Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2023.035

RESEARCH ARTICLE

Biosynthesis of *Salvia hispanica* Based Silver Nanoparticles and Evaluation of their Antibacterial Activity *in-vitro* and Rat Model

Barakat M ALRashdi[1], Mousa O. Germoush[1], Sayydah Suryya Sani[2], Iqra Ayub[2], Waqas Bashir[2], Bilal Hussain[2], Mahrukh Mazhar[2], Shahid Ali[2], Zartasha Zahid[2], Sana Ayesha[2] and Azhar Rafique[2*]

[1]Biology Department, College of Science, Jouf University, Sakaka 41412, Saudi Arabia
[2]Department of Zoology, GC University Faisalabad Pakistan
*Corresponding author: azharrafique96@gmail.com

ARTICLE HISTORY (23-123)

Received:	April 09, 2023
Revised:	May 24, 2023
Accepted:	May 26, 2023
Published online:	May 27, 2023

Key words:
Enterotoxigenic *Escherichia coli*
Green synthesis
Salvia hispanica
Silver Nanoparticles (AgNPs)
V. cholerae

A B S T R A C T

Green synthesis of nanoparticles is a latest and foremost technique to replace antibiotics against resistant strains of bacteria in forthcoming era. Nano particles-based nanomedicine is the future of pharmaceutical industries. This study was designed to evaluate the antibacterial activity of green synthesis-based silver nanoparticles against an infection in rats. *Salvia hispanica* (*S. hispanica*) plant was used to reduce the silver nitrate ($AgNO_3$) into silver nanoparticles (AgNPs). Reduction of silver nitrate into silver nanoparticles confirmed through the change of color from light yellow to deep brown of silver nitrate solution with *S. hispanica* seed extract. Characterization of silver nanoparticles was done through UV-Vis spectrophotometer, Atomic force Microscopy (AFM), Zeta size number, Zeta potential, Transmission Electron Microscopy and Scanning Electron Microscopy. *S. hispanica* based AgNPs were evaluated through agar well diffusion method, MIC (minimum inhibitory concentration) as well as against experimental infection in rats. UV-Vis spectrophotometer depicted wavelength at 420nm confirmed that particles were synthesized in Nano range. AFM showed spherical and square shapes nanoparticles with 80-120nm average size and 45mV charge. TEM were used to study spherical shape particle size 50-120nm at different resolution (100, 300, 500 nm). Similarly, SEM also depicted average particle size 80-130nm with spherical shape. Silver nanoparticles against enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* (*V. cholerae*) through a gar well diffusion method exhibited the greatest antibacterial activity against ETEC as compared to *V. cholerae*. The MIC against ETEC was 100µg/ml while MIC against *V. cholerae* was 25µg/ml. Finally, a single dose of AgNPs was administered orally to adult rats colonized with ETEC and *V. cholerae*, which significantly reduced the rate of colonization by the pathogens 75 or 100 folds, respectively. It was concluded that a *S. hispanica* based AgNPs have significant antibacterial effect, which may be considered as an effective alternate of antibiotics in future.

To Cite This Article: ALRashdi BM, Germoush MO, Sani SS, Ayub I, Bashir W, Hussain B, Mazhar M, Ali S, Zahid Z, Ayesha S and Rafique A, 2023. Biosynthesis of *Salvia hispanica* based Silver nanoparticles and evaluation of their antibacterial activity *in-vitro* and rat model. Pak Vet J, 43(2): 283-289. http://dx.doi.org/10.29261/pakvetj/2023.035

INTRODUCTION

Many technological innovations of 21st century are based on "Nanotechnology". It is mainly connected with synthesis of material at Nano scale with variable shape size and controlled disparity (Bhattacherjee *et al*., 2018). In this field, research and developments are increasing day by day globally. Several physical and chemical methods are used to synthesize nanoparticles but there are limitations of each method, pose potential harms on environment and humans due to use of chemical toxins.

The alternate of these methods is biological method, which is protective, eco-friendly and cost effective. There are various applications of AgNPs in pharmaceutical and biological industries due to their unique properties in compounds. In toothpaste, sanitizer spray and in dental resins, AgNPs are added as bactericidal agent (Mihailovi *et al*., 2023). The advanced method for synthesis of AgNPs is simpler, fast, reliable, less toxic, less harmful, easily handled and cost-effective as compared to chemical and physical methods known as Biosynthesis of AgNPs (Ahmad *et al*., 2019).

AgNPs are synthesized by reduction of *Salvia hispanica* (*S. hispanica*) seed extract with $AgNO_3$ following the modified methods reported by Khodaman *et al*. (2022). *S. hispanica* is also used as growth promotor (Uribe-Martínez *et al*., 2023). It is an annually grown herb belonging to family Lamiaceae that people began to be use as food in several civilization of Central America and preferred even on Amaranth (Ahmed *et al*., 2018). Plant seeds are very important due to its nutritional value. Chemically the seeds contain proteins, carbohydrates, fats, dietary fibers and ash with contents 15-25%, 26-41%, 30-39%, 18–30% and 4-5%, respectively. Vitamins and minerals and antioxidants are also present, which reduce the cardiovascular diseases (Rizwana *et al*., 2022). The essential property of chia seed, it is gluten free seed and mycotoxins are also not present, which spoil the food after few days therefore they can be preserved for long period of time in dry form (Ali *et al*., 2012; Asma *et al*., 2018). Synthesis of nanoparticles through plants utilization is an economical, toxins free and easily handle method. AgNPs have reported to have great antimicrobial efficacy too. These are used against multidrug resistant (MDR) bacteria, which resist broad spectrum class of antibiotics (Enrique *et al*., 2018). Silver nanoparticles are used to treat the pathogenic and drug resistant bacteria therefore called gray area. The nanoparticles may be used for the treatment of the infections caused both by the gram positive as well as gram negative bacteria, as a replacement of antibiotics in an era troubled for the vastly budding antibiotic resistance (Khodaman *et al*., 2022).

In the present study, we synthesized metallic AgNPs using *S. hispanica* seeds extract and characterized their antibacterial activity against ETEC and *V. cholerae* (Ali *et al*., 2012). These AgNPs showed the robust antibacterial efficacy against both pathogens throughout the study. Furthermore, the AgNPs reduced fitness of the bacteria in biofilms as well as *in-vivo*.

MATERIALS AND METHODS

Preparation of extract: 100 grams fresh seeds of *S. hispanica* plant were collected from the local market and authenticated by a qualified botanist (Coelho *et al*., 2014; Khodaman *et al*., 2022). Seeds were washed 2-3 times then dried and grinded to fine powder. 2 grams powder was used to make aqueous extract in 100ml distilled water by heating for 20 min and then filtered through Whatman sieve paper No.1. Filtrate was stored at 4°C for further use.

Preparation of 0.1Molar solution of $AgNO_3$: 100ml of 0.1M solution was prepared by dissolving 1.69g of $AgNO_3$ in 100ml distilled water (Carmen *et al*., 2018).

Synthesis of silver nanoparticles: Silver nitrate solution (45ml) was heated on a magnetic stirrer at 60°C for 15 min. *S. hispanica* seed extract (5ml) was added drop by drop until its color changed from yellow to brown as described by Akbari *et al*. (2018) and Ali *et al*. (2018).

Characterization of silver nanoparticles: The ocular property of AgNPs was determined through Ultraviolet Visible spectrophotometer. AFM is applied to obtain a 3D carbon copy of the silver nanoparticles. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to reveal the shape of nanoparticles. Moreover, the mean size of AgNPs is also determined. Zeta potential measurement and ZETA sizer were used to measure the charge and stability of nanoparticles.

Minimum inhibitory concentration (MIC): MIC is the minimum concentration of an agent to inhibit the bacterial growth. The value of MIC for synthesized AgNPs were measured using micro broth dilution method (Gnanajobitha *et al*., 2012; Enrique *et al*., 2018). It is standard method to determine the levels of susceptibility and resistance of antibacterial agent. These serial dilutions of antibacterial agents are made in a liquid media like broth with quantify number of bacteria and antibacterial agent and then incubated for 24 hours.

Synergistic effect of AgNPs with antibiotics: The test was performed in triplets for each concentration to get the average synergetic effect against respective concentrations as described by Haq *et al*. (2015).

***In-vivo* colonization assay:** *In-vivo* experiments were performed as previously described by Schild *et al*. (2007); Moisi *et al*. (2009); Leitner *et al*. (2013) with some modifications. Male rats of 3 months age were used in all the experiments. The rats were maintained in the cages of experimental animal house, which were offered food and water *ad-libitum*. The rats were monitored by a full-time staff before and after infection. Subsequently, the rats were anesthetized by isoflurane gas inhalation method and 50µl of *V. cholerae* or ETEC were inoculated by oral gavage (approx. 1×10^5 CFU/rat for both pathogens). The inocula were plated on LB agar plates to determine the exact inputs appropriate dilutions. The infected rats were divided into two groups 6 hours post infection. One group was treated orally with 50µl of AgNPs (1.2×10^8 NPs/ml), while the other group received 50µl saline solution. After 24h, the rats were sacrificed and the small intestine from each mouse was collected by dissection. The small intestine was mechanically homogenized in LB broth with 15% glycerol and appropriate 1:10 dilutions were plated on LB agar (Salem *et al*., 2015). After incubation at 37°C, the colonization rates in CFU/small intestine were determined by back-calculation to the original volume of the homogenized small intestine.

Statistical analysis: The data presented in the Tables and Histogram were analyzed by the mean zone of inhibition observed against AgNPs and antibiotics. All-Pair wise comparisons at 5% standard deviation were plotted in the bar diagram as shown in Fig. 2.

RESULTS

Characterization of silver nanoparticles

UV-Visible spectroscopy: UV-visible spectroscopy showed peak from 350 to 800nm which confirmed the formation of AgNPs. Absorption peak was observed at wavelength from 350 to 800nm. The peak value was taken on Y-axis and on X-axis and a graph was plotted (Fig. 2).

Table 1: Zones of inhibition of AgNPs against ETEC and *V. cholerae* at various concentrations of AgNPs

Bacteria	Zone of inhibition (mm)				
	50 ul (C1)	100 ul (C2)	200 ul (C3)	(+) (CO)	(-)
ETEC	11	13	14	26	00
V. cholerae	10	10	12	21	00

ETEC: Enterotoxigenic *Escherichia coli*; *V. cholerae*: *Vibrio cholerae*

Table 2: Effect of various concentrations of AgNPs against ETEC and *V. cholerae*

Well No.	AgNPs µg/ml	ETEC	*V. cholerae*
1.	+	☑	☑
2.	$400x10^{1}$	×	×
3.	$200x10^{1}$	×	×
4.	$100x10^{1}$	×	×
5.	$50x10^{1}$	×	×
6.	$25x10^{1}$	×	×
7.	$12.5x10^{1}$	×	☑
8.	$6.5x10^{1}$	☑	☑
9.	$3.5x10^{1}$	☑	☑
10.	$1.5x10^{1}$	☑	☑
11.	-	-	-

ETEC: Enterotoxigenic *Escherichia coli*: *V. cholerae*: *Vibrio cholerae* ☑ : bacterial growth present; X: growth absent (+): Positive control (Bacteria culture); (-): Negative control (Nutrient broth)

Table 3: Synergistic effect of AgNPs and antibiotics against ETEC and *V. cholerae*

Bacteria	Volume of *S. hispanica* based AgNPs			
	50µl	100µl	200µl	Positive control
	Zone of Inhibition (mm) AgNPs + Drug			
ETEC	14	19	22	26
V. cholerae	12	13	18	21

S. hispanica: *Salvia hispanica* commonly named as Chia seed ETEC: Enterotoxigenic *Escherichia coli*; *V. cholerae*: *Vibrio cholerae.*

Atomic Force Microscopy (AFM): AFM image showed that Silver nanoparticles are two types with spherical and square shape at average particle size height 8nm.

Scanning electron microscopy: Scanning Electron microscopy also showed the nanoparticles with average size of 80-130nm at 300 and 500 resolution power. Measurements at different resolution clarified the size of nanoparticles (Fig. 3).

Transmission electron Microscopy: Transmission Electron microscopy also showed the nanoparticles with average size of 50-120nm at 300 and 500 resolution power. Measurements at different resolution clarified the size of nanoparticles and support the image of Scanning Electron Microscopy (Fig. 3).

Zeta potential analysis: The charge repulsion/ attraction showed the stability of AgNPs with +45mV surface potential. This stability factor is elementary substance, which enables us to understand that AgNPs suspensions in an aqueous medium is stable at storage to be used for experimental studies.

Zeta size number: This is a crucial parameter to determine the cell uptake efficiency of AgNPs. The hydrodynamic size number of AgNPs was at 80-120nm range (Fig. 4).

A significant antibacterial effect was shown by AgNPs, and synergistic effect of AgNPs and antibiotics also gave good response against test strains as compared to use of separate drug and separate nanoparticles.

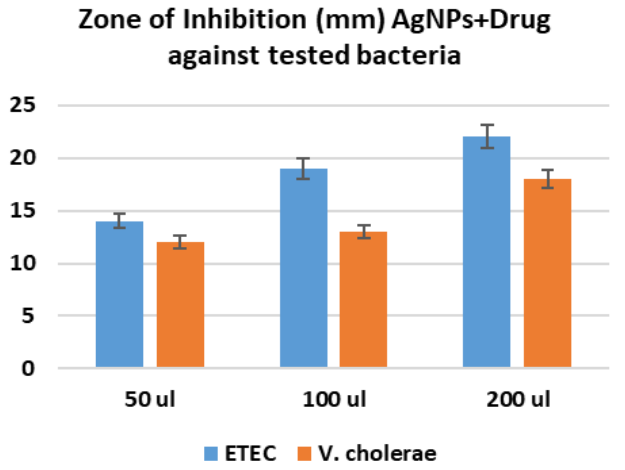

Fig. 1: Synergistic effects of AgNPs+Antibiotics tested at different concentrations against the tested bacteria (Y axis = the zone of inhibition measured in mm; X axis = different concentrations of AgNPs *i.e.*, 50, 100 and 200ul).

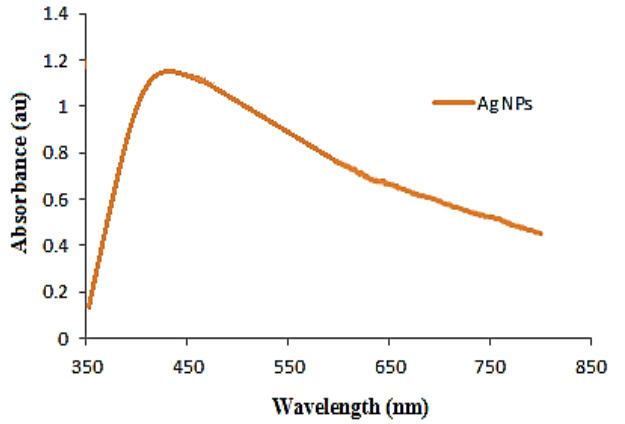

Fig. 2: Wavelength and absorbance analysis of the nano particles after synthesis.

DISCUSSION

Plants are nontoxic and healthier product of earth, especially medicinal plants. In the past, people have been using plants for the treatment of many infections (Campo *et al*., 2017). For the green synthesis of nanoparticles, plants are favorable component (Khodaman *et al*., 2022). There are different applications in nanobiotechnology by using various medicinal plants like *Dioscorea bulbifera*, *Aloe vera*, *Gnidia glauca*, *Gloriosa superba*, *Plumbago zeylanica*, *Pterocarpus santalinus* and *Adiantum philippense* L. Vajradanti scientifically called *Barleria prionitis* used against various infections including respiratory diseases, joint pains, fever, glandular swellings, ulcers, enlargement of scrotum and boils (Coelho *et al*., 2014). The whole plant may be used as therapy while parts of a plant like root, stem flower bark and leaf can also be used for this purpose (Rizwana *et al*., 2022). Plants are reported to possess different phytochemicals e.g., lupulinoside, acetyl barlerin, verbascoside, barlerinoside, pipataline prionisides, shanzhiside methyl ester, scutellare, barlerin, balarenone having a variety of therapeutic indications in the field of ethno-pharmaceutics. Some of these plant-oriented products are used as anti-inflammatory, anti-diabetes,

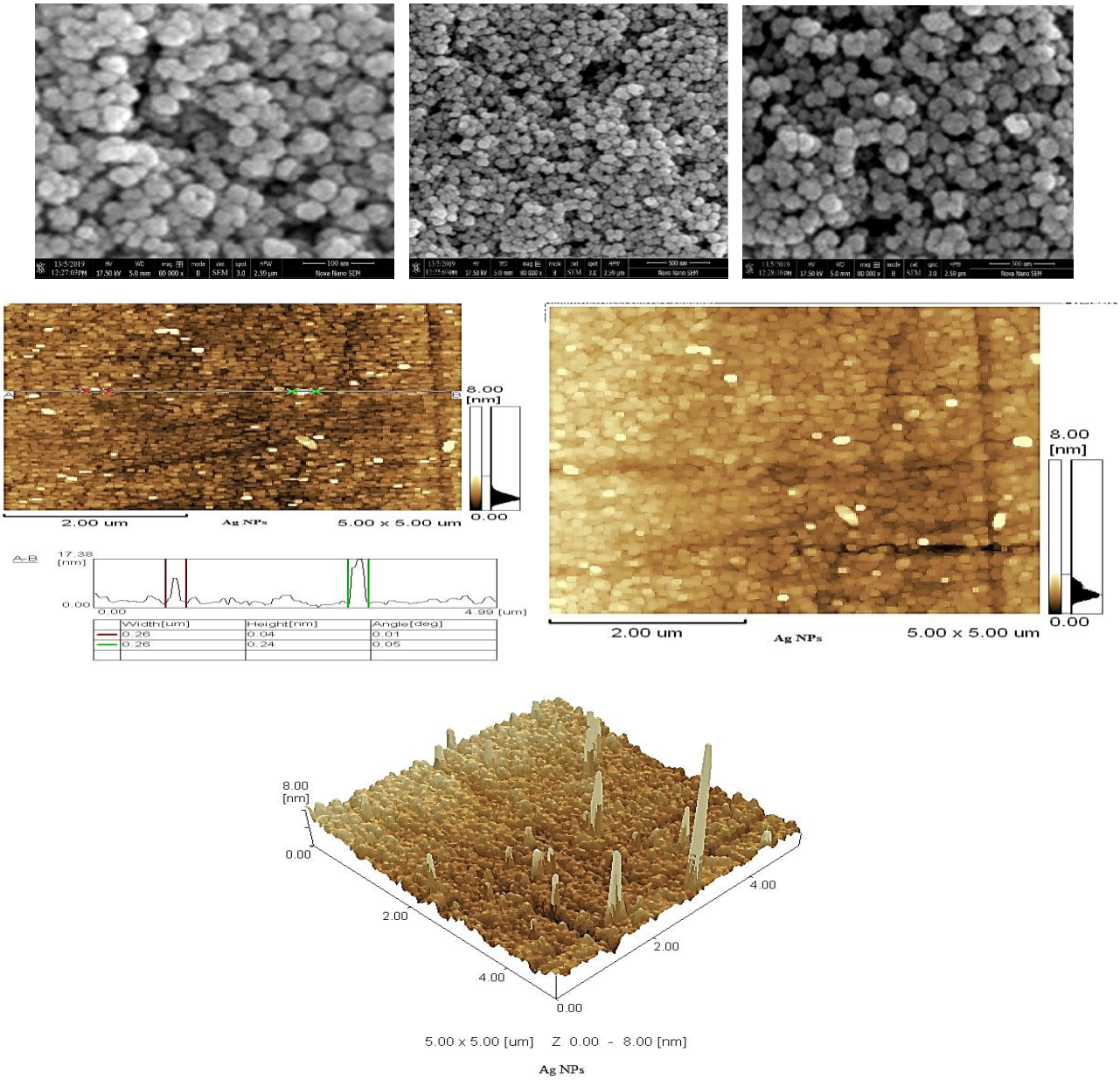

Fig. 3: Scanning Electron Microscopy and Transmission electron microscopy of the Silver Nanoparticles.

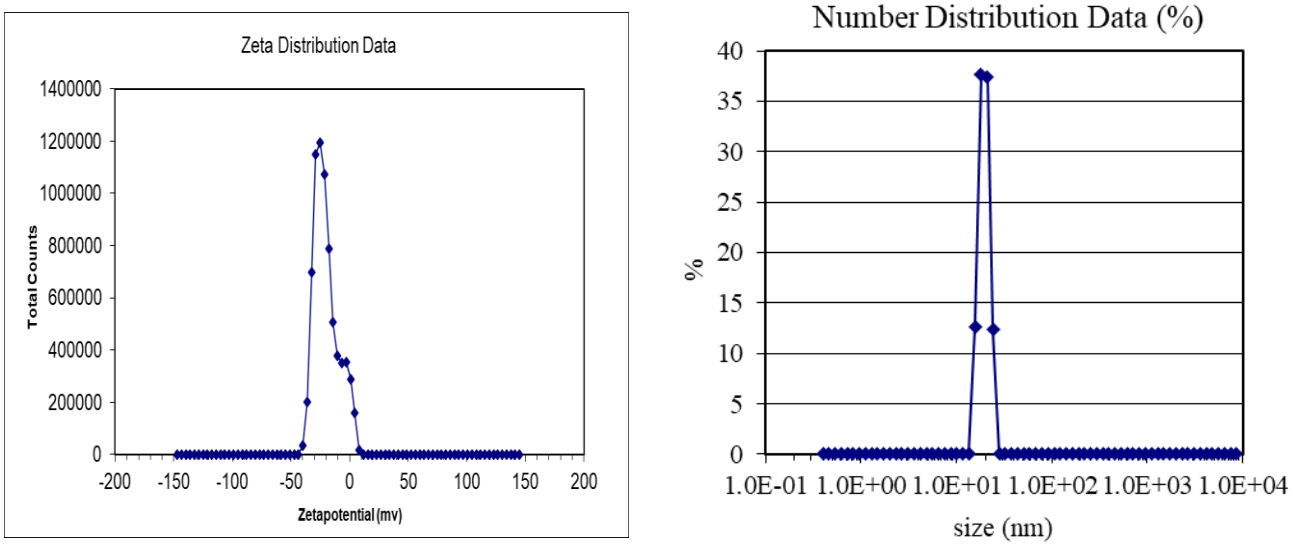

Fig. 4: Zeta sizer analysis deciphers the mean size of the nano particles.

arthritis and microbial infections. These products also reported to have hepatoprotective efficacy. Therefore it is interesting to use plant extracts as reducing agents for creation of AgNPs as reported by Ghosh *et al.* (2016). The AgNPs synthesized by using *S. hispanica* seed extract have been reported to be fastest, easily handled and nontoxic (Uribe-Martínez *et al*., 2023). Development of nanoparticles through green method was considered to be reliable and stable over a duration of 1 year at temperature 25°C and no clump formation was observed (Rajendran and Prabha, 2015).

Green synthesis of AgNPs by using *S. hispanica* seed extract at room temperature was conducted in the current study, it was a simple and cost effective method. Ibraheem *et al*. (2016) synthesized AgNPs by using *Acanthophora specifera* and investigated that preparation of AgNPs by green method was found to be eco-friendly, cheap, unwavering and steady. Various approaches are used for the synthesis of AgNPs but all of these methods are costly. Moreover, physical and chemical methods produce toxicity, which pollute not only the environment whereas also harmful for the person who handle (Rajamani *et al.*, 2018). Therefore, in this century, plant, flower, seeds, fruits and microorganisms are used for reduction of AgNPs for preparation of nanoparticles. AgNPs were prepared through *S. hispanica* seeds, which were dried at room temperature and ground to form powder by using pestle and motor. To make the aqueous extract 2g of seed powder was mixed in 100ml of distilled water. It was heated gently, and filtration was done through filter paper by using filtration assembly. The same extraction methods are reported by Rajendran and Prabha (2015). After seed extract preparation, 10mM solution of $AgNO_3$was prepared. For this purpose, 5ml of seed extract in a separate conical flask was taken and 45ml of $AgNO_3$ was added in this flask (Rosas-ramírez *et al*., 2017). Newly prepared AgNPs were stored at room temperature in Amber color bottle to avoid the oxidation of photo sensitized AgNPs (Segura-campos *et al*., 2014; Anjum *et al.*, 2018). The results obtained by green synthesis of silver nanoparticles were almost similar to that obtained by the Angeles *et al.* (2017).

Spectrophotometer was used to confirm the development of AgNPs. UV-VIS spectra showed AgNPs at the wavelength 420nm confirming that the synthesized particles were in Nano range and well dispersed without any clump formation (Shende *et al*., 2018). Moreover, to understand their intrinsic properties, AgNPs were biologically characterized such as size, net charge, aqueous stability and monodispersity. The current study provides the important information regarding AgNPs synthesis, Zeta potential analysis for charge repulsion/attraction, the stability of AgNPs with +45mV surface potential as performed by Hamad *et al.* (2018). Zeta sizer, a crucial parameter to determines the cell uptake efficiency of AgNPs was adapted in the current study. The hydrodynamic size number of AgNPs ranged from 80-120nm. Atomic force microscopy technique was used to determine the topography of prepared AgNPs. Scanning probe microscope was used to examine the AgNPs quite close to study of Zahir *et al.* (2015). Moreover, TEM analysis also showed the size and shape of AgNPs at 100, 300, 500nm resolution. The AgNPs synthesized in our study were relatively wavering and steady showing no clump formation upto 1 month owing to the use of stabilizing proteins. The same features of stability have been reported by Asad *et al.* (2017).

In addition, the scanning electron microscopy was used to observe the size and shape of AgNPs, the findings were similar to XRD that confirmed the texture of mixed crystals. SEM micrograph revealed by the reduction of silver nitrate into AgNPs through aqueous seed extract of *S. hispanica* plant. SEM image showed high population of NPs size range 80-130nm at different resolutions of 100, 300 and 500nm. The morphology of AgNPs is polymorphic like hexagonal, triangular, rod shaped and deformed spherical, while AgNPs prepared from *S. hispanica* seeds were spherical in shape. In powder form the AgNPs were stable for long period of time because in aqueous form clump formation due to the reaction, this aggregation decreased the biological activities of AgNPs, results of the present study showed resemblance to research findings of Chavan and Ghadage (2018).

AgNPs showed the great antibacterial activity against ETEC as compared to *V. cholerae:* AgNPs and other nanomaterial are used to replace these antibiotics, because they act as antimicrobial agent and inhibit the growth of bacteria, virus and fungi. Agar well diffusion method was used to perform the antibacterial action of AgNPs against ETEC and *V. cholerae*. Different concentrations of AgNPs of 50, 100 and 200µl were used to check the antibacterial activity against the selected pathogenic bacteria, three wells were filled for each concentration for each bacteria to determine the mean zone of inhibition against bacteria. Disc diffusion method was performed by the synthesized silver nanoparticles against pathogenic bacteria by Gnanajobitha, (2012) but aliquoted AgNPs enabled to diffuse in filter paper discs into the media plates containing the bacterial inoculum (Ajnum *et al*., 2018). Minimum inhibitory concentration of AgNPs against ETEC and *V. cholerae* were 50µg and 100ml, respectively.

In this work higher minimum inhibitory concentration of AgNPs was reported against ETEC and *V. cholerae* and found to be significantly more inhibitory against ETEC as compared to *V. cholerae*. These findings are in agreement with Roy and Anantharaman (2018), who report that AgNPs have more inhibitory effect against gram negative bacteria as compared to gram positive bacteria due the composition of plasma membrane. AgNPs killed the cells by forming the free radicals, which interfere the structure of plasma membrane and denature the protein of membrane, in this way cell death occurs, and this has been reported by many researchers (Paul *et al*., 2015; Moodley *et al*., 2018). It also deposits in cytoplasmic region and periplasm of bacteria and disorganized the cells (Rajamani *et al*., 2017; Roy and Anantharaman, 2017). The nanoparticles reduced through *S. hispanica* seed extract showed more antibacterial activity against hospital acquired bacteria as compared to antibiotics.

Besides *ex-vivo* application, the AgNPs were orally administered to assess lowered colonization levels of ETEC and *V. cholerae in-vivo*. Intake of NPs through

GIT may be undertaken through nano-technological food, packaging or medical applications (Klien and Godnic-Cvar, 2012; Iqbal *et al*., 2019). It was observed that there was no adverse side effects by single oral administration of AgNPs, which suggests that the colloidal AgNPs had no acute toxic effects, the same has been stated by Quang *et al*. (2013). The colloidal AgNPs are reported nontoxic when given orally, through ocular and dermal method in mice and guinea pigs (Pattwat *et al*., 2011). AgNPs have been used safely in the current study even to higher dose level. This safety interpretation is in line with a previous report in which dose toxicity was assayed in rats and mice given AgNPs for 28 days, which exhibited no significant changes in body weight, exposure to high doses of more than AgNPs (300 mg/kg for rats and 1 mg/kg in mice) resulted in adverse effects indicating slight liver or kidney damages(Kim *et al*., 2008; Eun-Jung *et al*., 2010). Commonly, the nanoparticles tend to accumulate in tissues which is directly related to dose levels given repeatedly (Quang *et al*., 2013). This tissue accumulation aspect is underway, which may be concluded in a coming manuscript. AgNPs may be designated as biocides, which may be targeting multiple sites within the host and or pathogen cell surface hence have a broad spectrum activity (Markowska *et al*., 2013).

Conclusions: It is concluded that the application of *Salvia hispanica* based silver nanoparticles (AgNPs) is an effective antimicrobial therapy. The findings of the present study suggest the use of AgNPs as an alternate to antibiotics, which may bout the emergence of antimicrobial resistance even after long-term usage.

Conflict of interest: The authors declare no conflicts of interest.

Authors contribution: SSS, IA, BMA, MOG and WB designed the study; MM, SA and ZZ synthesized the AgNPs and tested *in-vitro*; SA and SSS conducted *in-vivo* trials; SSS, IA, BMA, MOG drafted article; BH, BMA, MOG and AR supervised the work, read and approved the final draft of the manuscript.

Acknowledgements: The authors extend their appreciation to the Deanship of Scientific Research at Jouf University for funding this work through research grant No. DSR2020-06-3664.

REFERENCES

Ahmad N, Hussain SM, Ali Q, *et al*., 2019. Effects of chromium nanoparticles supplementation on body composition of *Cirrhinus mrigala* fingerlings fed sunflower meal based diet. Int J Biol Macromol 14:186-93.

Ahmed SH, Hameed RH, Thamir H, *et al*., 2018. Production of iron nanoparticle by using Aloevera gel and studying its effect on *Lepidium sativum* seed germination. Int J Biosci 13:248-55.

Akbari S, Foroughi MM and Ranjbar M, 2018. Nanomedicine & Nanotechnology Solvent-free Synthesis and Characterization of MnO_2 Nanostructures and Investigation of Optical Properties. J Nanomed Nanotechnol 9:3-7.

Ali I, Qiang TY, Ilahi N, *et al*., 2018. Green synthesis of silver nanoparticles by using bacterial extract and its antimicrobial activity against pathogens. Int J Biosci 13:1-15.

Ali NM, Yeap SK, Ho WY, *et al*., 2012. The Promising Future of Chia, *Salvia hispanica* L. J Biomed Biotechnol 2012:1-9.

Angeles MCL, Anandalakshmi K and Venugobal J, 2017. Green synthesis and characterization of Silver nanoparticles using Vitex negundo (Karu Nochchi) Leaf extract and its antibacterial activity. Med Chem (Los. Angeles) 7:218-225.

Anjum FR, Anam S, Rahman S, *et al*., 2018. Evaluation of antibacterial activity of plant based silver nanoparticles in synergism with antibiotics. Int J Biosci 13:250-261.

Asma S, Suryawanshi HH, Mundada PS, *et al*., 2018. Nanomedicine & Nanotechnology control of bacterial blight disease of Pomegranate using silver. J Nanomed Nanotechnol 9:2-5.

Bhattacherjee A, Ghosh T and Datta A, 2018. Green synthesis and characterisation of antioxidant-tagged gold nanoparticle (X-GNP) and studies on its potent antimicrobial activity. J Exp Nanosci 13:50-61.

Campo CDe, Pereira P, Maria T, *et al*., 2017. Nanoencapsulation of chia seed oil with chia mucilage (*Salvia hispanica* L.) as wall material : Characterization and stability evaluation. Food Chem 234:1-9.

Carmen S, Ruiz-torres CA, Niño-mart N, *et al*., 2018. Cytotoxic and bactericidal effect of Silver nanoparticles obtained by green synthesis method using annona muricata aqueous extract and functionalized with 5-Fluorouracil. Bioinorg Chem Appl 18:1-8.

Chavan JJ and Ghadage DM, 2018. Nanomedicine & nanotechnology biosynthesis , characterization and antibacterial capability of silver and copper nanoparticles using aqueous leaf extract of *Salacia chinensis* L. J Nanomed Nanotechnol 9:1-5.

Coelho MS and Salas-Mellado MDLM, 2014. Chemical characterization of Chia seed (*Salvia hispanica* L.) for use in food products. J Food Nutr Res 2:236-269.

Enrique C, Morales RMC and Carrazco JL, 2018. *In-vivo* antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using *Acacia rigidula* as a reducing and capping agent. Int J Nanomedicine 13:1-15.

Eun-Jung P, Eunjoo B, Jongheop Y, *et al*., 2010. Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environ Toxicol Pharmacol 30:162-8.

Ghosh S, Gurav SP, Harke AN, *et al*., 2016. Dioscorea oppositifolia mediated synthesis of gold and silver nanoparticles with catalytic activity Nanomedicine & Nanotechnology. J Nanomed Nanotechnol 7:1-7.

Gnanajobitha G, Annadurai G and Kannan C, 2012. Green synthesis of Silver Nanoparticle using *Elettaria Cardamomom* and assessment of its antimicrobial activity. Int J Pharma Sci Res Green 3:323-30.

Hamad ZS, Hashim A and Hashim AA, 2018. Biopolymer blend or titanium nitride nanoparticles : synthesis and pressure sensor characterization for environmental application contact. J Biodivers Environ Sci 13:1-7.

Haq M, Vandana R, Nanganagouda S, *et al*., 2015. Silver nanoparticles from mushroom Agricus bisporus and their activity against multi drug resistant strains of Klebsiella sps . Pseudomonas sp . Int J Nat Prod Res 5:20-6.

Ibraheem AE, Bee SW and Wa F, 2016. Green biosynthesis of silver nanoparticles using marine red algae acanthophora specifera and its antimicrobial activity nanomedicine & nanotechnology. J Nanomed Nanotechnol 7:7-10.

Iqbal S, Jabeen F, Sultana A, *et al*., 2019. The Nickel nanoparticles induced renal pathophysiological and histopathological alterations in male sprague dawley rats. Int J Biosci 14:497-504.

Khodaman E, Barzegar H, Jokar A *et al*., 2022. Production and evaluation of physicochemical, mechanical and antimicrobial properties of Chia (*Salvia hispanica* L.) mucilage-gelatin based edible films incorporated with Chitosan Nanoparticles. Food Measure 16:3547-56.

Kim YS, Kim JS, *et al*., 2008. Twenty-eight day oral toxicity, genotoxicity and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol 20:575-83.

Klien K and Godnic-Cvar J, 2012. Genotoxicity of metal nanoparticles: focus on *in-vivo* studies. Arhiv Za Hig Rada I Toksikol 63:133-45.

Leitner DR, Feichter S, Schild-Prufert K, *et al*., 2013. Lipopolysaccharide modifications of a cholera vaccine candidate based on outer membrane vesicles reduce endotoxicity and reveal the major protective antigen. Infect Immun 81:2379-93.

Markowska K, Grudniak AM and Wolska KI, 2013. Silver nanoparticles as an alternative strategy against bacterial biofilms. Acta Biochim Pol 60:523-30.

Mihailovi V, Sre-ckovi N, Nedi ZP, *et al*., 2023. Green synthesis of Silver nanoparticles using *Salvia verticillata* and *Filipendula ulmaria* extracts: Optimization of synthesis, biological activities, and catalytic properties. Molecules 28:808.

Moisi M, Jenul C, Butler SM, *et al*., 2009. A novel regulatory protein involved in motility of *Vibrio cholerae*.J Bacteriol 191:7027-7038.

Moodley JS, Babu S, Krishna N, *et al*., 2018. Green synthesis of silver nanoparticles from *Moringa oleifera* leaf extracts and its antimicrobial potential. Adv Nat Sci Nanosci Nanotechnol 9:1-10.

Pattwat MT, Wijit B, Chuchaat T, *et al*., 2011. An evaluation of acute toxicity of colloidal silver nanoparticles. Toxicol 73:1417-23.

Paul NS and Yadav RP, 2015. Biosynthesis of silver nanoparticles using plant seeds and their antimicrobial activity. J Biomed Pharmacutical Sci 5:26-8.

Quang HT, Van-Quy N and Anh-Tuan L, 2013. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Adv Nat Sci Nanosci Nanotechnol 4:2043-6262.

Rajamani M, Johney J and Ragunathan R, 2017. Detection of mec A gene associated with methicillin resistant *Staphylococcus aureus* and its alternatives using nanoparticles and Chia Seeds. Int J Biosci 6:67-75.

Rajamani RK, Bellan CS, Ravi PH, *et al*., 2018. Biosynthesis , characterization and remedical aspect of silver nanoparticles against pathogenic bacteria. MOJ Toxicol 4:103-9.

Rajendran R and Prabha AL, 2015. Silver nanoparticles from mushroom *Agricus bisporus* and their activity against multi drug resistant strains of Klebsiella sps. Pseudomonas sp. Int J Sci Res 5:1-6.

Rizwana H, Bokahri NA, Rashed SA, *et al*., 2022. Characterizing Silver nanoparticles biosynthesized from *Salvia rosmarinus* and assessing their *in-vitro* antifungal and cytotoxic activities against phytopathogens and cervical cells. J Animal Plant Sci 32:764-74.

Rosas-ramírez DG, Fragoso-serrano M, Escandón-rivera S, *et al*., 2017. Resistance-modifying activity in Vinblastine- resistant Human breast cancer cells by oligosaccharides obtained from mucilage of Chia Seeds (*Salvia hispanica*). Phyther Res 31:906-14.

Roy S and Anantharaman P, 2017. Nanomedicine & nanotechnology biosynthesis of Silver nanoparticles by *Chaetomorpha antennina* (bory de saint-vincent) kutzing with its antibacterial activity and ecological implication. J Nanomed Nanotechnol 8:310-5.

Roy S and Anantharaman P, 2018. Nanomedicine & nanotechnology biosynthesis of Silver nanoparticle by *Amphiroa anceps* (lamarck) decaisne and its biomedical and ecological implications. J Nanomed Nanotechnol 9:2-5.

Salem W, Leitner DR, Zingl FG, *et al*., 2015. Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxic *Escherichia coli*. Int J Med Microbiol 305:85-95.

Schild S, Tamayo R, Nelson EJ, *et al*., 2007. Genes induced late in infection increase fitness of *Vibrio cholerae* after release into the environment. Cell Host Microbe 2:264-77.

Segura-campos MR, Ciau-solís N, Rosado-rubio G, *et al*., 2014. Chemical and functional properties of Chia Seed (*Salvia hispanica* L.) Gum Int J Food Sci 14:1-5.

Shende S, Joshi KA, Kulkarni AS, *et al*., 2018. Nanomedicine & Nanotechnology *Platanus orientalis* Leaf Mediated Rapid Synthesis of Catalytic Gold. J Nanomed Nanotechnol 9:2-7.

Uribe-Martínez S, Rendón-Huerta JA, Hernández-Briones VG, *et al*., 2023. Effects of Chia Seeds on Growth Performance, Carcass Traits and Fatty Acid Profile of Lamb Meat. Animals 13:1005.

Zahir AA, Chauhan S, Bagavan A, *et al*., 2015. Green Synthesis of Silver and Titanium Dioxide nanoparticles using *Euphorbia prostrata* extract shows shift from apoptosis to G 0 / G 1 arrest followed by necrotic cell death in *Leishmania donovani*. Antimicrob Agents Chemother 59:4782-99.