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Parameters' Optimization for the Green Synthesis of Silver Nanoparticles from *Ziziphus spina-christi* Leaves and their Antibacterial Activities against Antibiotic Sensitive and Resistant Bacteria

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Abstract:
The present work aimed to optimize eco-friendly and rapid green method for the synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Ziziphus spina-christi* (ZSC) and evaluated their anti-bacterial activity against sensitive and resistant Gram-positive and Gram-negative bacterial strains. Factors influencing the rate of biosynthesis reaction (pH, reaction time, temperature, and reactants' ratio) were optimized. Various analytical techniques were used to confirm the formation of green synthesized AgNPs and to investigate their size and morphology. The UV-visible analysis of AgNPs solution revealed a single absorption peak at 418 nm indicating the formation of small particles. Fourier transform infrared spectroscopy (FTIR) analysis of the synthesized AgNPs confirmed the role of ZSC as reducing agent for (Ag^+) ions as well as a stabilizing agent. The synthesized AgNPs were found to be spherical in shape and crystalline in nature with an average size of 32 nm. AgNPs showed significant antibacterial activities against both sensitive and resistant pathogenic *S. aureus* and *E. coli* bacteria strains and even exceeded the activities of standard antibiotic drugs (vancomycin and ciprofloxacin) in the case of resistant bacteria. Based on the results of the present work, green synthesized AgNPs could be an alternative option to overcome the ever-increasing challenge posed by antibiotic-resistant bacterial strains taking into account the cost of production and antibacterial efficacy of the nanoparticles.

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INTRODUCTION

Over the last two decades, nanotechnology has underwent enormous and rapid development where a total number of publications exceeded 12000 articles in SAARC countries alone according to a recent survey (Borgohain *et al.*, 2021). The applications of nanotechnology have impacts on almost everyday materials and processes and revolutionized several fields of man's life including biomedicine, bioengineering, diagnostics, food production, water treatment, environment, cosmetics and material science.

Nanoparticles, one of many products of nanotechnology, have attract increasing attention due to their unique size (10^{-9} m), shape with dimension of (1D, 2D, and 3D) and potential applications where they can be easily synthesized, functionalized and used for various purposes (Khan *et al.*, 2019). In biomedicine, special consideration is given to silver nanoparticles (AgNPs) due to their potential applications to combat bacteria resistance to antibiotic (Halawani, 2016), fungi (Elamawi *et al.*, 2018), cancer cells (Akther *et al.*, 2019), viruses such as HIV (Singh *et al.*, 2017), Avian influenza (Saadh *et al.*, 2021) SARS-CoV-2 (Jeremiah *et al.*, 2020) and covid-19 (Behbudi, 2021; Jeremiah *et al.*, 2020; Rai *et al.*, 2020; Sarkar, 2020).

Green synthesis is a favorable approach for synthesizing AgNPs using natural resources such as plant extract due to various desirable characteristics such availability and diversity of the natural resources, easy, ecofriendly and cost effective synthesizing procedure for the large-scale production, stability and biocompatibility of the NPs for diagnostic and therapeutic purpose (Bhardwaj *et al.*, 2020). The chemical constituents of the plants such as proteins, phenols, terpenoids, flavonoids, alkaloids, ketones, saponins. aldehydes, amides, and carboxylic acids act as a reducing and capping agents (Panzella *et al.*, 2020).

Experimental conditions for the synthesis of AgNPs play a detrimental role on the final size and morphology of AgNPs which in turn control their final characteristics. These conditions include pH, reductant concentration, temperature, mixing ratio of the reactants, and time (Singh *et al.*, 2020). Thus, it is paramount to optimize these parameters and have control over the final properties of the nanoparticles.

Traditionally, *Zizyphus spina-christi* (ZSC) is one the many plants that is used as a natural remedy to treat inflammation and fungal infection. It is also used to treat ulcer, wounds, and relief digestive and urinary troubles (Mintah *et al.*, 2019). ZSC leaves have much content of alkaloids, flavonoids, sterols, butulinic acid and triterpenoidal saponin glycosides phenolic, tannins, and saponins. The availability of many phytochemical compounds containing plenty of OH-groups make them excellent reducing and anti-oxidant agents (Hussein, 2019).

In the last few years, several reports have published on the use of ZSC extract for the synthesis of AgNPs (El-Ansary *et al.*, 2018; Halawani, 2016; Hassan *et al.*, 2020; Mostafa *et al.*, 2020; Saleh *et al.*, 2021). The results of these reports revealed that the characteristics of the synthesized AgNPs varied according to the experimental conditions and the phytochemical species present in the plant extract which varies from region to region. Furthermore, optimization of the experimental condition is either done on a limited level or not considered at all. Thus, in this work, we reported the green synthesis of AgNPs using aquous leaf extract of ZSC species available in the capital of Yemen, Sana'a.

The current investigation was carried out for phytochemical screening of the ZSC leaves. Procedure conditions including pH, reaction time, temperature, and reactants ratio were fully optimized. Characterization of the synthesized AgNPs was done with assistance of UV-Vis, FT-IR, X-ray diffraction and SEM. And finally, the antibacterial activity of the synthesized AgNPs was tested against sensitive and resistant gram positive and gram negative bacterial strains.

MATERIALS AND METHODS

Plant and chemicals

The leaves of *Ziziphus spina-christi* were collected from the vicinity of the University of Science and Technology (UST) main campus at the capital Sana'a, Yemen. The plant was authenticated by a pharmacognosist at the Department of Pharmacognosy, Faculty of Pharmacy, UST. Silver nitrate ($AgNO_3$, 99%), ciprofloxacin and vancomycin antibiotics, disks (HiMedia), Mueller-Hinton Agar (MHA), Mueller-Hinton broth (MHB), H_2SO_4, copper acetate, ferric chloride, HCl, iodine, KOH, and potassium iodide were purchased from Sigma-Aldrich. All the chemicals were of analytical reagent grade and were used without further purification. Milli-Q water was prepared in-house and used in all the experiments.

Cultures

Standard cultures for antibacterial assays were obtained from the Hospital of UST, Sana'a, Yemen. These include sensitive and extended-spectrum β-lactamase-producing *Escherichia coli* and sensitive and methicillin-resistant *S. aureus* (MRSA) pathogens.

Preparation of *Ziziphus spina-christi* leaf extract

Fresh leaves of *Z. spina-christi* were thoroughly washed under tap water and then with Milli-Q water to remove the adhered dirt, soil, and undesirable particles. Then, the leaves were dried at room temperature (15min) to remove the water from the surface of the leaves. The aqueous leaf extract of *Z. spina-christi* was prepared as follows: About 25 g of plant's leaves were mixed with 100 mL deionized water in a 250 mL flask. The contents were heated at 70°C for about 30 min and then left to cool down to room temperature. The cold solution was filtered using Whatman No.1 filter paper. Then the extract was centrifuged for 10 min at 9,000 rpm to produce clear solution which was stored at 4–8 °C until the synthesis of AgNPs.

Phytochemical screening and the pH value of leaf extract

The aqueous extract of *Z. spina-christi* leaves was examined for the presence of biologically active constituents by following standard biochemical methods as described by (Harborne, 1998). The pH value of the aqueous extract was measured using pH meter electrode (Jenway, model 3510, UK) at 25°C.

Silver Nanoparticles (AgNPs) biosynthesis under Optimized Conditions

Parameters that could affect the size and morphology of AgNPs, which included reaction time, temperature, reactants ratio, and pH, were optimized one at a time.

With optimum values at hand, a volume of 95 mL of 1 mM $AgNO_3$ was transferred into a 250 mL Erlenmeyer flask. The pH was adjusted to 9 and the temperature was maintained 70 °C. ZSC extract (5 mL) was added dropwise to the silver nitrate solution using a sterilized syringe with a small needle in a dark under constant magnetic stirrer. Visual observations were monitored and UV–visible spectra were recorded for 24 Hrs. The synthesized nanoparticles mixture was concentrated using a rotary evaporator (Buchi Rotavapor R-200, Germany) to remove excess water, and centrifuged at 9,000 rpm for 10 minutes (Hettich, Universal 32) in order to obtain the pellets. The formed pellets were redispersed in Milli-Q water to remove any biological contaminant and sonicated for 10 min thoroughly for at least three times. The supernatant was discarded and the pellets were dried by a freeze-dryer (Freezone plus 2.5, Labconco, Uk). The obtained nanoparticles were stored in Eppendorf tube for further use.

Characterization of AgNPs

The characterization of the synthesized AgNPs was done using several techniques as follows:

UV-Vis Spectra Analysis

The UV-Vis spectra of AgNPs solution in deionized water were acquired using a double beam spectrophotometer (Analytical Jena-Specord 205, Germany) in the range between 190 nm and 750 nm at room temperature.

Scanning Electron Microscopy (SEM)

The size and morphology of green synthesized AgNPs were determined using scanning electron microscopy (JEOL, Tokyo, Japan) at the scanning electron microscopy unit, King Abdul-Aziz University, KSA.

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra were recorded in the 4000 - 400 cm^{-1} with a Perkin Elmer Spectrum 2 Spectrometer (Perkin Elmer Spectrum 2, USA), equipped with data station. Dried samples of about 1.0 mg was mixed with 100 mg of spectral grade KBr and pressed into discs under hydraulic pressure.

X-Ray Diffraction (XRD)

The purified AgNPs were analyzed to examine their crystallographic structure. The XRD grids were coated with dried biosynthesized AgNPs. The x-ray diffraction patterns were recorded using X-ray diffractometer (Bruker AXS, Germany) operated at 10 kV. The wavelength was set at 1.5406 Å in the 2**θ** range of 20° to 80.

Antibacterial Activity

Antibacterial activity of AgNPs against human pathogenic bacteria was examined on Mueller Hinton Agar plates using agar disc diffusion method (Netai *et al.*, 2017). Two different concentrations (100 µL and 150 µL) of AgNPs were used to carry out the test. Each volume was added to a sterilized filter paper disc (6 mm in diameter) under sterilized condition. The discs were kept to dry and tested against the two sensitive and multidrug resistant pathogenic bacteria *S. aureus* and *E. coli*. The pathogenic bacteria were spread on Mueller Hinton Agar plates and AgNPs-discs were placed on the surface of agar. Standard antibiotics (vancomycin and ciprofloxacin) were used as a positive control for Gram-positive *S. aureus* bacteria and as a positive control for Gram-negative *E. coli* bacteria, respectively. Antibiotic Discs were used as control in each plate. The petri dishes were incubated at 37°C for 24 hours. The reported inhibition's zone for each type of bacteria was the mean for three measurements.

RESULTS AND DISCUSSION

Phytochemical screening and the pH value of leaf extract

The results of qualitative phytochemical screening and pH value of *Ziziphus spina-christi* leaf extract were presented in Table (1). The results revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, and terpenes which was in agreement with previous report and considered to act as reducing, stabilizing and capping agents (Asgarpanah and Haghighat, 2012). Their presence was further confirmed by FTIR analysis. Nevertheless, the protein constituent was not detected by biuret qualitative test. The pH value for the leaf extract (pH = 8.03 ± 0.1) indicated the presence of some basic biologically active constituents.

Biosynthesis and Characterization of Silver Nanoparticles

The biosynthesis of AgNPs was achieved by the addition of 95 mL of 1 mM $AgNO_3$ solution to 5 mL ZSC leaf extract under dark conditions at 70°C, pH at 9.0, and constant magnetic stirrer. Immediate color change of the solution (from colorless to deep reddish) was easily observed indicating the formation of silver nanoparticles. The observed color change was attributed to the presence of phytochemicals reducing agents such as those shown in Table (1) which reduced Ag^+ ions to Ag°. The UV-Vis spectrum (Figure 1) of the reddish-color solution showed a single, sharp, and a narrow distinct peak at λ_{max} = 418 nm, which was attributed to surface plasmon resonance of a noble metal confirming the formation of AgNPs.

Our results were in agreement with previous studies (Halawani, 2016; Sanchooli *et al.*, 2018). Increasing the contact time between $AgNO_3$ and ZSC leaf extract as shown in Figure 2 caused an increase in the absorbance intensity which was associated with a blue shift of the λ_{max} until reaching maximum values at 24 hours indicating a complete reduction of silver ions and stabilization of the AgNPs. This was in-line with the results of previous reports (Netai *et al.*, 2017; Shehzad *et al.*, 2018).

Table 1. Phytochemical screening and pH value of *Z. spina-christi* leaf extract.

S. no.	Phytochemicals	*Ziziphus spina-christi*
1	Alkaloids	+
2	Tannins	+
3	Flavonoids	+
4	Phenols	+
5	Saponins	+
6	Proteins	–
7	Carbohydrates	+
8	Terpenes	+
9	PH	8.03 ± 0.1

+ sign indicates positive test and − sign indicates negative test.

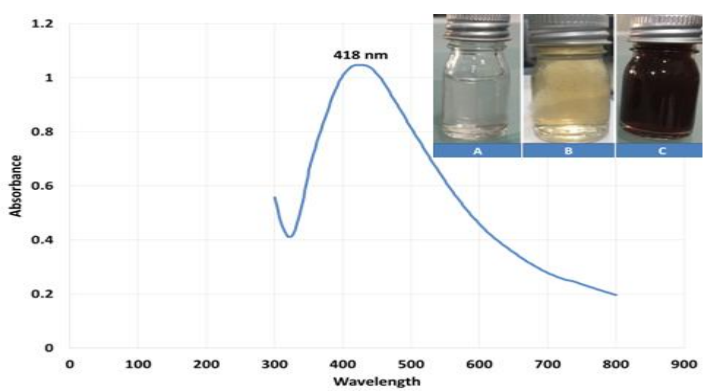

Fig. 1. UV- Vis spectrum and visual observation of green synthesized silver nanoparticles (AgNPs) synthesized under optimized conditions. A. 1 mM $AgNO_3$. B. *Z. spina-christi* leaf extract and C. AgNPs.

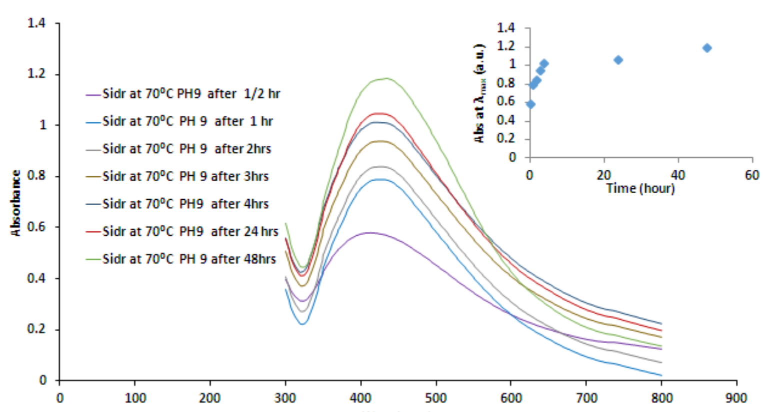

Fig. 2. UV- Vis spectra of Silver Nanoparticles (AgNPs) showing the effect of reaction time.

Parameters' Optimization for the Green Synthesis of AgNPs

Effect of Reactants' Ratio and Time contact

The synthesis of AgNPs using different ratios of aqueous extract of ZSC and 1 mM $AgNO_3$ (1:99, 5:95, 10:90 and 15:85) respectively was carried out at room temperature and contact time of 24 Hrs. The color change and UV-Vis spectra were monitored. The highest reddish-color intensity (Figure 3) was observed when the ratio of 5:95 (ZSC:1 mM $AgNO_3$) was used. This was confirmed by the UV-Vis spectra (Figure 4) where the maximum λ was located at 443 nm when the above-mentioned ratio was used. Higher reactants' ratios could result in the formation of large size AgNPs that were precipitated while lower ratios than 5:95 may not have enough bio-organic compounds required for complete reduction of Ag^+ and stabilization the biosynthesized AgNPs. Similar trends were reported by (Verma and Mehata, 2016). The effect of the contact time between $AgNO_3$ and ZSC aqueous extract was presented in Figure (5) and revealed that longer reaction time increased the formation yield of AgNPs. Furthermore, lower reaction time than 1 Hr. showed no sign of AgNPs formation. Our observations were in agreement with those reported previously (Qais *et al.*, 2019; Verma and Mehata, 2016).

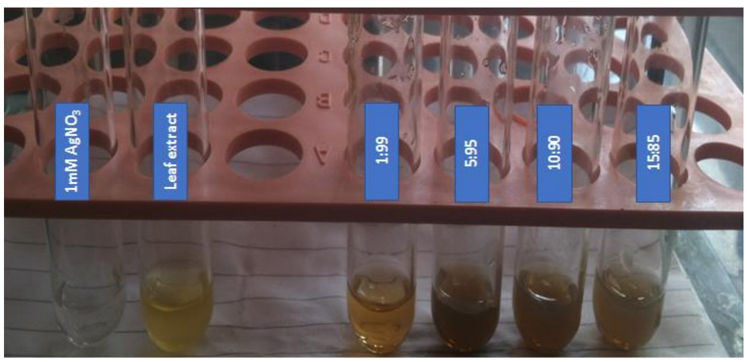

Fig. 3. Color change for Silver Nanoparticles (AgNPs) synthesized at room temperature with different ratios of leaf extract and 1 mM $AgNO_3$ after 24hrs.

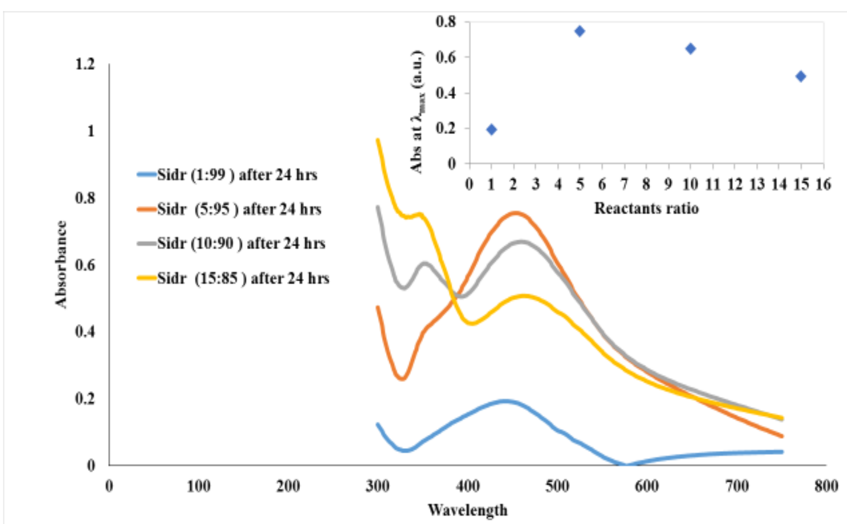

Fig. 4. UV- Vis spectra of Silver Nanoparticles (AgNPs) synthesized at room temperature with different ratios of leaf extract and 1mM $AgNO_3$ after 24hrs.

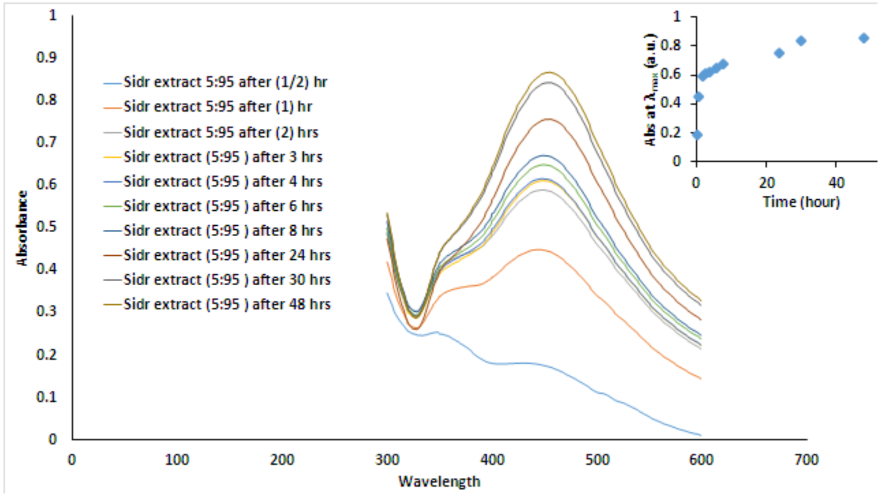

Fig. 5. Effect of the reaction time on the UV- Vis spectra of Silver Nanoparticles (AgNPs) synthesized at room temperature using (5:95) reactants' ratio.

Effect of Reaction Temperature

The effect of temperature on the synthesis of AgNPs was investigated at different temperatures (10, 30, 50, and 70ºC) using (5:95) ratio of leaf extract and 1 mM $AgNO_3$ respectively. UV–visible spectra and visual observation were monitored for 24 Hrs. The UV-Vis data (Figure 6) revealed that optimum AgNPs formation was seen when reaction temperature was maintained at 70°C. The maximum peak intensity was seen at λ_{max} at 434 nm. Moreover, a blue shift in the λ_{max} that was associated with narrower peak was observed with increasing temperature. This may attributed to an increase in the in kinetic energy of the active constituents of leaf aqueous extract, where the silver ions' consumption increases leading to the formation of small size and uniform AgNPs. Our results agreed with those published by (Verma and Mehata, 2016).

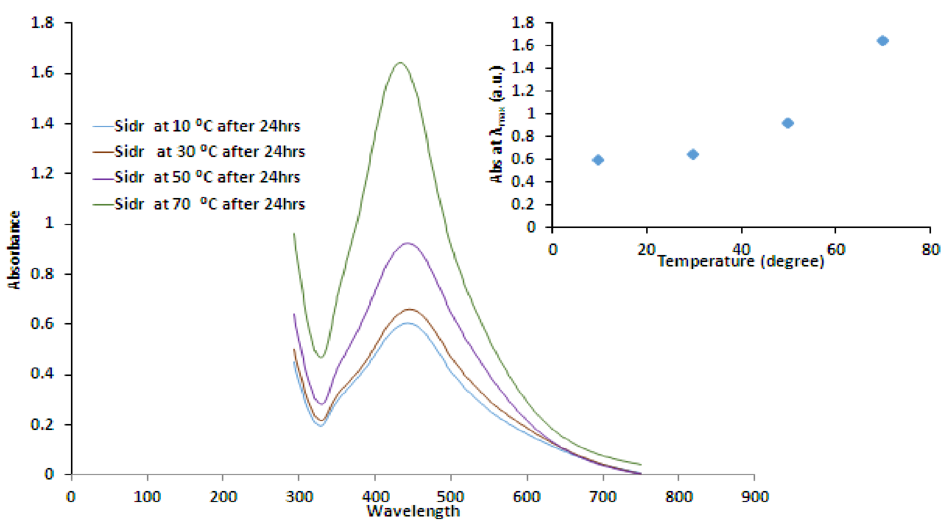

Fig. 6. Temperature effect on the UV- Vis spectra of Silver Nanoparticles (AgNPs) synthesized using (5:95) ratio of leaf extract and 1 mM $AgNO_3$ and a reaction time of 24 Hrs.

Effect of pH

The optimum value of pH for the synthesis of AgNPs was determined using acidic and alkaline pH (5.0, 7.0, 8.0, and 9.) under optimum conditions of time (24 Hrs), temperature (70°C), reactants' ratio (5 mL ZSC extract: 95 mL 1 mM $AgNO_3$). The visual color changed and UV-Vis spectra confirmed the formation of AgNPs as shown in Figure (7). The results revealed that the most favorable conditions for the biosynthesis of AgNPs were observed at alkaline pH 9.0. It is apparent that the rate of bioreduction process was improved with increasing pH from acidic (pH = 5.0) to alkaline (pH = 9.0) which could be attributed to formation of more negative anioizble groups of the natural biologically active constituents present in the ZSC extract. Our results were in a good correlation with those published earlier (Dubey *et al.*, 2010; Vanaja *et al.*, 2013).

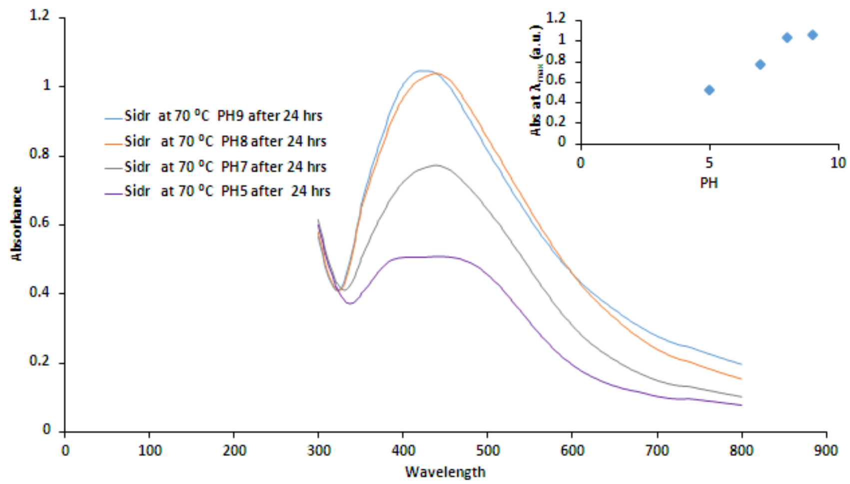

Fig. 7. UV- Vis spectra of Silver Nanoparticles (AgNPs) using different values of pH. Reactions were carried out using 5:95 (ZSC:1 mM $AgNO_3$) and left for 24 Hrs.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrum of the *Z. spina-christi* (ZSC) leaf extract and AgNPs were in presented in Figure (8). The spectrum of the leaf extract showed a band at 3367.50 corresponding to a stretching mode of OH groups that were possible present in phenols, carbohydrates, alkaloids, terpenoids and etc. This band exhibited a large shift from 3367.50 cm^{-1} (leaf extract) to 3392.33 cm^{-1} (AgNPs) indicating the involvement of the OH groups in the reduction of silver ions and capping the AgNPs. Similar bands were reported by other groups (Netai *et al.*, 2017). The leaf extract showed a strong absorption band at 1582.58 cm^{-1} that was shifted to to1558.96 cm^{-1} in the spectrum of AgNPs which could be due to the presence of aromatic -C=C- of phenolic and flavonoid present in biomolecules and involved in the synthesis of the AgNPs ((Shehzad *et al.*, 2018). The shift in (C - O) band from 1416.46 (leaf extract) to 1411.95 cm^{-1} (AgNPs) could another indication for the presence of phenolic compounds in leaf extract and their contribution in the synthesis and stabilization of AgNPs (Bin-Meferij and Hamida, 2019). The shift of the band at 1047.19 (leaf extract) to 1033.57 cm^{-1} (AgNPs) with decreasing intensity could be assigned to the participation of C-O-C of christinin-A (the major saponin component) found in leaf extract in the formation of silver nanoparticles (Asgarpanah and Haghighat, 2012; Bin-Meferij and Hamida, 2019). The peak observed at 872.09 cm^{-1} (AgNPs) may be assigned to the bending vibrations of C-H groups of phenyl rings (Coates, 2006). The final shift in the absorption peak from

660.17 (leaf extract) to 419.11 cm^{-1} (AgNPs), indicated the bond formation between silver nanoparticles and the oxygen of the hydroxyl group (Kokila *et al.*, 2015).

Previous work reported that *Z. spina-christi* contain chemical compounds such as christinin-A and quercetin which are considered potent reducing and capping compounds that play a key role in the biosynthesis of AgNPs and prevent them from aggregation (Makarov *et al.*, 2014). Moreover, quercetin with high reducing potential due to its ability of releasing reactive hydrogen atoms act as a strong reducing agent of Ag^+ to form AgNPs via enol to keto transformation process (Albers *et al.*, 2013).

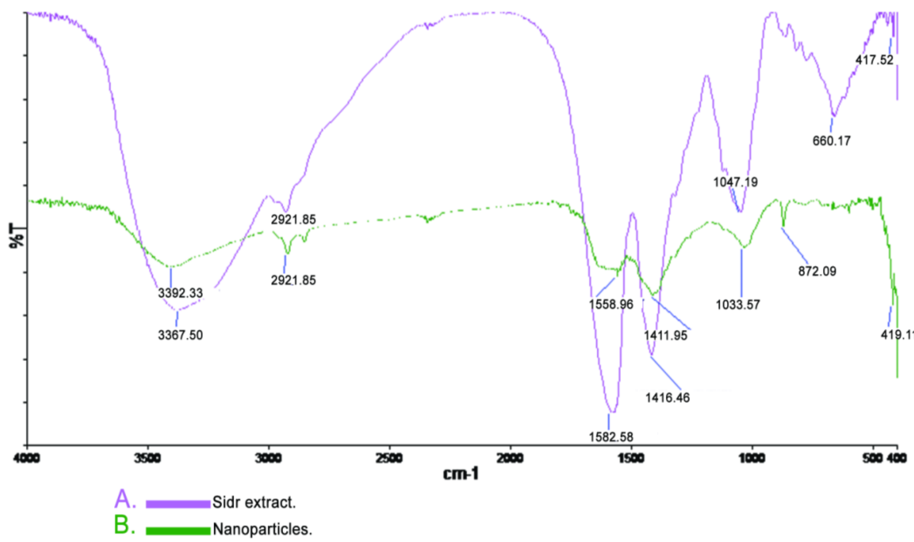

Fig. 8. FTIR spectra of (A) ZSC leaf extract and (B) AgNPs.

X-Ray Diffraction (XRD)

Typical XRD pattern of the AgNPs prepared by the reaction of *Z. spina-christi* leaf extracts and 1 mM $AgNO_3$ under optimum reaction conditions was shown in Figure (9). The XRD diffractogram showed main peaks at approximately 38.14°, 44.52°, 64.42°, and 77.42° were assigned to (111), (200), (220), and (311) planes of silver respectively indicating spherical shape AgNPs and crystalline in nature with face centered cubic (FCC) structure after matching with the standard data of the Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783 (El-Naggar *et al.*, 2018; Prakash *et al.*, 2013). More peaks were also noticed at 29.52°, 32.28°, 36.02° which may be due to organic compounds that were present the leaf extract and crystalized on the surface of the AgNPs. The peak corresponding to the 111 plane showed more intensity than the other planes, suggested that the 111 plane is the most main orientation (Netai *et al.*, 2017). The size of the silver nanoparticles was calculated to be ~41.9 nm using Debye-Scherrer equation (Dubey *et al.*, 2010). Our results were in agreement with those reported by Rajakumar (Rajakumar *et al.*, 2017).

Scanning Electron Microscopy (SEM)

For additional confirmation of the size and morphology of the synthesized AgNPs, scanning electron microscopy (SEM) images were recorded by using the JEOL SEM and shown in Figure (10). SEM analysis of AgNPs revealed that the particles were nearly spherical in shape, and had various sizes with an average value of 32 nm. The SEM images confirmed that the bioorganic constituents in the leaf' extract acted as bio-reducing and capping agents which helped also in preventing AgNPs from aggregation. Similar remarks were found in the literature (Bin-Meferij and Hamida, 2019; Shehzad *et al.*, 2018; Vanaja *et al.*, 2013).

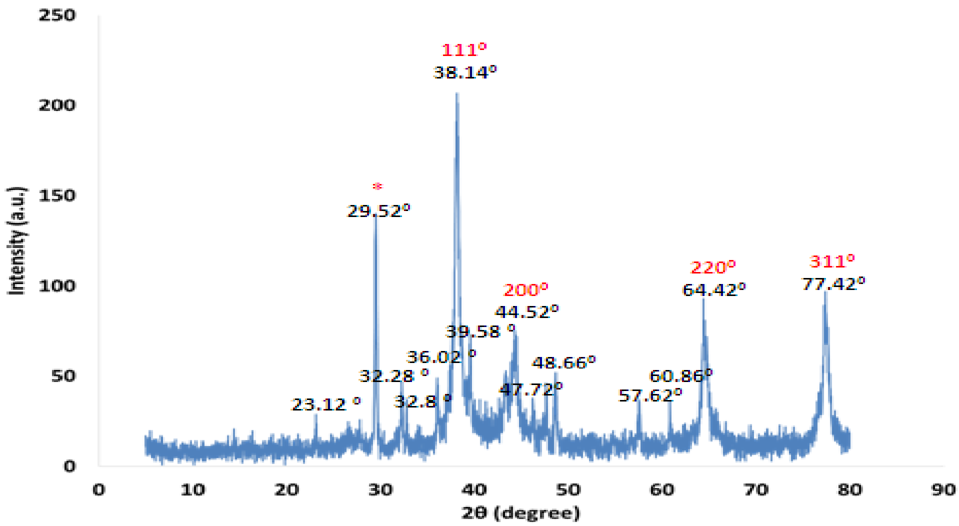

Fig. 9. XRD pattern of Silver Nanoparticles (AgNPs) synthesized by *Z. spina-christi*.

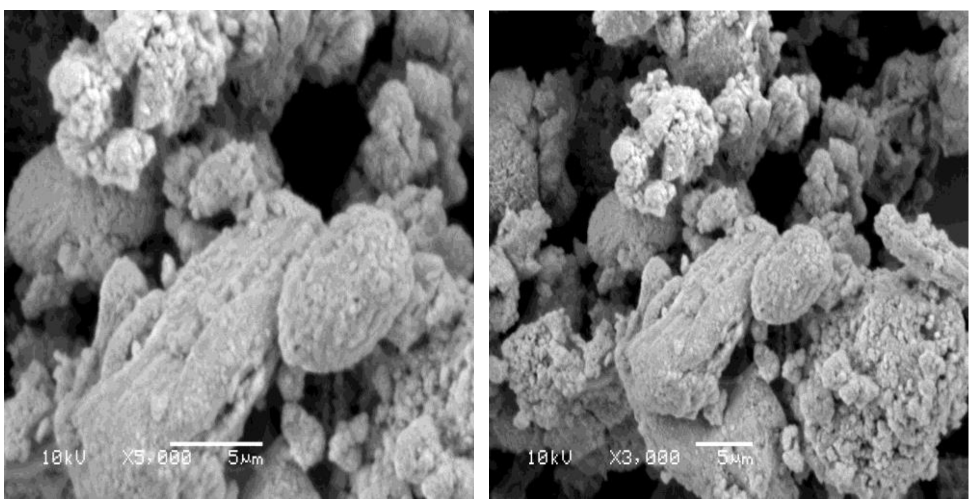

Fig. 10. SEM images of biosynthesized Silver Nanoparticles (AgNPs).

Antimicrobial activity

Antimicrobial activity of AgNPs against pathogenic antibiotic sensitive bacteria

The antibacterial effect of AgNPs at two levels of concentrations was tested on selected human pathogenic bacteria strains that were sensitive to vancomycin and ciprofloxacin antibiotics. Table (2) and Figure (11a and b) showed a comparison of the antibacterial activity of AgNPs (100 µg/disc and 150 µg/disc) along with that of $AgNO_3$, leaf extract, vancomycin and ciprofloxacin against *S. aureus* and *E.coli*. The results exhibited significant antibacterial activity of AgNPs that was increased with increasing AgNPs concentration compared to those of precursor and leaf extract. The antibacterial activity of AgNPs reached 68% compared to that of vancomycin standard (30 µg/disc) against *S. aureus* when the concentration of AgNPs increased from 100 µg/disc to 150 µg/disc while the activities of the precursor ($AgNO_3$) and leaf extract were 22% and non-detectable respectively. A previous report (Paling *et al.*, 2017) indicated that *S. aureus* posed as a risk factor for spreading infection in intensive care units (ICU) due to its colonization and disinfection equipment at ICU with biosynthesized AgNPs may reduce this risk as suggested in a previous research (El-Ansary *et al.*, 2018).

Table 2. Antibacterial activity of AgNPs, $AgNO_3$, *Z. spina-christi* (ZSC) leaf extract, and reference antibiotic drugs (ciprofloxacin and vancomycin) against *E. coli and S. aureus* pathogenic antibiotic sensitive bacteria (a) 100 µg/disc and (b) 150µg/disc of AgNPs.

Conc. of AgNPs	Pathogenic species	Mean zone of inhibition (mm ± SD)				
		AgNPs	$AgNO_3$	Leaf extract	Vancomycin 30µg	Ciprofloxacin 5µg
100 µg/disc	*S. aureus*	13.5±0.5	6.0±0.3	NA*	27.0±0.0	
	E. coli	14.2±0.3	6.5±0.5	NA*		30.0±0.0
150µg/disc	*S. aureus*	18.6±0.3	9.3±0.57	NA*	27.0±0.0	
	E. coli	20.5±0.5	9.3±0.57	NA*		30.0±0.0

NA* represent poor antibacterial activity was found in this work.

(a)

(b)

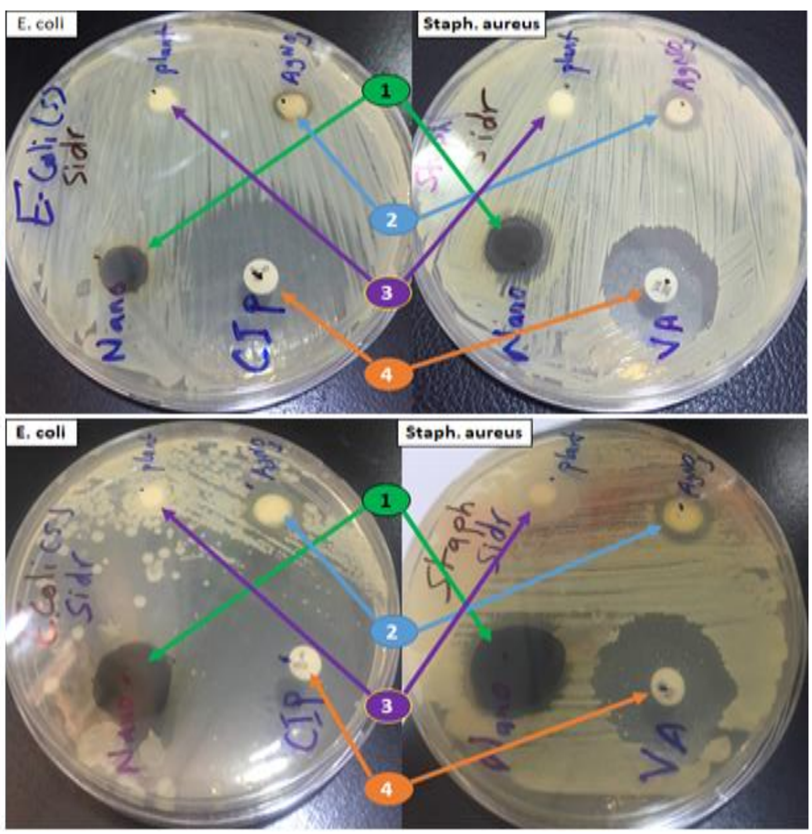

Fig. 11. The antibacterial activity of (1) AgNPs, (2) $AgNO_3$, (3) leaf extract, and (4) Standard reference antibiotic drugs (ciprofloxacin and vancomycin) against *E. coli and S. aureus* pathogenic antibiotic sensitive bacteria (a) 100µg/disc and (b) 150µg/disc of AgNPs.

Similar increase in the antibacterial activity of AgNPs was observed against *E.coli* that reached 60% at 150 µg/disc compared to that of 5 µg/disc ciprofloxacin standard. The antibacterial activities of the biosynthesized AgNPs indicated that the nanoparticles have the desirable sizes that were capable of penetrating the bacteria wall and damaging its structure. Our results were in agreement with those reported by (Sanchooli *et al.*, 2018). The enhanced antibacterial activity of the biosynthesized AgNPs compared to that of the leaf aqueous extract has to be highlighted. *Z. spina-christi* leaves and plants are traditionally used as a medical remedy for the treatment of various illness including inflammation, pain and antimicrobial infection (Zayed *et al.*, 2015). These desirable properties could be greatly enhanced when leaves' phytochemicals were incorporated onto the surface of the silver metals as shown by the results presented here.

Antimicrobial activity of AgNPs against pathogenic antibiotic resistance bacteria

The gram -ve *E. coli* and gram +ve *S. aureus* are among the bacteria that exhibit high multi drug resistant (MDR) that reach 75.7% and 100% respectively (Zayed *et al.*, 2015). For this reason, antibacterial effect of biosynthesized AgNPs were tested against strains of *S. aureus* and *E. coli* and the results were compared to the effect of vancomycin and ciprofloxacin antibiotic against the same bacteria strains.

Data in Tables (3) and Figure (12a and b) showed that the biosynthesized AgNPs exhibited significant antibacterial activity against both pathogenic antibiotic-resistant bacteria (*S. aureus* and *E.* coli) at the concentrations of 100 µg/disc and 150 µg/disc. Few conclusions could be drawn from these results. First, the biosynthesis AgNPs have great antibacterial efficacy that reached 12.6 ± 0.3 mm inhibition zone against the gram -ve *E. coli* which is totally resistant to the standard ciprofloxacin (5 µg/disc) antibiotic**.** Second, the biosynthesized AgNPs showed high antibacterial effect against the strains of the gram +ve *S. aureus* which were resistant to vancomycin antibiotic at the concentration of 30 µg/disc. Third, the results revealed that the antibacterial effect of the biosynthesized AgNPs is concentration dependent. Our data were in agreement with those reported by other investigators (Muthulakshmi *et al.*, 2018; Sanchooli and Yazdanpour, 2018).

CONCLUSION

The biosynthesis procedure of AgNPs from aqueous extract of *Z. spina-christi* leaves from species available in Yemen was optimized. Optimum conditions including reaction time, pH, temperature, volumes of 1 mM $AgNO_3$ precursor and leaf aqueous extract were determined. Characterization of the synthesized AgNPs were carried out using UV-Vis, FT-IR, X-ray diffraction and SEM were carried out and revealed that the particles were nearly spherical, unaggregated with an average size of 32 nm.

Table 3. Antibacterial activity of AgNPs, $AgNO_3$, *Z. spina-christi* (ZSC) leaf extracts, and reference antibiotic drugs (ciprofloxacin and vancomycin) against *E. coli and S. aureus* pathogenic antibiotic resistant bacteria (a) 100 µg/disc and (b) 150 µg/disc of AgNPs.

Conc. of AgNPs	Pathogenic species	Mean zone of inhibition (mm ± SD)				
		AgNPs	$AgNO_3$	Leaf extract	Vancomycin 30µg	Ciprofloxacin 5µg
100 µg/disc	*S. aureus*	11.5±0.5	6.5±0.5	NA*	14.0±0.0	
	E. coli	12.6±0.3	7.2±0.3	NA*		NA*
150µg/disc	*S. aureus*	15.3±0.3	9.5±0.5	NA*	14.0±0.0	
	E. coli	16.6±0.3	9.5±0.5	NA*		NA*

NA* represent poor antibacterial activity was found in this work.

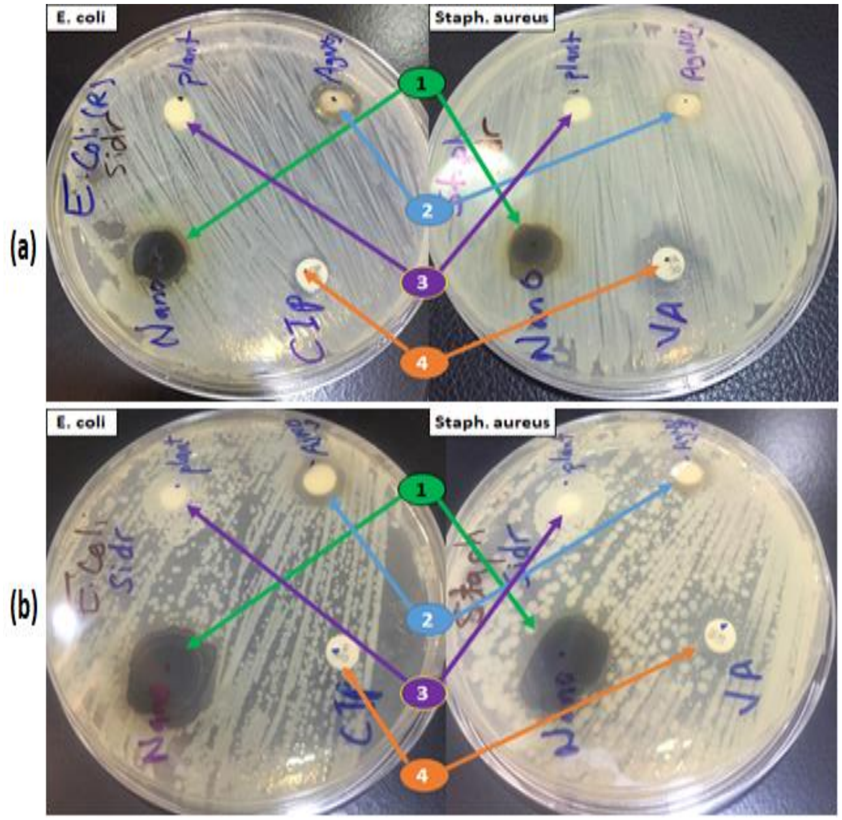

Fig. 12. The antibacterial activity of (1) AgNPs, (2) $AgNO_3$, (3) leaf extract, and (4) Antibiotic drugs (ciprofloxacin and vancomycin) against *E. coli and S. aureus* pathogenic antibiotic resistance bacteria (a) 100µg/disc and (b) 150µg/disc of AgNPs.

The antibacterial activity of the biosynthesized AgNPs were tested against sensitive and resistant strains of gram +ve *S. aureus* and gram -ve *E. coli* bacteria and proved to have significant antibacterial activity indicating that AgNPs could be an easy and cheap alternative to control bacterial infection in medical facilities and be used against multi-drug resistant bacterial in medical fields. The phytochemical content of the ZSC leaves were also determined.

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CONFLICT OF INTEREST

The authors declare that this article's content has no conflict of interest.

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REFERENCES

Akther, T., Vabeiryureilai, M., Nachimuthu Senthil, K., Davoodbasha, M., Srinivasan, H., 2019. Fungal-mediated synthesis of pharmaceutically active silver nanoparticles and anticancer property against A549 cells through apoptosis. Environ. Sci. Pollution Res., 26(13): 13649-13657.

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. Nanotoxicol., 7(1): 30-36.

Asgarpanah, J., Haghighat, E., 2012. Phytochemistry and pharmacologic properties of Ziziphus spina christi (L.) Willd. Afr. J Pharm. Pharmacol., 6(31): 2332-2339.

Behbudi, G., 2021. Effect of silver nanoparticles disinfectant on covid-19. Adv. Appl. NanoBio-Technol., 2(2): 63-67.

Bhardwaj, B., Singh, P., Kumar, A., Kumar, S., Budhwar, V., 2020. Eco-Friendly Greener Synthesis of Nanoparticles. Adv. Pharmaceut. Bull., 10(4): 566.

Bin-Meferij, M.M., Hamida, R.S., 2019. Biofabrication and antitumor activity of silver nanoparticles utilizing novel nostoc sp. Bahar M. Int. J. Nanomed., 14: 9019.

Borgohain, D.J., Mansor, A.N., Verma, M.K., 2021. Nanotechnology Research in SAARC Nations during 1996 to 2020: A Scientometric Assessment based on Scopus Database. Library Philosophy and Practice: 1-19.

Coates, J., 2006. Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry: applications, theory and instrumentation.

Dubey, S.P., Lahtinen, M., Sillanpää, M., 2010. Tansy fruit mediated greener synthesis of silver and gold nanoparticles. Process Biochem., 45(7): 1065-1071.

El-Ansary, A., Warsy, A., Daghestani, M., Merghani, N.M., Al-Dbass, A., Bukhari, W., Al-Ojayan, B., Ibrahim, E.M., Al-Qahtani, A.M., Bhat, R.S., 2018. Characterization, antibacterial, and neurotoxic effect of Green synthesized nanosilver using Ziziphus spina Christi aqueous leaf extract collected from Riyadh, Saudi Arabia. Materials Res. Expr., 5(2): 025033.

El-Naggar, N.E.-A., Hussein, M.H., El-Sawah, A.A., 2018. Phycobiliprotein-mediated synthesis of biogenic silver nanoparticles, characterization, in vitro and in vivo assessment of anticancer activities. Scient. Rep., 8(1): 1-20.

Elamawi, R.M., Al-Harbi, R.E., Hendi, A.A., 2018. Biosynthesis and characterization of silver nanoparticles using Trichoderma longibrachiatum and their effect on phytopathogenic fungi. Egy. J. Biol. Pest Contr., 28(1): 1-11.

Halawani, E.M., 2016. Rapid biosynthesis method and characterization of silver nanoparticles using Zizyphus spina christi leaf extract and their antibacterial efficacy in therapeutic application. J. Biomater. Nanobiotechnol., 8(1): 22-35.

Harborne, A., 1998. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science & Business Media.

Hassan, O.M., Ibraheem, I.J., Adil, B.H., Obaid, A., Salih, T.A., 2020. Synthesis of Silver Nanoparticles by ecofriendly nvironmental method using Piper nigrum, Ziziphus spina-christi, and Eucalyptusglobulus extract, Journal of Physics: Conference Series. IOP Publishing, pp. 012139.

Hussein, A.S., 2019. Ziziphus spina-christi: Analysis of Bioactivities and Chemical Composition, Wild Fruits: Composition, Nutritional Value and Products. Springer, pp. 175-197.

Jeremiah, S.S., Miyakawa, K., Morita, T., Yamaoka, Y., Ryo, A., 2020. Potent antiviral effect of silver nanoparticles on SARS-CoV-2. Biochem. Biophysic. Res. Communic., 533(1): 195-200.

Khan, I., Saeed, K., Khan, I., 2019. Nanoparticles: Properties, applications and toxicities. Arab. J. Chem., 12(7): 908-931.

Kokila, T., Ramesh, P., Geetha, D., 2015. Biosynthesis of silver nanoparticles from Cavendish banana peel extract and its antibacterial and free radical scavenging assay: a novel biological approach. Appl. Nanosci., 5(8): 911-920.

Makarov, V., Love, A., Sinitsyna, O., Makarova, S., Yaminsky, I., Taliansky, M., Kalinina, N., 2014. “Green” nanotechnologies: synthesis of metal nanoparticles using plants. Acta Naturae (англоязычная версия), 6(1 (20)).

Mintah, S.O., Asafo-Agyei, T., Archer, M.-A., Junior, P.A.-A., Boamah, D., Kumadoh, D., Appiah, A., Ocloo, A., Boakye, Y.D., Agyare, C., 2019. Medicinal plants for treatment of prevalent diseases. Pharmacogn.-Med. Plants: 1-19.

Mostafa, M., Al-Emam, A., Sayed, M., Alamri, S., Alghamdil, H., Shati, A., Alrumman, S., Alfaify, A.K., 2020. Silver nanoparticles from Ziziphus spina-christi (L.) Desf. fruits extract and their antimicrobial activity. Bangl. J. Bot., 49(4): 1127-1134.

Muthulakshmi, K., Uma, C., Sivagurunathan, P., Yoganathan, K., Satheeshkumar, S., 2018. Extracellular, biosynthesis of silver nanoparticles using Enterobacter cloacae (mk163462) and their antibacterial activity against certain multidrug resistant pathogens. J. Pharmaco. Phytochem., 7(6): 741-747.

Netai, M.-M., Stephen, N., Musekiwa, C., 2017. Synthesis of silver nanoparticles using wild Cucumis anguria: Characterization and antibacterial activity. Afr. J. Biotechnol., 16(38): 1911-1921.

Paling, F.P., Troeman, D.P., Wolkewitz, M., Kalyani, R., Prins, D.R., Weber, S., Lammens, C., Timbermont, L., Goossens, H., Malhotra-Kumar, S., 2017. Rationale and design of ASPIRE-ICU: a prospective cohort study on the incidence and predictors of Staphylococcus aureus and Pseudomonas aeruginosa pneumonia in the ICU. BMC Infect. Dis., 17(1): 1-6.

Panzella, L., Moccia, F., Nasti, R., Marzorati, S., Verotta, L., Napolitano, A., 2020. Bioactive phenolic compounds from agri-food wastes: An update on green and sustainable extraction methodologies. Front. Nutr., 7.

Prakash, P., Gnanaprakasam, P., Emmanuel, R., Arokiyaraj, S., Saravanan, M., 2013. Green synthesis of silver nanoparticles from leaf extract of Mimusops elengi, Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. Colloids and Surfaces B: Biointerfaces, 108: 255-259.

Qais, F.A., Shafiq, A., Khan, H.M., Husain, F.M., Khan, R.A., Alenazi, B., Alsalme, A., Ahmad, I., 2019. Antibacterial effect of silver nanoparticles synthesized using Murraya koenigii (L.) against multidrug-resistant pathogens. Bioinorg. Chem. Applicat., 2019.

Rai, P.K., Usmani, Z., Thakur, V.K., Gupta, V.K., Mishra, Y.K., 2020. Tackling COVID-19 pandemic through nanocoatings: Confront and exactitude. Curr. Res. Green Sustain. Chem., 3: 100011.

Rajakumar, G., Gomathi, T., Thiruvengadam, M., Rajeswari, V.D., Kalpana, V., Chung, I.-M., 2017. Evaluation of anti-cholinesterase, antibacterial and cytotoxic activities of green synthesized silver nanoparticles using from Millettia pinnata flower extract. Microb. Pathogen., 103: 123-128.

Saadh, M.J., Aggag, M.M., Alboghdadly, A., Kharshid, A.M., Aldalaen, S.M., Abdelrazek, M.A., 2021. Silver nanoparticles with epigallocatechingallate and zinc sulphate significantly inhibits avian influenza A virus H9N2. Microb. Pathogen., 158: 105071.

Saleh, M.M., Alnaddaf, L.M., Almuhammady, A.K., Salem, K.F., Alloosh, M.T., Al-Khayri, J.M., 2021. Applications of Plant-Derived Nanomaterials in Mitigation of Crop Abiotic Stress, Nanobiotechnology. Springer, pp. 201-238.

Sanchooli, A., Yazdanpour, Z., 2018. Synthesis of Silver Nanoparticles Using Verbena officinalis Plant Extract and Investigation of the Antimicrobial Activity Against Acinetobacter baumannii Bacteria. J. Nano Drug Sci., 1(1): 5-9.

Sanchooli, N., Saeidi, S., Barani, H.K., Sanchooli, E., 2018. In vitro antibacterial effects of silver nanoparticles synthesized using Verbena officinalis leaf extract on Yersinia ruckeri, Vibrio cholera and Listeria monocytogenes. Ir. J. Microbiol., 10(6): 400.

Sarkar, S., 2020. Silver Nanoparticles with Bronchodilators Through Nebulisation to Treat COVID 19 Patients. J. Curr. Med. Res. Opin., 3(04): 449–450.

Shehzad, A., Qureshi, M., Jabeen, S., Ahmad, R., Alabdalall, A.H., Aljafary, M.A., Al-Suhaimi, E., 2018. Synthesis, characterization and antibacterial activity of silver nanoparticles using Rhazya stricta. PeerJ., 6: e6086.

Singh, A., Gautam, P.K., Verma, A., Singh, V., Shivapriya, P.M., Shivalkar, S., Sahoo, A.K., Samanta, S.K., 2020. Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review. Biotechnol. Rep., 25: e00427.

Singh, S.P., Bhargava, C., Dubey, V., Mishra, A., Singh, Y., 2017. Silver nanoparticles: Biomedical applications, toxicity, and safety issues. International J. Res. Pharm. Pharmaceut. Sci., 4(2): 01-10.

Vanaja, M., Rajeshkumar, S., Paulkumar, K., Gnanajobitha, G., Malarkodi, C., Annadurai, G., 2013. Kinetic study on green synthesis of silver nanoparticles using Coleus aromaticus leaf extract. Adv. Appl. Sci. Res., 4(3): 50-55.

Verma, A., Mehata, M.S., 2016. Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. J. Rad. Res. Appl. Sci., 9(1): 109-115.

Zayed, M.F., Eisa, W.H., Abdel-Moneam, Y.K., El-Kousy, S.M., Atia, A., 2015. Ziziphus spina-christi based bio-synthesis of Ag nanoparticles. J. Industr. Engin. Chem., 23: 50-56.