Caliphate Journal of Science & Technology (CaJoST)

ISSN: 2705-313X (**PRINT**); 2705-3121 (**ONLINE**) **Research Article**

Open Access Journal available at: https://cajostssu.com and https://www.ajol.info/index.php/cajost/index

DOI: https://dx.doi.org/10.4314/cajost.v4i2.3

Article Info

Received: 30th November 2021

Revised: 10th February 2022

Accepted: 13th February 2022

Antioxidant and Antimicrobial Activities of Green Synthesized Silver Nanoparticle Using *Moringa Oleifera* Seeds Extracts

Mustapha Isah*, Sumayya A. Ayuba, and Zainab H. Bello

Department of Biochemistry, Sokoto State University, Sokoto-Nigeria

***Corresponding author's email:**

mustapha.isah@ssu.edu.ng

Nanotechnology deals with materials in nanoscale that exhibit incredible chemical, physical and biological properties. This study is aimed at synthesizing silver nanoparticles from *Moringa oleifera* seed using aqueous and ethanol as solvents and compare their antioxidant as well as antimicrobial activities of these two synthesized nanoparticles. The synthesized nanoparticles were subjected to preliminary characterization using UV- spectroscopy to ensure their formation which were confirmed by attaining the plasmon resonance surface of both particles at 320nm. Standard assay for antioxidant scavenging of ferric ion and DDPH were employed and standard methods for drop plate technique, minimum inhibition concentration and minimum bactericidal/fungicidal concentrations were used. The results indicate that silver nanoparticles synthesized from ethanol extract (EEMS) has excellent ferric ion scavenging activity compared to silver nanoparticles synthesized from aqueous extract (AEMS), while AEMS has 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging potential compared to EEMS. Both nanoparticles show the antimicrobial efficiency were dose dependent but more inhibition zone was observed on EEMS using drop plate technique at 25mg/dl against all the organisms used (*S. aureus*, *E. coli* and *C. albican*). And in minimum inhibition concentrations, the result shows both particles inhibit the growth of all organisms at 6.25 µg/dl except against *E. coli* which is at 12.5 µg/ml by EEMS. Similarly, minimum bactericidal / fungicidal concentrations were the same in all organisms at 12.5 µg/ml except against *C. albican* which was at 6.25 µg/ml by EEMS. The finding revealed that both EEMS and AEMS are good antioxidants and antimicrobial agents and their activities are concentration dependent.

Keywords: Antimicrobial; Antioxidant properties; *Moringa oleifera*; Seed extracts; Silver nanoparticle.

Cite this: *CaJoST*, 2022, 2, 142-150

1. Introduction

Nanotechnology involve the study of tiny objects and their applications which can be applied across diverse fields like chemistry, physics, biology, engineering [11]. Nano stands for 10^{-9} or billionth units. Its size begins from 1 - 100nm because of this size it can occupies position in numerous fields in nanoscience and technology [3] [19] [43]. Silver particles have remarkable applications in diagnostics, high sensitivity in bio-molecular detection, therapeutics, antimicrobials, catalysis and microelectronics [11]. Currently, several methods for the synthesis of nanoparticles are available either physical or chemical methods [5]. Many of the nanoparticle synthesis methods involve the use of hazardous chemicals, low material conversions and high energy requirements. Therefore, there is need to develop an environmental friendly process for nanoparticle synthesis without using toxic chemicals. Biosynthetic methods employing either microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. Certain plant parts such as fruits, leaves, roots, seeds, stem, have been utilized for synthesizing various nanoparticles owing to the occurrence of the phytochemicals in their extracts which serves as reducing and stabilizing agent [22]. For instance, *C. sinensis* extracts was used as reducing and stabilizing agent for the synthesis of gold nanoparticles and silver nanostructures [21]. Silver nanoparticles (AgNPs) are among the most widely used nanoparticles that show a broad spectrum of antibacterial activity. Studies have shown that silver nanoparticles prevent replication of the AIDS virus (HIV) and their impact is much greater than that of gold nanoparticles [17].

Moringa oleifera is a native to South Asia but now can be found all over the tropics. The treatment of different diseases using Moringa leaves was estimated to efficiently curing about 300 various ailments [16]. He further added that most of the developing countries use traditional medicine as therapeutics for maintenance of good health. Moringa is regarded as one of the important crop in Saharan and sub-Saharan countries [14]. All of its parts are believed to have been safe for consumption ranging from leaves, pods to its flowers [24] [4]. Specifically, the leaves are known to have healing properties and can be consumed in different ways [25] [23]. Reports show that leave extracts possess high antioxidant properties [14] [2].

Antioxidants are substances that protect cells from the damages caused by unstable molecules known as free radicals. They play an important role in eliminating these free radicals prior to cellular attack thereby preventing diseases cause as a result of their attack [10]. Examples of these antioxidants include Vitamin A, C and E, lycopene, beta-carotene among others [15] [7]. Antioxidant can be enzymatic or non -enzymatic.

The free radicals are otherwise known as reductive oxygen species (ROS) which are the cellular respiration by-products affecting cellular oxidative functions and play an important in cell signaling [9]. Reactive oxygen species possess an unpaired valence electron and are responsible for many diseases due to their consequences which include but not limited to cancer, cataract, aging, diabetes mellitus and brain dysfunction [20]. Most of the researches focus on the synthesis of silver nanoparticles using aqueous extract for antimicrobial and antioxidant activities. In this finding, the silver nanoparticles were synthesized from *Moringa oleifera* seed extracts using aqueous and ethanol solvents and compare their antimicrobial and antioxidant activities. The cytotoxic analysis of these nanoparticles need to be investigated to ensure proper safety.

2. Materials and Methods

2.1 Collection of Plant Material

The *Moringa oleifera* seed (unpeeled) were obtained, peeled and air dried at room temperature. Pestle and mortar were used for grinding the dried seed to powder form. The powder was stored for further analysis.

2.2 Preparation of Aqueous Extract

Thirty gram (30 g) of the powder was weighed using a weighing balance. It was then transferred into the 1000 mL beaker, and 400 mL of H_2O was added into the beaker containing the powder and allowed to soak for 72 hours after which the extract was filtered using whatman No. 1 filter paper, the filtrate was used for synthesis of silver nanoparticles.

2.3 Preparation of Ethanol Extract

Thirty gram (30 g) of the powder was weighed using a weighing balance. It was then transferred into the 1000 mL beaker, and 200 mL of ethanol was added into the beaker containing the powder and allowed to soak for 72 hours after which the extract was filtered using whatman No. 1 filter paper.

2.4 Preparation and Characterization of Silver Nanoparticles

Silver nitrate $AgNO_3$ was obtained from Sigma-Aldrich chemical company, all glass wares were washed with distilled water and dried in oven. A stock solution of $AgNO_3$ 2×10^{-3} M were prepared by dissolving 0.34 g/1000 ml of de-ionized water. 100 mL of extract were added to 900 mL of the $AgNO_3$ stock solution and were kept in the dark and allow to change colour to brown or dark brown within 30 minutes. After that, the absorbance was taken using UV spectrophotometer (model 752N) at different wavelength of 20 nm intervals from 200nm to 620 nm. Then, the content was evaporated to obtain the powder for antioxidant properties and antimicrobial screening by constituting different concentrations according to Moodley *et al.*, [26] with little modification.

2.5 Ferric Reducing Antioxidant Power Assay

The ferric reducing Antioxidant power of the extract was determined using potassium ferricyanide, ferric chloride method [6]. 2.5 mL of various concentration of the extract was added to 2.5mL of potassium ferricyanide, then, incubated at 50 ºC for 20 minutes. (i) 2.5 mL of 10% Trichloroacetic acid (TCA) was added to the mixture which was then centrifuged at 300 rpm for 20 minutes. (ii) 2.5 mL of upper layer was mixed with 2.5 mL of distilled water, then 0.5 mL of fresh prepared ferric chloride was added (0.5%). The control was prepared using the same procedure with distilled water. Then absorbance was measured at 700 nm. Ascorbic acid of various concentration was used as standard. Antioxidant reducing power was determined using the formula:

$$\text{Antioxidant Reducing Power} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

2.6 2, 2-diphenyl-1-picrylhydrazyl (DPPH*)*

The effect of Butyl hydrogen acetate (BHA) was also assessed for comparison with that of plant extract dilutions (0.2, 0.4, 0.6, 0.8, and 1.0) mg/ml and increase the volume to 4 mL with distilled water. 1 mL DPPH radical (1 mmol) was

added to each tube and same procedure as in DPPH scavenging experiment was followed, the absorbance was measured at 517nm [10]. The percentage free radical scavenging activity was calculated using the formula:

$$\%SA = \frac{\text{Absorbance of control - Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

2.7 Source of Microorganisms

The test organisms were selected based on their availability. Hence, two bacteria and one fungus isolates were used for the test. The organisms were collected from the Microbiology laboratory, Sokoto State University, Sokoto, Nigeria. The organisms were; *Staphylococcus aureus*, *E. coli* and *Candida albican*. The bacteria and fungus were maintained on nutrient agar(NA) and potato dextrose agar (PDA) and stored in the refrigerator at 4^{o}C. The bacteria were sub-cultured into fresh media at regular interval until it was used for the test.

2.8 Sterility Test of the AgNPs from Seed Extracts

The AgNPs synthesized from aqueous and ethanol extracts were tested for growth. This was carried out by inoculating 1ml of the AgNPs on nutrient agar and incubated at 37^{o}C for 24hours. The plates were observed for growth. No growth in the AgNPs inoculated after incubation indicates that the AgNPs was sterile. The AgNPs was then accessed for antimicrobial activity.

2.9 Antimicrobial assay

2.9.1 Agar Well Diffusion Method

The antimicrobial properties of the AgNPs were determined using the agar well diffusion method Hilton Mueller Agar (HMA) and Potato Dextrose Agar (PDA) plates were prepared, sterilized and solidified. After solidification, sterile cork borer of 6mm diameter was used to punch wells on the agar on each petri dish. The holes were filled with different concentrations of AgNPs. Control experiments were also carried out where the holes were filled with 0.5ml of silver nitrate. Each hole was labeled representing a particular sample. In the agar well diffusion method, the petri dishes containing HMA and PDA were seeded throughout with the twenty-four hours old test organisms in the case of bacteria and seventy-two hours against the *C. albican*. The culture plates were incubated at 37 ^{o}C for 24 hours (*S. aureus* and *E. coli*), 25 ^{o}C for 72 hours (*C. albican*) after which the zones of inhibition were measured where obtainable in accordance with the procedure employed in [18].

2.9.2 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined against bacteria and fungi after the antimicrobial test have been performed. This shows the lowest concentration of an antimicrobial agent that could inhibit the visible growth of a microorganism after overnight incubation. The isolates were cultured on Mueller Hinton agar (MHA) and PDA and agar diffusion method was used for this purpose. Sterile cork borer of diameter 6mm was used to bore holes on the plates after seeding the plates with the microbial strains being tested. It was left for about one hour at room temperature and subsequently incubated at 37 ^{o}C. while fungus at 25 ^{o}C. Results were read after incubation in 24hours for bacteria and 72 hours for fungus.

2.9.3 *Determination of Minimum Bactericidal / fungicidal Concentration Tests*

Minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) tests were undertaken by immediately sub culturing the organism by spreading a loopful evenly from the tube containing solution of 6 mg/mL, 3mg/mL and 1.5 mg/mL over MBC media Muller Hinton agar solid. Then incubated at 37 °C for 24 hours. After 24 hours, while 72 hours for the fungus. The lowest concentrations that did not show any growths of the test organisms on the solid media was considered as the MBC/MFC of the organism.

3. Results and Discussion

3.1 Results

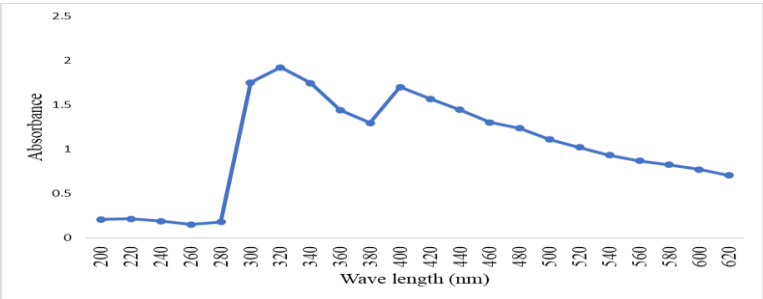

Fig.1: UV-Visible Spectroscopy of AgNPs using *Moringa oleifera* Aqueous Extract

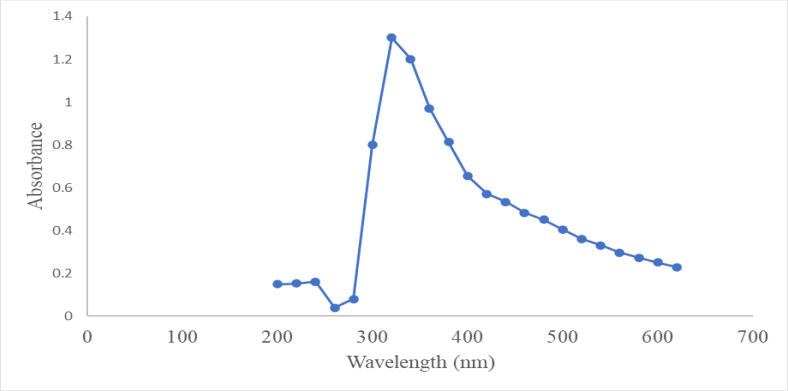

Fig. 2: UV-Visible Spectroscopy of AgNPs using *Moringa oleifera* Ethanol extract

Table 1: Ferric ion scavenging activities of silver nanoparticles synthesized from *M. oleifera seed* extracts

	Concentration (mg/mL)				
Silver nanoparticles/ Control	25	50	75	100	125
EEMS	4.45	7.66	8.45	11.67	67.98
AEMS	27.74	28.03	58.30	79.78	86.37
Control (Vitamin C)	61.08	69.47	79.72	94.16	96.64

EEMS = Silver nanoparticles synthesized from ethanol extract of *M. oleifera* seed
AEMS = Silver nanoparticles synthesized from aqueous extract of *M. oleifera* seed

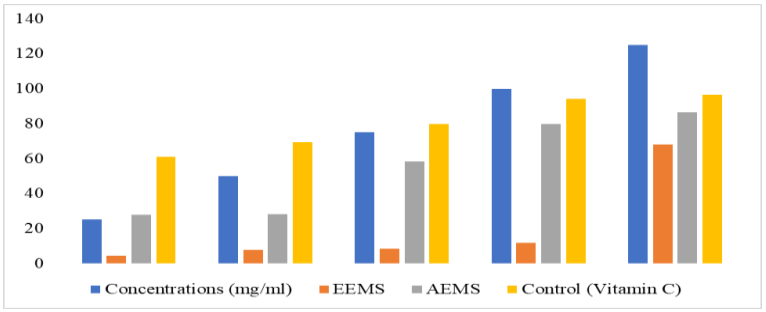

Fig.3: Ferric ion scavenging activity of silver nanoparticles synthesized from seed extracts of *M. oleifera*

Table 2: Level of DPPH scavenging activities of AgNPs synthesized from EEMS and AEMS

	Scavenging activity (%)	
Free radical scavenging	**EEMS**	**AEMS**
DPPH	73.4 ± 1.07	62.8 ± 0.86

Represented as mean ± SEM of the three replicates

Table 3: Zone of inhibition (mm) of green synthesized silver nanoparticles using *Moringa oleifera* seed of ethanol and aqueous extracts on bacterial and fungal

		Concentration (mg/dl)					
Organism	Extracts	10	15	20	25	+	-
E. coli	EEMS	7.3±0.3	9.0±0.00	13.0±0.6	14.6±0.3	3.0±0.03	0±0.0
	AEMS	3.9±0.4	5.9±0.4	9.2±0.4	11.3±0.2	2.3±0.2	0±0.0
S. aureus	EEMS	10.5±0.3	11.4±0.2	13.0±0.6	14.6±0.3	6.9±0.03	0±0.0
	AEMS	0±0.00	2.6±0.2	5.4±0.4	7.3±0.4	6.3±0.2	0±0.0
C. albican	EEMS	9.1±0.05	10.5±0.6	12.5±0.3	14.3±0.3	3.0±0.5	0±0.0
	AEMS	0±0.00	2.2±0.2	6.5±0.06	8.4±0.2	2.3±0.2	0±0.0

Values are presented as means ± SEM of three determinations

KEY: EEMS- Ethanol extract of Moringa seed; AEMS-Aqueous extract of Moringa seed
+ Positive control ($AgNO_3$) - Negative control (DH_2O)

Table 4: Minimum Inhibitory Concentration

Microorganisms	Concentration (µg/mL)	
	EEMS	AEMS
S. aureus	6.25	6.25
E. coli	12.5	6.25
C. albican	6.25	6.25

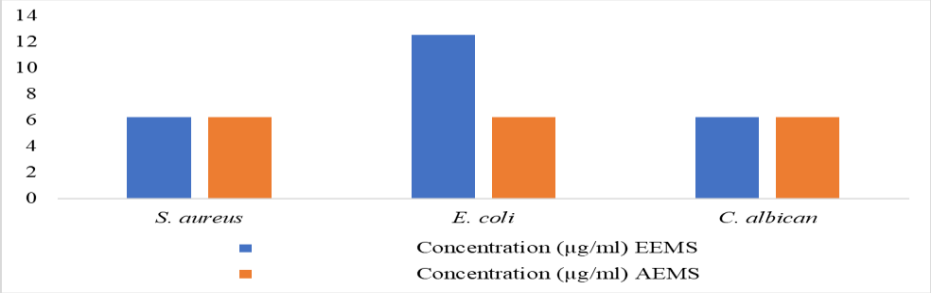

Fig. 4: Result of minimum inhibitory concentration of AgNPs from ethanol extract (EEMS) and AgNPs from aqueous extract (AEMS) using *M. oleifera* seed

Table 5: Bactericidal/Fungicidal Concentration

Microorganisms	Concentration in (µg/mL)	
	EEMS	AEMS
S. aureus	12.50	12.50
E. coli	12.50	12.50
C. albican	6.25	12.50

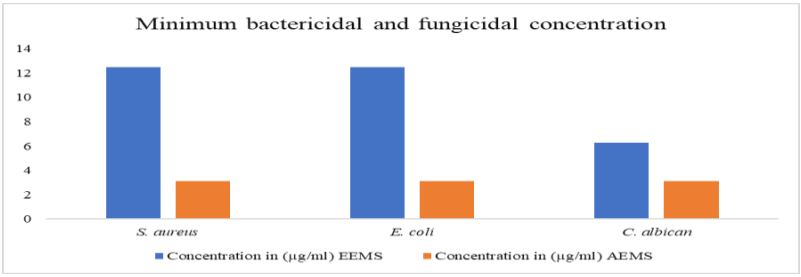

Fig. 5: Result of the minimum bactericidal and fungicidal concentration of AgNPs from ethanol extract (EEMS) and aqueous extract (AEMS) using *M. oleifera* seed

3.2 Discussion

Given the wide ranging applications of AgNPs in recent years, many researchers have focused on the development of modified or novel synthetic strategies for AgNPs as opposed to the use of conventional methods which are strongly associated with toxic environmental footprints [13].

The primary indication of silver nanoparticle formation is represented by a reaction solution colour change to dark brown [13]. Therefore, preliminary characterization of synthesized AgNPs was done using UV-Visible spectrophotometer model- 752N and the recorded UV-absorption spectra while attaining its plasmon resonance vibration which was observed at 320nm for both aqueous and ethanol extracts of *Moringa oleifera* seed. Which is tallied with the report by Kalugendo & Kousalya, [11], in which the UV-absorption spectra exhibited the highest absorbance starting from 320nm up to 460nm. This can be possible due to the differences in phytochemistry component in plants which are responsible for the conversion. Other reports believed that the peak of synthesized silver nanoparticles can be observed from 390nm to 420nm or more [40].

The result in table 1 of ferric ion scavenging activities of both AgNPs were good but it shows clearly that the activity was less in ethanol extract of *Moringa oleifera* seed (EEMS) when compared with that of aqueous extract of *Moringa oleifera* seed (AEMS). The radical scavenging activity shows that its directly proportional to the concentration of both silver nanoparticles synthesized from ethanol and aqueous extract respectively.

It was reported that the percentage free radical scavenging activity of AgNPs is found to be high,

due to its capability of being good oxidant, electron loosing and capping agents present on AgNPs surface [8]. Ferric ion radical is not very reactive and it is a weak oxidizing agent; biologically, it acts as a toxicant to the cell by converting itself into hydroxyl radical in the presence of metal ions in the living system resulting in initiation and propagation of lipid peroxidation [8]. Based on the result obtained it indicates that AgNPs synthesized from aqueous extract of the seed are excellent ferric ion scavengers compared to AgNPs synthesized from the ethanol extract. The ferric ion scavenging power assay of both EEMS and AEMS revealed that the percentage scavenging potential of both AgNPs are dose dependent in which the concentration of EEMS at 125 mg/mL observed to have 67.98% whereas AEMS at same concentration possesses 86.37%. vitamin C was used as a control with 96.64% scavenging effect. Therefore, the AEMS has an increased level of scavenging activity compared to EEMS

The result in Table 2 of DPPH radical scavenging, the result obtained was reported in percent and shows that there is an increase percentage activity between the EEMS and AEMS. EEMS has 73.4 $\pm$ 1.07 % while AEMS has 62.8 $\pm$ 0.86% and therefore, DPPH radical scavenging potential is more pronounced in EEMS compared to AEMS. This result is closely related with the finding of Nagaich *et al.,* [40] where the DPPH scavenging potential of synthesized silver nanoparticles using apple extract provided the excellent scavenging of 75.16 $\pm$ 0.045%.

The result in table 3 of antimicrobial assay, showed that there is significant growth inhibition in which *E. coli*, *S. aureus* and *C. albican* show high zone of in AgNPs synthesized from aqueous extracts at 6.25 µg/mL while in ethanol extract synthesized AgNPs is the same in *S. aureus* and *C. albican* but differs in inhibition of *E. coli* which was at 12.5 µg/mL.

The effective inhibition of both gram-negative and gram-positive bacteria by AgNPs derived from *M. oleifera* seed is of great significance as it demonstrates their broad-spectrum as compared the silver nitrate used as positive control. It also indicates that the mode of action is not affected by the difference in membrane stabilities of the bacteria since gram-positive bacteria contain a thick peptidoglycan layer whereas gram-negative bacteria possess a rigid outer membrane structure composed of lipids and lipoproteins. Contrasting results were observed in a similar study where AgNPs (5–35 μgmL^{-1}) synthesized from reducing agents D-glucose and hydrazine, displayed effective activity towards gram-negative bacteria but minimal activity against gram-positive bacteria [8]. Another contrasting studies were presented in which a greater sensitivity was recorded in gram-negative when compared to gram-positive bacteria [13].

The result in table 5 of MBC and MFC indicated that there was a homogenous concentration of 12.50 μg/mL that kill all the microorganisms by AgNPs synthesized from aqueous extract whereas a concentration of 6.50 μg/mL kills *C. albican* while 12.50 μgmL^{-1} kills both Gram-positive *S. aureus* and Gram-negative *E. coli* in AgNPs synthesized from ethanol extract of *Moringa oleifera* seed.

The AgNPs synthesized from seed of *M. oleifera* displayed inhibition of gram-negative and gram-positive bacteria. The results of this study showed that AgNPs prepared in this study are not only more effective at lower concentrations but also display a broader susceptible antibacterial spectrum range more specifically in ethanol extract synthesized silver nanoparticles. The results indicate that the synthesized nanoparticles show strong inhibition against the tested organisms. This result is comparable with the result obtained by testing gram-positive, gram-negative and fungal strains using silver nanoparticles [27] [26]. The AgNPs were report to have shown no toxicity towards humans but highly toxic to microorganism [28]. The antibacterial activity shown by AgNPs is related to the concentration of $AgNO_3$. This means that the lesser the concentration, the more the antimicrobial activity in that other [29]. The reason that, the smaller particles possess large surface area and hence interact freely with the microbes and provide more effect compared to the larger ones [41]. Although the exact mechanism to which it operates still not clear but many mechanisms were proposed. One the proposal reveal that a positive charge of the Ag ion plays crucial role for its antimicrobial effect [29]. It derived due to the electrostatic attraction occurring between the negatively-charged on the microbial cell membrane and the positive charged of nanoparticles [33]. Another related proposal reported the microbial cell membrane is negatively charged while AgNPs possess positive charge and when the positively charged AgNPs accumulate around the negatively charged surface of cell membrane, it would change the conformation of the membrane and become easily permeable leading to the cell death [36] [34]. Mubarak Ali *et al.,* [38], revealed that, once AgNPs are in the bacterial cytoplasm, there would be an interference in the bacterial growth pathway due to the modulation of phosphorylated tyrosine of putative peptides which is critical for cell division and viability. Also according to Zodrow *et al.,* [41], AgNPs are probably attacking the electron transport chain and also cell division which eventually leads to

the cell death. Amro *et al.,* [30], reported that the depletion of metal may be the cause for the formation of irregular shape pits at the outer membrane thereby changing its permeability leading to the continuous release of the membrane and lipopolysaccharides. Or possibly DNA may lose its replication capability and affect the expression of ribosomes and other essential proteins and inactivation of enzymes for ATP synthesis [37]. Danilczuk *et al.,* [31] and Kim *et al.,* [35] also proposed that the reason for AgNPs exhibiting the antimicrobial property is because they induce the free radicals and subsequently causing the membrane damage. The other mechanism suggested that the interaction of AgNPs with macromolecules like DNA and enzyme via electro-release [29]. The sulfur containing proteins at the bacterial membrane and the AgNPs interact with such proteins and the phosphorus containing substance like DNA which may cause the DNA and proteins damage causing in cell death. When Ag^+ binds the proteins functional groups it would be denatured [32].

4. Conclusion

Silver nanoparticles were successfully synthesized from ethanol and aqueous *Moringa oleifera* seed extracts, and the AgNPs synthesized from ethanol extracts exhibit an excellent antimicrobial activity against the enteric bacteria (*Escherichia coli, Staphylococcus aureus*) and fungus (*Candida albican*), with very good antioxidant scavenging potential.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] Acharya, D., & Shrivastava, A. (2008). Indigenous herbal medicines. Aavishkar Publishers, Distributors.

[2] Aviles-Gaxiola, S., Leon-Felix, J., Jimenez-Nevarez, Y.B., Angulo-Escalante, M.A., Ramos-Payan, R., Colado-Velazquez III, J., & Heredia, J.B. (2021). Antioxidant and anti-inflammatory properties of novel peptides from Moringa oleifera Lam. Leaves. South African Journal of Botany, 141, 466-473. https://doi.org/ 10.1016/j.sajb.2021.05.033

[3] Bauer, A.W., Kirby, W.M., Sherris, J.C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal Clinical Pathology 45(1), 493-496.

[4] Bernstein, N., Akram, M., Yaniv-Bachrach, Z., & Daniyal, M. (2021). Is it safe to consume traditional medicinal plants during pregnancy? Phytotherapy Resarch, 35(4), 1908-1924. https://doi.org/ 10.1002/ptr.6935

[5] Bindhani, B. K., & Panigrahi, A. K. (2015). Biosynthesis and characterization of silver nanoparticle (snps) by using leaf extracts of Ocimum sanctum L. (Tulsi) and study of its antibacterial activities. Journal of Nanomedicine Nanotechnology. 1, S6.

[6] Chang, L. W., Yen, W.J., Huang, S.C., & Duh, P.D. (2002). Antioxidant activity of sesame coat. Food Chemistry, 78(3), 347-354. https://doi.org/10.1006/S0308-8146(02)00119-X

[7] Fernandez-Lazaros, C.I., Martinez-Gonzalez, M.A., Aguilera-Buenosvinos, I., Gea, A., Ruiz-Canela, M., Romanos-Nanclares, A., & Toledo, E. (2021). Diatary antioxidant vitamins and minerals and breast cancer risk: prospective results from the SUN cohort. Antioxidants, 10(3), 340. https://doi.org/10.3390/antiox10030340

[8] Gurav, A.S., Kodas, T.T., Wang, L.M., Kauppinen, E.I., & Joutsensaari, J. (1994). Generation of nanometer-size fullerene particles via vapor condensation. Chemical. Physics Letters. 218(4), 304–308. https://doi.org/10.1016/0009-2614(93)E1491-X

[9] He, L., He, T., Farrar, S., Ji, L., Liu, T., & Ma, X. (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cellular Physiology and Biochemistry, 44(2), 532-553. https://doi.org/10.1159/000485089

[10] Kumar, B., Smita, K., Cumbal, L., & Debut, A. (2014). Synthesis of silver nanoparticles using Sacha inchi (Plukenetia volubilis L) leaf extracts. Saudi Journal of Biological Sciences. 21(6), 605-609. https://doi.org/10.1016/j.sjbs.2014.07.004

[11] Kalugendo, E., & Kousalya, P. (2019). Synthesis of silver nanoparticles using Moringa oleifera seeds, Glycyrrhiza glabra stems, and its anti-methicillin resistant Staphylococcus aureus activity. Synthesis, 12(2). https://dx.doi.org/10.22159/aipcr.2019.v12i2.28863

[12] Madukwe, E.U., Ugwuoke, A. L. & Ezeugwu, J. O. (2013). Effectiveness of dry Moringa oleifera leaf powder in the treatment of anemia. International Journal of Medicine and Medical Sciences, 5(5), 226-227. https://doi.org/10.5897/IJMMS2013.0884

[13] Ryan, K., Nordeide, M.B., Hatly, A., Folling, S., & Ray. C. (2004). Medical Microbiology an Introduction to infectious Diseases. 4th edition, 179.

[14] Siddhuraju, P., & Becker, K. (2003). Antioxidant Properties of various solvent extracts of Total phenolic constituents from three different agro-climate origins of drumstick three (Moringa oleifera Lam.). Journal of Agriculture and Food Chemistry, 15(8), 2144-2155. https://doi.org/10.1021/jf020444+

[15] Sies, H. (1997). Oxidative stress: oxidants and antioxidants. Experimental Physiology: Translation and Integration, 82(2), 291-295. https://doi.org/10.1113/expphysiol.1997.sp004024

[16] Singh, A., Sharma, R. K., Agrawal, M., & Marshall, F.M. (2010). Risk Assessment of Heavy Metal Toxicity through Contaminated Vegetables from Waste Water Irrigated Area of Varanasi, India. Tropical Ecology, 51(2), 375-387.

[17] Sun, R. W. Y., Chen, R., Chung, N.P.Y., Ho, C. M., Lin, C. L. S., & Che, C.M. (2005). Silver nanoparticles fabricated I Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. Chemical Communications, (40), 5059-5061. https://doi.org/10.1039/B510984A

[18] Sundararajan, B., & Kumari, B. R. (2014). Biosynthesis of silver nanoparticles in Lagerstroemia speciosa (L.) Pers and their antimicrobial activities. International Journal of Pharmacy and Pharmaceutical Sciences 6(3), 30-34.

[19] Vlerick, M. (2021). Calibrating the balance: The ethics of regulating the production and the use of nanotechnology applications. https://doi.org/10.1515/9783110669282

[20] Zimmerman, M.C., & Zucker, I. H. (2009). Mitochondrial dysfunction and mitochondrial-produced reactive oxygen species: new targets for neurogenic hypertension? https://doi.org/10.1161/HYPERTENSIONAHA.108.125567.

[21] Nestor, A.R.V., Mendieta, V.S., Lopez, M.A.C., Espinosa, R.M.G., Lopez, M.A.C. & Alatorre, J.A.A. (2008). Solventless synthesis and optical properties of Au and Ag nanoparticles using Camiellia sinensis extract. Material Letters. 62(1), 3103–3105. https://doi.org/10.1016/j.matlet.2008.01.138

[22] Narayanan, K. B., & Sakthivel, N. (2011). Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Advances Colloid Interface Science, 169(2), 59–79. https://doi.org/10.1016/j.cis.2011.08.004

[23] Kumari, P., Sharma, P., Srivastava, S., & Srivastava, M. M. (2006). Biosoption studies on shelled Moringa oleifera Lamarck seed powder: removal and recovery of arsenic of aqueous system. International Journal of Mineral Processing, 78(3), 131- 139. https://doi.org/10.1016/j.minpro.2005.10.001

[24] Ramachandran, C., Peter, K. V., & Gopalakshnan, P. K. (1980). Drumstick (Moringa oleifera) a multipurpose Indian vegetable. Economic Botany, 276-283. https://www.jstor.org/stable/4254186

[25] Makkar, H.P.S., & Becker, K. (1997). Nutrients and antiquality factors in different morphological parts of Moringa oleifera tree. The Journal of Agricultural Science, 128(3), 311- 322. https://doi.org/10.1017/S0021859697004292

[26] Moodley, J.S., Krishna, S.B.N., Pillay, K., & Govender, P. (2018). Green synthesis of silver nanoparticles from Moringa oleifera leaf extracts and its antimicrobial potential. Advances in Natural Sciences: Nanoscience and nanotechnology, 9(1), 015011.

[27] Gupta, A., Bonde, S. R., Gaikwad, S., Ingle, A., Gade, A.K., & Rai, M. (2014). Lawsonia inermis-mediated synthesis of silver nanoparticles: activity against human pathogenic fungi and bacteria with special reference to formulation of an antimicrobial nanogel. IET nanobiotechnology, 8(3), 172-178. https://doi.org/10.1049/iet-nbt.2013.0015

[28] Nilanjuna, G., Samrat, P., & Piyali, B. (2014). Silver nanoparticles of Moringa oleifera- Green synthesis, Characterization and its antibacterial efficacy. Journal of Drug Delivery and Therapeutics, 11, 20-25. https://doi.org/10.22270/jddt.v0i0.906

[29] Dexit, S., & Tripathi, A. (2019). A review on biosynthesis, characterization and antimicrobial effect of silver nanoparticles of Moringa oleifera (MO-AgNPs). Journal of Pharmaceutical Sciences and Research, 11(5), 1937-1943. https://www.jpsr.11051947

[30] Amro, N. A., Kotra, L. P., Wadu-Mesthrige, K., Bulychev, A., Mobashery, S., & Liu, G.Y. (2000). High-resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability. Langmuir, 16(6), 2789-2796. https://doi.org/10.1021/la991013x

[31] Danilczuk, M., Lund, A., Saldo, J., Yamada, H., & Michalik, J. (2006). Conduction electron spin resonance of small silver particles. Spectrochimaca Acta Part A: Molecular and Biomolecular Spectroscopy, 63(1), 189-191. https://doi.org/10.1016/j.saa.2005.05.002

[32] Guzman, M., Dille, J., & Godet, S. (2012). Synthesis and antibacterial activity of silver nanoparticles against gram positive and gram negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine, 8(1), 37-45. https://doi.org/10.1016/j.nano.2011.05.007

[33] Hamouda, T., & Baker, J. R. (2000). Antimicrobial mechanism of action of surfactant lipid preparations in enteric gram-negative bacilli. Journal of Applied Microbiology, 89(3), 397-403. https://doi.org/10.1046/j.1365-2672.2000.01127.x

[34] Hamouda T., Myc, A., Donovan, B., Shih, A., Reuter, J.D., & Baker, J.R. (2000). A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. Microbiological Research, 156 (1), 1-7. https://doi.org/10.1078/0944.5013-00069

[35] Kim , J. S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J., …& Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology, and Medicine, 3(1), 95-101. https://doi.org/10.1016/j.nano.2006.12.001

[36] Ramamurthy, C.H., Padma, M., Mareeswaran, R., Suyavaran, A., Kumar M., Premkumar, K., &Thirunavukkarasu, C. (2013). The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. Colloids and Surfaces B: Biointerfaces, 102, 802-815. https://doi.org/10.1016/j.colsurfb.2012.09.025

[37] Sanghi, R., & Verma, P. (2009). Biomimetic synthesis and characterization of protein capped silver nanoparticles. Bioresource Technology, 100(1), 501-504. https://doi.org/10.1016/j.biortech.2008.05.048

[38] MubarakAli, D., Thajuddin, N., Jeganathan, K., & Gunasekaran, M. (2011). Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids and Surfaces B: Biointerfaces, 85(2) 360-365. https://doi.org/10.1016/j.colsurfb.2011.03.009

[39] Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27(1), 76- 83. https://doi.org/10.1016/j.biotechadv.2008.09.002

[40] Nagaich, U., Gulati, N., & Chauhan, S. (2016). Antioxidant and antibacterial potential of silver nanoparticles: biogenic synthesis utilizing apple extract. Journal of Pharmaceutics, 2016. https://doi.org/10.1155/2016/7141523

[41] Baker, C., Pradhan, A., Pakstis, L., Pochan, D. J., & Shah, S.I. (2005). Synthesis and antibacterial properties of silver nanoparticles. Journal of Nanoscience and Nanotechnology, 5(2), 244-249. https://doi.org/10.1166/jnn.2005.034

[42] Zodrow, K., Brunet, L., Mahendra, S., Li, D., Zhang, A., Li, Q., & Alvarez, P. J. (2009). Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. Water Research, 43(13), 715 – 723. https://doi.org/10.1016/j.watres.2008.11.014

[43] Mechauche, M. S., Merouane, F., Messaad, C. E. H., Golzadeh, N., Vasseghian, Y., & Berkani, M. (2022). Biosynthesis, characterization, and evaluation of antibacterial and photocatalytic methylene blue dye degradation activities of silver nanoparticles from Streptomyces tuirus strain. Environmental Research, 204, 112360. https://doi.org/10.1016/j.envres.2021.112360

