Green synthesis of silver nanoparticles from leaf extract of *Tetrapleura tetraptera* and its antimicrobial activity

B O Ogunsile*1, D O Seyinde2 and B A Salako1

1Department of Chemistry, University of Ibadan, Ibadan, Nigeria
2Department of Chemical Sciences, Anchor University, Lagos, Nigeria

*E-mail: ogunsile@yahoo.com.

Abstract

Plants have very high potential as alternative source of bio-reducing and capping agents in nanoparticles (NPs) synthesis. Silver nanoparticles (AgNPs) were synthesized from *Tetrapleura tetraptera* leaf extract. The UV-vis spectrophotometer was used to observe the formation of the NPs at various time intervals and ratios of leaf extract to AgNO₃ solution. The properties of the AgNPs synthesized were characterized by FTIR and Scanning Electron Microscope (SEM) equipped with Energy Dispersive X-ray Spectroscopy (EDX). Antimicrobial activities of the AgNPs synthesized were evaluated against ten human pathogens using agar well diffusion method. The rate of formation of the AgNPs was shown to increase with respect to time and ratio of leaf extract to AgNO₃ solution. The EDX result showed signal energy peak for silver atom at 3.0-3.8 KeV. The AgNPs was characterized with FTIR peaks around 3280, 1620, 1400 and 1040 cm⁻¹ assigned to O-H, C=O, C–O–H and C-C groups respectively, as possible stabilizing and capping groups. The AgNPs showed maximum inhibitory activity against *Staphylococcus aureus, Escherichia coli* and *Salmonella* sp.

1 Introduction

The synthesis of nanoparticles using environmentally friendly biosynthetic methods is fast emerging [1]. Biosynthetic methods is currently being used in the production of large quantities of nanoparticles that are free from contamination with well defined size and morphology while reducing the chance of failure with greater ease of characterization [2]. Green synthesis of nanoparticles is an alternative approach to the more complex chemosynthetic procedures. Most significantly is its advantage that conditions of high temperature, pressure, energy and toxic chemicals are not required in the biosynthesis [3]. Biologically synthesized methods employ
Nanotechnology Applications in Africa: Opportunities and Constraints

IOP Conf. Series: Materials Science and Engineering 805 (2020) 012032
IOP Publishing
doi:10.1088/1757-899X/805/1/012032

microorganisms, marine algae, live plants, plant tissues, fruits and plant extracts for the production of various nanoparticles [4-6]. Although nanoparticles can be synthesized using different biogenic routes, their production using simple and less expensive plant extract to a rather complex and expensive methods based on plant tissue and microbial processes is an increasing focus of attention [3,7-16]. The synthesis of nanoparticles using plant extract is advantageous in that it is easily scalable and can be used as both a bio-reducing and capping agents. Plants are known to contain different concentrations and compositions of bioreducing agents [17]. The quality and quantity of the nanoparticles produced are affected by the source and nature of the plant extract and other operational variables like pH, reaction time, and volume ratio of plant extract to the metallic precursor [18].

Silver nanoparticles are of great interests to nanotechnologist because of their unique physiochemical properties when compared to their nano-scaled counterparts [19], especially in its role in nanomedicine as therapeutic and diagnostic agents [20-23]. Silver nanoparticles have been produced from various plant extracts such as tea extract [24], beet juice [25], Mussaenda glabrata leaf extract [26], Thymbra spicata extract [27], Pedalium murex leaf extract [22], aqueous extract from geranium leaf [28], blackberry, blueberry, pomegranate, and turmeric extracts [29].

Tetrapleura tetraptera (known locally as Aridan) is a species of flowering plant in the pea family normally found in Western Africa. It contains high amount of essential phytochemicals and nutrients needed for proper body functioning [30]. Due to its high vitamin content, it is used as a dietary supplement, a spice and as medicine. Studies have been done to discover its molluscicidal [31], anticonvulsant [32], hypotensive, anti-inflammatory [33] and antipathogenic properties [34] but its silver nanoparticles potential had not be reported. This paper is aimed at synthesizing silver nanoparticles from T. tetraptera leaf extract and evaluation of its antimicrobial activity against ten selected human pathogens.

2 Materials and methods

2.1 Selection and collection of plant material

The leaves of T. tetraptera were collected from the botanical garden at the University of Ibadan campus, Ibadan, Nigeria. The leaves were sliced and washed thoroughly with de-ionized water.
2.2 Biosynthesis of silver nanoparticles

About 60 g of the sliced leaves was boiled in 600 ml of de-ionized water on the hot plate to obtain the leaf extract. The extract obtained was filtered into a 250 ml Erlenmeyer flask and kept in the refrigerator. Equal volume (20 ml) of 1 mM AgNO₃ solution was added to the extract in a conical flask and the mixture was incubated on a hot plate at 70 °C under agitation for 10-45 min. The change in colour as a function of the bioreduction of silver ion to metallic silver nanoparticles was monitored.

2.3 Effect of time

The effect of time on the bioreduction of silver ion by the leaf extract was investigated. Exactly 30 ml of T. tetraperta leaf extract was added to 90 ml of 1 mM AgNO₃. The absorbance of the reaction mixture was read on UV-visible spectrophotometer at 15 min intervals for 45 min.

2.4 Effect of volume ratio

The effect of varying the volume of 1 mM AgNO₃ solution used to a fixed volume of the leaf extract in the bioreduction process was monitored using UV-visible spectrophotometer. Approximately 5 ml of 1 mM AgNO₃ solution was added to 5 ml of leaf extract and incubated on a hot plate at 70 °C for 10 min, after which a color change was observed. Ratios 1:2 and 1:3 of AgNO₃ solution to leaf extract were also carried out.

2.5 Characterization of the synthesized silver nanoparticles

Four characterization techniques were used for the synthesized AgNPs. The formation of the NPs with respect to time and ratios of leaf extract to AgNO₃ solution was monitored using the UV-vis spectrophotometer (Perkin Elmer, Lamda 25, Germany). The bioreduction of silver ions was observed by measuring the UV-visible spectrum of the solutions at wavelength range of 300-900 nm. Organic functional groups present in the leaf extract and AgNPs were detected using FTIR (Perkin Elmer LS-55-Luminescence spectrometer). The elemental composition of metal nanoparticles was established using energy dispersive x-ray spectroscopy (EDX), while scanning electron microscope (SEM) was employed for morphological characterization.

2.6 Antimicrobial activity by well diffusion method

The synthesized AgNPs from T. tetrapleura was screened for its antimicrobial activity by well diffusion method against ten (10) pathogenic organisms (six bacteria and four fungi). The pure cultures of organism were sub-cultured on sterile nutrient agar at 45 °C. Each strain was swabbed uniformly on the individual plates using sterile cotton swabs. Then, 8 mm well size was made on
sterile Petri dishes using sterile cork borer. Using micropipette, the nanoparticles solution was released into the wells on all plates. The negative and positive control used for bacteria and for fungi are solvent, gentamycin and ticonazole. After incubation at 37 °C for 24 h, the levels of zones of inhibition were measured.

3 Results and discussion

The aqueous silver ions were reduced to zero valent silver nanoparticles when added to natural leaf extract of *T. tetrapleura* as observed by the color change from faint yellow to reddish brown. The color change has been attributed to the surface plasmon resonance phenomenon (SPR) [35]. The synthesized nanoparticles showed a spectrum range around 390 to 450 nm. It is reported in literature that AgNPs show spectrophotometric absorption measurements in the wavelength ranges of 400-450 nm [36]. The aggregation of the silver nanoparticles in the solution is indicated by the presence of broad resonance (Figure 1).

The bioreduction of the silver ions occurs fairly rapidly with more than 90% of reduction of Ag⁺ ions completed less than 1 h after its addition to the leaf extract. Higher SPR peaks intensities were observed at the higher time intervals which indicate the increase in the production of smaller silver nanoparticles (Figure 2). After addition of the leaf extract to 1 mM AgNO₃ solution at different ratios, the curve shows increased absorbance at higher volume ratio. This apparently indicates the extracellular bioreduction of the Ag⁺ ions [35].

FTIR spectroscopic studies of both the leaf extract and the AgNPs produced were carried out to investigate the possible bioreducing and capping agents present in the leaf extract. The spectra of the *T. tetraplera*-AgNPS showed FTIR peaks around 3280, 1620, 1400 and 1040 cm⁻¹ assigned to O-H, C=O, C–O–H and C-C groups absorption bands as the possible stabilizing and capping groups (Figure 4). The result revealed that the silver nanoparticles were synthesized due to the bioreducing property of the leaf extract.
Figure 1. UV-vis spectra of *T. tetraperta* extract and its AgNPs

Figure 2. UV-vis spectra of *T. tetraperta* AgNPs at various time intervals
Figure 3. UV-vis spectra of *T. tetraaptera* AgNPs at different volume ratio

The morphology of the AgNPs as revealed by SEM showed the formation of spherical, crystalline AgNPs (Figure 5). The EDX result (Figure 6) confirmed the elemental silver signal energy peak at 3.0-3.8 KeV. The presence of signals from other trace element like carbon and oxygen is likely as a result of the direct usage of the synthesized AgNPs from the native reaction solution prior to further purification.

Figure 4. FTIR spectra of *T. tetraaptera* plant and AgNPs obtained from *T. tetraaptera* extract

Figure 5. SEM images of the AgNPs synthesized by *T. tetraaptera* extract
The AgNPs showed the greatest antimicrobial activity against *S. aureus* (14 mm), *E. coli* (14 mm) and *Salmonella* sp (14 mm) and no inhibitory effect against *B. subtilis*, *Pseudomonas* sp, *Rhizopus* sp and *P. aeruginosa* (Table 1). The synthesized AgNPs showed greater antimicrobial property towards bacteria pathogens compared to fungi pathogens under this study [36].

**Table 1.** Zone of inhibition of the synthesized silver nanoparticles from *T. tetraperta* plant extract against various microbes

| Organism/concentration     | Zone of inhibition (mm) |
|----------------------------|-------------------------|
|                            | 100mg/ml | 50 mg/ml | 25mg/ml | 12.5mg/ml |
| *S. aureus*                | 14        | 12       | 10      | -         |
| *E. coli*                  | 14        | 10       | -       | -         |
| *B. subtilis*              | -         | -        | -       | -         |
| *Pseudomonas* sp           | -         | -        | -       | -         |
| *Salmonella* sp            | 14        | 12       | 10      | -         |
| *Klebsiella pneumoniae*    | 12        | 10       | -       | -         |
| *Candida albicans*         | 12        | 10       | -       | -         |
| *Aspergillus niger*        | 12        | 10       | -       | -         |
| *Rhizopus* sp              | -         | -        | -       | -         |
| *P. aeruginosa*            | -         | -        | -       | -         |
4 Conclusion

The study presents an easy, economical and efficient route for the production of AgNPs using T. tetraptera leaf extract. The synthesized AgNPs were characterized and confirmed using UV-vis spectroscopy, FTIR and SEM equipped with EDX. The silver ions were believed to be reduced to metallic AgNPs by the phytochemicals present in T. tetraptera leaf extract. The synthesized AgNPs showed efficient antifungal and antibacterial activities.

References

[1] Gour A, Jain N K 2019 Advances in green synthesis of nanoparticles. Artif. Cells Nanomed. Biotechnol. 47 (1) pp 844-851.

[2] Abdelghany T M, Al-Rajhi A M H, Al Abboud M A, Alawlaqi M M, Ganash Magdah A, Helmy E A M and Mabrouk A S 2018 Recent advances in green synthesis of silver nanoparticles and their applications: about future directions- A review. BioNanoSci. 8 (1) pp 5-16.

[3] Singh A, Jain D, Upadhyay M, Khandelwal N and Verma H 2010 Green synthesis of silver nanoparticles using Argemone mexicana leaf extract and evaluation of their antimicrobial activities. Dig. J. Nanomater. Biostruct. 5 (2) pp 483-489.

[4] Chandran S P, Chaudhary M, Pasricha R, Ahmad A and Sastry M 2006 Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnol. Prog. 22 (2) pp 577-583.

[5] Luangpipat T, Beattie I R, Chisti Y and Haverkamp R G 2011 Gold nanoparticles produced in a microalga. J. Nanopart. Res. 13 (12) pp 6439-6445.

[6] Rajesh S, Raja D P, Rathi J M and Sahayaraj K 2012 Biosynthesis of silver nanoparticles using Ulva fasciata (Delile) ethyl acetate extract and its activity against Xanthomonas campestris pv. malvacearum. J. Biopest. 5 pp 119-128.

[7] Dhillon G S, Brar S K, Kaur S and Verma M 2012 Green approach for nanoparticle biosynthesis by fungi: current trends and applications. Crit. Rev. Biotechnol. 32 (1) pp 49-73.

[8] Li X, Xu H, Chen Z S and Chen G 2011 Biosynthesis of nanoparticles by microorganisms and their applications. J. Nanomater. Article ID 270974. https://doi.org/10.1155/2011/270974.
[9] Ali D M, Thajuddin N, Jeganathan K and Gunasekaran M 2011 Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. *Colloids Surf. B Biointerf.* **85** (2) pp 360-365.

[10] Bankar A, Joshi B, Kumar A R and Zinjarde S 2010 Banana peel extract mediated novel route for the synthesis of silver nanoparticles. *Colloids Surf. A: Physicochem. Eng. Aspects* **368** (1-3) pp 58-63.

[11] Baskaralingam V, Sargunar C G, Lin Y C and Chen J C 2012 Green synthesis of silver nanoparticles through *Calotropis gigantea* leaf extracts and evaluation of antibacterial activity against *Vibrio alginolyticus*. *Nanotechnol. Dev.* **2** (1) e3. https://doi.org/10.4081/nd.2012.e3.

[12] Castro L, Blázquez M L, Muñoz J A, González F, García-Balboa C and Ballester A Biosynthesis of gold nanowires using sugar beet pulp. *Process Biochem.* **46** (5) pp 1076-1082.

[13] Daisy P and Saipriya K 2012 Biochemical analysis of *Cassia fistula* aqueous extract and phytochemically synthesized gold nanoparticles as hypoglycemic treatment for diabetes mellitus. *Int. J. Nanomed.* **7** pp 1189-1202.

[14] Kaler A Nankar R Bhattacharyya M S and Banerjee U C 2011 Extracellular biosynthesis of silver nanoparticles using aqueous extract of *Candida viswanathii*. *J. Bionanosci.* **5** (1) pp 53-58.

[15] Lee H J, Lee G, Jang N R, Yun J H, Song J Y and Kim B S 2011 Biological synthesis of copper nanoparticles using plant extract. *Nanotechnol.* **1** (1) pp 371-374.

[16] Park Y, Hong Y N, Weyers A, Kim Y S and Linhardt R J 2011 Polysaccharides and phytochemicals: a natural reservoir for the green synthesis of gold and silver nanoparticles. *IET Nanobiotechnol.* **5** (3) pp 69-78.

[17] Mukunthan K and Balaji S 2012 Cashew apple juice (*Anacardium occidentale* L.) speeds up the synthesis of silver nanoparticles. *Int. J. Green Nanotechnol.* **4** (2) pp 71-79.

[18] Dwivedi A D and Gopal K 2010 Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. *Colloids Surf A: Physicochem. Eng. Aspects* **369** (1-3) pp 27-33.
[19] Gong P, Li H, He X, Wang K, Hu J and Tan W 2007 Preparation and antibacterial activity of Fe$_3$O$_4$@Ag nanoparticles. *Nanotechnol.* 18 (28) pp 604-611.

[20] Ghiută I, Cristea D, Croitoru C, Kost J, Wenkert R, Vyrides I, Anayiotos A and Munteanu D 2018 Characterization and antimicrobial activity of silver nanoparticles, biosynthesized using *Bacillus* species. *Appl. Surf. Sci.* 438 pp 66-73.

[21] Khatami M, Varma R S, Zafarnia N, Yaghoobi H, Sarani M and Kumar V G 2018 Applications of green synthesized Ag, ZnO and Ag/ZnO nanoparticles for making clinical antimicrobial wound-healing bandages. *Sustain. Chem. Pharm.* 10 pp 9-15.

[22] Anandalakshmi K, Venugobal J and Ramasamy V 2016 Characterization of silver nanoparticles by green synthesis method using *Pedaliun murex* leaf extract and their antibacterial activity. *Appl. Nanosci.* 6 (3) pp 399-408.

[23] Shankar S and Rhim J-W 2015 Amino acid mediated synthesis of silver nanoparticles and preparation of antimicrobial agar/silver nanoparticles composite films. *Carbohydr. Polym.* 130 pp 353-363.

[24] Nadagouda M N and Varma R S 2008 Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. *Green Chem.* 10 (8) pp 859-682.

[25] Kou J and Varma R S 2012 Beet juice – induced green fabrication of plasmonic AgCl/Ag nanoparticles. *ChemSusChem.* 5 (12) pp 2435-2441.

[26] Francis S, Joseph S, Koshy E P and Matthew B 2017 Green synthesis and characterization of gold and silver nanoparticles using *Mussaenda glabrata* leaf extract and their environmental applications to dye degradation. *Environ. Sci. Pollut. Res. Int.* 24 (21) pp 17347-17357.

[27] Veisi H, Azizi S and Mohammadi P 2018 Green synthesis of the silver nanoparticles mediated by *Thymbra spicata* extract and its application as a heterogeneous and recyclable nanocatalyst for catalytic reduction of a variety of dyes in water. *J. Clean Prod.* 170 pp 1536-1543.

[28] Rivera-Rangel R D, Gonzalez-Munoz M P, Avila-Rodriguez M, Razo-Lazcano T A and Solans C 2018 Green synthesis of silver nanoparticles in oil-in-water microemulsion and nano-emulsion using geranium leaf aqueous extract as a reducing agent. *Colloids Surf. A: Physicochem. Eng. Aspects* 536 pp 60-67.
[29] Nadagouda M N, Iyanna N, Lalley J, Han C, Dionysiou D D and Varma R S 2014 Synthesis of silver and gold nanoparticles using antioxidants from blackberry, blueberry, pomegranate, and turmeric extracts. *ACS Sustain. Chem. Eng.* 2 (7) pp 1717-1723.

[30] Enema O J, Umoh U F, Thomas P S, Adesina S K and Eseyin O A 2019 Phytochemical and antioxidant studies of leaf of *Tetrapleura tetraptera* (Schumand Thon) Taubert (Mimosaceae). *Br J. Pharm. Med. Res.* 04 (03) pp 1865-1875.

[31] Akin-Idowu P E, Ibibo O D, Ademoyegum O T and Adeniyi O T 2011 Chemical composition of the dry fruit of *T. tetraptera* and its impact on human health. *J. Herbs Spices Med. Plants* 17 (2) pp 52-61.

[32] Akintola O O, Bodede A I and Ogunbanjo O R 2015 Nutritional and Medicinal Importance of *Tetrapleura tetraptera* Fruits (Aridan) *Afr. J. Sci. Res.* 6 (4) pp 33-38.

[33] Aderibigbe A O, Iwalewa E O, Adesina S K, Adebanjo A O and Ukponmwan O E 2007 Anti-convulsant, analgesic, and hypothermic effects of aridanin, isolated from *Tetrapleura tetraptera* fruit in mice. *J. Biol Sci.* 7 (11) pp 1520-1524.

[34] Awofisayo S O, Udoh I E and Mbagwu H O C 2010 Antibacterial effects of the aqueous and ethanolic extracts of *Tetrapleura tetraptera* pods on the pathogens in nosocomial wound infections. *J. Pharmacogn. Herbal Formul.* 1 (2) pp 18-23.

[35] Logeswari P, Sivagnanam S and Jayanthi A 2012 Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. *J. Saudi Chem. Soc.* 19 (3) pp 311-317.

[36] Mittal A K, Kaler A and Banerjee U C 2012 Free radical scavenging and antioxidant activity of silver nanoparticles synthesized from flower extract of *Rhododendron dauricum*. *Nano Biomed. Eng.* 4 (3) pp 118-124.