SYNTHESIS, CHARACTERISATION, CYTOTOXICITY AND ANTIBACTERIAL STUDIES OF GREEN SYNTHESISED SILVER NANOPARTICLES USING LEAVES OF *POLYALTHIA SCLEROPHYLLA*

Mustafa Mudhafar[1,*], Hasan Ali Alsailawi[2], Ismail Zainol[3] and Che Nor Aiza Jaafar[4]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Ahl Al Bayt, 56001, Karbala, Iraq
[2]College of Dentistry, University of Ahl Al-Bait, 56001, Karbala, Iraq
[3]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Proton City, 35900 Tanjung Malim, Perak, Malaysia.
[4]Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43000 Serdang Selangor, Malaysia

*almosawy2014@gmail.com

Abstract. The study was initiated to prepare silver nanoparticles (AgNPs) by using the green method with the objective to examine the biomedical properties of AgNPs from cytotoxicity and antibacterial properties against MG-63 human cell line and *Staphylococcus Aureus* (*S. aureus*) as Gram-positive and *Escherichia Coli* (*E. coli*), respectively. The rapid green method was performed by using silver nitrate ($AgNO_3$) and crude extract of leaves of *Polyalthia sclerophylla* (CEPS) as the initiator and reducer, respectively. Two different concentrations of $AgNO_3$ were used to prepare two AgNPs sizes; the prepared samples were labelled as AgNPs-a and AgNPs-b respectively. AgNPs-a and AgNPs-b were determined for their chemical, physical and biomedical properties by using various characterisation techniques including Energy Dispersive X-Ray Analysis (EDX) and ultraviolet-visible spectroscopy (Uv-vis). Other techniques included Scanning Transmission Electron Microscopy (STEM) and Scanning Electron Microscopy (SEM). The results showed that the wavelength of AgNPs-a was 436 nm, while the AgNPs-b showed 441 nm. Prepared samples were shown spherical in their shapes according to SEM and STEM images. The size of the particles was not the same, whereas, the diameter size range of AgNPs-a was from 48nm to 68 nm, while the AgNPs-b showed a range from 59 nm to 77 nm. Prepared samples showed availability for the MG-63 cells of more than 83 % for all concentrations after 24 hours. It was found that AgNPs had more inhibition for the bacteria growth against both of the bacteria. The study had demonstrated that non-toxic prepared samples have the ability to use as an antibacterial agent, with nano sizes that can be used safely in the medical and biological fields.

Keywords: AgNPs, *polyalthia sclerophylla,* green synthesis, cytotoxicity study, antibacterial

Article Info

Received 21st October 2022
Accepted 14th December 2022
Published 23rd December 2022

ISSN: 1823-7010, eISSN: 2600-7444

Introduction

In the past few years, researchers have turned their attention to the synthesis of nanomaterials due to their unique properties [1]. The field of nanosynthesis of materials includes many metals such as gold (Au), silver (Ag), lead (Pb) and others [2-4]. One of the important metals that have been focused on by the researchers was Ag due to its unique properties, which is used in many applications such as medical, industrial, biological etc. [5,6]. The study was conducted to examine the synthesised AgNPs due to its unique properties as mentioned above.

Nanomaterials were synthesised using three types of methods including physical, chemical and green synthesis. However, there are many challenges that researchers faced during the synthesis of nanomaterials, as well as when using them in vital fields. The physical and chemical methods of the nanomaterials are needed requirements and one of the important ones is the cost of the preparation is very high. However, the green chemistry compared to the chemical and physical methods, has many advantages [9,10]. The advantages of green chemistry include eco-friendly, cheaper and appropriate to synthesise large-scale from scaled-up. Green synthesised method was chosen due to its advantages.

In the previous studies, the methods that were used to synthesise AgNPs were developed by the researchers through the green chemistry approach according to three steps including: stability, stenosis and reduction. Green chemistry methods are used extracts from natural sources from many biological materials including amino acids, vitamins, plants, enzymes etc. [7,8]. The method of the synthesised AgNPs in the present study was done using green chemistry through with non-toxic and environmentally friendly biological materials [11,12]. Medicinal plants considered as very important source to be used as a reducing agent due to easy to be collected, economically cheaper and non-toxic [13].

Polyalthia sclerophylla (PS) is a species of *Polyalthia* genus, which belongs to Annonaceae family and it is distributed in the tropics and subtropics areas. *Polyalthia* genus is very rich in terms of its medicinal values and applied in the properties like antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, antihyperglycemic, hepatoprotective and anti-HIV-1 activity from the leaves and twigs [14-18]. According to previous studies, there was only one study about twigs and leaves of PS to evaluate its properties as anti-HIV-1 and the study showed significant results [19]. However, there was no study reported synthesising AgNPs from CEPS. The leaves of PS were used due to its biological properties, which are used to enhance the ability of AgNPs to inhibit bacteria growth. Furthermore, the leaves are eco-friendly, non-toxic, easy to get and to be used.

Materials and Methods

Preparation of Crude Extract of PS's Leaves

The leaves of PS (LPS) were collected from Tanjong Malim, Perak Malaysia. The leaves were cleaned with water for several times to remove all dust and fungus from the leaves. Then, the LPS were sun-dried for seven days, subsequently crushed and pounded completely to obtain the powder of LPS. The dried LPS were extracted by using hot-extraction method with the application of Soxhlet extractor and distilled water (DW) as a solvent. CEPS were prepared and approximately, 10 g of LPS powder have been placed in

the round bottom flask of the Soxhlet with 100 ml DW. The temperature of the Soxhlet was set at 80 ºC for half an hour. CEPS were filtered and stored in at -4 ºC for the following research work.

Synthesis Method of AgNPs

Silver nitrate ($AgNO_3$) was purchased from the Bendon brand (Malaysia). Two concentrations of silver nitrate ($1x10^{-3}$ M and $1x10^{-4}$ M) were used to synthesise two type of AgNPs samples namely AgNPs-a and AgNPs-b. Briefly, $AgNO_3$ with concentration of $1x10^{-3}$M solution were dissolved in 100 ml of DW in conical flask and placed in hot-bath at 80 ºC for 40 min. About 10 ml of CEPS were then added gently to the solution, and the solution's colour transformed from colourless to yellow-brown, which indicated the conversion of the Ag ion to Ag^0. The AgNPs solution was centrifuged at 14,000 rpm for 15 minutes. The bottom part of the micro tube was collocated, which represented as AgNPs-a, with the upper part has been discarded. The same procedure was repeated for $AgNO_3$ with concentration of $1x10^{-4}$ M to prepare AgNPs-b.

Ultraviolet-visible Spectrophotometer (Uv-Vis)

The Uv-Vis spectrophotometer (Agilent, Cary 60) was used to confirm the present of nano Ag in the sample solution. The leave extract (CEPS), argentum nitrate ($AgNO_3$) and two synthesized nano Ag solutions (AgNPs-a and AgNPs-b) were prepared and analyzed using Uv-Vis spectrophotometer from 200 nm to 800 nm using Uv-Vis.

Scanning Electron Microscopy (SEM).

The SEM, Nova NanoSEM 450 was used to observe the morphology of AgNPs. Prior to imaging, the samples were mounted on aluminum stubs and coated with Pt for better conductivity using Quorum Q150RS cryocooler. EDX (Bruker X Flash 6110) was used to prove the liberation of silver ions, as well as the presence of free silver in the sample

Scanning Transmission Electron Microscope (STEM) Analysis

A scanning transmission electron microscope (STEM) is a type of transmission electron microscope (TEM). FESEM model Hitachi SU8020 was used to obtain STEM analysis of samples. This analysis was used to confirms the 2-dimensional structure of AgNPs and helps in measuring the lateral dimensions of nano samples. To obtain the images for AgNPs samples, a drop of sample solution was diluted in acetone and dropped 13 casts on a copper TEM grid and allowed to dry for several minutes before scanning.

Cytotoxicity of AgNPs

Alamar blue assay was used to examine the cytotoxicity of the prepared samples (AgNPs-a and AgNPs-b) against MG-63 human cells. The samples were immersed in the complete media 200 mg/ mL for 24 hours at 37 ℃ without agitation, with a weight-volume ratio of 200 mg/ml. Meanwhile, the present study did not include any materials in the media which is represented for the negative control. The pure extracts were diluted with media to make a weight-volume ratio of 100, 75, 50, and 25 mg/ml dilutions. The pure extract and diluted extracts were added into healthy monolayer MG-30 cell which seeded with 1×10^5 cells/mL in 24-multiwell plates for 24 hours in CO_2 incubator at 37 °C. Upon the completion

of incubation process, the next step was to determine the cell viability, which was done using the Alamar blue assay (Invitrogen, USA). Cultures were stained in CO_2 incubator for 24 hours at 37 °C. After staining, the stained culture was detected by absorbance at 570 nm using universal microplate reader (Bio-Tek Instruments, USA).

Antibacterial Activity of AgNPs

Paper disk diffusion test was employed to examine antibacterial activities of the prepared AgNPs against *E. coli* and *S. aureus*. Nutrient agar media was used to cultivate bacteria. 10 µg of AgNPs-s and AgNPs-b were saturated in 1 ml of DW and tested against the bacteria. CEPS was also examined. Next, a total of 10, 20, 40, and 80 µM/ml of AgNPs-a, as well as AgNPs-b were dissolved in DW and poured onto a 6 mm disk filter. Upon 24 hours incubation, inhibited zones were measured and the magnitude of antibacterial impacts on *S. aureus* and the *E. coli* were determined. Water was used as a negative control and ampicillin was used as a positive control.

Results and Discussion

The study was conducted to green-synthesise and characterise the Ag nano particles (AgNPs) by using $AgNO_3$ as starting materials and CEPS as reducing agent. The first indication of conversion of silver ion into silver nano particle was the colour change of the solution from colourless to yellow-brown. The changes of were due to the effect of surface plasmon resonance (SPR) of the synthesised Ag nano particles in the solution. This observation was also reported by other researchers even though they were using different type of reducing agent [20-24] Uv-vis spectrophotometer was used to affirm the fashioning of AgNPs, SPR of the AgNPs attributed to the optical properties [20,21]. SPR band formation due to the occurrence of the light on the surface of particles, which led to the absorption of the waves in the visible light [22,23]. It was reported that AgNPs were demonstrated to absorb and showed peak in a range of 390- 460 nm [24].

Figure 1 shows absorbance peaks of raw materials used i.e $AgNO_3$, CEPS and the two nano silver samples i.e AgNPs-a and AgNPs-b. As expected, extract of the leaves and $AgNO_3$ did not show any absorbance peaks in wavelength range 200-600 nm. However, the effect of $AgNO_3$ concentrations were clearly observed, the absorbance peak was maximum at 436 nm for AgNPs-a ($1x10^{-3}$ M) sample and at 441 nm for AgNPs-b ($1x10^{-4}$ M) sample. The results indicated that, increase the concentrations of the $AgNO_3$ may results decreased the absorbance peak. As reported earlier, the shift in absorbance peak may be attributed to many reasons, such as size and shapes of the particles [25]. The similar effect for the different concentrations of silver nitrite was also documented in the study of Karimi et al. [26]. They found that the peak of the wavelength have been shifted from red (higher wavelength) to blue (lower wavelength) with the increase in the concentration of the silver nitrite. The relationship between the size of the silver particles and absorbance peak have been demonstrated previously. The red shift indicated bigger size, compared to blue shift [27]. Our study affirmed the reason of the shift was due to the size of the particles according to SEM and STEM analysis.

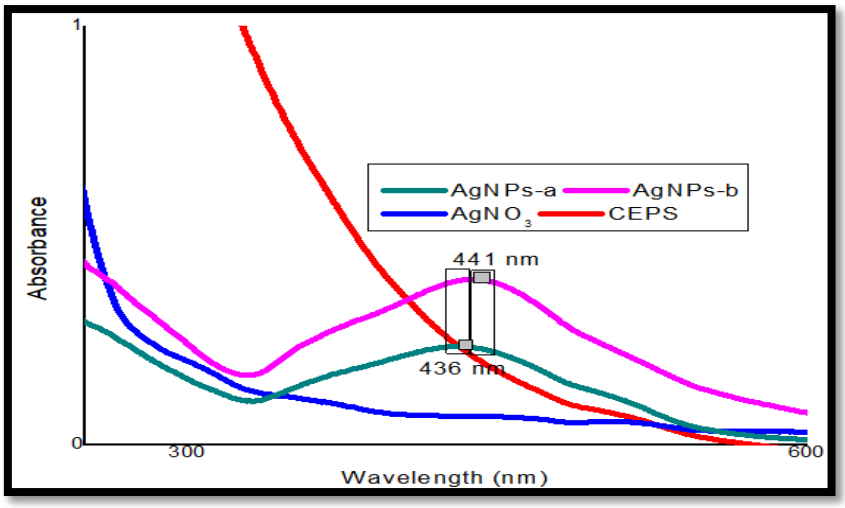

Figure 1: Uv-vis Spectrum of the a) AgNPs-a, b) AgNPs-b and c) $AgNO_3$ and CEPS

Figure 2 shows the SEM images of AgNPs-a, and AgNPs-b, both of the samples had shown same surface morphology and the shapes of the particles for the samples were spherical. Meanwhile, the sizes of the particles were decreased with the increase in the concentrations of $AgNO_3$. Whereas the images of SEM showed the range of the AgNPs-a particles were from 48 nm to 68 nm, while the AgNPs-b particles were from 59 nm to 77 nm. Study of Htwe et al. [25] reported that the effect of the silver nitrate concentrations on the morphology of AgNPs was identified under SEM image analysis. The results of the SEM image analysis showed spherical shape with homogenous surface for the AgNPs, while the sizes of the particles of silver were not same. On another note, the size of the particles was decreased along with the increase of the concentration of silver nitrate. The outcome from their study supported our results.

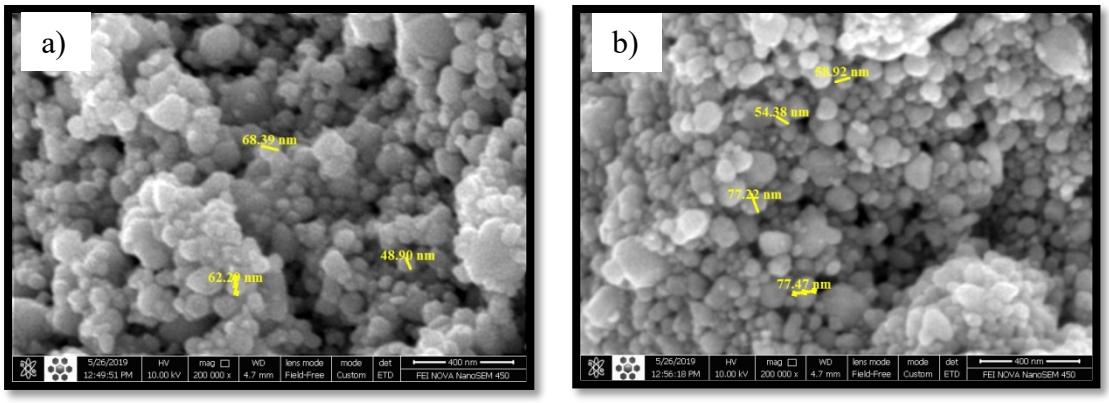

Figure 2: SEM Images of a) AgNPs-a and b) AgNPs-b

EDX technique has been used to emphasise the peaks of the AgNPs, the strong peak for the Ag has been obtained at 3 keV. Previous studies documented the peak of silver nanoparticles in 3 keV for silver nanoparticles from 5-250 nm [28].

Figures 3(a) and (b) show the EDX spectrum of AgNPs-a and AgNPs-b, respectively. The presence of silver element along with oxygen and carbon being observed in both spectra. The AgNPs-b spectrum showed carbon with higher percentage than the silver element, while

the AgNPs-a spectrum showed carbon with lower percentage than carbon Oxygen and carbon peaks were observed as a result of the presence of oxygen and carbon in the CEPS. These results indicated that the presence of silver nanoparticles is surrounded by carbon and oxygen [29]. The EDX profile showed a spectral signal in the silver region (Ag) approximately at 3 keV, which corresponded to the absorption of metallic silver due to surface plasmon resonance (SPR) of AgNPs with size from 20-350 nm [30].

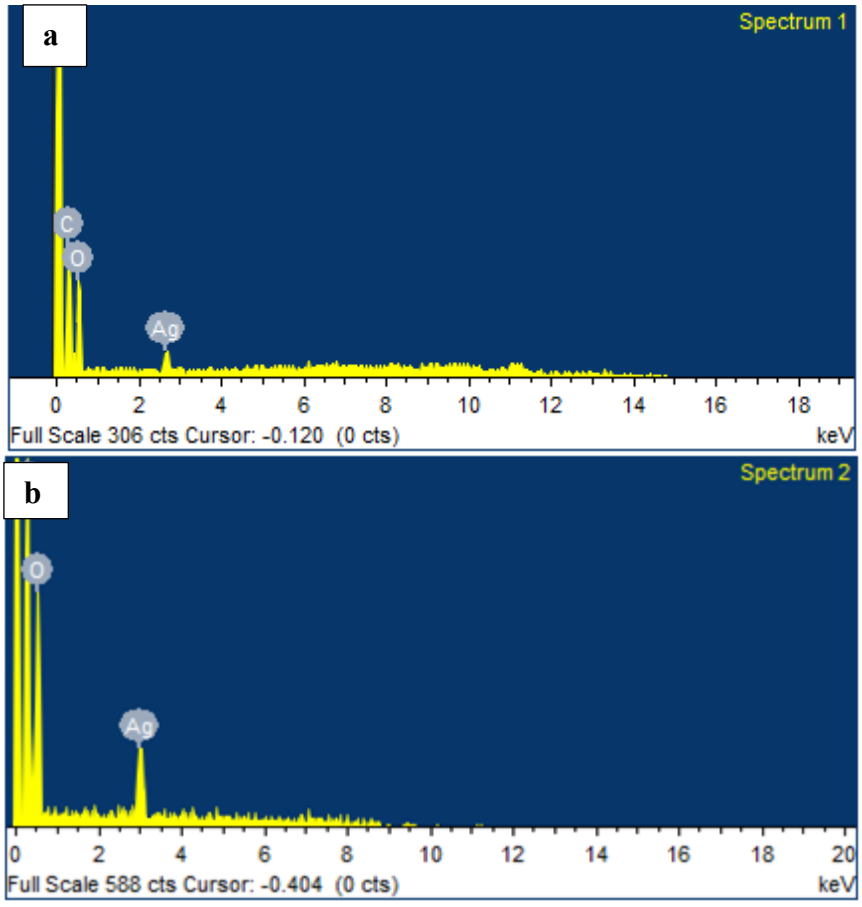

Figure 3: EDX Spectrum of a) AgNPs-a and b) AgNPs-b

STEM analysis was used to confirm the shape and morphology of the synthesized silver nanoparticles. Figures 4(a) and (b) shows the STEM image of AgNPs-a and AgNPs-b samples respectively. The shape of the synthesized silver nanoparticles was spherical, which is identical to the obtained results that were mentioned in the SEM analysis. The size of the particles was measured in Figure 4, which the maximum particles in the size ranged from diameter of 46 to 56 nm for AgNPs-a, while the particles size of AgNPs-b was from 42 nm to 70 nm. These results are aligned with the SEM obtained results. In AgNPs-b there were some particles with more than 100 nm, which might be due to the particles were clumped together while being thawed and put on a copper grid before the examination.

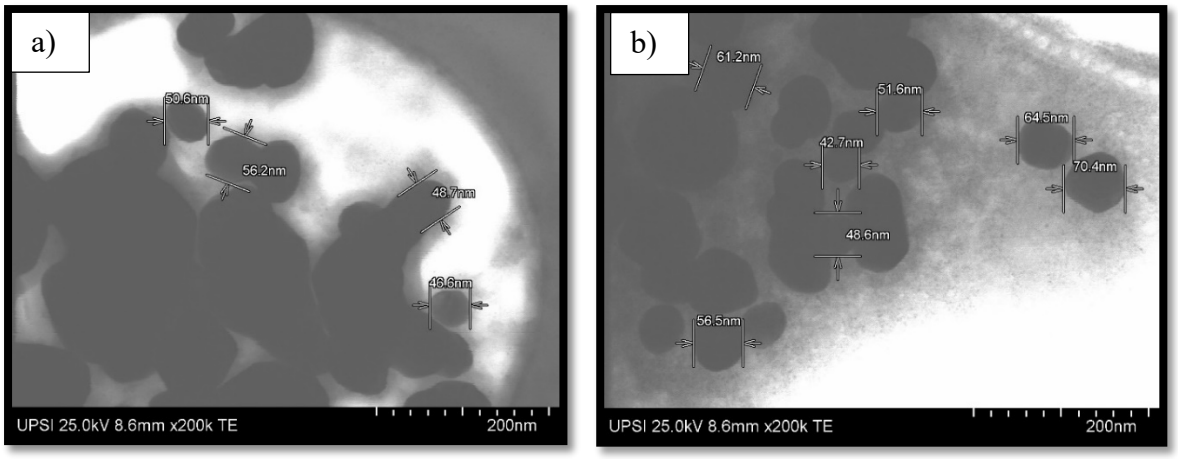

Figure 4: STEM image of a) AgNPs-a and b) AgNPs-b

Cytotoxicity Study of AgNPs-a and AgNPs-b

MG-63 human cells were used to study the cytotoxicity of the AgNPs using the Alamar blue assay. Based on Figure 5, the viability of the cell treated with the AgNPs-a and AgNPs-b in all concentrations was greater than 83 %. Alamar blue assay was used to determine the cell viability of MG-63 with the presence of AgNPs. Figure 5 shows the cell viability of MG-63 after treated with different concentration of extracted samples. Both samples show cell viability for more than 83 % at all concentrations. The outcomes of this study showed there was no toxicity for the prepared samples even at the higher concentrations, which demonstrated it can be used safely and can be applied in the medical and biological fields. The results were aligned with the findings reported by Albers et al. [31]. According to literature, AgNPs maybe toxic at the higher concentrations, which indicates the possibility to use AgNPs in the human body with control dose in order to take advantage of the unique properties of silver nanoparticles and avoiding the possible possibilities of side effects by using a few ratios of it. The studies had demonstrated a range of concentrations, which is non-toxic 10 µg/ml [32], 25 µg/mL [33], 40 µg/mL [34], 50 µg/mL [35], 100 µg/mL [36], 160 µg/mL, 320 µg/mL [37] and 300 µg/m. There are many researchers reported the toxicity of AgNPs as indicated below when it is synthesised through the use of natural material in order to reduce the potential of silver nanoparticles cytotoxicity [13].

Table1: The cell viability of Mg-63 attained after exposure to the AgNPs-a and AgNPs-b For 24 h

Concentrations	Control	AgNPs-a	AgNPs-b	Cell viability	
25 mg/ml	0.253	0.21	0.223	83.0	88.1
50 mg/ml	0.277	0.259	0.241	93.5	87.0
75 mg/ml	0.304	0.277	0.267	91.1	87.8
100 mg/ml	0.238	0.213	0.212	89.5	89.1
200 mg/ml	0.305	0.267	0.346	87.5	113.4
AVERAGE				**89.0**	**93.6**

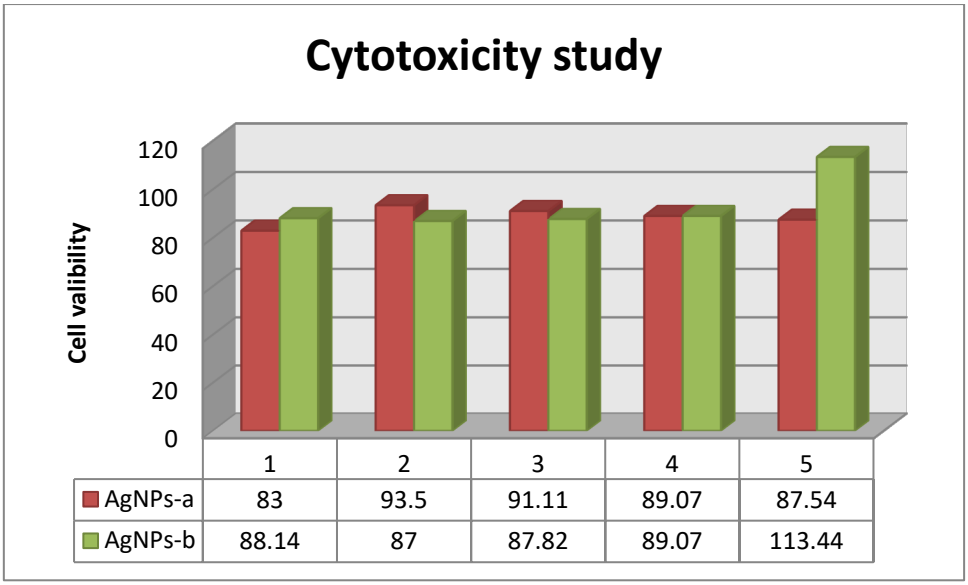

Figure 5: The cell viability of MG-63 percentages at several concentrations of a) AgNPs-a and b) AgNPs-b

Antibacterial Activities of AgNPs-a and AgNPs-b

Table 2 shows two parts; the first part explains the positive (ampicillin) and negative (DW) controls along with the CEPS while the second part illustrates the ability of AgNPs-a and AgNPs-b in the inhibitation of bacteria growth against *E. coli* and *S. aureus*. The first part shows clearly the ability of CEPS to be used as an antibacterial agent that demonstrated significant activity against both of the bacteria reaching 8.8 mm and 7 mm at higher concentrations. This ability was increased in the AgNPs-a and AgNPs-b. The outcomes for this study showed a significant effect on the growth of the bacterial, whereas the inhibition growth of AgNPs-a reached 24 mm and 22 mm against *E. coli* and *S. aureus* respectively. Meanwhile, the AgNPs-b showed 20 mm and 18 mm against both pathogens respectively.

Table 2: Antibacterial activities of AgNPs-a, AgNPs-b and CEPS, with controls

No	Conic	CEPS		Ampicillin		DW	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
1	10 µM/ml	6 mm	5 mm	21 mm	18 mm	0	0
2	20 µM/ml	7 mm	5.4 mm	24 mm	19 mm	0	0
3	40 µM/ml	8.4 mm	6.2 mm	28 mm	24 mm	0	0
4	80 µM/ml	8.8 mm	7 mm	33 mm	29 mm	0	0

No	Conic	AgNPs-a		AgNPs-b	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
1	10 µM/ml	14 mm	15 mm	13 mm	12 mm
2	20 µM/ml	16 mm	16 mm	16 mm	15 mm
3	40 µM/ml	20 mm	20 mm	18 mm	16 mm
4	80 µM/ml	24 mm	22 mm	20 mm	18 mm

Results showed that AgNPs-a had more effect than AgNPs-b, which was due to the dissimilarity of the particle size of them. As mentioned previously, the smaller size of the nanoparticles can show a higher effect against bacteria [29]. The reason for that can be explained by two or three mechanisms but the most acceptable reason is the small particle size have more surface area, which has more advantage to these particles to penetrate the cell wall of the bacteria than leading to accumulating particles then damage the reactive oxygen species (ROS), which makes it dead. The inhibition of the bacteria zone in *E. coli* was more than the inhibition of *S. aureus,* that due to the structures of the bacteria cell wall, the most acceptable mechanisms to inhibition of the bacteria growth, says that, the nanoparticles is congregate and then pierce the wall down to the ROS of the bacteria, the cell wall structure play important role in that matter.

Conclusions

This study had successfully synthesized the AgNPs by applying the green synthesis method. The leave extract of *polyalthia sclerophylla* successfully used to reduce the AgNO3 into silver nano particles. The size of AgNPs can be altered by varying the concentration of $AgNO_3$. The higher the concentration of $AgNO_3$ the smaller the AgNPs particles size. The shape of AgNPs produced in this study was spherical. The smaller AgNPs particles gives more effective effect on the antibacterial properties. This study was demonstrated the AgNPs produced was non-toxic and can be used safely in the medical and biological fields.

Acknowledgements

The financial support for this research was provided by Ministry of Higher Education Malaysia under FRGS Grant no. 2019-0147-103-02.

Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards

References

[1] Mudhafar, M., Zainol, I., Jaafar, C. N. A., Alsailawi, H. A. & Majhool, A. A. (2020). Microwave-Assisted Green Synthesis of Ag Nanoparticles using Leaves of Melia Dubia (Neem) and its Antibacterial Activities. *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, 65(1), 121-129.

[2] Tailor, G., Yadav, B. L., Chaudhary, J., Joshi, M. & Suvalka, C. (2020). Green synthesis of silver nanoparticles using Ocimum canum and their anti-bacterial activity. *Biochemistry and Biophysics Reports*, 24, 100848.

[3] Zainurin, M. A. N., Zainol, I. & Mudhafar, M., (2022). Biogenic Synthesis Of Silver Nanoparticles Using Neem Leaf Extract As Reducing Agent And Hydrolyzed Collagen As Stabilizing Agent. *Malaysian Journal of Microscopy*, 18(1).

[4] Shahid, M., Farooq, A. B. U., Rabbani, F., Khalid, S. & Dumat, C. (2020). Risk Assessment and Biophysiochemical Responses of Spinach to Foliar Application of Lead Oxide Nanoparticles: A Multivariate Analysis. *Chemosphere*, 245, 125605.

[5] Alharbi, N. S., Alsubhi, N. S. & Felimban, A. I. (2022). Green synthesis of silver nanoparticles using medicinal plants: characterization and application. *Journal of Radiation Research and Applied Sciences*, 15(3), 109-124.

[6] Naganthran, A., Verasoundarapandian, G., Khalid, F. E., Masarudin, M. J., Zulkharnain, A., Nawawi, N. M. & Ahmad, S. A. (2022). Synthesis, Characterization and Biomedical Application of Silver Nanoparticles. *Materials*, 15(2), 427.

[7] Ijaz, M., Zafar, M. & Iqbal, T. (2020). Green Synthesis of Silver Nanoparticles by Using Various Extracts: A Review. *Inorganic and Nano-Metal Chemistry*, 51(5), 744-755.

[8] Yaqoob, A. A., Umar, K. & Ibrahim, M. N. M. (2020). Silver Nanoparticles: Various Methods of Synthesis, Size Affecting Factors and Their Potential Applications–A Review. *Applied Nanoscience*, 10(5), 1369-1378.

[9] Prasad, B. S. & Padmesh, T. V. N. (2014). Common Duckweed (Lemna minor) Assisted Green Synthesis of Silver Nanoparticles as Potent Anti-Fungal Nanomaterial. *Research Journal of Pharmacy and Technology*, 7(9), 955-958.

[10] Krishnaraj, C., Harper, S. L. & Yun, S. I. (2016). In Vivo Toxicological Assessment of Biologically Synthesized Silver Nanoparticles in Adult Zebrafish (Danio rerio). *Journal of Hazardous Materials*, 301, 480-491.

[11] Bose, S., Shinde, H., Karikalan, K., Lalitha, P. & Mandal, A. K. A. (2016). Antibacterial, Anti-inflammatory and Antiproliferative Activity of Silver Nanoparticles Synthesized from Leaf Extract of Azadirachta Indica A. Juss. *Research Journal of Pharmacy and Technology*, 9(12), 2422-2426.

[12] Asha, S., Thirunavukkarasu, P. & Rajeshkumar, S. (2017). Green Synthesis of Silver Nanoparticles using Mirabilis Jalapa Aqueous Extract and Their Antibacterial Activity Against Respective Microorganisms. *Research Journal of Pharmacy and Technology*, 10(3), 811-817.

[13] Mudhafar, M., Zainol, I., Jaafar, C.N.A., Alsailawi, H. A. & Desa, S. (2021). A Review Study on Synthesis Methods of AgNanoparticles, Considering Antibacterial Property and Cytotoxicity. *International Journal of Drug Delivery Technology*, 11(2), 635-48.

[14] Sudhakar, T., Premkumar, J., Sapkota, R. & Rijal, S. (2017). Antimicrobial Activity of Silver Nanoparticles Synthesized from Ficus Benghalensis against Human Pathogens. *Research Journal of Pharmacy and Technology*, 10(6), 1635-1640.

[15] Chen, Y. C., Chia, Y. C. & Huang, B. M. (2021). Phytochemicals from Polyalthia Species: Potential and Implication on Antioxidant, Anti-inflammatory, Anti-cancer, and Chemoprevention Activities. *Molecules*, 26(17), 5369.

[16] Mumtaz, S., Nadeem, R., Sarfraz, R. A. & Shahid, M. (2022). Mechanism Study of Green Synthesis and Antibacterial Attribute of Polyalthia longifolia Based Gold Nanoparticles. In *Journal of Nano Research*. Vol. 75. (Trans Tech Publications Ltd.), pp. 1-16.

[17] Mudhafar, M. & Zainol, I. (2019). Medical Values, Antimicrobial and Anti-Fungal Activities of Polyalthia Genus. *International Journal of Pharmaceutical Research*, 11(1).

[18] Firdous, S. M., Ahmed, S. N., Hossain, S. M., Ganguli, S., & Fayed, M. A. (2022). Polyalthia longifolia: phytochemistry, ethnomedicinal importance, nutritive value, and pharmacological activities review. *Medicinal Chemistry Research*, 31(8), 1252-1264

[19] Saepou, S., Pohmakotr, M., Reutrakul, V., Yoosook, C., Kasisit, J., Napaswad, C. & Tuchinda, P. (2010). Anti-HIV-1 Diterpenoids from Leaves and Twigs of Polyalthia sclerophylla. *Planta Medica*, 76(07), 721-725.

[20] Hamedi, S. & Shojaosadati, S. A. (2019). Rapid and Green Synthesis of Silver Nanoparticles using Diospyros Lotus Extract: Evaluation of Their Biological and Catalytic Activities. *Polyhedron*, 171, 172-180.

[21] Daphne, J., Francis, A., Mohanty, R., Ojha, N. & Das, N. (2018). Green synthesis of Antibacterial Silver Nanoparticles using Yeast Isolates and its Characterization. *Research Journal of Pharmacy and Technology,* 11(1), 83-92.

[22] Dangi, S., Gupta, A., Gupta, D. K., Singh, S. & Parajuli, N. (2020). Green Synthesis of Silver Nanoparticles using Aqueous Root Extract of Berberis Asiatica and Evaluation of Their Antibacterial Activity. *Chemical Data Collections*, 28, 100411.

[23] Bala, R., Madaan, R. & Arora, S. (2017). Green Synthesis and Characterization of silver nanoparticles using Kinnow mandarian peels extract and its application in Shampoo Formulation. *Research Journal of Pharmacy and Technology*, 10(8), 2461-2466.

[24] Mudhafar, M., Zainol, I., Alsailawi, H. A. & Jaafar, C. N.A. (2021). Green Synthesis of Silver Nanoparticles using Neem and Collagen of Fish Scales as a Reducing and Stabilizer Agents. *Jordan Journal of Biological Sciences*, 14(5).

[25] Htwe, Y. Z. N., Chow, W. S., Suda, Y. & Mariatti, M. (2019). Effect of silver nitrate concentration on the production of silver nanoparticles by green method. *Materials Today: Proceedings*, 17, 568-573.

[26] Karimi, M. A., Mozaheb, M. A., Hatefi-Mehrjardi, A., Tavallali, H., Attaran, A. M. & Shamsi, R. (2015). A new simple method for determining the critical micelle concentration of surfactants using surface plasmon resonance of silver nanoparticles. *Journal of Analytical Science and Technology*, 6(1), 1-8.

[27] Darroudi, M., Ahmad, M. B., Abdullah, A. H., Ibrahim, N. A. & Shameli, K. (2010). Effect of accelerator in green synthesis of silver nanoparticles. *International Journal of Molecular Sciences*, 11(10), 3898-3905.

[28] Bai, X., Wang, Y., Song, Z., Feng, Y., Chen, Y., Zhang, D. & Feng, L. (2020). The basic properties of gold nanoparticles and their applications in tumor diagnosis and treatment. *International Journal of Molecular Sciences*, 21(7), 2480.

[29] Mudhafar, M., Zainol, I., Jaafar, C. N. A., Alsailawi, H. A. & Majhool, A. A. (2020). Two green synthesis methods to prepared nanoparticles of Ag: two sizes and shapes via using extract of M. dubia leaves. *Journal of Computational and Theoretical Nanoscience*, 17(7), 2882-2889.

[30] Tyagi, S., Alam, T., Khan, M. A., Tarannum, H. & Chauhan, N. (2018). Biosynthesis of silver nanoparticles using medicinal plant Anacyclus Pyrethrum and its antibacterial efficacy. *Asian Journal of Research in Chemistry*, 11(3), 515-520.

[31] Albers, C. E., Hofstetter, W., Siebenrock, K. A., Landmann, R. & Klenke, F. M. (2013). In vitro cytotoxicity of silver nanoparticles on osteoblasts and osteoclasts at antibacterial concentrations. *Nanotoxicology*, 7(1), 30-36.

[32] Reddy, N. J., Vali, D. N., Rani, M. & Rani, S. S. (2014). Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit. *Materials Science and Engineering: C*, 34, 115-122.

[33] Arokiyaraj, S., Arasu, M. V., Vincent, S., Prakash, N. U., Choi, S. H., Oh, Y. K. & Kim, K. H. (2014). Rapid green synthesis of silver nanoparticles from Chrysanthemum indicum L and its antibacterial and cytotoxic effects: an in vitro study. *International Journal of Nanomedicine*, 9, 379.

[34] Huang, Z., Xu, P., Chen, G., Zeng, G., Chen, A., Song, Z. & Hu, L. (2018). Silver ion-enhanced particle-specific cytotoxicity of silver nanoparticles and effect on the production of extracellular secretions of Phanerochaete chrysosporium. *Chemosphere*, 196, 575-584.

[35] Kanagavalli, U., Sadiq, A. M. & Rajeshkumar, S. (2016). Plant assisted synthesis of silver nanoparticles using boerhaavia diffusa leaves extract and evolution of antibacterial activity. *Research Journal of Pharmacy and Technology*, 9(8), 1064.

[36] Chahardoli A, Karimi N. & Fattahi A. (2017). Biosynthesis, characterization, antimicrobial and cytotoxic effects of silver nanoparticles using Nigella arvensis seed extract. *Iranian Journal of Pharmaceutical Research: IJPR*, 31167.

[37] Saravanakumar, K. & Wang, M. H. (2018). Trichoderma based synthesis of anti-pathogenic silver nanoparticles and their characterization, antioxidant and cytotoxicity properties. *Microbial Pathogenesis*, 114, 269-273.