

Study Of Biosynthesis, Characterization And Antibacterial Activity Of Silver Nanoparticles Against Pseudomonas Aeruginosa Isolated From Wounds From Some Baghdad Hospitals

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ABSTRACT

The aim of this research is to study the biosynthesis of silver nanoparticles using the aqueous extract of rosemary leaf (*Rosmarinus officinalis*) because it is cost-effective and contains many active biocompounds. Biosynthesized AgNPs were characterized using UV-Visible Scanning spectrophotometer to measure absorption peaks, λ_{max} was 465nm. FTIR technology was used to detect effective groups in plant which are responsible for nanoparticles formation. Scanning electron microscope (SEM) used to investigate the shape and size of nanoparticles, the size of particles was 74.7nm. X-ray diffraction meter (XRD) was used for analysis of nanoparticle structures, particle qualitative analysis as well as particle size, the size measured was 24.3nm. Atomic force microscope (AFM) used for detection of dispersion and agglomeration of nanoparticles, in addition to size, shape, and composition, the average of size was 67.15nm. zeta potential measurement device was used to measure the stability of AgNPs, Surface zeta potential value was -8.01mV. Particle size analysis was used for the purpose of determining the size of AgNPs, the size of nanoparticles was 82.4nm. The effectiveness of silver nanoparticles was studied against *Pseudomonas aeruginosa* isolated from wounds. The results showed that AgNPs were effective against these bacteria.

Keywords: Rosmarinus officinalis, Zeta potential, particle size analysis, Well agar diffusion method.

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1- Uv-visible scanning spectrophotometer (UV-VIS)

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1ml of AgNPs was diluted with 2ml of de-ionized water then characterized using UV-Visible scanning spectrophotometer. at wavelengths of 190 - 1100 nm. The instrument type was UV-1800 Series which was manufactured by Shimadzu company, Japan. (10).

2- Fourier Transform Infrared Spectrometry (FTIR) Analysis

Drops of AgNPs sample of and another of plant extract sample were put on a slide and dried in an electrical oven at 100°C until the thickness of dried material became 0.5 mm. FTIR spectrometer with scanning range 400-4000cm⁻¹ was used in the characterization process (10).

3- Scanning Electron Microscopy

AgNPs was dissolved in ethyl alcohol was taken for the purpose of detecting AgNPs created using a microscope Characterization of the (SEM) Nanoparticles and measure their size and shape instrument from Nikon, Japan. (3, 6).

4- X-ray Diffraction Instrument (XRD)

AgNPs was diluted with de-ionized water and put on a slide and dried in an electrical oven at 100°C until the thickness of dried material became 0.5 mm then the dispersions of them were examined using X-ray diffraction Instrument (XRD).; instrument from Shimadzu, Japan (6).

5- Atomic Force Microscopy (AFM)

Nano extract was put in a tube then the dispersion and aggregation was examined using Atomic Force Microscopy (AFM) which is also used to investigate their size, shape, sorption as well as the structure of the particles (20).

6- Zeta Potential analyzer

The physical property of Zeta potential gives the net surface charge of the NPs. The stability of NPs is measured when zeta potential values ranged from higher than +30 mV to lower than -30 mV. Surface zeta potentials were measured using zeta potential analyzer Brookhaven Instruments. 5ml of Liquid NPs samples were diluted with 50 ml of double de-ionized water and the suspending electrolyte solution was using NaCl with 2 x10⁻²M. The value of pH was then adjusted and the samples were shaken for 30 min. pH of equilibrium was recorded after shaking and the value of zeta potential of AgNPs was measured(15, 8).

7- Particle size analysis device

Particle size analysis was done in order to determine the size of the AgNPs formed (15).

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Isolation and identification of of Pseudomonas aeruginosa

28 of the bacterial isolates which were collected, isolated from patient's wounds at Laboratories Al-Kindi Teaching hospital and Medical city in Baghdad, the capital of Iraq and diagnosed at the hospitals laboratories from 20/1/2019 to 15/3/2019. The isolated colonies were cultured in sterile Petri plates using cetrimide agar medium (12) and diagnosed again using biochemical tests as oxidase, catalase, imvc, urease, nitrate reduction, growth at 42°C tests (19).

Studying the antibacterial effects evaluation of Nano silver by well agar diffusion method

The antibacterial effects evaluation of AgNPs was measured using different concentrations then these concentrations were used with 1 ml of de-ionized water by well agar diffusion method. the serial dilutions of (5000, 2500, 1250, 625, 312.5, 156.5, 78.125, 39.06) µg/ml were prepared, the sample was exposed to ultrasonic wave device for about 4 hours until it became homogeneous. Meanwhile the bacterial aqueous was prepared by taking a swab from the recently grown isolated bacteria and diluted it using normal saline solution then it was compared with McFarland solution, and were cultured on nutrient agar using sterilized swabs of cotton. Wells with 6 mm diameter were made in plates. 100µl of each dilution containing AgNPs was put in wells then the plates were put in an incubator for 24 hours at 37°C. The results were observed by determining the zones of inhibition formed around wells (8, 9)

RESULTS AND DISCUSSION

The change of color to deep brown when adding plant extract solution to 2mM of AgNO₃ solution within 24 hours at room temperature shown the percentage of silver nitrate solution of plant extract was (10:90), PH (6.1) in (fig.1), the color change indicates the formation of AgNPs (1). The change in color was due to the reduction of Ag⁺ to Ag⁰ by the biomolecules present in the Leaf extract such as alkaloids, flavonoids, phenol, resins, tannins, saponins, etc (15)

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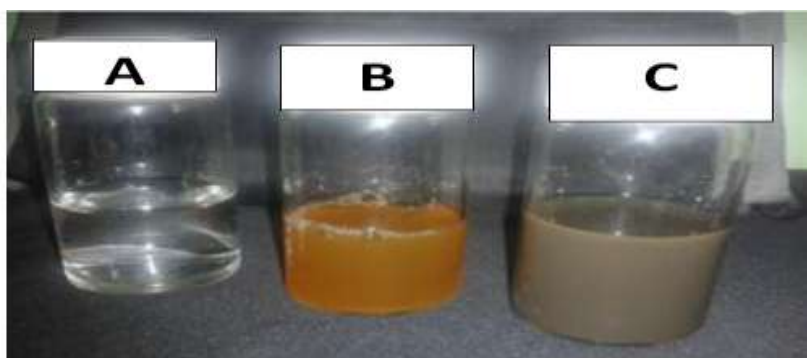


Figure 1. Reaction solutions. (A): AgNO_3 (2mM) without *Rosmarinus officinalis* extract. (B): Aqueous solution for *Rosmarinus officinalis* extract. (C): Color change from yellow to deep brown after 24 hours.

AgNPs biosynthesized using AgNO_3 solution mixed with *Rosmarinus officinalis* Leaf extract were examined by UV-Visible Scanning spectrophotometer shown in fig. (2). The spectrum showed that the maximum absorption band (λ_{max}) was at 465 nm which indicating that AgNPs were scattered in the solution and weren't agglomerate in it, It also well known that the color change of solutions is because of the excitation of the surface Plasmon Resonance (SPR) with the AgNPs(6). This spectroscopy is compatible with other studies and researches which stated that AgNPs can be synthesized using plant extract (10, 6, 7).

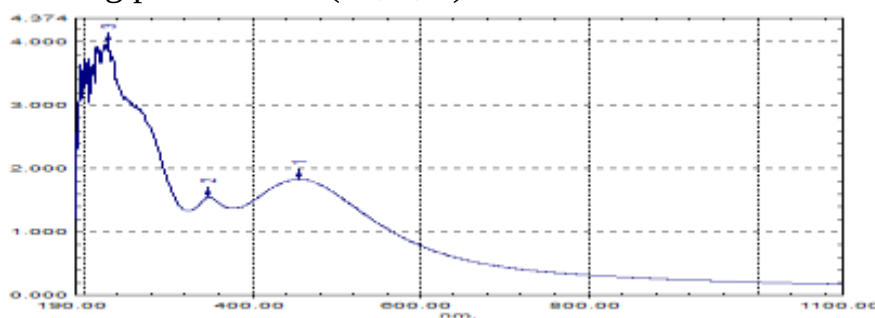


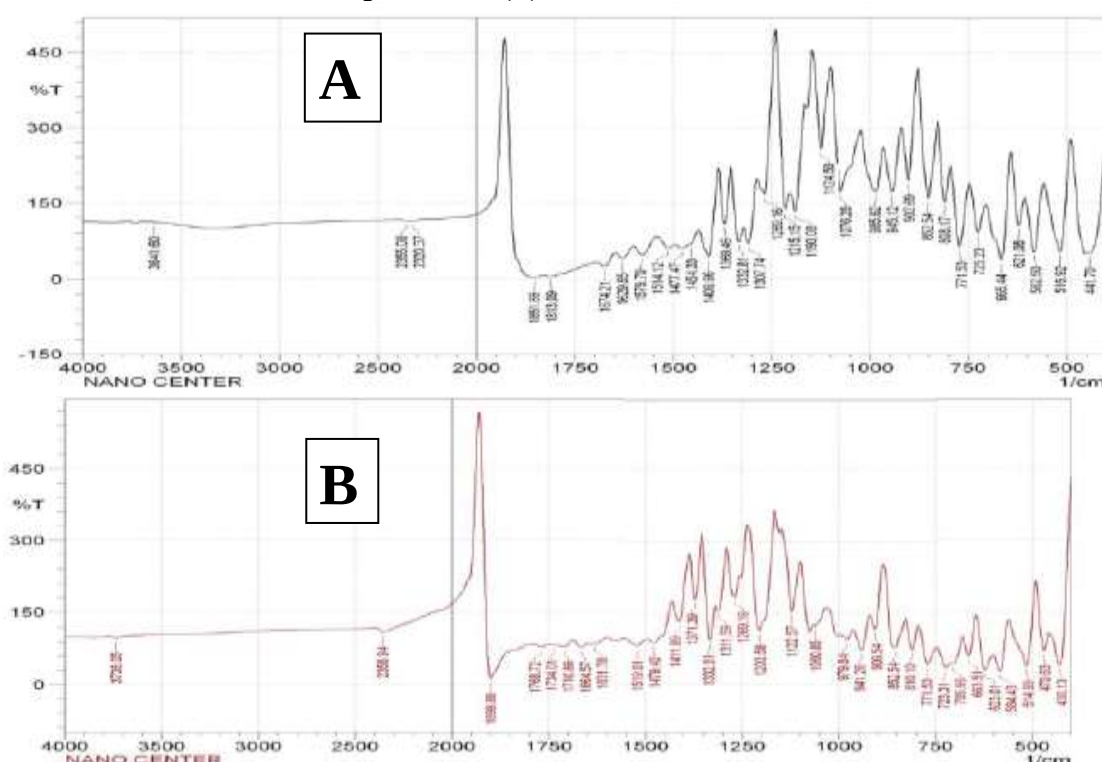
Figure 2. UV-VIS scanning spectroscopy shows the absorption spectrum of AgNPs

The obtained AgNPs using *Rosmarinus officinalis* Leaf extract was studied using Fourier Transform Infrared Spectroscopy (FTIR). It was used to identify the biomolecules which is responsible for reduction Ag^+ ions (7). FTIR spectrum of AgNPs biosynthesized from plant extract is shown in (Fig.3). The IR band that appeared in the plant extract in the range 1851.66 cm^{-1} was C=O of carbonyl group had shifted to the range 1899.8 cm^{-1} in the nano extract, the band that appeared in the plant extract in the range 1409.96 cm^{-1} was referred to benzoil ring had shifted to the range 1411.89 cm^{-1} in the nano extract, also the band C-H3 that appeared in the plant extract in the

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range 1369.46 cm^{-1} had shifted to the range 1371.39 cm^{-1} in the nano extract, band that appeared in the plant extract in the range 1332.81 cm^{-1} was NO_2 aromatic stretch of nitro group had stayed in the same range in the nano extract, the band that appeared in the plant extract in the range 1307.74 cm^{-1} was C-N stretch (alkyl) of amines had shifted to the range 1311.59 cm^{-1} in the nano extract, band that appeared in the plant extract in the range 1269.16 cm^{-1} was C-C stretch ketones and was appeared in the same range in the nano extract, the band that appeared in the plant extract in the range 1190.08 cm^{-1} was C-C(O)-C stretch of ester had disappeared in the nano extract, the band that appeared in the plant extract in the range 1124.50 cm^{-1} was C-O stretch of alcohol had shifted to the range 1122.57 cm^{-1} in the nano extract, the band that appeared in the plant extract in the range 1076.28 cm^{-1} was C-O had disappeared in the nano extract, the band C-X of alkyl halides that appeared in the plant extract in the range 985.62 cm^{-1} had shifted to 941.26 cm^{-1} . The change that happened to the bands may be because of using them as a capping agent during biosynthesis of AgNPs or using some of them for the stabilization of the nanoparticles (7).



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Figure 3. The FTIR spectrum: (A): Plant extract alone. (B): Plant extract with AgNO₃

Scanning electron microscopy (SEM) was used to characterize and determine the particles size, microstructure and shape of AgNPs as shown in (fig.4). The results showed that the particles were semi spherical and the average size of the particles was 74.7 nm which is compatible with the size limits of nanoparticles (1-100nm) (16).

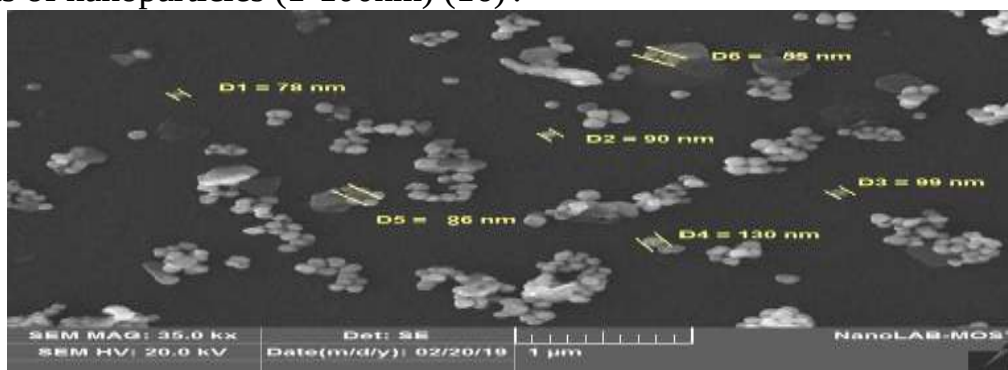


Figure 4. The SEM image shows shape and size of AgNPs

The XRD spectrum of the silver nanoparticles are demonstrated in fig.(5), four diffraction peaks were shown, locating at $2\theta = 38.3^\circ, 44.5^\circ, 64.65^\circ$ and 77.5° , which were indexed to the reflections (111), (200), (220) and (311) crystal planes of pure silver with face centered cubic symmetry (JCPDS file No.04-0783). The diffraction peaks are clearly enlarged as compared with bulk silver, indicating the formation of AgNPs.

The sizes of the AgNPs (D_p) is calculated by using Scherrer's formula (4) as follows:

$$D_p = \frac{0.9\lambda}{\beta \cos \theta} \quad (1)$$

Where β is the full width at half maximum (radians), λ is the wavelength in (nm) of employed radiation, θ is the Bragg diffraction angle in degree and (0.9) is the shape factor value. The size of the silver nanoparticles was calculated for dominate plane of (111), it is determined as 24.3 nm. The size of the silver nanoparticles which were determined from the XRD data are smaller than that estimated from the SEM images, this can be attributed to the SEM images indicate the size of the aggregated nanoparticles (4).

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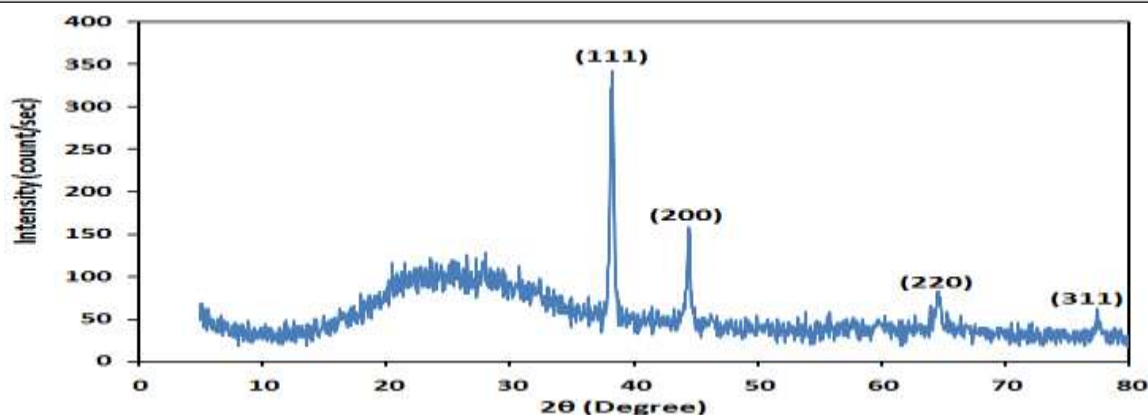


Figure 5. The XRD spectrum of AgNPs

Atomic Force Microscopy (AFM) was used to investigate the dispersion and aggregation of AgNPs as well as their sorption, size, shape, and structure as shown in (fig. 6). The size of the particles measured using AFM was between (40nm-140nm) and the average of the size was 67.15 nm. (Fig. 7) shows 2D and 3D picture for the particles.

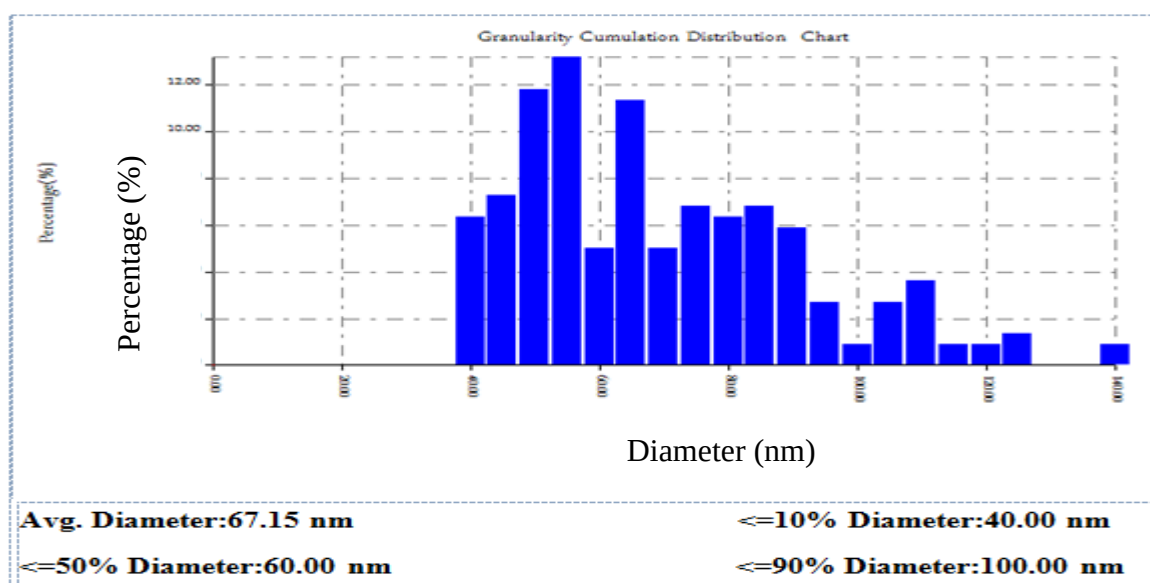


Figure 6. The AFM spectrum of AgNPs

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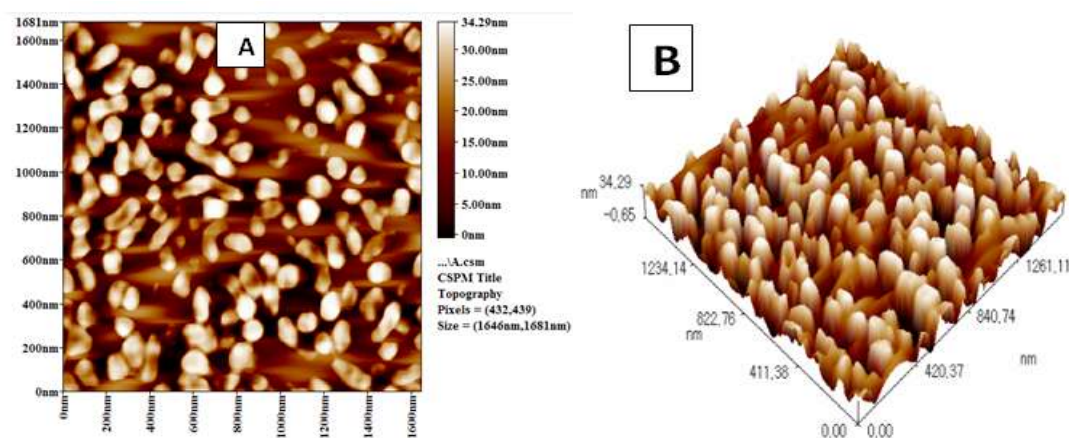


Figure 7. shows: A: 2D picture for AgNPs, B: 3D picture for AgNPs

The stability of nanoparticles was studied using zeta potentials measurements device, surface zeta potential value was found to be (-8.01mV) as shown in (fig.8). This negative sign in the result indicated the stability of AgNPs and it showed that there was no agglomeration in these biosynthesized nanoparticles (15).

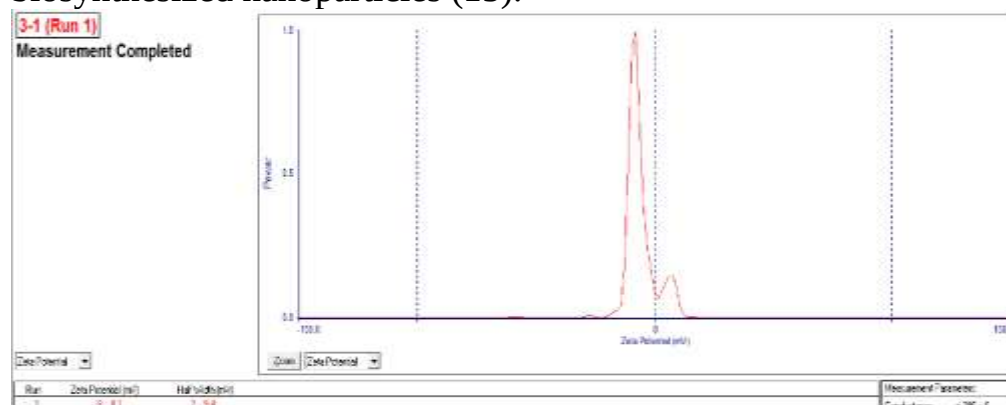


Figure 8. Shows spectrum of zeta potential

In the current study, we had also used the Particle size analysis to determine the size of the AgNPs formed. The distribution curve of the particle size of synthesized AgNPs is shown in (fig.9). The measurement showed various sizes of the particles ranging from 30.9 nm to 168.4 nm. The average of particle size was 82.4 nm. The difference between the largest measured size and the lowest measured size was 137.5 nm which was used in the distribution curve.

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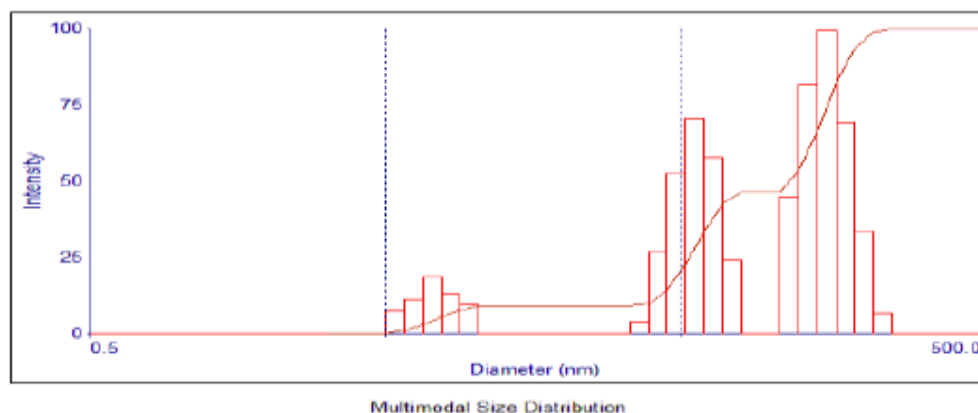


Figure 9. Shows the distribution curve of the particle size of synthesized AgNPs

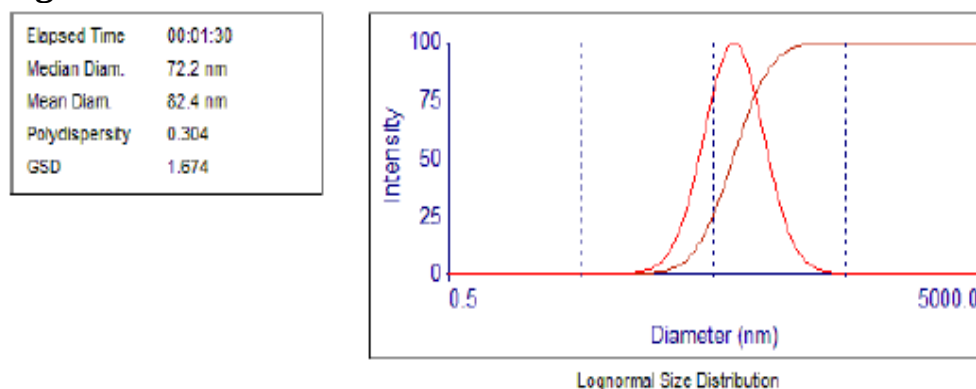


Figure 10. Shows spectrum of particle size analysis

In this study it was clear that only 25 isolates belongs to Pseudomonas aeruginosa bacterial. After diagnosis using biochemical tests as shown in the table (1).

Table 1. The biochemical tests of Pseudomonas aeruginosa

Test	Result
Oxidase	+
Catalase	+
Indole test	-
Methyl Red	-
Voges – Proskauer	-
Citrate Utilization	+
Urease test	-
Nitrate Reduction	+
Growth at 42°C	+

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The results showed that the highest inhibition area was at concentration were (312.5µg/ml) 14 isolates of P.aeruginosa with 6 resistive isolates as shown in table (2). It appeared that the increase in the concentration of the AgNPs causes the enlargement of the diameter for the inhibited growth zone of the P.aeruginosa. The reason may be the inhibited dispersion of solid nanoparticles. The diameter of the inhibited growth zone depends on the concentrations of AgNPs used (11).

Table 2. Antibacterial activity values of AgNPs for 25 isolates of Pseudomonas aeruginosa

Isolate no.	5000 µg/ml	2500 µg/ml	1250 µg/ml	625 µg/ml	312.5 µg/ml	156.25 µg/ml	78.125 µg/ml	39.06 µg/ml
P.a (1)	14	13	12	11	9	-	-	-
P.a (2)	14	11	-	-	-	-	-	-
P.a (3)	14	12	10	-	-	-	-	-
P.a (4)	15	14	13	12	10	-	-	-
P.a (5)	-	-	-	-	-	-	-	-
P.a (6)	-	-	-	-	-	-	-	-
P.a (7)	-	-	-	-	-	-	-	-
P.a (8)	16	13	12	11	10	9	-	-
P.a (9)	14	13	12	11	10	-	-	-
P.a (10)	16	13	12	11	10	-	-	-
P.a (11)	14	13	12	11	10	-	-	-
P.a (12)	-	-	-	-	-	-	-	-
P.a (13)	14	13	11	-	-	-	-	-
P.a (14)	16	15	14	13	11	9	-	-
P.a (15)	-	-	-	-	-	-	-	-
P.a (16)	17	16	14	13	12	9	-	-
P.a (17)	16	15	14	13	11	9	-	-
P.a (18)	16	15	14	13	10	-	-	-
P.a (19)	15	14	13	11	9	-	-	-
P.a (20)	14	11	-	-	-	-	-	-
P.a (21)	-	-	-	-	-	-	-	-
P.a (22)	15	14	13	12	10	-	-	-
P.a (23)	16	15	14	12	10	-	-	-
P.a (24)	16	15	14	13	11	-	-	-
P.a (25)	14	12	-	-	-	-	-	-

* p.a = Pseudomonas aeruginosa

** - = there is no inhibition

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CONCLUSION

In this study we had found that Rosmarinus officinalis Leaf extract considered as a good natural source for the biosynthesis of silver nanoparticles (AgNPs). These nanoparticles had a good and effective antibacterial activity against Pseudomonas aeruginosa isolated from wounds. It is also economical and environment friendly.

REFERENCES

- 1- Abd El-Rahman, A.F. and Mohammad, Tahany.G.M. 2014. Green synthesis of silver nanoparticle using Eucalyptus globulus leaf extract and its antibacterial activity. Journal of applied sciences research. 9 (10): pp. 6437-6440.
- 2- Agrawal, Pushpa; Mehta, Karan; Vashisth, Pooja; Sudarshan; Bhat, Preethi And Vishnu, B V Goutham. 2014. Green Synthesis Of Silver Nanoparticles And Their Application In Dental Filling Material. International Journal Of Innovative Research In Science, Engineering And Technology, Vol. 3, Issue 6.
- 3- Alagumuthu, G. Kirubha, R. 2012. Synthesis and Characterisation of Silver Nanoparticles in Different Medium. Open Journal of Synthesis Theory and Applications, 1,pp. 13-17.
- 4- Alwan AM, Yousif AA, Wali LA. 2017.The growth of the silver nanoparticles on the mesoporous silicon and macroporous silicon: A comparative study. Indian Journal of Pure & Applied Physics (IJPAP). 55:813-20.
- 5- Annamalai, Jayshree and Nallamuthu, Thangaraju. 2016. Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency. Appl Nanosci.6 : pp 259-265.
- 6- Das, Balaram; Dash, Kumar Sandeep; Mandal, Debasish; Ghosh, Totan; Chattopadhyay, Sourav; Tripathy, Satyajit; Das, Sabyasachi; Dey, Sankar Kumar; Das, Debasis; Roy, Somenath 2017. Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage. Arabian Journal of Chemistry. 10, Pp: 862-876
- 7- Fierascu, Radu Claudiu; Bunghez, Ioana Raluca; Somoghi, Raluca; Fierascu, Irina And Ion, Rodica Mariana. 2014. Characterization Of Silver Nanoparticles Obtained By Using Rosmarinus Officinalis Extract And Their Antioxidant Activity, revue roumaine de Chimie, 59(3-4),Pp:213-218.
- 8- Haider, Mohammed J and Mehdi, Mohammed S. 2014. Study of Morphology and Zeta Potential Analyzer for the Silver Nanoparticles.

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International Journal of Scientific & Engineering Research, Volume 5, Issue 7, Pp: 381- 387.

9- Jackson, Tenderwealth Clement; Uwah, Timma Otobong; Ifekpolugo, Nonye Linda and Emmanuel, Nsikak Anthony. 2018. Comparison of Antimicrobial Activities Of Silver Nanoparticles Biosynthesized From Some Citrus Species. American Journal Of Research And Applications. 6(2), Pp: 54-59.

10- Kareem, Payman A. 2018. Silver Nanoparticles Synthesized by Using Matricaria Chamomilla Extract and Effect on Bacteria Isolated from Dairy Products .Diyala Journal For Pure Sciences, Vol: 14 NO: 4, Pp: 176- 187.

11- Karimipour, S. N and Tanomand A. 2016. Evaluating The Antibacterial Activity Of The Nanoparticles Of Silver On Pseudomonas aeruginosa, International Journal Of Medical Research & Health Sciences, 5, 11, PP:424-430

12- Kathiravan, T; Marykala, J; Sundaramanickam, A; Kumaresan, S And Balasubramanian, T. 2012. Studies on Nutritional Requirements of Pseudomonas aeruginosa For Lipase Production. Advances In Applied Science Research. 3 (1), Pp: 591-598.

13- KHAN, M. Z. H; TAREQ, F. K; HOSSEN, M. A. and ROKI, M. N. A. M. 2018. green synthesis and characterization of silver nanoparticles using coriandrum sativum leaf extract. Journal of Engineering Science and Technology, Vol. 13, No. 1 ,pp 158-166.

14- Okafor, Florence; Janen, Afef; Kukhtareva, Tatiana; Edwards, Vernessa and Curley, Michael. 2013. Green Synthesis of Silver Nanoparticles, Their Characterization, Application and Antibacterial Activity. International Journal of Environmental Research and Public Health. 10, pp 5221-5238.

15- Raja, Selvaraj; Ramesh, Vinayagam and Thivaharan, Varadavenkatesan. 2017. Green Biosynthesis Of Silver Nanoparticles Using Calliandra Haematocephala Leaf Extract, Their Antibacterial Activity And Hydrogen Peroxide Sensing Capability. Arabian Journal of Chemistry. 10, Pp: 253-261.

16- Rigo, Chiara; Ferroni, Letizia; Tocco, Ilaria; Roman, Marco; Munivrana, Ivan; Gardin, Chiara; Cairns, Warren R. L; Vindigni, Vincenzo; Azzena, Bruno; Barbante, Carlo And Zavan, Barbara. 2013. Active Silver Nanoparticles for Wound Healing. International Journal of Molecular Sciences. Pp: 4817-4840.

17- Supraja, S; Mohammed, Ali S ; Chakravarthy N ; Jayaprakash, Priya A ; Sagadevan E; Kasinathan M. K.; Sindhu S And Arumugam P. 2013. Green

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Synthesis of Silver Nanoparticles From Cynodon Dactylon Leaf Extract. International Journal Of Chemtech Research, Vol.5, No.1, Pp: 271-277.

18- Thamer, Neran Ali; AL-Mashhedy, Lamia A. Ahmed and Basim.Sh. 2016. Green Synthesized Silver Nanoparticles using Crocus sativus L Extract after reduces Prehepatocellular Carcinoma In Rats. Iraqi Journal of Cancer and Medical Genetics (IJCMG). Vol.: 9, No.2. pp.181-186.

19- Varghese, Naveena; Joy, P.P. 2014. Microbiology Laboratory Manual. <https://www.researchgate.net/publication/306018042>

20- Zhang, Xi-Feng; Liu, Zhi-Guo; Shen,Wei and Gurunathan, Sangiliyandi. 2016. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. International Journal of Molecular Sciences. 17, 1534, pp1-34.

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دراسة التخليق الحيوي، التوصيف، الفعالية المضادة للبكتيريا لدقائق الفضة النانوية ضد بكتريا الزائفة الزنجارية Pseudomonas aeruginosa المعزولة من الجروح من بعض المستشفيات في بغداد

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المستخلص

يهدف هذا البحث الى دراسة التخليق الحيوي لدقائق الفضة النانوية باستعمال المستخلص المائي الحار لاوراق نبات اكليل الجبل (*Rosmarinus officinalis*) لكونه ذو تكلفة مناسبة، ويحتوي العديد من المواد الفعالة. تم توصيف دقائق الفضة النانوية (AgNPs) المخلقة حيويًا باستعمال المطياف الضوئي الماسح UV-Visible Scanning spectrophotometer لقياس قمم الامتصاص اذ كان اقصى قيمة للطول الموجي λ_{max} عند 465 نانومتر، تقنية مطياف الاشعة تحت الحمراء FTIR للكشف والاستدلال عن المجاميع الفعالة في النبات والمسؤولة عن تكوين الدقائق النانوية، المجهر الالكتروني الماسح SEM لملاحظة شكل الدقائق النانوية ومعرفة حجمها حيث بلغ 74.7 نانومتر، مقياس حيود الاشعة السينية XRD لتحليل تراكيب الدقائق النانوية، التحديد النوعي للدقائق وكذلك معرفة حجم الدقائق حيث بلغ حجم الدقائق 24.3 نانومتر، مجهر القوة الذرية AFM لغرض التحري عن التشتت والتكتل للدقائق النانوية فضلاً عن الشكل، التركيب والحجم وكانت قيمة معدل الحجم 67.15 نانومتر، جهاز قياس زيتا الكامنة لمعرفة استقرارية دقائق الفضة النانوية التي بلغت قيمتها -8.01mV ، جهاز تحليل حجم الدقائق لغرض حساب حجم دقائق الفضة النانوية المخلقة حيويًا وكان حجم الدقائق 82.4 نانومتر. تمت دراسة فعالية دقائق الفضة النانوية ضد بكتيريا *Pseudomonas aeruginosa* المعزولة من الجروح. أظهرت النتائج كفاءة دقائق الفضة النانوية ضد هذه البكتيريا.

الكلمات المفتاحية : اكليل الجبل، التخليق الحيوي لدقائق الفضة، تقنية قياس زيتا الكامنة، تقنية تحليل حجم الدقائق، طريقة انتشار الحفر بالاكار