Trop J Nat Prod Res, May 2024; 8(5): 7285-7289 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Characterization and Evaluation of Potential Antibacterial Activity of Green Synthesized Silver Nanoparticles from *Guiera senegalensis* Leaf Extract

Ameh B. Amedu[1]*, Ahmad Uba[2], Muhammad Chika[1], Wasagu R. S. Umar[3]

[1]*Department of Energy and Applied Chemistry, Faculty of Chemical and Life Science, Usmanu Danfodiyo University, Sokoto, Nigeria*
[2]*Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.*
[3]*Department of Biochemistry and Molecular Biology, Faculty of Chemical and Life Science, Usmanu Danfodiyo, University, Sokoto, Nigeria.*

ARTICLE INFO

Article history:
Received 13 November 2023
Revised 28 April 2024
Accepted 30 April 2024
Published online 01 June 2024

ABSTRACT

This study investigates *Guiera senegalensis* J.F. Gmel (Combretaceae), a widely acknowledged herb in Africa for its medicinal properties against various ailments, including fever, diarrhea, diabetes, dysentery, eczema, malaria, cough, tuberculosis, and its potential to enhance milk production in lactating women. The research focuses on utilizing *Guiera senegalensis* for synthesizing silver nano-particles (GS-AgNPs), due to its abundance in primary and secondary metabolites. The primary aim is to produce GS-AgNPs and assess their antibacterial efficacy. The synthesis of GS-AgNPs involved mixing a silver salt solution with the water leaf-extract. Characterization of the nanoparticles was conducted using Ultraviolet-visible spectroscopy with surface-plasmon-resonance (S-P-R) analysis at 390 nanometers (nm). Fourier transform infrared spectroscopy (F-T-IR) investigated the identity of the phytochemicals responsible for the green reduction of silver ions. X-ray diffraction (X-RD) confirmed the face-centered-cubic crystallinity of the GS-AgNPs with a mean size (33 nm). The antibacterial potential of GS-AgNPs was evaluated against both gram-negative (*Escherichia coli* and *Salmonella typhi*) and gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) using the Agar-well method. Results demonstrated the inhibitory effect of GS-AgNPs on microbial growth, with zone-of-inhibition (Z-OI) ranging from 4-7 millimeters (mm) to 16-19 mm at concentrations of 10 mg/cm³ and 20 mg/cm³, respectively. In comparison, the aqueous leaf extract exhibited Z-OI between 1.3-6 mm. GS-AgNPs displayed superior antibacterial activity against both Gram-positive and Gram-negative bacteria when compared to the aqueous leaf extract.

Keywords: Antibacterial, Green synthesis, *Guiera senegalensis*, Silver-nanoparticles, Gram-positive, Gram-negative

Introduction

Nano-particles (NPs) have found widespread use across various scientific fields such as pharmaceutics, biomedicine, agriculture, textiles, electronics, and medicine, garnering significant interest in recent years.[1,2] Notably, NPs including gold, copper, zinc, iron, and silver have gained substantial attention.[2,3,4,5] Silver-based nanoparticles, in particular, have emerged as one of the most extensively researched nano-materials due to their diverse applications including antimicrobial, drug delivery, anti-inflammatory, and wound dressing properties. [6,7] Previous studies have explored the antimicrobial, antioxidant, and anti-cancer potential of NPs. [8,9,10] One efficient technique gaining traction is the clean synthesis method, which replaces harmful chemicals with natural compounds. Plants, including their roots, leaves, and bark, have been identified as valuable resources for nanoparticle synthesis. [2,9,11,12] Green production of silver nanoparticles (AgNPs) using plant extracts has garnered significant attention due to their nonpathogenic, environmentally friendly, cost-effective, and straightforward synthesis process. [14]

*Corresponding author. E mail: abamedu@yahoo.com
Tel: +2348038092132

Citation: Amedu AB, Uba A, Chika M, Umar WRS. Characterization and Evaluation of Potential Antibacterial Activity of Green Synthesized Silver Nanoparticles from *Guiera senegalensis* Leaf Extract. Trop J Nat Prod Res. 2024; 8(5): 7287-7291. https://doi.org/10.26538/tjnpr/v8i5.34

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Guiera senegalensis, commonly known as "Sabara" by the Hausas and other tribes in Northern Nigeria, is a shrub native to the Sahel region spanning from Mauritania to Northern Nigeria and Sudan. [14,15] This plant is rich in phenols and flavonoid chemicals and is traditionally used to supplement breast milk in lactating women and to treat diarrhea and fever. [16] In African traditional medicine, *Guiera senegalensis* leaves (GS-L) are renowned as a "cure-all" and are utilized to treat various ailments including malaria, asthma, diabetes, dysentery, eczema, cough, and tuberculosis. [17] GS-L, chosen for its high content of phenols and flavonoids, holds promise for the fabrication of silver phytochemical nano-particles due to their nonpathogenic nature, ecological safety, low cost, and simplicity of production. [14,17] However, there is a notable gap in the literature regarding the clean synthesis of silver nanoparticles using *Guiera senegalensis* leaf extract. Therefore, this study aims to synthesize Ag-NPs from *Guiera senegalensis leaves* and evaluate their antibacterial potential.

Materials and Methods

Materials
Dimethyl sulfoxide and silver nitrate were procured from Loba Chemie, India, and Central Drug House, India, respectively. All chemicals used were of analytical grade.

Collection and identification of Plant material
Healthy leaves of *Guiera senegalensis* were collected in Wamakko, Sokoto-Nigeria, in February 2023 and identified by Musa Magagi, a herbarium expert at the Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. A voucher specimen

Trop J Nat Prod Res, May 2024; 8(5): 7285-7289 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

(PCG/UDUS/COMB/0002) was deposited in the herbarium. The leaves were washed, shade-dried, and powdered for further use.

Preparation of the plant-extract

The preparation of the plant-extract followed the method recommended by Liaqat *et al*. and Halilu *et al*. [20,21] with slight modifications. 30 g of the leaf powder was soaked in 300 cm^3 of distilled water (1:10 ratio) and heated for 10 minutes at 60 °C in a water bath (Memmert 854, W-Germany). After 24 hours of soaking, the mixture was filtered, and the supernatant was saved for Ag-NPs production. [20,21]

Solution of silver nitrate (1 mM)

A 1 mM (0.001 M) solution of silver nitrate ($AgNO_3$) was prepared by dissolving 0.085 g of $AgNO_3$ in 500 cm^3 of distilled water in a volumetric flask. [14,20]

Green synthesis of silver nano-particles from aqueous-leaf-extract of Guiera senegalensis (GS)

The formation of GS-AgNPs was carried out as per the method described by Liaqat *et al.*, with minor adjustments. 10 cm^3 of the aqueous leaf extract was added dropwise to 90 cm^3 of silver-nitrate solution (1 mM) and stirred continuously for 2 hours using a magnetic stirrer (SH-2, India), kept in the dark at room temperature. The change in colour of the mixture from yellow to dark-brown indicated the formation of GS-AgNPs. The particles were separated from the mixture by centrifugation (Hanil Science Industrial MF 80, Korea) for 20 minutes at 4000 revolutions per minute (r-p-m) and dried (Thermostat Oven DHG-9023A, China) for further analysis.[20]

Characterization of GS-AgNPs

UV-vis spectrophotometer analysis of GS-AgNPs

The UV-vis spectra of GS-AgNPs were recorded using a UV-Vis spectrometer (Double Beam, ATICO-ATE 4331, India) with a wavelength range of 200-750 nm.

F-T-IR Analysis of GS-AgNPs

F-T-IR spectra (Cary 630 Agilent Technologies, Malaysia) of GS-AgNPs and *Guiera senegalensis* leaf-extract were recorded between 4000–650 cm^{-1} for functional group identification,

X-ray diffraction analysis of GS-AgNPs

X-RD analysis (Rigaku, Miniflex 600-C, Tokyo, Japan) of GS-AgNPs was performed in the angular range of 3° and 90° with operating current of 15 mA and voltage of 40 kV to confirm crystallinity.

Antibacterial studies

Test microorganisms

Clinical isolates of Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria were acquired from Usmanu Danfodiyo University Teaching Hospital' medical microbiology laboratory at, Sokoto Nigeria.

Media preparation and growth of microorganisms

Nutrient agar (NA) and Mueller Hinton agar (MHA) plates were prepared, and bacterial cultivation was carried out as per standard protocols. Autoclaving (Dixon Surgical Instrument ST 19T, UK) for 15 min at 121 ˚C was performed for sterilization. Briefly, five (5) and twenty-five (25) plates were prepared by dissolving 3.8 and 28.5 g of the NA and MHA with 100 and 750 cm^3 of distilled-water. The prepared microbe was placed in an incubator (Memmert DIN 12880-K1, W-Germany) for 24 hours. The growth of the various bacteria was observed. The media preparation was determined by using equation 1 below:

$$\frac{\text{Weigh of the medium in directions (g)}}{\text{volume of D.W in directions (cm3)}} = \frac{\text{Weigh of powder should we weight (g)}}{\text{volume of media (D.W) we need (cm3)}}$$

(1)

Preparation of McFarland turbidity standard scale and organism suspension

A McFarland standard turbidity scale number 0.5 was prepared by combining 99.5 cm^3 of 1% sulfuric acid with 0.5 cm^3 of 1% barium chloride solution. The organism suspension was diluted using sterile distilled water until its turbidity matched that of the McFarland scale, achieving a concentration of approximately 1.5 x 10^8 colony forming units (CFU/cm^3). The mixture was covered with foil and stored at room temperature. [22]

Determination of the antibacterial activity of G.S leaf extract and GS-AgNPs

The antibacterial assay was conducted using the agar well diffusion method following procedures outlined by Yusuf *et al.* and Aziz *et al.* Sterilized agar medium was poured into sterile petri dishes in two layers, inoculated with the organism suspension, and two wells were bored at the side of each inoculated medium. The wells were filled with the extract solutions using a sterile syringe and allowed to diffuse. After incubation at 37°C for 24 hours, the zones of inhibition were observed and measured. The experiments were performed 3 times, and the zone-of-inhibition (Z-OI) mean recorded. [23,24]

Analysis of data

The mean value along with the standard error of the mean (SEM) was computed utilizing Minitab 17 software. The X-RD graph was generated using GraphPad Prism 10, whereas the Z-OI graph was created using MS Excel 16.

Results and Discussion

Synthesis of GS-AgNPs

The change in colour from yellow to dark-brown confirmed the synthesis of GS-AgNPs. The reduction of silver ions (Ag^+) to zero-valence silver (Ag^0) was achieved by GS leaf-extract, resulting in surface plasmon resonance (SPR) of Ag-NPs. This observation aligns with previous studies by Sarwer *et al.*,[25] and Veerasamy *et al*.,[26] The color change is depicted in Figure 1.

Characterization of GS-AgNPs

Ultra-violet visible (UV-vis) spectroscopy analysis

Analysis using ultraviolet-visible (UV-vis) spectroscopy revealed a distinct absorption peak at 390 nm in the UV spectrum, which provided confirmation of the formation of GS-AgNPs. This peak signifies the reduction of silver-ions and the existence of surface plasmon resonance (S-P-R) as documented in prior research.[28,29] Additional details of the UV spectra can be found in Table 1 and Figure 2.

Table 1: UV-spectroscopy analysis

S/N	Sample	Wavelength (nm)
1	Plant extract	410
2	Silver nitrate solution	280
3	silver nanoparticles solution (AgNPs)	390

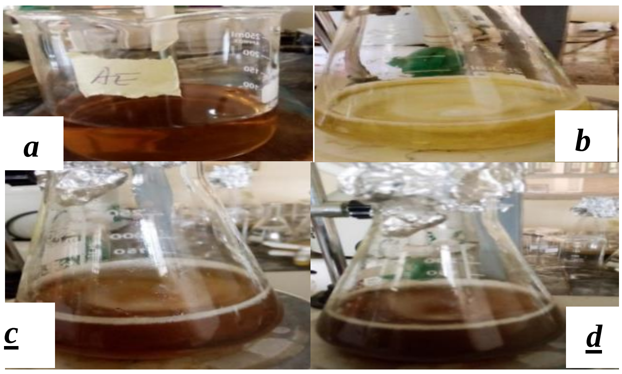

Figure 1: colour change of (a) aqueous leaf extract (AE) (b) GS-AgNPs after adding the silver-nitrate solution with the leaf-extract and (c) GS-AgNPs after 1 hour and (d) GS-AgNPs after 24 and 72 hours.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

F-T-IR analysis of the GS-L-E and silver-nanoparticles (GS-AgNPs)

F-T-IR analysis of *Guiera senegalensis* leaves-extract (GS-L-E) revealed absorption peaks at 3306.1, 1634.4, and 1004.5 cm^{-1} corresponding to N-H stretch, -C=C-, and -C-C functional groups, respectively. Conversely, the I-R peaks of the GS-AgNPs exhibited absorption bands at 3311.7, 2160, 1960.6, 1627, and 693.3 cm^{-1} attributed to N-H/O-H, -C-H, -C=C-, and C-Cl functional groups. These peaks indicate the presence of alkyl ether and phenol groups, serving as capping and stabilizing agents. F-T-IR technique was employed to identify phyto-compounds, providing evidence of Ag-NPs capping. [31] Upon analyzing the F-T-IR spectra of both the plant extract and GS-AgNPs, similar absorption bands were observed. Notably, the GS-AgNPs spectra exhibited O-H stretching of carbohydrates at 3311.7 cm^{-1}. [32] The observed shift in different bands of the GS-AgNPs compared to the leaf extract (L-E) suggests a crosslinking between the Ag-NPs and the L-E, confirming the capping of GS-AgNPs by the organic molecules of the L-E. [33] Considering the utilization of GS-L-E, it can be inferred that flavonoids, alkaloids, and terpenoids are present. The F-T-IR data of GS-L-E and GS-AgNPs indicated that the phytochemicals were responsible for the reducing and stabilizing power of nano-particles. [34] Additionally, another peak was observed nearby at 693 nm, potentially indicating the initiation of Ag-NPs agglomeration. [14] The FTIR spectra are illustrated in Figure 3.

X-ray diffraction (X-RD) of GS-AgNPs

X-RD analysis peaks confirmed the crystalline nature of GS-AgNPs with 2θ = 15.34°, 22.48°, 27.00°, 32.54°, and 41.33° corresponding to specific planes (111), (210), (220), (222), and (3-11). The results matched those reported in the literature (COD-DB card no. 1509518 standard database), [14,31,35] confirming the nano-crystalline form of the synthesized nanoparticles. X-RD spectrum is shown in Figure 4.

Antibacterial assay

GS-AgNPs and GS-L-E exhibited significant antibacterial activity against human pathogenic microorganisms, with higher concentrations (20 mg/cm^3) resulting in larger zones of inhibition (16.33-19.33 nm) compared to the extract (4-9 nm). The results in Table 2 indicate promising antibacterial properties of GS-AgNPs, especially against *Escherichia coli*. The mechanism of bactericidal action of GS-AgNPs involves their interaction with bacterial cell membranes, disrupting permeability and energy functions. The bactericidal activity is influenced by factors such as particle size distribution and surface area. These findings are consistent with previous research. [14,18,36]

Conclusion

In conclusion, silver nano-particles synthesized using *Guiera senegalensis* leaf-extract displayed antibacterial efficacy against both gram-positive and gram-negative bacteria. The nano-particles exhibited superior antibacterial properties compared to the leaf extract alone. This study lays the groundwork for further exploration of *Guiera senegalensis* in nanoparticle synthesis and biomedical applications. Future research avenues include toxicity studies, antifungal activity, antioxidant properties, and in silico ADMET analysis of the synthesized nano-particles.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors expressed their sincere appreciation to Christiana Simon, Mal. Sabiu (Laboratory Technicians, Microbiology PG Lab), Ibrahim Nasir (Pharmaceutical Chemistry Lab technician) Usmanu Danfodiyo University Sokoto, Nigeria for their immense support during the research.

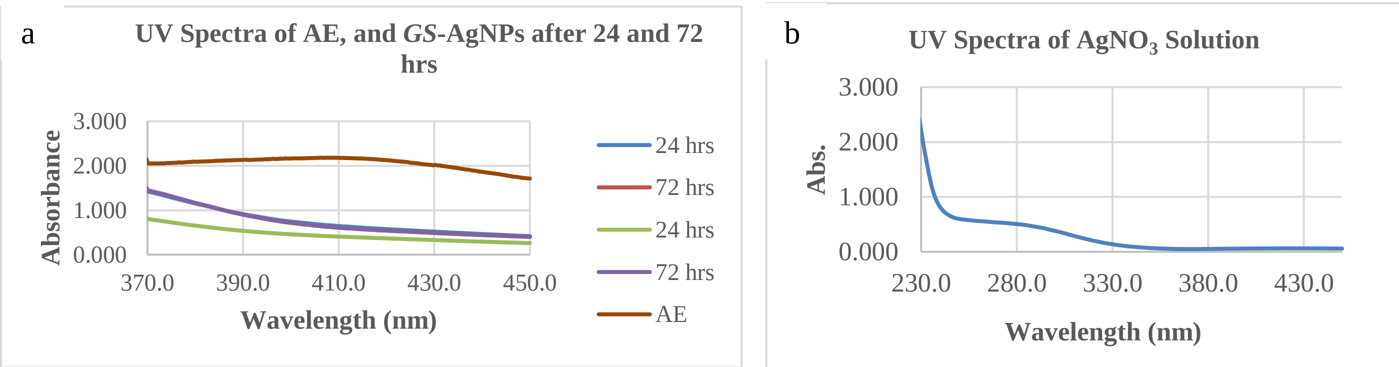

Figure 2: Graph of UV spectra of (a) silver nanoparticles from *Guiera senegalensis* (GS-AgNPs) and aqueous extract (AE) (b) solution of $AgNO_3$ solution

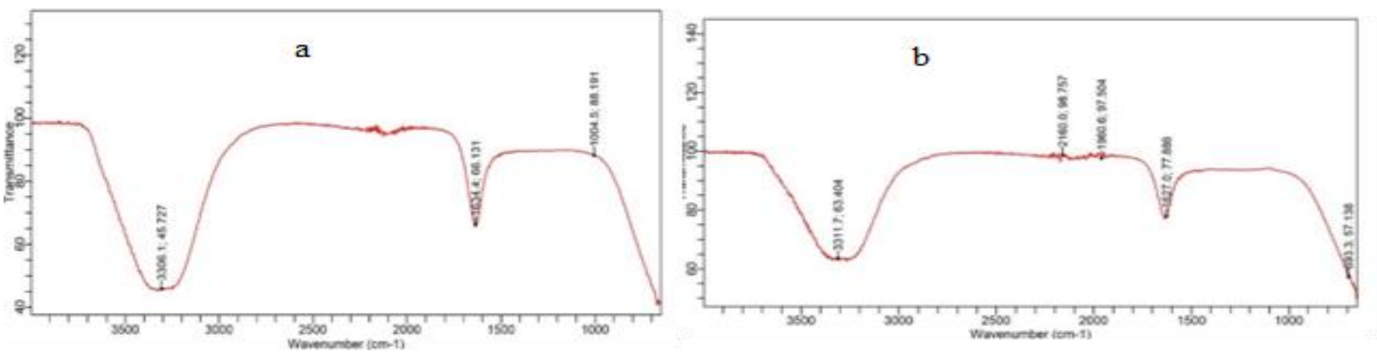

Figure 3: FTIR spectra of (a) *Guiera senegalensis* aqueous-extract and (b) silver-nanoparticles

Table 2: Mean zone-of-inhibition (Z-O-I) of growth in mm (n=3)

S/N	Test Organism	E 1	E 2	N 1	N 2	C 1	C 2
1	*S. Typhi*	2.33	5.67	ND	16.33	41.17	2.17
2	*E. coli*	3.67	5.67	7.67	19.33	28.84	9.67
3	*S. aureus*	1.33	4.00	4.00	17.00	40.17	2.00
4	*B. Subtilis*	3.00	9.00	7.00	16.33	34.17	9.17

E1: Extract (10 mg/cm^3), E2: Extract (20 mg/cm^3), N1: Nanoparticle at (10 mg/cm^3), N2: Nanoparticle at (20 mg/cm^3), C1: Ciproflaxacin, C2: Silver Nitrate solution

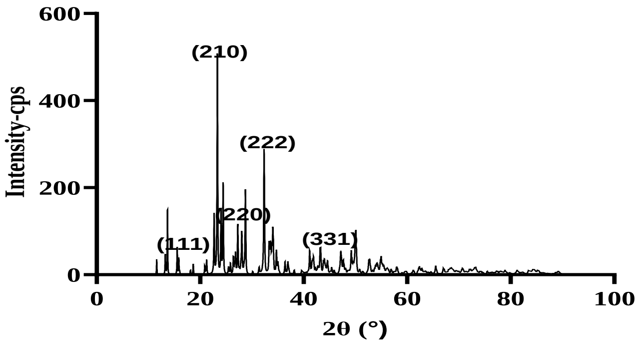

Figure 4: X-ray diffraction of GS-AgNPs

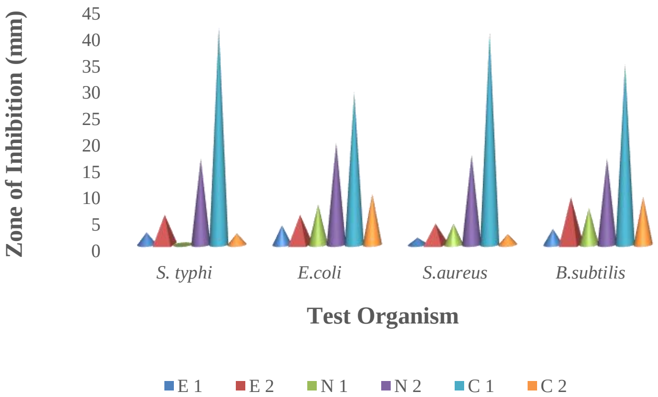

Figure 5: Zone-of-inhibition against tested organism (mean ± SEM, n=3)

References

1. Bachheti RK, Fikadu A, Bachheti A, Husen A. Biogenic Fabrication of Nanomaterials from Flower-based Chemical Compounds, Characterization and their various Applications: A review. Saudi J Biol Sci. 2020; 27(10):2551–2562.
2. Akter S, Huq MA. Biologically Rapid Synthesis of Silver Nanoparticles by Sphingobium sp. MAH-11 T and their Antibacterial Activity and Mechanisms investigation against Drug-resistant Pathogenic Microbes. Artif Cells Nanomed Biotechnol. 2020; 48 (1):672–682
3. Hamouda RA, Hussein MH, Abo-Elmagd RA, Bawazir SS. Synthesis and Biological Characterization of Silver Nanoparticles Derived from the *Cyanobacterium oscillatoria limnetica*. Sci Rep. 2019; 9(1): 13071
4. Kulkarni N, Muddapur U. "Biosynthesis of Metal Nanoparticles: A Review", J Nanotechnol. 2014; 8 pages,
5. Jamkhande PG, Ghule NW, Bamer AH, Kalaskar MG. Metal Nanoparticles Synthesis: An Overview on Methods of Preparation, Advantages, Disadvantages, and Applications. J Drug Deliv Sci Technol. 2019; 53(7):101174.
6. Huq MA, Ashrafudoulla M, Rahman MM, Balusamy SR, Akter S. Green Synthesis and Potential Antibacterial Applications of Bioactive Silver Nanoparticles: A Review. Polymers. 2022; 14(4):742.
7. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry, M. "Extracellular Biosynthesis of Silver Nanoparticles using the Fungus *Fusarium oxysporum*," Colloids Surf B: Biointerfaces. 2003; 28, (4):313–318. https;//doi.org/10.1016/S0927-7765(02)001174-1
8. Huq MA. Green synthesis of silver nanoparticles using *Pseudoduganella eburnean* MAHUQ-39 and their Antimicrobial Mechanisms Investigation against Drug-Resistant Human Pathogens. Int J Mol Sci. 2020; 21(4):1510
9. Kedi PBE, Meva FE, Kotsedi L. Eco-friendly Synthesis, Characterization, *in vitro* and *in vivo* Anti-inflammatory Activity of Silver Nanoparticle-mediated *Selaginella myosurus* Aqueous Extract. Int J Nanomedicine. 2018; 13:8537–8548.
10. El-Naggar NE, Hussein MH; El-Sawah AA. Bio-fabrication of Silver Nanoparticles by Phycocyanin, Characterization, in vitro Anticancer Activity against Breast Cancer cell line and in vivo Cytotoxicity. Sci Rep. 2017; 7 (1):10844
11. Du J, Sing H, Yi TH. Antibacterial, Anti-biofilm, and Anticancer Potentials of Green Synthesized Silver Nanoparticles using *Benzoin gum* (Styrax benzoin) extract. Bioprocess and Biosyst Eng. 2016; 39:1923–1931
12. Vigneshwaran N, Kathe AA, Varadarajan PV. Silver-protein (core-shell) Nanoparticle Production using Spent Mushroom Substrate. Langmuir. 2007; 23(13):7113–7117.
13. Anees-Ahmad S, Sachi-Das S, Khatoon A, Ansari MT, Afzal M, Saquib-Hasnain M, Kumar-Nayak A. Bactericidal Activity of Silver nanoparticles: A Mechanistic Review. Mat Sci Energy Technol. 2020; 3: 756–769
14. Jemal K, Sandeep BV, Sudhakar P. Synthesis, Characterization, and Evaluation of the Antibacterial Activity of *Allophylus serratus* Leaf and Leaf Derived Callus Extracts Mediated Silver Nanoparticles. J Nanomaterials. 2017; Article ID 4213275, 11 pages
15. Dirar AI, Devkota HP. Ethnopharmacological uses, Phytochemistry and Pharmacological Activities of *Guiera senegalensis* J.F. Gmel. (Combretaceae) J Ethnopharmacol. 2020; 267:113-433.
16. Hassan MG, Mohammed MF, Mogoro UJ, Omotainse SO, Ali AS, Malami AI, Shamsuddeen Y and Ugochinyere P.C. Phytochemical Analysis, Cytotoxcity and Antifungal Activities of *Guiera senegalensis* Leaves Extract Review. Chem Pharm Res. 2020; 2(1): 1-4.
17. Yahaya T, Kasimu S, Hanan I, Oladele E, Shemishere U. Toxicological Evaluation of the Leaves of *Guiera senegalensis* (J.F. Gme), *Cassia occidentalis* (Linn), and *Ziziphus mauritiana* (Lam). Beni-Suef Univ J Basic Appl Sci. 2019; 8:14. 9 pages.
18. Hamadnalla HMY, Hamad MAB, Adam AAI. Phytochemical Investigation, Antimicrobial, Antioxidant and Anti-Diabetic Potential of *Guiera senegalensis* Leaves Extracts. J Pharmacol Pharmaceut Pharmacovig. 2020; 4: 015.

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

19. Ravichandrana V, Vasanthib S, Shalinic S, Syed A, Shahd A, Tripathyd M, and Paliwal N. Green Synthesis, Characterization, Antibacterial, Antioxidant and Photocatalytic Activity of *Parkia speciosa* Leaves Extract Mediated Silver Nanoparticles. J Results Phys.2019; 15 (2019): 102565.
20. Liaqat N, Jahan N, Khalil R, Anwar T and Qureshi, H. Green Synthesized Silver Nanoparticles: Optimization, Characterization, Antimicrobial Activity, and Cytotoxicity Study by Hemolysis Assay. Front Chem. 2022; 10:952006.
21. Halilu EM, Ngweh VA, Airemwen CO. Green Synthesis of Silver Nanoparticles from *Parinari curatellifolia* Methanol Stem Bark Extract and Evaluation of Antioxidant and Antimicrobial Activities. Trop J Nat Prod Res. 2023; 7(3):2498-2505
22. Acharya T. Preparation of McFarland Turbidity Standards [online]. 2022 (cited 2023 June 14) from: https://microbeonline.com/preparation-mcfarland-turbidity-standards/index
23. Aziz N, Fatma T, Varma A, Prasad R. Biogenic Synthesis of Silver Nanoparticles using *Scenedesmus abundans* and Evaluation of their Antibacterial Activity. J. Nanoparticles. 2014; 1–6. doi:10.1155/2014/689419
24. Yusuf AJ, Abdullahi MI, Haruna AK, Musa, AM, Abdullahi, MS, Ibrahim ZYY, Halilu E, Odiba OJ. Phytochemical and Antimicrobial Evaluations of the Methanol Stem Bark Extract of *Neocarya macropylla*. J Chem Pharmaceut Res. 2015; 7(1):477-481
25. Sarwer Q, Amjad MS, Mehmood A, Binish Z, Mustafa G, Farooq A, Qaseem MF, Abasi F, Pérez de la Lastra JM. Green Synthesis and Characterization of Silver Nanoparticles using *Myrsine africana* Leaf Extract for Their Antibacterial, Antioxidant and Phytotoxic Activities. Molecules. 2022; 27(21):7612.
26. Veerasamy R, Sethu V, Sivadasan S, Syed AAS, Rajak, H. Green Synthesis of Silver Nanoparticles using *Atrocarpus altilis* Leaf Extract and the Study of their Antimicrobial and Antioxidant Activity, Mat Lett. 2016.
27. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of Silver Nanoparticles using *Acalypha indica* Leaf Extracts and its Antibacterial Activity against Water-borne Pathogens. Colloids Surf B: Biointerfaces. 2010; 76 (1): 50–56
28. Jyoti K, Baunthiyal M, Singh A. Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. Leaves and their Synergistic Effects with Antibiotics. J Rad Research App Sci. 2016; 9(3);217–227
29. Ravikumar S, Angelo RU. Green Synthesis of Silver Nanoparticles *Using Acacia Nilotica* Leaf Extract and Its Antibacterial and Anti-Oxidant Activity. Int J Pharmaceut Chem Sci. 2015 4 (4)
30. Rakesh B, Srinatha N, Rudresh KKJ, Madhu A, Suresh, KMR, Praveen N. Antibacterial Activity and Spectroscopic Characteristics of Silver Nanoparticles Synthesized via Plant and Invitro Leaf-derived Callus Extracts of *Mucuna pruriens* (L.) DC. South Afri J Bot. 2022; 148 (2022): 251-258.
31. Olufunto TJ, El Hadji MS, Sundararajan P, Vuyo M, Oluwatobi SO. Green Synthesis of Silver Nanoparticles using *Combretum erythrophyllum* Leaves and its Antibacterial Activities. J Colloids Interface Sci Commun. 2019; 31: 100191.
32. 6Shankar PD, Shobana S, Karuppusamy I, Pugazhendhi A, Ramkumar VS, Arvindnarayan S, Kumar G. A Review on the Biosynthesis of Metallic Nanoparticles (Gold and Silver) using Bio-components of Microalgae: Formation Mechanism and Applications, Enzy Microbial Technol. 2016; 95: 28–44
33. Rhede DT, Guilger M, Bilesky JN, Germano-Costa T, Pasquoto-Stigliani G, Grillo, TBB, Carvalho RCDS, Fraceto LF, Lima R. Synthesis of Biogenic Silver Nanoparticles using *Althaea officinalis* as Reducing Agent: Evaluation of Toxicity and Ecotoxicity. Sci Rep. 2018; 8:1–11
34. Gopinath V, Priyadarshini S, Priyadharsshini NM, Pandian K, Velusamy P. Biogenic Synthesis of Antibacterial Silver chloride Nanoparticles using Leaf Extracts of *Cissus quadrangularis* Linn. Mat Let. 2013; 91: 224–227.
35. Ahmad N, Sharma S. Green Synthesis of Silver Nanoparticles using Extracts of *Ananas comosus*. Green Sustain Chem. 2012; 2: 141-147.
36. Panacek A, Kvytek L, Prucek R, Kolar M, Vecerova R. Silver Colloid Nanoparticles: Synthesis, Characterization, and their Antibacterial Activity. J Physical Chem B. 2006; 110:16248–53.