RJMP Research Journal of Medicinal Plants

OPEN ACCESS

Biosynthesis of Silver Nanoparticles from Aqueous Extract of *Salvia officinalis* Aerial Parts and its Antibacterial Activities

Amal Saad Yousif and Ahlam Salih Eltahir

Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan

ABSTRACT

Background and Objective: Medicinal plants used to combat diseases, they are of great importance for primary healthcare due to of their lesser costs, high safety margin and large biological activities. This study for *Saliva officinalis* L. aimed to synthesize silver nanoparticles from an aqueous extract of aerial parts as a reducing agent and evaluate its antibacterial potential. **Materials and Methods:** About 1 mL of 0.01 mM of silver nitrate was added to 5 mL of the prepared aqueous extract. The green synthesized silver nanoparticles were characterized by using UV-vis Spectroscopy and Fourier Transform Infrared Spectroscopy, antibacterial activity was evaluated by disk diffusion method against two Gram-negative (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) and one Gram-positive (*Staphylococcus aureus*) and was compared with the antibacterial activity of the aqueous extract. **Results:** Formation of silver nano particles by reduction of silver ions into silver element was indicated by the change of colour of the reaction mixture from pale yellow to dark brown colour. The UV-vis Spectroscopy absorption peak is obtained in the visible range at 410 nm confirmed the formation of silver nanoparticles. Fourier Transform Infrared Spectroscopy confirmed the presence of ingredients in *Salvia* extract which are responsible for the capping and reduction of the silver. The disk diffusion method results showed potent antibacterial activity against the three bacteria species, showing higher antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and medium activity against *Klebsiella pneumonia*. **Conclusion:** Aqueous silver nanoparticle extracts are found to be more effective against all bacteria studied than the normal aqueous extract so it can improve the activity of the plant extract and it can be further investigated for biomedical applications.

KEYWORDS

Saliva officinalis, silver, nanoparticles, antibacterial activity, klebsiella *pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus*

INTRODUCTION

The use of plant-based materials including herbal or natural health products with supposed health benefits is increasing in developed countries. Medicinal plants have been used for many centuries to combat diseases, they are of great importance for primary healthcare because of their lesser costs, high safety margin and large biological activities[1]. Microbial diseases are increasing day by day and becoming

ISSN: 2151-7924 (Online)
ISSN: 1819-3455 (Print)
https://doi.org/10.17311/rjmp.2022.20.28

Received: 15 Feb. 2022
Accepted: 03 Aug. 2022
Published: 01 Oct. 2022

a big problem for human health. There are many known diseases which are transmitted by bacteria, fungi, viruses and other microbes to the human being. The development of antibiotic resistance makes scientists around the world seek new drugs that would be more effective. The use and search for drugs obtained from plants and other natural products have increased in recent years. Plants-derived silver nanoparticles can be a good substitute for antimicrobial agents and can help in fighting the problem of drug resistance as nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology[2]. Nanotechnology is the science that manipulates matter at the scale of 1 billionth of a meter and studies matter at the atomic and molecular scale. Metallic nanoparticles have different physical and chemical properties which prove disarmingly in different industrial applications[3]. Silver nanoparticles are interested in many areas because they have a large number of applications[4]. *Salvia* genus includes about 900 species cultivated in many countries for its traditional importance in folk medicine and domestic implementations[5]. *Salvia officinalis* L. (sage) extract and essential oil are used in pharmaceutical industries and food preparation as flavouring agents[6]. *Salvia officinalis* has been used for the treatment of many kinds of diseases like seizure, gout, ulcers, rheumatism, dizziness, inflammation, tremor, diarrhoea, paralysis, hyperglycemia anti-inflammatory, anti-cancer, anti-antioxidant, nociceptive, antimutagenic, antimicrobial, anti-dementia, hypolipidemic effects and hypoglycemic[7]. This study for *Saliva officinalis* L. aimed to synthesize silver nanoparticles from aqueous extract of aerial parts as a reducing agent, characterize the synthesized silver nanoparticles using UV absorbance and FTIR and treat them against one gram-positive and two gram-negative bacteria to compare them with the antibacterial activity of its aqueous extract.

MATERIALS AND METHODS

Study area: The study was carried out in Khartoum Central Sudan the period from May, 2017 to May, 2018.

Materials

Plant materials: *Saliva officinalis* L. aerial parts were purchased from a local market in Saudi Arabia. The identification of plant material had been carried out at the Department of Botany Faculty of Science and Technology, Omdurman Islamic University, Sudan.

Microorganisms: The bacteria *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Gram-negative) were isolated and identified at the Department of Bacteriology (Factually of Veterinary, University of Khartoum, Sudan).

Methods

Preparation of the aqueous extract: The aerial parts of *S. officinalis* were air dried under shade and ground to a fine powder. About 50 g of the powder was macerated in 100 mL boiling distilled water at room temperature. After 24 hrs the solution was filtered using man filter paper (No.1) and kept in the refrigerator for further use.

Synthesis of aqueous silver nanoparticles: About 1 mL of 0.01 mM of silver nitrate ($AgNO_3$) was added to 5 mL of the prepared aqueous extract. After 20 min, the colour of the solution (aqueous extract+$AgNO_3$) changed from light yellow to dark brown, indicating the formation of aqueous AgNPs[8].

Characterization of silver nanoparticles by UV-VIS: The formation of AgNPs was confirmed by measuring its optical properties with UV-Vis spectroscopy (Shimadzu UV 1800) in the range of 200 to 800 nm. To determine the phytochemical compounds involved in the reduction of $AgNO_3$ to AgNPs as well as those involved in the stabilization of AgNPs.

Characterization of silver nanoparticles by Fourier Transform Infra-Red: Fourier Transform Infrared (FTIR) Spectroscopy Binding properties of AgNPs synthesized by *S. officinalis* L. aqueous extract were investigated by Fourier Transform Infrared Spectroscopy (FTIR) analysis. The FTIR measurements were taken on Bruker vertex 70 in the range of 4000-400 cm^{-1}. Dried and powdered AgNPs were palliated with potassium bromide (KBr) (1:1 proportion). The spectra were recorded in the wave number range of 450-2500 cm-k and analyzed by subtracting the spectrum of pure KBr.

Antimicrobial activity

Preparation of standard bacterial suspension: Mueller Hinton agar powder 2.8 g was dissolved into 1000 mL distilled water and allowed to soak for 10 min. Then each 20 mL of the prepared solution was put in 5 bottles. These were sterilized by autoclaving at 121°C for 15 min, after which they were cooled at room temperature.

Disk diffusion method: The antimicrobial activity was evaluated by paper disc diffusion[9] and dilution methods[10]. The disk diffusion assay was used to determine the antibacterial activity of the aqueous extract and the silver nanoparticles of the aqueous extract of *S. officinalis*[11]. Overnight bacterial cultures of *Staphylococcus aurous*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* which were incubated at 37°C in broth media, were spread or swabbed onto the surface of Mueller Hinton agar. *S. officinalis* extracts were applied to 10 mm disks (what man filter paper NO. 1) and then placed onto the inoculated dishes and after 24 hrs of incubation at 37°C, the antibacterial activity was assessed by measuring the diameter of inhibition zones. The control disks were immersed in test tubes containing AgNPs and were placed on agar plates. The zones of inhibition around the disks were measured after one-night incubation at 37°C.

RESULTS AND DISCUSSION

Formation of silver nano particles: Formation of Silver Nano Particles (AgNPs) by reduction of silver ions into a silver element using aqueous extract of *S. officinalis* is indicated by the change of colour of the reaction mixture from pale yellow to dark brown colour in Fig. 1, the colour darkened with time. The $AgNO_3$ solution and leaf extract have not shown any visible colour changes during the same incubation period under similar conditions. Other study[12] stated that silver nanoparticles are synthesized using plant extract showing yellowish-brown colour in an aqueous solution due to excitation of Surface Plasmon vibrations in silver nanoparticles.

Characterization using UV-visible spectrophotometers: The formation of silver nanoparticles in UV-vis absorption spectroscopy analysis is one of the most sensitive, efficient and simple techniques that is commonly used at the preliminary stage to confirm the synthesis of AgNPs. The absorption peak is obtained in the visible range at 410 nm in Fig. 2. Generally metal nanoparticles such as in Ag, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a Surface Plasmon Resonance (SPR) absorption band occurring due to the collective oscillation of electrons of Ag-NPs in resonance with the light wave[13,14]. The UV-vis Spectra depict the surface plasmon resonance peak for *Salvia splendens* found previously at 437 nm[15].

Characterization by Fourier Transform Infra-Red: To identify the phytochemical compounds from *S. officinalis* extract responsible for the reduction of $AgNO_3$ and those involved in the capping and stabilizing of AgNPs. Fourier Transform Infra-Red (FTIR) analysis was carried out and the result is depicted in Fig. 3. FTIR analysis revealed that the biomolecules present in the leaf extract could be responsible for the reduction of silver ions as well as prolonged stability of nanoparticles. In this study, *S. officinalis* extract showed several spectra that indicate the complex nature of the plant extract and our findings corroborate with the previous results[16]. The spectra of *S. officinalis* extracts displayed a broad and strong absorbance peak at 3248 cm^{-1} that corresponds to O-H vibration, from phenolic compounds or carboxylic group in

Fig. 1: Color change after formation of aqueous silver nanoparticles
1: $AgNo_3$ solution, 2: Aqueous extract+$AgNo_3$ after 5 min reaction and 3: After 2 hrs reaction

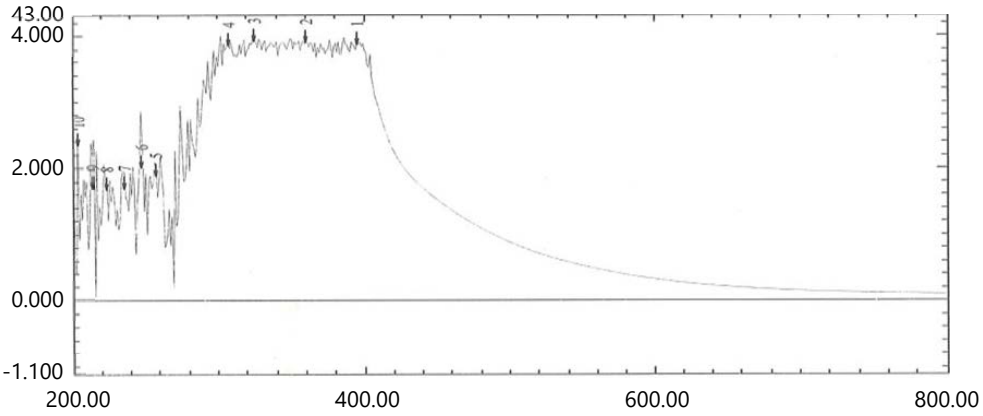

Fig. 2: UV spectrophotometer of the silver NPs aqueous extract of *S. officinalis*

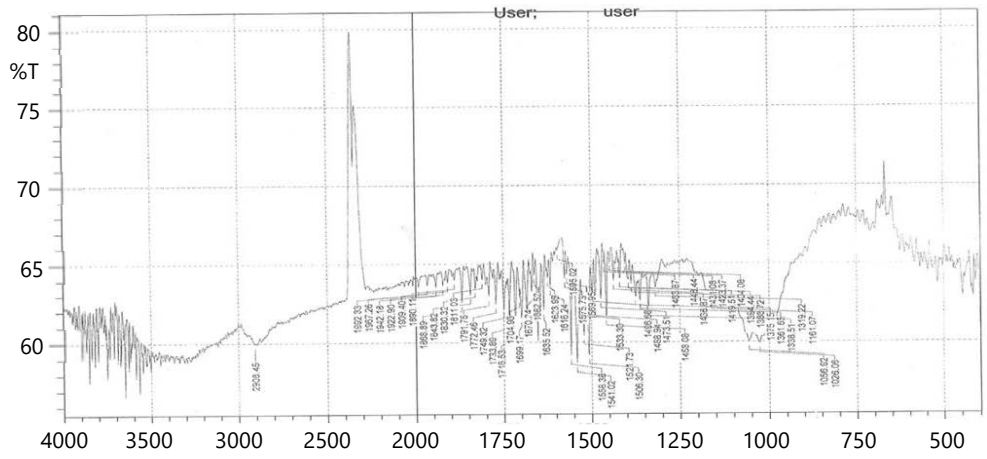

Fig. 3: FTTR spectra of synthesized AgNPs of aqueous extract of *S. officinalis*

the extract[17]. The peak spectrum at 2918.9 cm^{-1} is assigned to the stretching vibration of CH_2 group[18], vibration peak at 1683 cm^{-1} indicates C=O stretching or C-N bending 0f the amide group[16]. The spectra peaks of AgNPs at 3895, 3739, 2842, 2312, 2102, 1900,1683, 1566, 1215 and 1006 cm^{-1} indicate a shift in absorption bands of the phytochemical compounds from aqueous extract of *S. officinalis* stabilizing it. These observations agreed with the reports of other researchers in the literature[19,20]. The FTIR revealed some key phytochemical compounds acting as capping and stabilizing agents.

Antibacterial activity: Antibacterial activities of the aqueous extract and synthesized aqueous AgNPs were determined using the disc diffusion assay method, they were screened against three bacterial strains *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Table 1 shows that the extracts inhibited the growth of bacteria at different levels, the inhibition zone of the concentrations 25 g mL^{-1} against *S. aureus* was 11.07 and 14.3 for aqueous extract and aqueous nanoparticles, respectively in Fig. 4, whereas the inhibition zones against *P. aeruginosa* was 9.77 and 21.00 for aqueous extract and aqueous nanoparticles respectively in Fig. 5 and against *K. pneumonia* were 9.73 and 10.00 for aqueous extract and aqueous nanoparticles respectively in Fig. 6. The inhibition zones of concentrations 50 g mL^{-1} against *S. aureus* were found to be 9.77 and 17.00 for aqueous extract and aqueous nanoparticles respectively in Fig. 7, whereas the inhibition zones against *P. aeruginosa* was 12.73 and 19.67 for aqueous extract and aqueous nanoparticles, respectively in Fig. 8 and against *K. pneumonia* were 9.87 and 11.67 for aqueous extract and aqueous nanoparticles, respectively (Fig. 9). The inhibition zones of concentrations 75 g mL^{-1} were found to be 16.33 and 18.00 against *S. aureus* for aqueous extract and aqueous nanoparticles, respectively in Fig. 10, whereas the inhibition zones against *P. aeruginosa* were 10.67 and 10.30 for aqueous extract and aqueous nanoparticles, respectively in Fig. 11 and against *K. pneumonia* were 10.30 and 10.33 for aqueous extract and aqueous nanoparticles, respectively in Fig. 12. From the present study it has been observed that the aqueous and aqueous silver nano particle extracts are active and the inhibition zones are different for different concentrations and for the different bacteria. *Staphylococcus aureus* is sensitive followed by *Pseudomonas aeruginosa* but *Klebsiella pneumoniae* is resistant. Aqueous silver nanoparticle extracts are found to be more effective than the normal aqueous extract which means that nanoparticles can improve the activity of the plant extract. In previous studies, it was found that the aqueous extract of *S. officinalis* concentration of 100 mg mL^{-1} was most effective against *Staphylococcus aureus*[21]. Other studies with different results[22] higher antibacterial activity against

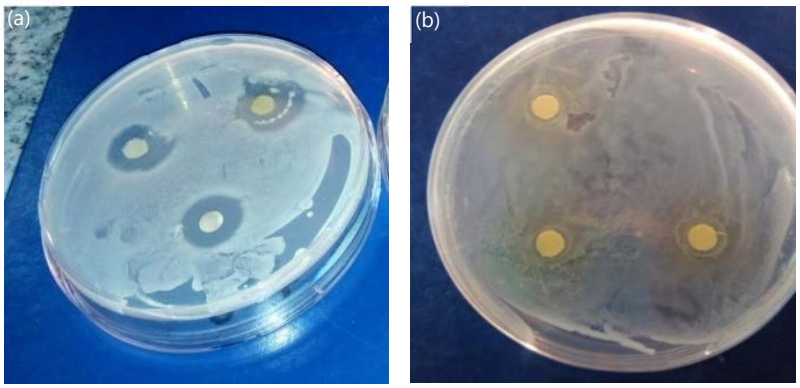

Fig. 4(a-b): Inhibition zones of extracts of concentration 25 g mL^{-1} against *S. aureus*, (a) Aqueous extract and (b) Silver nanoparticle

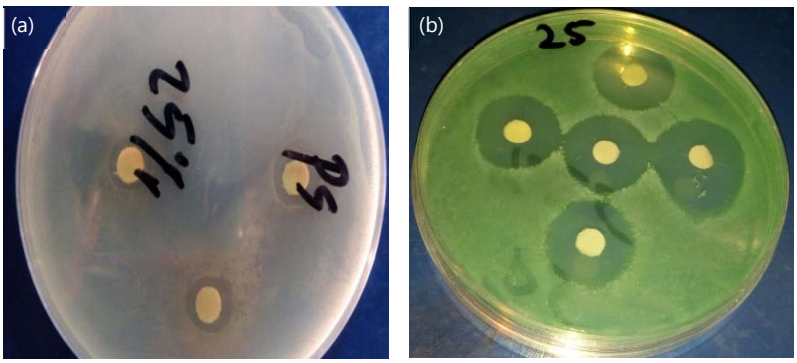

Fig. 5(a-b): Inhibition zones of extracts of concentration 25 g mL^{-1} against *P. aeruginosa*, (a) Aqueous extract and (b) Silver nanoparticle

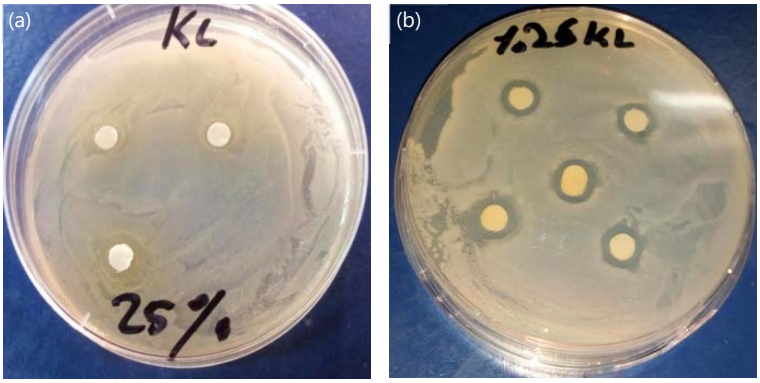

Fig. 6(a-b): Inhibition zones of extracts of concentration 25 g mL^{-1} against *K. pneumoniae*, (a) Aqueous extract and (b) Silver nanoparticle

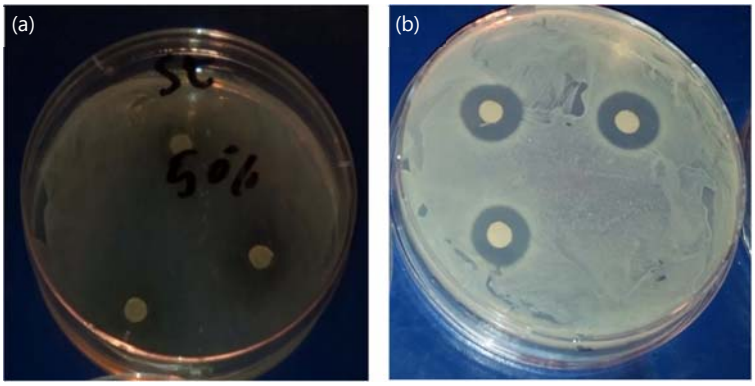

Fig. 7(a-b): Inhibition zones of extracts of concentration 50 g mL^{-1} against *S. aureus*, (a) Aqueous extract and (b) Silver nanoparticle

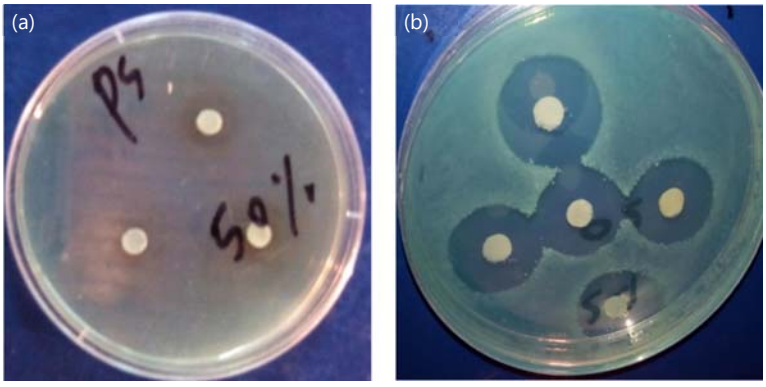

Fig. 8(a-b): Inhibition zones of extracts of concentration 50 g mL^{-1} against *P. aeruginosa*, (a) Aqueous extract and (b) Silver nanoparticle

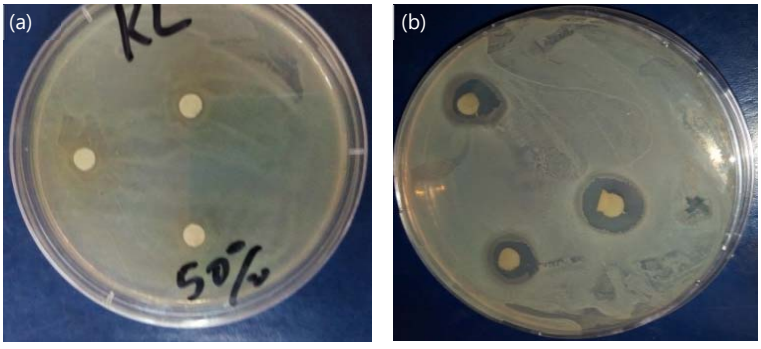

Fig. 9(a-b): Inhibition zones of extracts of concentration 50 g mL^{-1} against *K. pneumoniae*, (a) Aqueous extract and (b) Silver nanoparticle

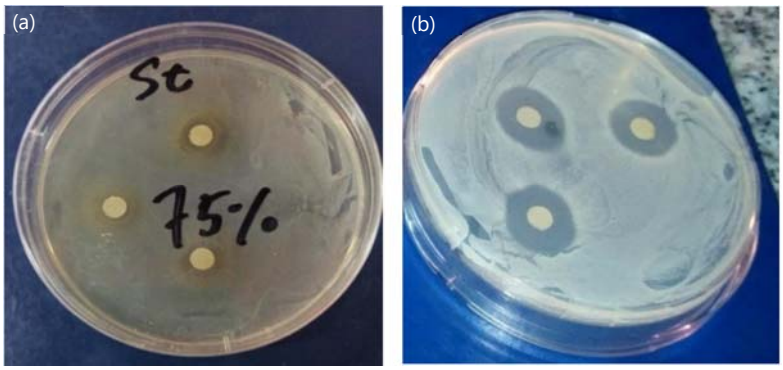

Fig. 10(a-b): Inhibition zones of extracts of concentration 75 g mL^{-1} against *S. aureus*, (a) Aqueous extract and (b) Silver nanoparticle

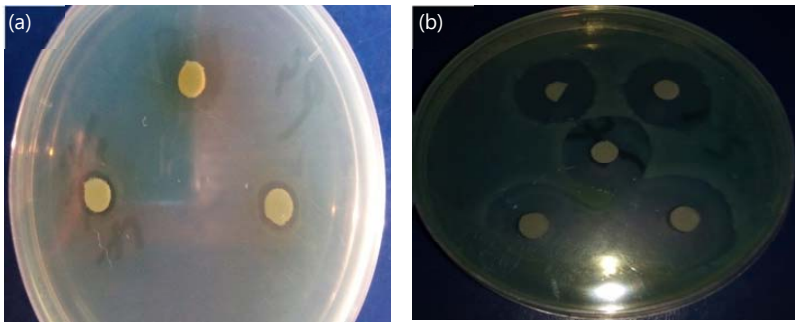

Fig. 11(a-b): Inhibition zones of extracts of concentration 75 g mL^{-1} against *P. aeruginosa*, (a) Aqueous extract and (b) Silver nanoparticle

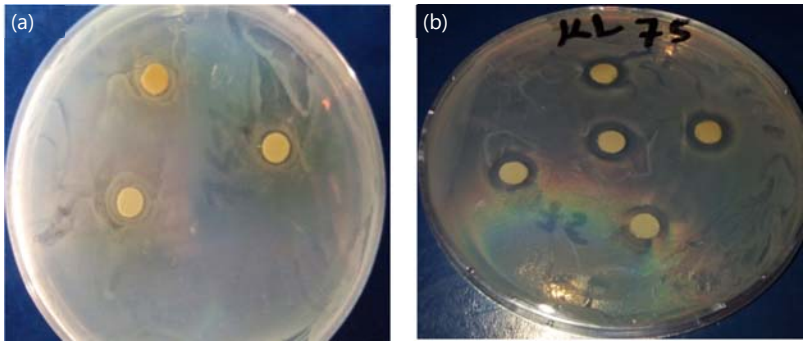

Fig. 12(a-b): Inhibition zones of extracts of concentration 75 g mL^{-1} against *K. pneumoniae*, (a) Aqueous extract and (b) Silver nanoparticle

Table 1: Mean inhibition zones of different concentrations of aqueous extract and aqueous nanoparticles against the tested bacteria

	Bacteria					
	Staphylococcus aureus		*Pseudomonas aeruginosa*		*Klebsiella pneumonia*	
Treatment	Aqueous extract	Aqueous Nanoparticles	Aqueous extract	Aqueous Nanoparticles	Aqueous extract	Aqueous Nanoparticles
25 g mL^{-1}	11.07	14.33	9.77	21.00	9.73	10.00
50 g mL^{-1}	9.17	17.00	12.73	19.67	9.87	11.67
75 g mL^{-1}	16.33	18.00	10.67	15.00	10.30	10.33
±SD	1.32		3.09		0.79	

SD: Standard deviation

Gram-negative bacteria than Gram-positive by silver nanoparticles using *S. officinalis* aqueous extract, no activity against *Staphylococcus aureus*[23] and other results showed weak activity against *Staphylococcus aureus*[24]. The aqueous extract was most effective against *Pseudomonas aeruginosa*[25]. In the aqueous extract of *S. officinalis* all concentrations are very weak. The effect in this study is very strong concentrations of 75 and 25 g mL^{-1} increased with increased concentration, results of aqueous nanoparticles following previous studies concluded that the effect is medium.

CONCLUSION

From this work, it had been concluded that *S. officinalis* aqueous extract was successfully used to produce AgNPs as a reducing and stabilizing agent. The AgNPs possessed good anti-bactericidal activity against Gram-negative bacterial strains. Therefore, it can be taken into consideration as a promising agent for the production of antibacterial drugs in the future.

SIGNIFICANCE STATEMENT

This study discovered that *S. officinalis* extract can be successfully used to produce AgNPs as a reducing and stabilizing agent that can be beneficial for the synthesis of silver nanoparticles, this study will help the researchers to uncover the promising agent for the production of the antibacterial drugs in the future that many researchers were not able to explore.

ACKNOWLEDGMENTS

Thanks to Mr. Tajaldin Mustafa for his assistance and help in evaluating the antibacterial activity. Great thanks to Dr. Khalid El-Tayeb for his assistance and help in the preparation of the extracts. Thanks also to Mr. Mahjoub Mohamed Al Said.

REFERENCES

1. Kumar, A., D. Singh, H. Rehman, N.R. Sharma and A. Mohan, 2019. Antibacterial, antioxidant, cytotoxicity and qualitative phyto-chemical evaluation of seed extracts of *Nigella sativa* and its silver nanoparticles. Int. J. Phar. Sci. Res., 10: 4922-4931.
2. Ramamurthy, C.H., M. Padma, I.D.M. Samadanam, R. Mareeswaran and A. Suyavaran *et al*., 2013. The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. Colloids Surf. B: Biointerfaces, 102: 808-815.
3. Horikoshi, S. and N. Serpone, 2013. Microwaves in Nanoparticle Synthesis Fundamentals and Applications. 1st Edn., Wiley-VCH, Weinheim, Germany, ISBN: 978-3-527-33197-0, Pages: 340.
4. Chung, I.M., I. Park, K. Seung-Hyun, M. Thiruvengadam and G. Rajakumar, 2016. Plant-mediated synthesis of silver nanoparticles: Their characteristic properties and therapeutic applications. Nanoscale Res. Lett., Vol. 11. 10.1186/s11671-016-1257-4.
5. Kamatou, G.P.P., N.P. Makunga, W.P.N. Ramogola and A.M. Viljoen, 2008. South African *Salvia* species: A review of biological activities and phytochemistry. J. Ethnopharmacol., 119: 664-672.
6. Mitic-Culafic, D., B.S. Vukovic-Gacic, J.B. Knezevic-Vukcevic, S. Stankovicand and D.M. Simic, 2005. Comparative study on the antibacterial activity of volatiles from sage (*Salvia officinalis* L.). Arch. Biol. Sci., 57: 173-178.
7. Ghorbani, A. and M. Esmaeilizadeh, 2017. Pharmacological properties of *Salvia officinalis* and its components. J. Traditional Complementary Med., 7: 433-440.
8. Saifuddin, N., C.W. Wong and A.A.N. Yasumira, 2009. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. J. Chem., 6: 61-70.
9. Balouiri, M., M. Sadiki and S.K. Ibnsouda, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal., 6: 71-79.
10. Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods-A review. Int. J. Food Microbiol., 94: 223-253.

11. Reller, L.B., M. Weinstein, J.H. Jorgensen and M.J. Ferraro, 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin. Infect. Dis., 49: 1749-1755.
12. Baharara, J., F. Namvar, M. Mousavi, T. Ramezani and R. Mohamad, 2014. Anti-angiogenesis effect of biogenic silver nanoparticles synthesized using *Saliva officinalis* on chick chorioalantoic membrane (CAM). Molecules, 19: 13498-13508.
13. Mohanty, S., S. Mishra, P. Jena, B. Jacob, B. Sarkar and A. Sonawane, 2012. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. Nanomed.: Nanotechnol. Biol. Med., 8: 916-924.
14. Markowska, K., A.M. Grudniak and K.I. Wolska, 2013. Silver nanoparticles as an alternative strategy against bacterial biofilms. Acta Biochim. Pol., 60: 523-530.
15. Okaiyeto, K., H. Hoppe and A.I. Okoh, 2021. Plant-based synthesis of silver nanoparticles using aqueous leaf extract of *Salvia officinalis*: Characterization and its antiplasmodial activity. J. Cluster Sci., 32: 101-109.
16. Al-Sheddi, E.S., N.N. Farshori, M.M. Al-Oqail, S.M. Al-Massarani and Q. Saquib *et al*., 2018. Anticancer potential of green synthesized silver nanoparticles using extract of *Nepeta deflersiana* against human cervical cancer cells (HeLA). Bioinorg. Chem. Appl., Vol. 2018. 10.1155/2018/9390784.
17. Carmona, E.R., N. Benito, T. Plaza and G. Recio-Sánchez, 2017. Green synthesis of silver nanoparticles by using leaf extracts from the endemic *Buddleja globosa* hope. Green Chem. Lett. Rev., 10: 250-256.
18. Ding, J., G. Chen, G. Chen and M. Guo, 2019. One-pot synthesis of epirubicin-capped silver nanoparticles and their anticancer activity against Hep G2 cells. Pharmaceutics, Vol. 11. 10.3390/pharmaceutics11030123.
19. Kathiravan, V., S. Ravi and S. Ashokkumar, 2014. Synthesis of silver nanoparticles from *Melia dubia* leaf extract and their *in vitro* anticancer activity. Spectrochim. Acta Part A: Mol. Biomol. Spectrosc., 130: 116-121.
20. Masum, M.M.I., M.M. Siddiqa, K.A. Ali, Y. Zhang and Y. Abdallah *et al*., 2019. Biogenic synthesis of silver nanoparticles using *Phyllanthus emblica* fruit extract and its inhibitory action against the pathogen *Acidovorax oryzae* strain RS-2 of rice bacterial brown stripe. Front. Microbiol., Vol. 10. 10.3389/fmicb.2019.00820.
21. Stanojević, D., L. Čomić, O. Stefanović and S. Solujić-Sukdolak, 2010. *In vitro* synergistic antibacterial activity of *Salvia officinalis* L. and some preservatives. Arch. Biol. Sci., 62: 167-174.
22. Mie, R., M.W. Samsudin, L. Din, A. Ahmad, N. Ibrahim and S. Adnan, 2014. Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum*. Int. J. Nanomed., 9: 121-127.
23. Abdelkader, M., B. Ahcen, D. Rachid and H. Hakim, 2014. Phytochemical study and biological activity of sage (*Salvia officinalis* L.). Int. J. Biol. Life. Agric. Sci., 8: 1253-1257.
24. Mohamed, A.Y. and A.A. Mustafa, 2019. *In vitro* anti-microbial activity of essential oils and other extracts from *Salvia officinalis* against some bacteria. Preprints, 10.20944/preprints201904.0012.v1.
25. Rami, K. and L.Z. Guo, 2011. Antimicrobial activity of essential oil of *Salvia officinalis* L. collected in Syria. Afr. J. Biotechnol., 10: 8397-8402.