparticles for medical application will be a better option (Leela1 and Vivekanandan, 2008; Jannathul, and Lalitha, 2015; Selvam, *et al.,* 2017). In this work the author intends to use fluted pumpkin (*telfairiaoccidentalis*)as both reducing agent and capping material for the production of pure AgNPs that can be applied as coating for various medical components to stop infections; including the treatment of wounds. No wonder the objective of the work which is the biosynthesis of AgNPs using eco-friendly approach of reducing with plant extract.

2. Materials and Method

2.1 Materials

The materials used for this work were; *TelfairiaOccidentalis* (Fluted Pumpkin), the extracts from the leaves were used as reducing agent, and 0.01M solution silver nitrate ($AgNO_3$.) was used as the source of silver ions. Also used was milli-Q-water. The leaves used can be seen in fig. 1 below:

Fig. 1: *TelfairiaOccidentalis* (Fluted Pumpkin)

2.1.1 Equipment

The following equipment were used for the research work; 50 ml measuring cylinder, 250 ml beaker, 250 ml conical flask, filter paper, 20 ml micropipette, micropipette tip, mortar and pestle, laser pointer. Digital camera, spatula, magnetic stirrer, hot plate (heater), 250 ml reagent bottles, digital weighing balance; energy dispersive x-ray fluorescence (ED-XRF), Scanning Electron Microscope (SEM), blender, kimwipes, Buchner funnel, 50 ml glass vials, and oven.

2.2 Method

2.2.1 Dried leaves extract preparation

Here the procedure varied slight from that of preparing extracts from fresh leaves. Fresh leaves of fluted pumpkin were collected from the University of Uyo, Biological Garden, the leaves were sun-dried after thoroughly washing them. They were again dried in the oven at 40^{o}C for 24hrs. The leaves were then blended using a blender. From the dried-processed leaves, 5g was measured and transferred into 250 ml beaker to which was poured 50 ml milli -Q- water and boiled on the heating plate. The suspension was allowed to cool before it was poured into the funnel with filter paper to filter out the suspension. The extract was collected as filtrate in the beaker. See fig. 2 below:

Fig. 2: Extract from Fluted Pumpkin *(TelfairiaOccidentalis)* Dried Leaves

2.2.2 Bio-Synthesis of AgNPs using Extract of the Dried Leaves

180 mL of the dried leave extract of *T. occidentalis* was poured into 250 mL reagent bottle. Then 20mL of 0.01 M $AgNO_3$ was poured into the reagent bottle containing 180 mL of the driedleave extract of *telfairiaoccidentalis* to synthesize the Ag NPs. After that, the mixture of the leave extract and $AgNO_3$ was gently shaken for 2 min to have a uniform solution of the mixture. After shaking, the mixture was kept still and observed for any colour change after interval of 15 min for 60 min. Laser beam from a laser pointer was used to observe if there was scattering of the light on the mixture as the beam of light passes through the

bottle containing the $AgNO_3$ solution to the bottle containing the mixture of the extract and the $AgNO_3$ Solution..After observation for 60 mins the solution was allowed to stay for 24 hrs resulting in more particles being formed; noticed through change of colour and quantity of residue on the filter paper. Fig. 3 captures the biosynthesis process of AgNPs using dried leaves of *telfairiaoccidentalis* extract.

(a) (b)

Fig.3. *(a) TelfairiaOccidentalis*dried leaves extract (b) the laser light is slightly scattered by the mixture of *telfariaoccidentalis* and 0.01M $AgNO_3$.

The mixture of the synthesized Ag-NPs using the extracts from the dried leaves of *telfairiaoccidentalis* was put in a dark cupboard for 24 hrs and was later filtered using filter paper. The residue that was deposited on the filter paper was dried in an oven at a temperature of 110^{o}C for 6 hours to obtain powdered Ag NPs. The process is captured in fig. 4.

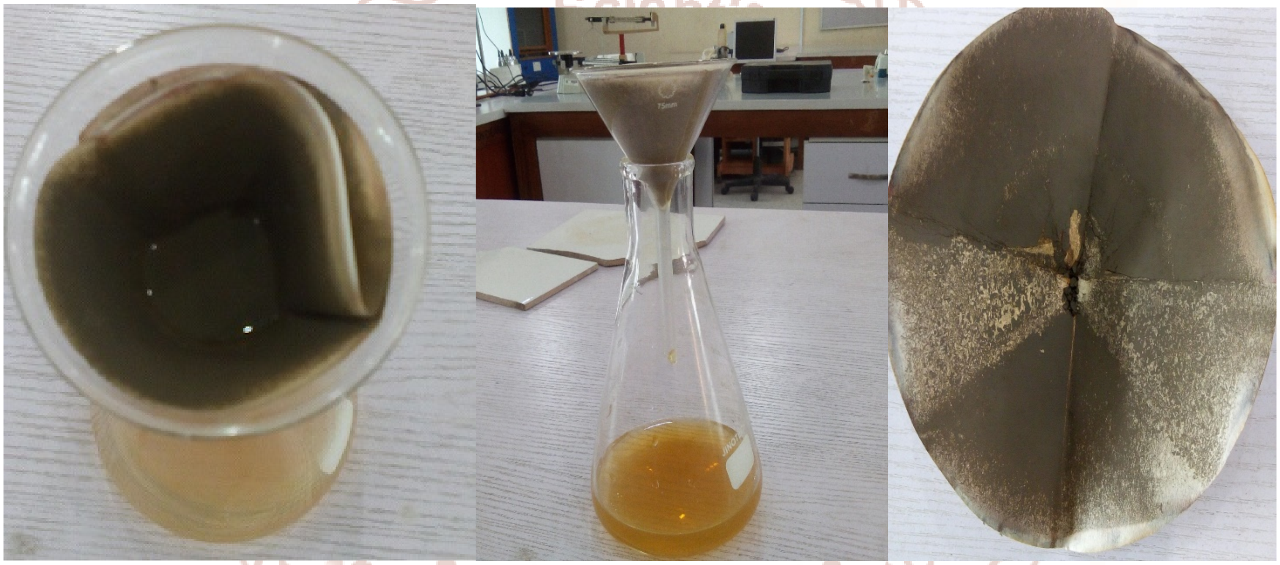

Fig. 4: Silver Nanoparticles from Dried Leaves of *TelfairiaOccidentalis* Extract without any Capping Agent.

2.2.3 Characterisation of AgNPs Produced using T*elfairiaOccidentalis (*Fluted Pumpkin*)*

SEM Analysis was carried out, with Phenom SEM Model Pro x, on the AgNPs produced to identify the morphology, size and shape of the produced AgNPs. The chemical analysis of the AgNPswas performed by using Energy Dispersive X-Ray Flourescent, Mini pal4 ED-XRF model. The two tests were carried out at Nigeria Geological Survey Agency, Zaria, Kaduna.

3. Results and Discussion

3.1 Results

The results of the work are displayed as below; Table 1 gives the result of the AgNPs biosynthesis process, fig. 5 indicates colour at start of biosynthesis and colour change after 1 hour, Table 2 shows some properties during the biosynthesis process, Table 3 shows the chemical analysis of the AgNPs using ED-XRF, and fig.6 shows the SEM micrographs of the AgNPs.

Table 1: Biosynthesis of Silver Nanoparticles using Dried *TelfairiaOccidentalis*Leaves Extract

PROCEDURE	OBSERVATIONS	COMMENTS
20 mL of 0.01 M $AgNO_3$ was added to 180 mL of *T. Occidentalis* leaves extract	No notable colour change	Initial time of reaction
15 min	Slight colour change	Reduction reaction taking place.
30 min	Solution continue to become darker	Reduction reaction continues, which indicates the formation of Ag NPs.
45 min	Slightly darker colour change	Formation of Ag NPs.
60 min	No further colour change	Reduction reaction on
Testing with Laser pointer Light	Scattering of Laser light was observed	Confirming formation of Ag-NPs.

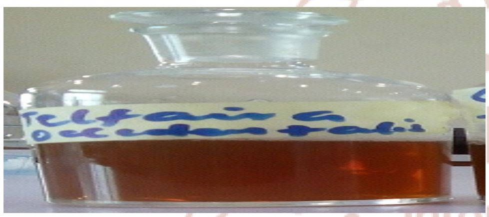

Fig.5a: Initial (Light Brown)

Fig.5b: Colour Change (Dark brownish)

Fig.5a and 5b Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Dried Leave Extract of *T. Occidentalis.*

3.1.1 Some Properties of the Aqueous Solution during Biosynthesis

Table 2: Electronic Conductivity, pH and Temperature during the Biosynthesis Process.

S/N	LEAVES EXTRACT	ELECTRONIC CONDUCTIVITY (µS)/Temp. (^{O}C)		pH (mV)/Temp. (^{O}C)	
		Before Addition of $AgNO_3$	**After Addition of $AgNO_3$**	**Before Addition of $AgNO_3$**	**After Addition of $AgNO_3$**
1	*T. Occidentalis*	11051(25.3^{o}C)	1002 (25.5^{o}C)	4.90 (25.7^{o}C)	4.95 (25.7^{o}C)

3.1.2 AgNPs Analysis using ED-XRF

Table 3: Result of ED-XRF analysis

CHEMICAL COMPOSITION (OXIDES)	(%) COMPOSITION
SiO_2	25.00
Cl	2.18
MnO	0.098
Fe_2O_3	0.35
NiO	0.035
CuO	0.076
Ag_2O	71.80
Yb_2O	0.05
Ga_2O_3	0.007
RuO_2	0.14
Er_2O_3	0.03
HfO_2	0.003
OsO_4	0.03
IrO_2	0.028
PbO	0.05

3.1.3 Scanning Electron Microscope Analysis

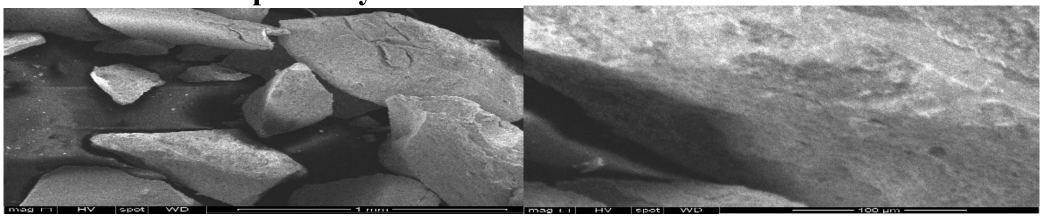

Fig.6: AgNPs Size and Shape at different Magnifications

3.2 Discussion

Table1 gives the result of the biosynthesis of AgNPs using dried leaves extract of *telfairiaOccidentalis (*fluted pumpkin*)*. The initial colour of the biosynthesis solution was light brown; this colour continued to change after the interval of 15 minutes up to 1 hr, when it was completely changed to dark brown. See fig.5a and 5b. As the colour change was going on, precipitates were also sighted in the biosynthesis solution indicating that the extract was reducing the Ag+ ions into Ag^0 metal (http:// www.nanowerk.com 2012;Elnashaie*et al.,* 2015; Manigandan, *et al*., 2017). The Laser light pointed at the solution revealed scattering in the biosynthesis solution. According to Poinern, (2015), one interesting property of colloidal particles, because of their shape and size, is that they scatter white light in a process called the Tyndall effect. Named after the nineteenth, Century Physicist John Tyndall, the effect is the process of light being scattered and reflected by colloidal particles or NPs in suspension. The presence of a colloidal suspension can be easily detected by the scattering/ reflection of a laser beam from the NPs as the beam of light passes through the solution. In contrast, when the laser beam is shined through a normal solution (i.e. silver nitrate solution) without colloids or NPs, the beam passes through without scattering. The Tyndall effect can only be used to determine if there are colloids/ NPs in that solution. Thus, it acts as a qualitative tool in the rapid determination of AgNPs in this instance because the human eye cannot directly see individual NPs in the solution.(Poinern, 2015) The method used in determining the formation of AgNPs agrees with above.

Table 2 gives the electronic conductivity, pH, and temperature change before and during the biosynthesis of the AgNPs using *telfairiaoccidentalis* as the reducing agent.The electronic conductivity was 11051µS and this dropped to 1002µS in the biosynthesis solution; indicating that reduction of Ag+ ions to Ag^0 was ongoing in the solution. The pH of the reducing agent which was 4.9 when added to the $AgNO_3$ solution; the pH of the biosynthesis solution became 4.95; reducing in acidity. The temperature change was not significant during the biosynthesis process. The potentials of leave extracts as a reducing agent for biosynthesis of AgNPs has been confirmed by several authors. In some plants, the acidic components can easily aid the reduction of the metallic ions. Furthermore, these studies showed that AgNPs created this way possesses good antimicrobial activity. The fact that no capping agent or templating agent is needed makes this chemical route an attractive one (Leela1 and Vivekanandan, 2008;Kuldeep, *et al.,* 2012; Jannathul, and Lalitha, 2015; Biswas, and Dey, 2015;Benakashani, *et al.,* 2016; Bansal, *et al.,* 2017). No capping agent was used in this work except the*telfairiaoccidentalis* extract which served as both reducing and capping agent.

The biosynthesis solution was hid in the cardboard for 24 hours before it was removed and filtered. The residue on the filter paper was dried in the oven at 110^0C to produce dry AgNPs before it was sent for characterization. See Table 3 for the chemical composition of the AgNPs and Fig.6 for the morphology, size and shape of the silver nanoparticles which were produced using *telfairiaoccidentalis* as a reducing agent for silver nitrate solution. The optical property of silver NPs is tunable through the visible and near-infrared region of the spectrum as a function of NP size, shape, aggregation state, and local environment. The size and shape of the Silver NPs and the viewing conditions (transmitted light or reflected light) determine the colour of the silver solution that is ultimately obtained (Poinern, 2015). The average size of the NPs were 30 nm and they were mostly angular and cubicle in shape. The chemical composition showed that $Ag_2O$was 71.8%, $SiO_2$was 25% and Clwas 2.18%, the other compounds were trace compounds. The instrument used for the analysis was calibrated to measure oxides and may have picked the oxygen from the surface of the NPs and not from the core of the NPs.The purity of the NPs can further be enhanced if for instance it is to be used for wound bandages.

4. CONCLUSION

This work has examined and studied the research work ‘’Biosynthesis of Eco-friendly Silver Nano-Particles using Dried Fluted Pumpkin (*telfairiaoccidentalis*) Leaves Extract as Reducing Agent’’ the details of the conclusions drawn from this study are here presented:

i. The work has shown that dried fluted pumpkin leave extract can be used as a reducing agent without any capping to produce eco-friendly AgNPs.
ii. Laser light pointer, colour change and precipitates formation were qualitative proofs that the dried fluted pumpkin leave extract reduced the Ag+ ions to AgNPs.
iii. The chemical composition of the AgNPs showed that it contained 71.8% Ag_2O; the purity of which can be increased by removing the 25% SiO_2, 2.18% Cl and other trace elements.
iv. The AgNPs produced were of the average size of 30 nm
v. The AgNPs produced were angular and cubicle in shape.
vi. Given the friendly manner in which the particles were synthesized with further washing and purification they can be used on wound bandage.

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