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FORMULATION AND EVALUATION OF HYDROGEL INCORPORATED WITH BIOSYNTHESIS SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *SALVIA OFFICINALIS* L. AGAINST *S. AUREUS* ISOLATED FROM WOUNDS FROM SOME BAGHDAD HOSPITALS

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Abstract

In this study, silver nanoparticles (AgNPs) were synthesized via biological reduction of silver nitrate using plant extract of *Salvia officinalis* (Green chemistry principle). Researcher use bio nanotechnology techniques as eco- friendly and cost effective routes to fabricate nanoparticles nanomaterials, AgNPs were synthesized by mixing plant extract with $AgNO_3$ 1Mm, the characteristic surface plasmon resonance (SPR) of nano silver was observed at 468 nm, SEM images showed the formation of spherical nanoparticles with mean particle size 73.4 nm, and zeta potential value – 25.56 mV XRD patter agreed with fcc crystalline stricter of silver nanoparticles. In the recent year, silver nanoparticles have gained much attention due to their unique antimicrobial properties and we detected that by formulation a hydrogel using carpobol 934 and evaluated for PH, spreadability, viscosity, then loaded AgNPs which synthesized against Gram positive bacteria *S. aureus in vitro* which isolated from wounds and characteristic by biochemical test, the inhibition zone and result of evaluation indicated that this AgNPs – hydrogel can be used potentially for biomedical application.

Keywords : Silver nanoparticles, FRTIR, XRD, Hydrogel, Green synthesis, Zeta potential.

Introduction

Nanotechnology is a field of applied science and can be termed as the synthesis, characterization, exploration and application of nanosized (1-100 nm), substances for improvement of science (Garg, 2012) it offers with materials whose constructions show off notably novel and elevated physical, chemical and biological properties ,and functionality due to their nano scaled measurement and have a larger floor location than macro-sized substances (Pandian *et al.*, 2013). Synthesized of AgNPs were executed by usage physical ,chemical and biological methods .and last method have arises to overcome all the issues of another methods, silver nanoparticles were synthesized with precis dimension the use of extraordinary organic systems together with microorganisms (like bacteria, fungi) plant extracts and small bioactive molecules like vitamins and amino acids as an alternatively chemical techniques not solely for silver nanoparticles, however additionally for the synthesis of some different nanoparticles.

Green synthesis of silver nanoparticles by using plant extract have a large interest according to its rapid, eco-friendly, nonpathogenic and providing a single step way to the biosynthesis processes (Zhang *et al.*, 2016) biomolecules which present in the plant extract such as proteins, enzyme, amino acids, polysaccharides, alkaloids, tannins, phenolics, terpenoids, are accountable for reduction and stabilization of silver ions (Bagyalakshmi and Haritha, 2017), *S. officinalis* is native to Middle East and Mediterranean areas. (Ghorbani and Esmaeilizadeh, 2017). The nanoparticles get attached to the cell membrane and penetrate interior the bacteria. The bacterial membrane contains of sulfur-containing proteins and the $AgNO_3$ interaction with these proteins of cell as well as with the phosphorus-containing compounds such as DNA. When SNPs became in the bacterial cell, it forms a low molecular weight region in the core of the mocroorganism leading to cell death. The nanoparticles launch silver atoms in the bacterial cells, which assess their bactericidal activity. (Prabhu and Poulose ,2012) the antimicrobial activity of AgNPs against *S.aureus* which isolated from wounds evaluated by formulation Hydrogels loaded with metal nanoparticles and this hydrogel can serve as a reservoir for the sustained release of silver ions and maintain a moist environment to aid in wound healing. Antimicrobial activity of some such gels has been proven, with wound healing mentioned as a possible application, it have obtained great importance for many applications in the biomedical and biotechnological fields. Hydrogel are biodegradable, biocompatible and non-toxic formulations and the unique physical properties of gel have sparked particular interest in their use in drug delivery application, thus extend solutions to some of the challenges in biology, medicine and material science (Saha *et al.*, 2017). The purpose of this study was fashioned to discover systematically and synthesis silver nanoparticle by using plant extract then formulate hydrogel containing silver nanoparticle and evaluate for its physicochemical residences and antibacterial undertaking.

Materials and Methods

Methods

Planet Collection : *Salvia officinalis* was collected from local market in Baghdad city. The dried leaves were grinding and stored at sterile condition until use and confirmed by Dr. Sahar Alsaadi Assistant professor dept of biology, Al-Basrah university.

Preparation of *S. officinalis* Aqueous Extract : The leaves of *Salvia officinalis* were cut into fine pieces then 10 gm of powder boiling in a 100 ml of distilled water and filtered using whatmann No. 1 filter paper (pore size 18 µm) (Indra *et al*., 2017) and the aqueous extract kept in 4°C until use. (Kokila *et al*., 2015).

Synthesis of Silver Nanoparticles : To synthesized of Silver nanoparticles 10 ml of aqueous extract of *Salvia officinalis* was added to 90 ml of aqueous silver nitrate solution 1mM, (Kumar *et al*., 2017). And heating the mixture to 70°C, changing color from pale yellow brown to reddish green indicated the reduction of $AgNO_3$ to metallic silver (Arrieta *et al.*, 2018).

Characterization of The Silver Nanoparticles

UV-Visible spectrophotometer : UV-vis absorption was analyzed after diluted 2 ml of silver nanoparticles solution in quartz cuvette with 1 ml distilled water, the absorption ranges from 190 -800 nm by using Chrome Tech spectrophotometer model UV-1100

Particles Size Analysis : Mean particle size diameter and polydispersity were all measured in solution form after synthesis, silver nanoparticles 2 ml were added to the quartz by using photon correlation spectroscopy, the measurement were taken.

Zeta Potential Measurements : The zeta potential was measured by an electrophoretic light - scattering apparatus (zeta sizer - nano series malvern instruments) to examine the charge of silver nanoparticle colloidal and stability.

X-RAY Diffraction Measurement : Crystalline metallic pattern of AgNPs powder was analyzed using X-ray diffraction to obtain a pellet of pure Nps, for XRD analysis silver nanoparticles solution was centrifuged at 10000 rpm for 20 minutes followed washed the deposit with distilled water three times, The X-RD pattern was operated at a voltage of 40 kv with cu-kα radiation. The scanning was done from 20° to 80.

FTIR analysis : The FTIR spectra was on record in the range of 4000- 400 cm^{-1} at a resolution of 4 cm^{-1}.then sample slide was prepared and placed in fourier transform infrared for the analysis of the nanoparticles.

SEM analysis : After preparing the nanoparticles, the suspension of nanoparticles was centrifugation on 10000 rim/min and let it to dry. The powder used under electronic microscope.

Bacteria strains

S. aureus isolated from wounds obtained from Baghdad hospital ,bacteria were cultured at 37 ºC on mannitol salt agar, and identified by biochemical test like catalase test, oxidase test, IMVC, Nitrate reductase, sugar fermentation test and characteristics of the isolates by light microscopic.

Preparation of hydrogel formulation

Hydrogel formulation were prepared by cold mechanical method with quantity of carbopol 934 polymer, (2g) of polymer were dissolved in distilled water and left overnight, then a quantity of glycerol (2g) was added and mixed well (Prabu *et al*., 2017). Then a preservatives was added sodium benzoate 0.25 gm, methyl paraben 0.03 gm, after that silver nanoparticles were added (Table 1) and volume made up to 100 ml distilled water, (Mohammed *et al*., 2018).

Table 1 : The preparation of three formulas using different concentration of AgNPs

Ingredients	A	B	C
Carbopol 934	2	2	2
Glycerol	2	2	2
Sodium benzoate	0.25	0.25	0.25
Methylparaben	0.03	0.03	0.03
AgNPs	50 µg	100 µg	200 µg
Distilled water	100	100	100

Characterization of Hydrogel Formulation Appearance : The hydrogel formulated were spotted for their visible appearance, colour, structure, feel up on application such as grittiness, stickiness and smoothness (Soni and Singhai, 2012).

pH : The pH of hydrogel formulated was determined by using pH meter, 1gm of hydrogel was diluted (dissolved) in 100 ml distilled water and stored in 4°C two hours, the PH was measured (Prusty and Parida, 2015).

Spreadability : Spreadability of hydrogel was determined by using glass slide fixed on the block and 2 gm was put on it, the hydrogel was sandwiched by using another glass slide and weight 20 g was placed on upper slide to form a thin gel layer between slides, weight movable, then the time taken to separate the upper slide away from the lower slide was noted, spreadability was calculated by using the equation:

$$S = ml / t$$

Where :

m = weight tide upper slide

l = length moved to the glass slide

t = time taken to separate from each other (Prabu *et al*., 2017).

Viscosity : The viscosity measurement of the gel was performed with a Viscometer and for this purpose, spindle NO. 04 was used. by rotation of 20 rpm / min.

Antibacterial Activity : The antibacterial activity of the hydrogel examined against *Staphylococcus aureus* which isolated from wounds by agar diffusion technique, the agar plates were prepared just like manufactures specification and 0.1ml from bacterial suspension having turbidity (10^5 cfu/ml) was spreading over the surface of medium, the wells were made with cork-borer 6mm diameter and put in this wells 0.1ml of hydrogel which loaded different Concentration of silver nanoparticles. Then the plates were incubated at 37°C for 24 hours and the inhibition zones were measured. Hydrogel without silver nanoparticles used as control (Mohammed *et al*., 2018).

Results

Evaluation of plant extract

The color of the extract was located to be brown .and the extract was tested for its phytochemical analysis like alkaloids, glycosides, saponin, phenolics, tannin, and flavonoids. The result of phytochemical analysis of the plant extract is show in table (1)

Table 1 : Chemical components analysis for aqueous extracts of *Salvia officinalis*

Components	Reagents	Result
Tannins	Lead acetate%1	+
Saponins	Fast stirring	+
Flavonoids	1% Aqueous ferric chloride	+
Alkaloids	Mayer's reagent Wagner reagent	- -
Proteins		+
Phenolics	1%Aqueous ferric chloride	+
Oil		-
Glycosides	Molish test	+

Green synthesis of silver nanoparticles

Green synthesis of AgNPs used to be prepared from plant extract, by mixing aqueous plant extract with silver nitrate solution and heated the mixture to 70 C° changing in solution color from faded yellow brown to reddish green inexperienced was once observed and suggests the reduction silver ions and component of AgNPs. This component is shown in (figure 1).

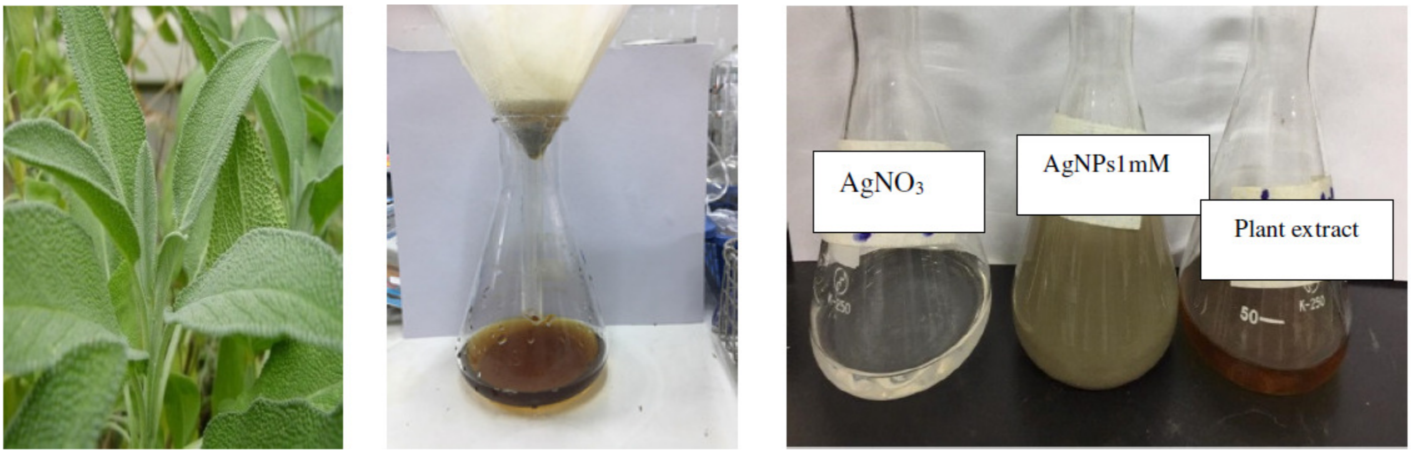


Fig. 1 : Formation of silver nanoparticles (a)*Salvia officinalis* plant, (b)*Salvia officinalis* aqueous extract (c)Reaction mixture(leaf extract +nitrate solution =AgNPs).

Characterization of silver nanoparticles

UV-Visible Spectroscopy Analysis : The response between the metal ions and the plant extract were examined by UV-Vis spectrophotometer. The silver nanoparticles (AgNPs) have free electrons that stimulate surface plasmon resonance absorption (SPR) band, (figure 2) suggest the UV-vis spectra of AgNPs recorded the wavelength range from 190 to 800 nm and the maximum absorbance of silver nanoparticles occurred at 468 nm. The appearance of the peaks, assigned to surface plasmons, are well-documented for more than a few metal nanoparticles (Yu *et al.*, 2019). The actual mechanism of the extracellular biosynthesis of Ag NPs is not well understood, It was once suggested that NADH coenzyme was once working as an electron shuttle to neutralize Ag+ ion (Okafor *et al.*, 2013).

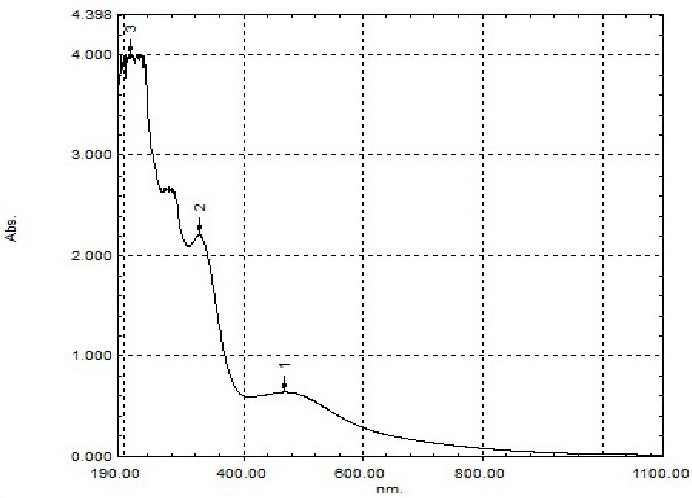


Fig. 2 : UV–vis spectrum of silver nanoparticles AgNPs 1mM.

Particle size analysis : Particle size analyzer has been used to detect the size of nanoparticles, (figure.3) shows PSA for silver nanoparticles synthesized by green method and it has a diameter 73.4 nm with poly dispersity index PDI 0.322 indicating that synthesized particles are good and uniform sizes. (Roy *et al.*, 2013).

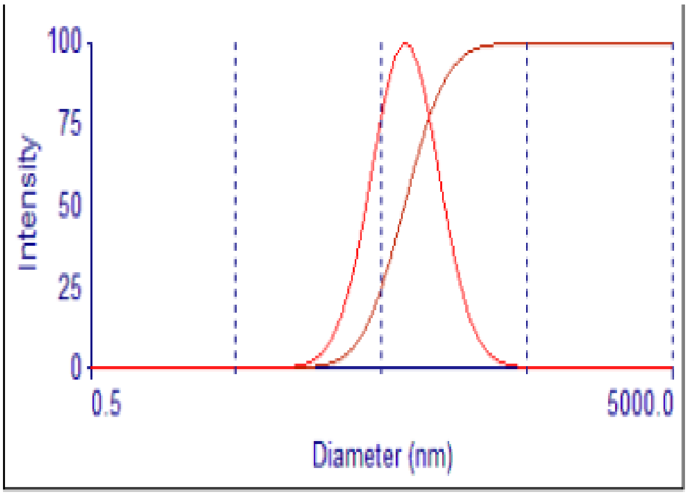


Fig. 3 : Particle size analyzer graph of Ag Nanoparticles using *Salvia officinalis* L. extract

Zeta potential analysis : Zeta Analysis used to painting the surface charge and stability of silver nanoparticles as proven in (figure 4). The biosynthesized AgNPs had a negative charge with zeta potential value - 25.66 mv, which that falls inside the vary of (- 20) to (- 30) mv is viewed as relatively moderately stable (Ardani *et al.*, 2017).

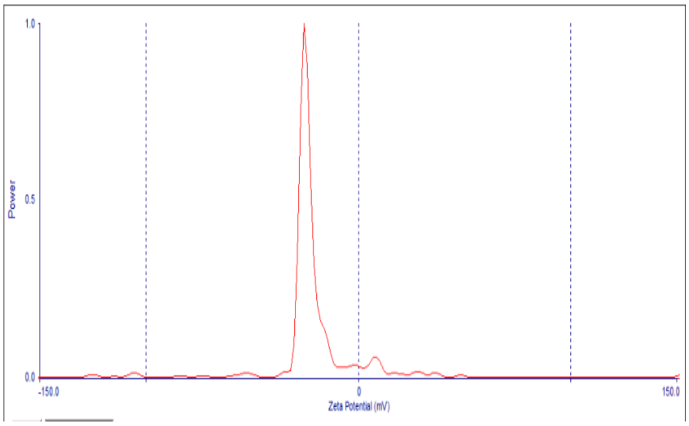


Fig. 4 : Zeta potential distribution of *Salvia officinalis* silver nanoparticles

X-ray diffraction spectroscopy : The crystalline nature of silver nanoparticles which prepared in this study were verified via XRD pattern in as show in (figure 5).

The XRD sketch genuinely shows 4 peaks at 2θ=38.19°, 44.37°, 64.65°,77.47° represented the synthesis AgNPs with fcc structure respectively, the reflex of crystalline planes (111),(200),(220),(311). The average size of the synthesized AgNPs was once determined by way of usage the Debye scherrer equation it was to be = 24.08 nm. These peaks are due to the natural compounds which are present in the extract and accountable for silver ions reduction and stabilization of AgNPs. our finding is line with (Mie *et al.*, 2013).

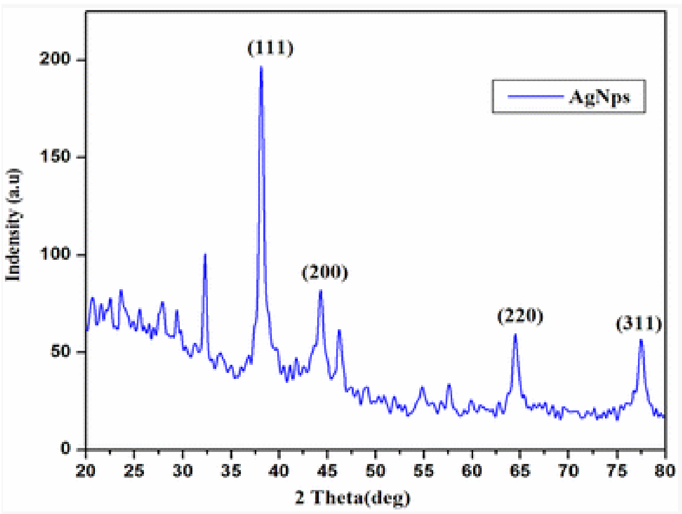


Fig. 5 : X-ray diffract gram of AgNPs.

Fourier infrared spectroscopy analysis : FTIR measurements are very vital for probing the chemical composition of the surface of silver nanoparticles and to become aware the feasible interactions between Ag and biomolecules (protein), responsible for the capping and efficacious stabilization of the silver nanoparticles (Umoran *et al.*, 2014; Prabakaran *et al.*, 2012). Figure (6a) and (6b) present the FTIR of the *Salvia officinalis* aqueous extract and of the silver nanoparticles respectively. The comparison of FTIR spectrum between the *Salvia officinalis* leaf extract and silver nanoparticles showed that the peaks of FTIR spectrum of silver nanoparticles located at 514.99, 582.5, 769.6, 902.64, 1122.57, 1159.22, 1192.01, 1263.37, 1309.67, 1334.74, 1371.34, 1624.08, 1660.71, 1770.65, 1826.59, 1905.67, 1955.82, 2357.01 cm^{-1}, can be comparable with that of plant extract located at 516.92, 580.57, 773.46, 902.69, 1120.64, 1157.29, 1190.08, 1267.23, 1313.52, 1334.74, 1373.32, 1629.85, 1665.5, 1768.72, 1826.59, 1903.74, 1955.82, 2358.94 cm^{-1} respectively. The peaks at 516.92 and 580.57 cm^{-1} refer to aromatic compounds in the plants (Kumar *et al.*, 2012). The peaks at 773.46 and 902.69 cm^{-1} refer to CH out-of-plane bending vibrations (Kumar *et al.*, 2012). The peaks at 1120.67, 115729 and 1190.08 cm^{-1} correspond to C-O stretching vibrations mode (Phanjom and Ahmed, 2015). The peak at 1267.23 cm^{-1} refers to to C–H deformation vibrations (Lin *et al.*, 2010). The peaks at 1313.52, 1334.74 and 1373.32 cm^{-1} refer to C–N stretch vibrations as well as to the amide I bands of proteins (Umoren *et al.*, 2014). The peaks at 1629.85 and 1665.5 cm^{-1} correspond to amide I and amide II and arises due to carbonyl stretch and –N–H stretch vibrations in the amide linkages of the proteins, respectively (Phanjom and Ahmed, 2015; Balagi *et al.*, 2009; Song *et al.*, 2009). The peak at 1768.72 cm^{-1} refers to carbonyl groups (Song *et al.*, 2009). The peaks at 1826.59, 1903.74 and 1955.82 cm^{-1} refer to aromatic –CH stretching (Prabakaran *et al.*, 2012). The peaks at 2358.94 cm^{-1} corresponds to primary amine group of protein (Phanjom and Ahmed, 2015). The carbonyl group from amino acid residues and peptides of proteins has the enhanced capacity to bind metal (Balaji *et al.*, 2009), so proteins can probable covering like coat AgNPs to forestall aggregation of the nanoparticles and stabilize the medium. Thus, the biological molecules can be operate the feature for the formation and stabilization of the silver nanoparticles in medium. Gole *et al.* (2001) said the proteins can bind to silver nanoparticles through free amine companies in the proteins. Hence, the proteins which existent on the surface of the silver nanoparticles act as capping agent for stabilization.

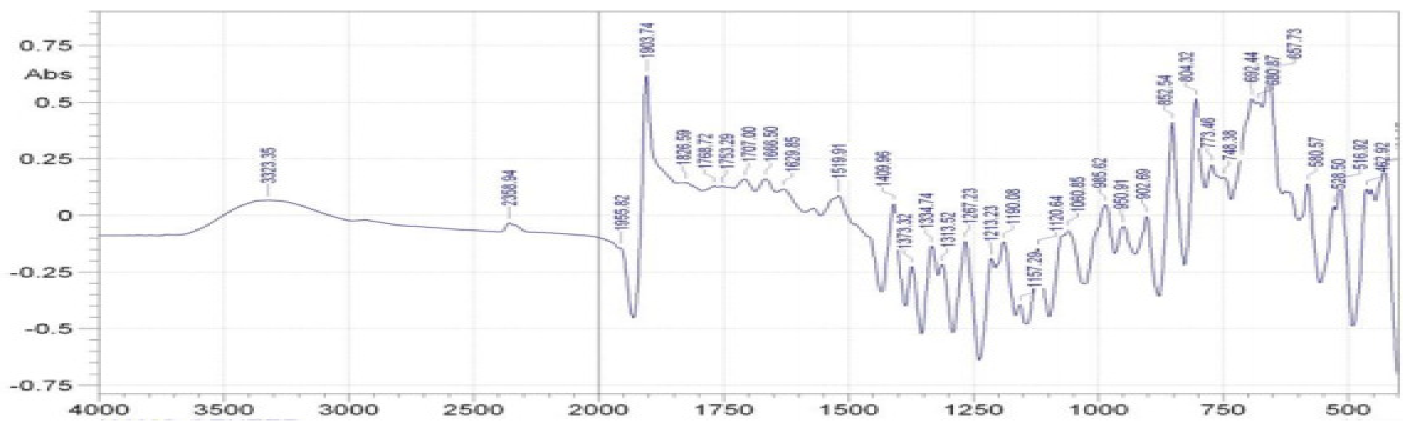


Fig. 6a : FTIR spectrum of *Salvia officinalis* leaf extract

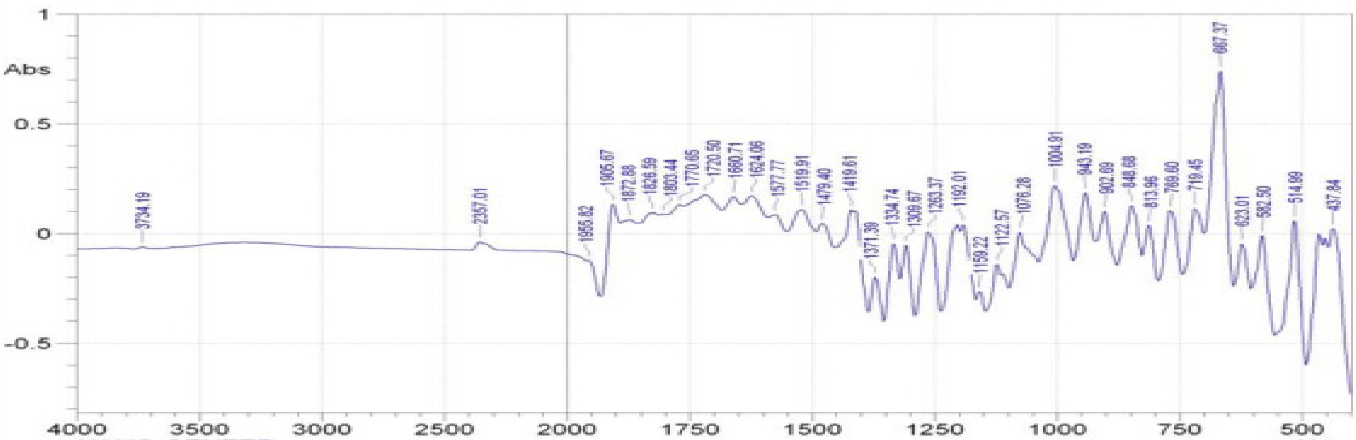


Fig. 6b : FT-IR spectrum of synthesized AgNPs using *Salvia officinalis* extract

SEM analysis : SEM used to be signify the shape, measurement and morphology of green synthesized of silver nanoparticles AgNPs. It reveals that silver nanoparticles have dispersed nearly spherical shape , with particle size in the range of 70nm to 80nm which that shows in (figure7). (Saha *et al.*, 2017).

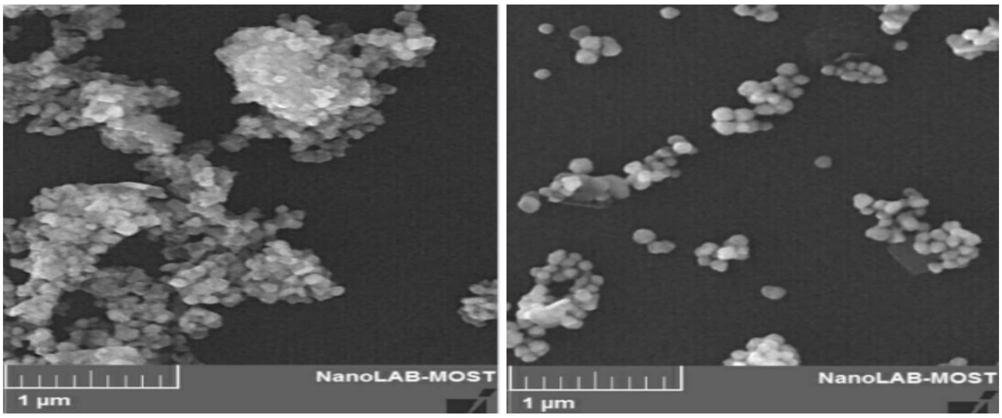
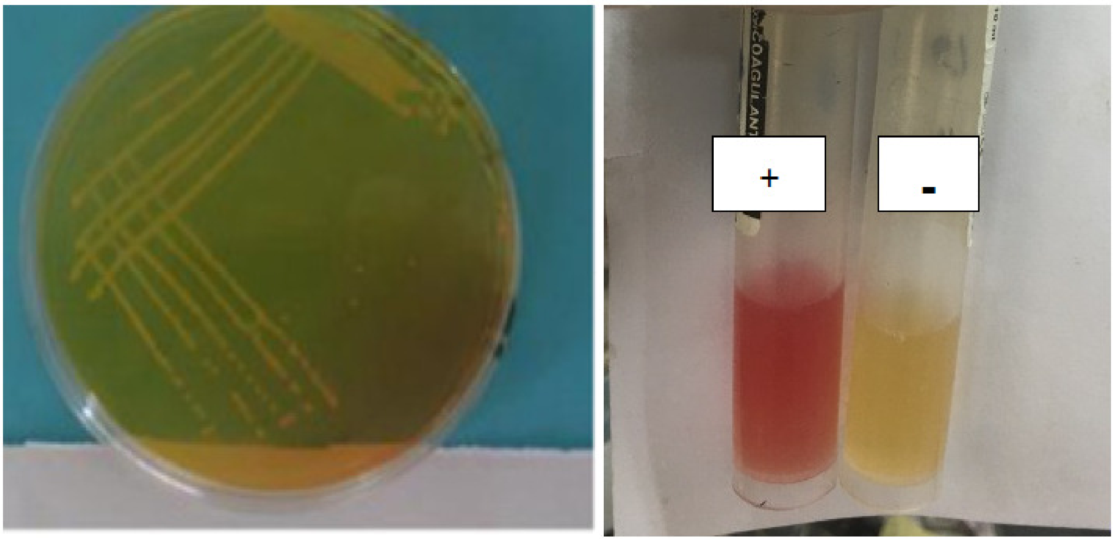


Fig. 7 : SEM image of AgNPs using *Salvia officinalis* L. extract.

Characterization of bacteria isolates : When the isolates cultured on mannitol salt agar the colony were pale yellow or deep yellow pigments on mannitol salt agar and the results of biochemical test revealed that all isolates were positive to catalase test and nitrate reduction test , further all the isolates were negative to oxidase test and citrate utilization and the isolates produced bright red color in methyl red test and red color in vogas –proskauer test ,and the bacteria isolates was gram positive, staining with purple coloured cocci in clusters (figure. 8).



(a) Colony of *S. aureus* on mannitol salt agar (b) Methyl red test for *S. aureus*

Physicochemical evaluation of Hydrogel formulation : Among the more than a few topical formulation, gel is desired both in cosmetic and in pharmaceutical preparations due to its quicker release rate of drug substance, easily spreadable and removable, physicochemical parameters such as homogenous of color, presence of any foreign particle and fibers, pH, visual examination results indicate that prepared topical gel formulation has uniform gray shade distribution and free from any lumps, fibers and any particle (Prabu *et al*., 2017).

The pH for the gel organized by means of carbopol 934 used to be located 5.43 which is near to be compatible with skin. (Soni and Singhai, 2012).

Spreadability : The therapeutic property of the topical formulation depends upon the spreadability, the result of spreadability was found to be 5.7 which truly signify that the hydrogel is convenient to spread on the skin. The result of viscosity of gel was found to be 28000 cp ,viscosity play an important role in controlling drug penetration, also when the spreadability is high the viscosity is decreased, and at the same time has another property of remaining at the application site without leak.

In vitro antibacterial activity : The inhibitory zone diameter for all three hydrogel formulation which prepared with different concentration of silver nanoparticles indicates that all three salvia officinalis leaf extract nanoparticles loaded hydrogel produced very significant zone inhibition and no zone of inhibition was obtained with control gel formulation (without AgNPs),in figure 8. it can be illustrated that at 24 hour incubation time the diameters of inhibition zones values were between 11-14 mm for the concentration of AgNPs 50 ,100 µm/ml , and 12-15 mm for the concentration 200 µm/ml against Gram positive bacteria S. aureus which used in this study.as show in (fig.10)

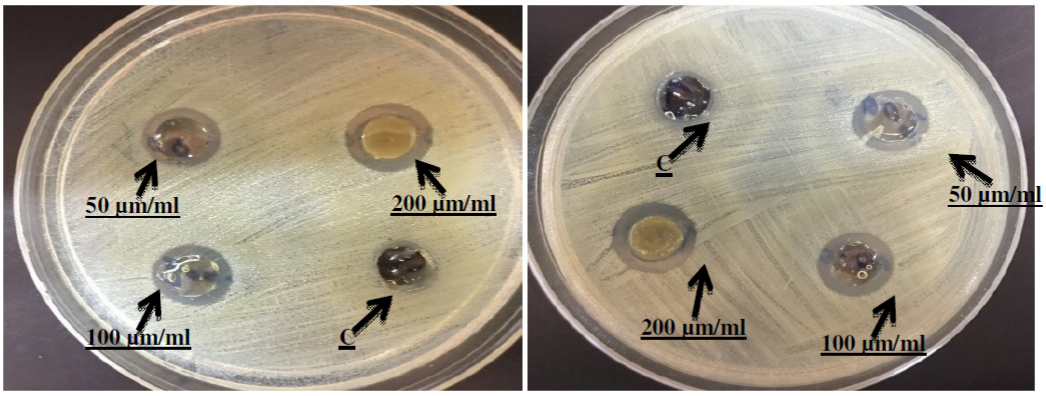


Fig. 8 : Antibacterial activity of AgNPs in Carbopol gel base against *S. aureus*. (C)control of hydrogel without AgNPs

Discussion

The problem of bacterial resistance to antibiotic traditionally has been solved by develop an appropriate antimicrobial drug by a mechanism using natural resources, in this study the rapid reducing agent for silver nitrate solution due to bioactive molecules which present in plant extract of *Salvia officinalis*, the progress of AgNPs green synthesis was observed by UV-Vis spectroscopy, appearance of peaks assigned to surface Plasmon resonance are well documented for more than a few metal nanoparticles (Yu *et al*., 2019), the mechanism of the extracellular biosynthesis of AgNPs is not well understood, it was once suggested that NADH coenzyme was working as an electron shuttle to neutralize Ag^{+} ion (Okafor *et al*., 2013). The particle size with PDI value 0.322 indicating that this particles are good and uniform size (Roy et al., 2013), crystalline structure of silver nanoparticles was identified by XRD showing planes due to natural compounds presenting in the plant extract and accountable for silver ions reduction and stabilization of AgNPs, our finding is line with (Mie *et al*., 2013) and the value of zeta potential was viewed as relatively moderately stable (Ardani *et al*., 2017). FTIR analysis revealed that the protein can probable covering like coat AgNPs to forestall aggregation of the nanoparticles and stabilization in medium, thus the biological molecules can be operate the feature for the formulation and stabilization of silver nanoparticles in aqueous medium. Gole *et al*. (2001) said the proteins can bind to the AgNPs through free amine companies in the protein. Hence, the proteins which existent on the surface of silver nanoparticles act as capping agent for stabilization. By definition gels are tridimensional polymeric networks that contain a large amount of water inside their binding and swell without dissolving (Wang *et al*., 2004). This skill that they have the capability to absorb exudates,which continues moisture on the wound surface. Besides, gels have high water vapor and oxygen permeablilty, as well as mechanical homes that resemble physiological gentle tissues, evaluation of hydrogel shows good pH which is near to be compatible with skin and spreadability signify that gel is high spread on the skin, at the same time when spreadability is high the viscosity is decreased to remaining at the application site without leak (Sezer *et al*., 2008).

In this work carbopol 934 was the material used to form the gels due to its suitable gel forming property. On the other hand, three different concentration of silver nanoparticles 200 µg, 100µg, 50µg were selected to create three types of samples, and agar well diffusion results indicate that increase of the concentration of AgNPs leads to more positive antibacterial effect. these observations also reconfirm the results from the previous study (Nguyen *et al*., 2019).

Conclusion

From the above study work, it was accomplished that green silver nanoparticles is a feasible choice to avoid toxic materials resulting from chemical and physical method, non-toxic effect on human cells and encourage as to use it in the formulation gel (as a drug delivery) due to antibacterial activity, Hence, to conclude it is one of the future novel delivery system in the field of skin therapeutic for the effective treatment of bacterial infection due to its properties.

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