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Biosynthesis of Eco-Friendly Silver Nano-Particles: The Efficiency of Fresh Leaves and Dried Leaves in the Synthesis of Silver Nanoparticles

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ABSTRACT

Biosynthesis of Eco-friendly Silver Nano-Particles: The Efficiency of Fresh Leaves and Dried Leaves in the Synthesis of Silver Nanoparticles were studied. The work started with the sourcing and preparation of the dried and fresh leave extracts, which was followed by the preparation of 0.01M $AgNO_3$ solution. The biosynthesis process was carried out for 1 hr. however, for the production of the AgNPs for analysis and characterization; the biosynthesis process was allowed to continue for 24 hours, after which it was filtered and the residue on the filter paper was dried in the oven at 110^oC to remove water from the particles. The chemical composition of the AgNPs was analysed using ED-XRF. The result showed 71.8% Ag_2O; the oxygen must have been surface-attached and not chemically attached in the structure of the particles. Qualitative analysis using laser light pointer, observation of precipitates formation and colour change were used to establish NPs formation. During the biosynthesis process both the measured pH and electronic conductivity of the solutions changed; indicating that reduction process was going on. The work clearly and concisely showed that the four dried leaves extracts (*azadirachtaindica*, *telfairiaoccidentalis, vernoniaamydalina, and camellia sinensis*) used produced better NPs in terms of quantity and quality than their fresh leaves extract counterparts. *A. indica* fresh leave extract was however, better than the other two fresh leaves of *telfairiaoccidentalis* and *vernoniaamydalina.* The heavy and thick chlorophyll may have interfered in the effectiveness of the fresh leaves. This is because the dried leaves extract had less chlorophyll and were very effective in reducing silver ions to Ag^0 nanoparticles in $AgNO_3$ solution.

Keywords: *Biosynthesis, AgNPs, Efficiency, Fresh Leaves, Dried Leaves, Reduction, Eco-friendly*

1. INTRODUCTION

Nanotechnology is envisioned to constitute a significant part of the next technological revolution that is the fourth generation of industrialization to happen in this modern era; industry 4.0. Hence, the production and analysis of nanoparticles, which is the building block of nanotechnology, and their properties has become one of the most active subjects of substantial research in modern material sciences. Typically, the methods used for the formulation of metal nanoparticles can be classified into two different categories Top-down and Bottom-up (Preetha, and Rani, 2012; Essien and Otobong, 2017).

In the manufacture of and fabrication of NPs and nanometer-scale structures, several synthesis parameters must be effectively controlled to deliver a satisfactory outcome (Katsnelson, 2007; Obikwelu, 2012; http:// www.nanowerk.com/). Just being below the 100nm mark is not enough, and the synthesis parameters must be controlled so that the following conditions are met: (1) identical NPs are made every time (i.e., same diameter and shape), (2) they have the same morphology, (3) the same crystal and chemical bonding occurs whether on the surface or inside the NPs and (4) the synthesis process must be stable; if these four conditions are met, then the synthetic

process can be considered as reproducible and is a reliable technique.

Today, there are many techniques capable of manufacturing NPs and nanometer-scale structures from solids, liquids, and gases. The solid-base techniques used to manufacture NPs is straightforward and is usually done by attrition. Liquid-phase based techniques include hydrothermal synthesis, co-precipitation, sol-gel processing, microemulsion, reverse micelle synthesis, microwave synthesis, ultrasound synthesis, and template methods. Gas-phase based techniques are generally carried out by vaporizing a precursor material in a suitable atmosphere. This step is then followed by rapid cooling, which produces supersaturation and condensation to produce NPs and nanometer-scale structures (Kuldeep, *et al.,* 2012;Poinern, 2015;Khatoon*et al.*, 2017).

In nano-technology, there are two main methodologies used to design and manufacture NPs and nanometer-scale structures the first is the top-down method, and the second is the bottom up method. The second method is commonly used. For instance, NPs and nanometer-scale structures can be made by either homogeneous or heterogeneous nucleation from liquids or vapours. For example, one widely used chemical method is to use micelles or reverse micelles to contain the chemical reactions with nanometer-scale or micrometer-size volumes. Within these confined volumes nucleation and growth of NPs and nanometer-scale structure take place. The advantage of this technique are that it can be done at ambient conditions, and it can be easily scaled up to produce macroscopic quantities of nanometer material (Poinern, 2015).

A recent and novel green chemical approach to synthesize NPs involves the use of natural biological molecules as reducing and capping agents. Plant extracts from leaves, stems, and roots have been used to synthesize a variety of metallic NPs, such as plates, rods, cubes, and even pyramids. In addition, both fungus and bacteria have shown the potential to synthesize NPs and appear to be low-cost and energy efficient ways to create NPs (Shreya, *et al.,* 2015; Jannathul, and Lalitha, 2015; Biswas, and Dey, 2015;Benakashani, *et al.,* 2016; Bansal, *et al.,*2017).

Green chemistry is one of the new branches of chemistry, and it involves the design of products and processes that reduce or eliminates the use or generation of hazardous substances. Green synthetic routes for manufacturing Nps and nanostructures are an emerging branch of nanotechnology as the biomolecules around us are safer generally and offer a cost-effective alternative in many cases. For example, today one would be rather reluctant to undertake Michael Faraday's 1857 method of reducing gold chloride with red phosphorus in a volatile, toxic carbon disulphide solution as a technique to create gold NPs. In many conventional methods, there is a tendency to use expensive chemicals and processes that use toxic materials that present hazards such as environmental toxicity and carcinogenic activity. There has been a push toward an alternative pathway of minimizing the use and production of hazardous materials in chemical research (Poinern, 2015; Selvam, *et al.,* 2017).

Sustainable or green technique pathways that creates materials utilizing relatively nontoxic chemicals to create nanomaterial are well favoured and are welcomed avenues of R & D efforts around the world. Following initial reports showing the feasibility of reducing silver ions to Ag NPs, there has been a general move to explore plant extract as a means of reducing, silver to produce NPs and nanostructures of this metal. In some plants, the acidic components can easily aid the reduction of the metallic ions. Furthermore, these studies showed that Ag NPs 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. For instance the biogenesis of Ag NPs by extracts such as those from the neem (azadirachtaindica), geranium leaves (pelargonium graveolens), and alfalfa (medicago sativa) has already been proven, and the list of plants capable of this reducing effect on silver ions is increasing (Shreya, *et al.,* 2015; Jannathul, and Lalitha, 2015; Biswas, and Dey, 2015;Poinern, 2015; Benakashani, *et al.,* 2016; Bansal, *et al.,* 2017; Selvam, *et al.,* 2017).

In this present work extracts of fresh leaves*AzadirachtaIndica*(Neem leaves), *TelfairaOccidentalis* (Fluted Pumpkin), and*VernoniaAmydalina*(Bitter leave)and their dried versions were used including dried *Camellia Sinensis* (Green Tea) to establish the comparative efficacy of the fresh leaves with respect to the dried version in the biosynthesis of Ag NPs. The objective of this work is to establish the comparative efficacy of the above-mentioned fresh leaves with respect to dried leaves extract in the biosynthesis of Ag NPs.

2. Materials and Method

2.1 Materials

The materials used for this work were; *AzadirachtaIndica* (Neem leaves), *TelfairiaOccidentalis* (Fluted Pumpkin), and *VernoniaAmydalina*(Bitter leave). *Camellia sinensis* (green tea) was only used in the dry form. The extracts from the leaves were used as reducing agent. 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 figs. 1- 5 below:

***Fig. 1: Azadirachtaindica* (Neem leaves)**

Fig.2: *VernoniaAmigdalina* (Bitter leaves)

Fig. 3: *TelfairiaOccidentalis* (Fluted Pumpkin leaves)

Fig.4: Fresh *Camellia Sinensis* (Green Tea)

Fig.5: Processed dry pure*Camellia Sinensis*

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 Fresh Leaves Extracts Preparation

The process of synthesizing silver nanoparticles from both fresh and dried leaves started with the preparation of the leave extracts. 5 g each of neem, bitter leaf, and fluted pumpkin were weighed. Each was transferred into a mortar to which was added 50 ml of milli-Q water and ground into paste. The paste was then filtered to obtain the leave extracts. Figs. 6-8 captures fresh leaves extracts prepared.

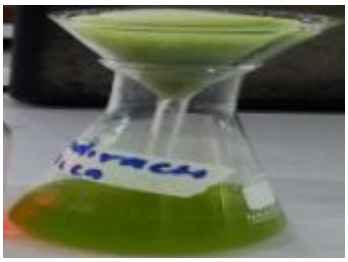

Fig. 6: Neem (*AzadirachtaIndica*) Fresh Leaves Extract

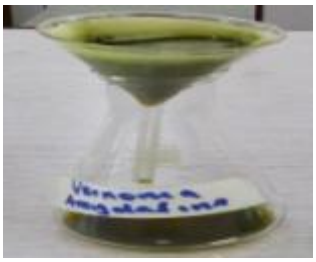

Fig. 7: Bitter Leaf *(VernoniaAmigdalina)* Fresh Leaves Extract

Fig.8: Fluted Pumpkin *(TelfairiaOccidentalis)* Fresh Leaves Extract

2.2.2 Dried Leaves Extracts Preparation

Here the procedure varied slight from that of preparing extracts from fresh leaves. Fresh leaves of neem, bitter leaf, and 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 40^0C for 24hrs. The leaves were then blended using a blender. In the case of the green tea this process was not necessary since the dry processed green tea was used. From each dry 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. Figs. 9- 12 captures the dry extract preparation process.

Fig. 9: Extract from Dried Neem Leaves (*AzadirachtaIndica*) Leaves

Fig.10: Extract from Dried Bitter Leaf *(VernoniaAmigdalina)*

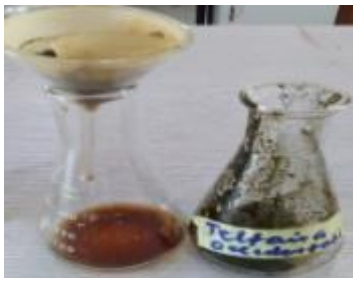

Fig. 11: Extract from Fluted Pumpkin *(TelfairiaOccidentalis)* Dried Leaves

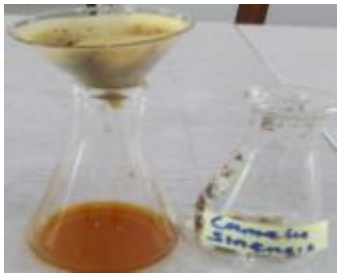

Fig. 12: Extract from Green Tea*(Camellia Sinensis)* dried processed leaves

2.2.3. Biosynthesis of AgNPs using Fresh Leaves Extract

50 mL of each of the leaves extract of *A. indica, V. amigdalina, and T. occidentalis* were poured into 250 mL reagent bottles. Then 10mL of 0.01 M $AgNO_3$ were poured into each of the reagent bottles containing 50 mL of *each of the* leave extract to synthesize the Ag NPs. For the production of AgNPs for characterization, the extracts were increased to 180mL each, while the silver nitrate solution was increased to 20 mL. 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. 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. 13 captures the biosynthesis process for AgNPs using fresh leaves extract

Fig. 13 (a.) *AzadirachtaIndica*(b) laser light pointed through 0.01M $AgNO_3$ solution and a mixture of *azadirachtaindica* and 0.01$AgNO_3$ solution. The laser light goes straight through the $AgNO_3$ solution, but was scattered in the mixture of*azadirachtaindica* and $AgNO_3$ solution. (c) *VernoniaAmigdalina*(d) the laser light is scattered by the mixture of *vernoniaamigdalna* and 0.01M $AgNO_3$. (e)*TelfairiaOccidentalis* (f) the laser light is scattered by the mixture of *telfariaoccidentalis* and 0.01M $AgNO_3$. Color change occurs in all the cases where 0.01M $AgNO_3$ solution was added to the extracts.

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

180 mL of the dried leave extract of*A. indica, V. amigdalina, T. occidentalis* and *Camellia Sinensis* were each poured into 250 mL reagent bottles. Then 20mL of 0.01 M $AgNO_3$ were poured into the reagent bottles containing 180 mL of *the dried* leave extract 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.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. 14 captures the biosynthesis process of AgNPs using dried leaves extracts.

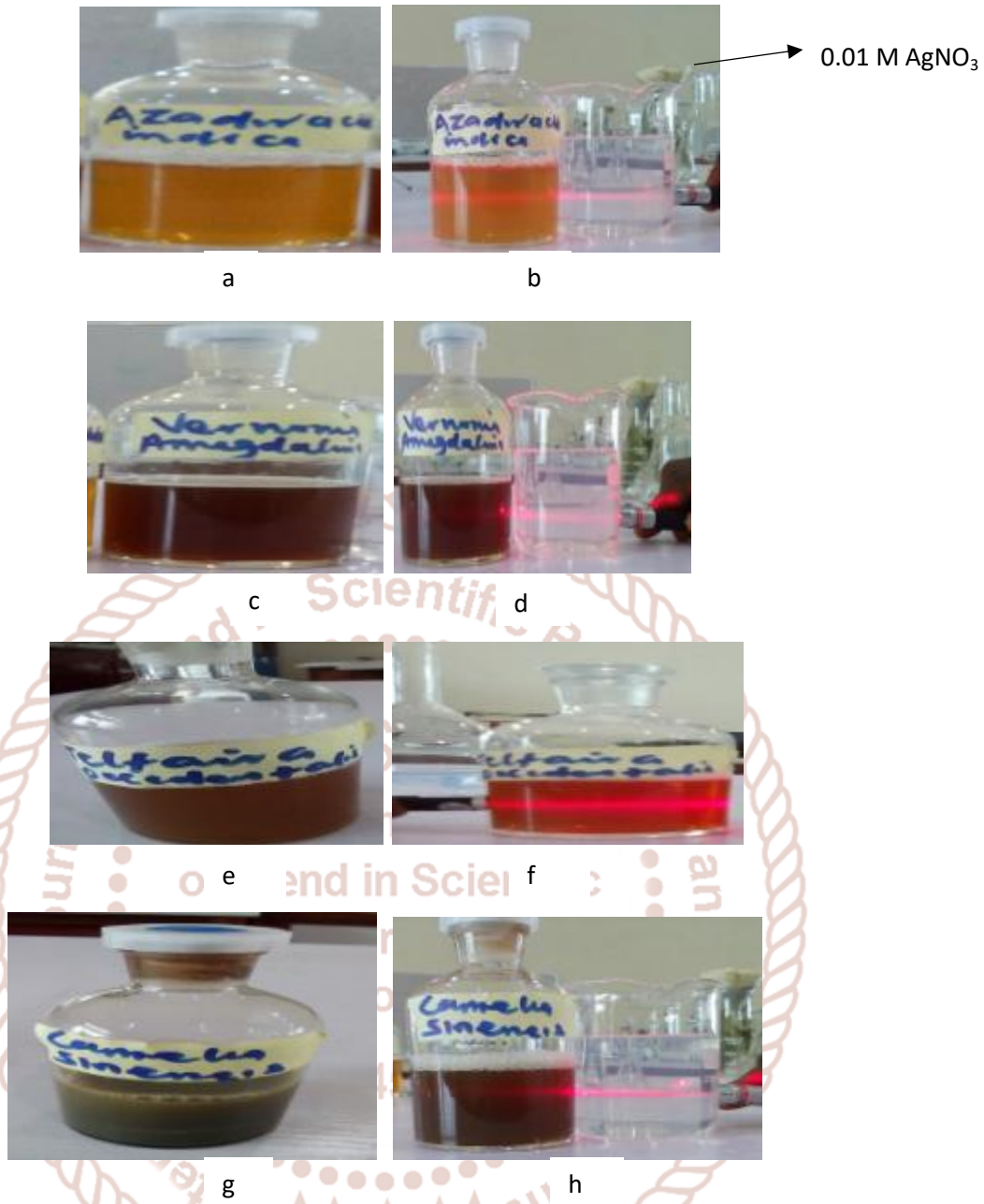

Fig. 14 (a.) *AzadirachtaIndica*extract from dried leaves(b) laser light pointed through 0.01M $AgNO_3$ solution and a mixture of *azadirachtaindic*

a dried leaves extract and 0.01M $AgNO_3$ solution. The laser light goes straight through the $AgNO_3$ solution, but was slightly scattered in the mixture of*azadirachtaindica* and $AgNO_3$ solution. (c) *VernoniaAmigdalina*dried leaves extract(d) the laser light is slightly scattered by the mixture of *vernoniaamigdalna* and 0.01M $AgNO_3$. (e)*TelfairiaOccidentalis*dried leaves extract (f) the laser light is slightly scattered by the mixture of *telfariaoccidentalis* and 0.01M $AgNO_3$. (g) *Camellia Sinensis*dried leaves extract(h) some scattering of the laser light occurs in the mixture of *camellia sinensis* and 0.01M $AgNO_3$ solution. Color change occurs in all the cases where 0.01M $AgNO_3$ solution was added to the dried leaves extracts.

The mixture of the synthesized Ag NPs using the extracts from the dried leaves 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. 15.

Fig. 15: Silver Nanoparticles from Dried Leaves Extract without any Capping Agent.

3. Results and Discussion

3.1 Results

The results of the research work are as displayed below:

3.1.1 AgNPs Bio-Synthesis using Dried and Fresh Leaves Extract

Table 1: Dried *AzadirachtaIndica*Leaves Extract and 0.01M $AgNO_3$ Solution

PROCEDURE	OBSERVATIONS	COMMENTS
20 mL of 0.01 M $AgNO_3$ was added to 180 mL of *A. indica* leaves extract	No notable colour change	Initial time of the reaction
15 min	Slight change in the colour from light brown to dark brown	Reduction of Ag ion to AgNPs by reducing agent.
30 min	Slight change in the colour from light brown to dark brown	More formation of AgNPs
45 min	More change in the colour from light brown to dark brown	Formation of AgNPs.
60 min	Dark brown, no more colour change	Indicating reduction reaction after 1 hr.
Testing with Laser pointer Light	Scattering of Laser light was observed	Confirmation of AgNPs was indicated (Tyndall effect).

Fig.16a Dried *A. Indica*, Initial (yellowish)

Fig.16b Dried *A. indica*, after I Hour (Brownish)

Fig.16a and 16b Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Dried Leave Extract of *A. Indica.*

Table 2: Fresh *AzadirachtaIndica*Leave Extract and 0.01M $AgNO_3$ Solution

PROCEDURE	OBSERVATIONS	COMMENTS
20 mL of 0.01 M $AgNO_3$ was added to 180 mL of *A. indica* leaves extract	No notable colour change	Initial time of the reaction
15 min	Colour changes gradually from light green to pale green	Reduction of Ag ion to AgNPs by reducing agent.

30 min	Pale green solution turns brown.	Reduction of Ag ion to AgNPs by reducing agent.
45 min	Slight formation of precipitates	Reduction of Ag ion to AgNPs by reducing agent
60 min	No further colour change	Reduction reaction after 1 hr
Testing with Laser pointer Light	Scattering of Laser light was observed	Confirmation of AgNPs was indicated (Tyndall Effect).

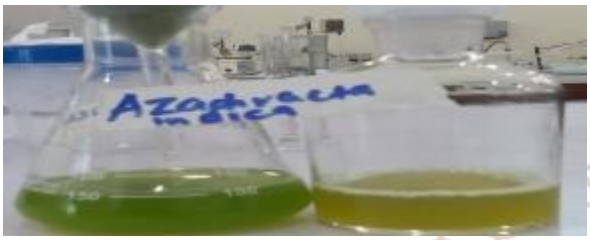

Fig. 17.a: Fresh *A. Indica,* Initial (Pale Green)

Fig.17.b: Fresh *A. Indica*, after 1 hr. (Brownish)

Fig.17a and 17b: Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Fresh Leave Extract of *A. Indica.*

Table 3: Dried *VernoniaAmigdalina*Leaves Extract and 0.01M $AgNO_3$ Solution

PROCEDURE	OBSERVATIONS	COMMENTS
20 mL of 0.01 M $AgNO_3$ was added to 180 mL of *V. Amigdalina* leaves extract	No notable colour change.	Initial time of the reaction
15 min	No notable colour change	No reduction reaction yet.
30 min	Slight colour change was observed from coffee brown to dark brown	Reduction reaction starts to occur.
45 min	Solution continues to become more dark brown	Reduction of Silver ion to Ag NPs.
60 min	No further colour change	Reduction reaction after 1hr.
Testing with Laser pointer light	Scattering of Laser light was observed	Confirmation AgNPs was indicated (Tyndall Effect).

Fig. 18a Dried *V. Amigdalina*, Initial (Light brown)

Fig.18b: Dried *V. Amigdalina,* after 1hr.(Dark brown)

Fig.18a and 18b Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Dried Leaves Extract of *V. Amigdalina*.

Table 4: Fresh *VernoniaAmigdalina*Leaves Extract and 0.01M $AgNO_3$ Solution

PROCEDURE	OBSERVATIONS	COMMENTS
20 mL of 0.01 M $AgNO_3$ was added to 180 mL of *V. Amigdalina* leaves extract	No notable colour change.	Initial time of the reaction
15 min	No notable colour change. The chlorophyll was thick.	No reduction reaction yet.
30 min	Formation of precipitates makes the solution slightly lighter.	Reduction reaction starts to occur.
45 min	Formation of precipitates makes the solution slightly lighter	Reduction of Silver ion to Ag NPs.
60 min	No detectable colour change	Reduction reaction after 1 hr.
Testing with Laser pointer Light	Scattering of Laser light was observed	Confirmation of AgNPs was indicated (Tyndall Effect).

Fig. 19a Fresh *V. Amigdalina,* Initial (Brownish) Fig.19b Fresh *V. Amigdalina,* after 1 hr(Dark brownish)

Fig. 19a and 19b Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Fresh Leave Extract of *V. Amigdalina*

Table 5: Dried *TelfairiaOccidentalis*Leaves Extract and 0.01M $AgNO_3$ Solution

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 becomes 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 after 1hr.
Testing with Laser pointer light	Scattering of Laser light was observed	Confirmation of AgNPs was indicated (Tyndall Effect).

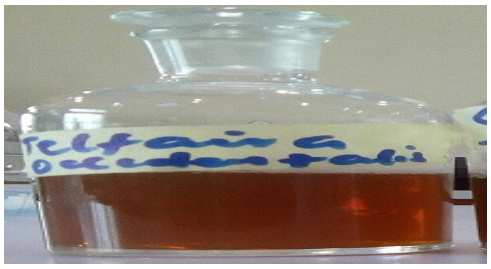

Fig. 20a Dried *T. Occidentalis.* Initial(Light brown)

Fig. 20b Dried *T. Occidentalis.*(Dark brownish)

Fig. 20a and 20b: Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Dried Leave Extract of *T. Occidentalis.*

Table 6: Fresh *TelfairiaOccidentalis*Leaves Extract and 0.01M $AgNO_3$ Solution

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 and precipitate were observed	Reduction reaction taken place.
30 min	More precipitates continue to form, making the solution clearer. Solution was chlorophyll ridden.	Reduction reaction continues which indicates the formation of AgNPs.
45 min	More precipitates continue to settle at the bottom	Formation of Ag NPs.
60 min	No further colour change. Level of precipitates continues to increase in volume.	Reduction reaction after 1 hr.
Testing with Laser pointer Light	Scattering of Laser light was observed	Confirmation of AgNPs was indicated (Tyndall Effect).

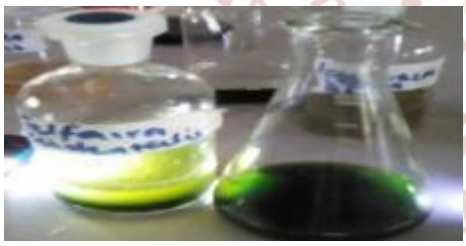

Fig. 21a Fresh *T.Occidentalis,* Initial (Dark Green)

Fig.21b Fresh *T. Occidentalis,* after 1hr.(Light Green)

Fig. 21a and 21b Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Fresh Leave Extract of *T. Occidentalis.*

Table 7: Dried *Camellia Sinensis* Leaves Extract and 0.01M $AgNO_3$ Solution

PROCEDURE	OBSERVATIONS	COMMENTS
20 mL of 0.01 M $AgNO_3$ was added to 180 mL of *C. sinensis* leaves extract	No notable colour change	Initial reaction time
15 min	Rapid colour change of the solution from light brown to dark brown	Reduction of Ag NPs started indicating the formation of Ag NPs.

30 min	Rapid colour change of the solution From dark brown to milky deep brown	Reduction reaction still in progress indicating the formation of Ag NPs.
45 min	Rapid colour change	Reduction of Ag ion to AgNPs by reducing agent.
60 min	Milky deep brown	Reaction after 1hr.
Testing with Laser pointer Light	Scattering of Laser light was observed	Confirmation of AgNPs was indicated (Tyndall Effect).

Fig.22a Dried *C. Sinensis* Initial stage of reaction (yellowish) Fig.22b Dried *C. Sinensis* after 1 hr. (Darkbrown)

Fig. 22a and 22b Indicates the Colour Change of the Reaction Mixture of Silver Nitrate and Dried Leave Extract of *C. Sinensis.*

3.1.2 Some Properties of the Leave Extract during Biosynthesis of AgNPs

Table 8: Electronic Conductivity, pH and Temperature of the leave extract and $AgNO_3$ Solution

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	***A.indica***	**2.40 (25.3 ^{o}C)**	**2.05 (25.4^{o}C)**	**6.82(25.9C)**	**7.00(25.7^{o}C)**
2	***V. Amigdalina***	**1504 (26.2^{o}C)**	**1338 (26.1^{o}C)**	**7.84(25.9C)**	**6.83(25.8^{o}C)**
3	***T. Occidentalis***	**11051(25.3^{o}C)**	**1002 (25.5^{o}C)**	**4.90(25.7^{o}C)**	**4.95(25.7^{o}C)**
4	***C. Sinensis***	**957 (28.1^{o}C)**	**519 (27.6^{o}C)**	**6.76(27.0^{o}C)**	**6.05(26.1^{o}C)**

3.1.3 Result of Characterization of the AgNPs Produced

Table 9: Result of ED-XRF analysis

CHEMICAL COMPSITION (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

3.2 Discussion

Tables 1-2 gives the result of the biosynthesis ofAgNPs using Dried and Fresh Leaves Extract of *Azadirachtaindica.* The result showed that the colour change was faster with the dried leaves than with the fresh leaves extract. Precipitates formation obviously were more using dried leaves extract than fresh leaves extract; indicated by a deep brown colour after one hour. The solution with the dried leave extract produced more AgNPs on the filter paper after 24 hours than the solution with fresh leave extract. Laser light pointed at the two solutions revealed more scattering in the biosynthesis solution containing dried *azadirachtaindica* leave extract. 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. The method used in determining the formation of AgNPs agrees with above.

Tables 3-7 captures the biosynthesis process of AgNPs from dried and fresh leaves extract. The results showed colour change after 1 hr of monitoring the changes in colour when the extracts were added to the 0.01M $AgNO_3$ solution. The dried leaves extracts reacted faster by changing the colour of the solutions. The fresh leaves of bitter leaf (*vernoniaamydalina)* and fluted pumpkin (telfairiaoccidentalis) were less effective in effecting quick colour change after 1 hr. this may not be unconnected to the thick chlorophyll content of the extracts from these fresh leaves. According to researchers; the colour change and precipitates formation is the indication of nanoparticles formation and reduction of the 0.01M $AgNO_3$ solution by the leave extracts (Poinern, 2015; Essien and Otobong, 2017).

Table 8 shows the change in electronic conductivity, temperature, and pH before and as the extracts were added to the 0.01M $AgNO_3$ solution. In all the mixtures of the extracts and the $AgNO_3$ solution the electronic conductivity dropped indicating that reduction process of $Ag+$ ion to Ag^o was taking place and there were less active radicals or ions in the solution (Essien and Otobong, 2017). The temperature change was not too significant in most cases. The reduction process was not too exothermic in nature. There was change in pH value of the mixture as the extracts were mixed with $AgNO_3$ solution. In the case of *A. indica* the pH slightly increased. For *vernoniaamydalina*, the pH decreased; for *telfairiaoccidentalis,* the pH increased and for *camellia sinensis*, the pH dropped from 6.76 to 6.05. This changes in pH were indicators of change in chemical composition of the mixtures. The work also showed that the pH of the leave extracts varied from that of the dried leaves. Several authors have shown that the acidic components of plants extracts can easily aid in reduction of metallic ions (Poinern, 2015; Shreya, *et al.,* 2015; Jannathul, and Lalitha, 2015; Biswas, and Dey, 2015;Benakashani, *et al.,* 2016; Bansal, *et al.,* 2017; Essien and Otobong, 2017).

Table 9 shows the chemical composition of the characterized AgNPs which were produced using plants extracts. The machine used for the characterization was calibrated to measure oxides (compounds) and not elements; and that explains why the result was in oxide form. The nanoparticles for the analysis were obtained by allowing the mixture to stay for 24 hrs before filtering and drying the residue in the oven to obtain dry AgNPs. The dried leave extracts of *A. indica,T.occidentalis, V. amydalina*and *C. sinensis* produced higher quality and quantity of AgNPs than their fresh leaves counterparts. The performance of *A.indica* fresh leave was better than the other fresh leaves in the biosynthesis of AgNPs. The chemical analysis showed that AgNPs produced contained 71.8% Ag_2O, 25.0% SiO_2, 2.18% Cl, and other trace elements.

4. CONCLUSION

The research work titled ‘’Biosynthesis of Eco-friendly Silver Nanoparticles: The Efficiency of Fresh Leaves and Dried Leaves in the Synthesis of Silver Nanoparticles’’ was investigated and the following conclusions drawn from the work:

i. The work has established that leaves extracts of plants can be used to reduce AgNPs from $AgNO_3$ solution.

ii. The work has established that dried leaves extracts of the four plants used were more efficient and effective in the biosynthesis of AgNPs from $AgNO_3$ than their fresh leaves extract counterparts.

iii. The AgNPs produced contained 71.8% Ag_2O which can be improved upon by further purification. The oxygen may have been attached on the surface and not the core of the particles.

iv. Qualitative analysis using laser light pointer, observed colour change and precipitates formation clearly indicated the biosynthesis of AgNPs using the leave extracts as reducing agents.

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