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Synthesis of silver nanoparticles using green method to studying the effect of extract on the crystal size and antibacterial activity

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Abstract

In this work, used the green method to preparing the silver nanoparticles (AgNPs) by using Citrus Limon Extract. The novel approach has been carried for the green synthesis of AgNPs by using different parts from Limon. Using X- RD to study the structural properties of samples and calculate the crystallite size then study the effect of deference parts of Citrus Limon plant. appear from the results change in the crystallite size, that because the extract influence, and found changed depending on the type of extract (12.6,15.4,17.2 nm) when use (Seeds, Leaves, peels extract) respectively, also the results were analysis by use (AFM) atomic force microscopy, the results appear improving in structural properties by using the plant extract. Optical properties (Absorption and Transmittance) also investigated, appear dependence the optical properties of the prepared samples on the extract type, higher absorption when used seeds extract to preparing Ag Nps. Appeared from the results high antibacterial activity of AgNPs to both positive and negative gram. The inhibition zone of AgNPs depends on crystal size and the concentration of AgNPs, for that the inhibition zone radius increases with decrease the grain size. To evaluation the antibacterial activity of AgNPs against three kind of Gram-positive bacteria (E. faecalis, Staph.hominis and Staph.aureus), proved that Citrus Limon extract improved the performance of nanoparticles and found the highest inhibition diameter was 45 mm against Staph.hominis , wheen against types of Gram-negitive bacteria (Entero.cloacae, E.coli and K.pneumoniae) found the highest inhibition zon 40mm againstStaph.hominis, wheen less inhibition zone 14 mm against E.coli.

Introduction

Nanotechnology involves the prepare of nanoparticles of various shapes and sizes with properties to enhance human life [1-3]. Nanotechnology is one of important fields, dealing wiesign, synthesis, and manipulation of particles and dealing with very small size materials (1-100 nm). Nanoparticles (NPs) have wide scope of applications in different fields, as health care, mechanics, optics, biomedical sciences, chemical industries, catalysis, light emitters, and photo-electrochemical applications [4-5]. The metal nanoparticles have been used in biomaterials design that becuse antimicrobial activity [6-8]. AgNPs have antibacterial properties and therefore play an important role in many applications as biomedical field [9].

AgNPs can be prepared by different techniques such as chemical and biological, etc [10-11]. Green techniques use principles to minimize the hazardous substances [12]. The plants using to manufacture of NPs is very low-cost techniques, for that used as an economic technique to production of NPs [13]. AgNPs manufacturer based on the green synthesis methods become very interesting because their use organic precursors materials eco-friendly and nontoxic [14]. to improved antioxidant and antibacterial properties of AgNps used green manufactured to prepared it, that because extracts contain reducing compounds [15-16]. AgNPs have an effective effect on the positive and negative Gram bacteria [17]. AgNPs has antibacterial properties that because it's strong inhibition, and it has an effect protect from bacterial infections. [18]. AgNPs are known to product reactive oxygen types (ROS), which act to killing the bacterial cell [19]. one from the important properties of AgNPs was release of silver ions, which are very toxic to the bacterial [20]. nanoparticles appear different range of efficiency versus different kinds of bacteria. From the research showed AgNPs are major effective on Gram positive bacteria, that because the structural properties of cell membranes, which allow to AgNPs to inter the bacteria. the interactions of AgNPs with another type of bacteria, Gram-negative are complex interactions with external membrane proteins [21-22]. The ability of Ag NPs to release Ag+ ions potent antimicrobial and antifungal, one from the important properties of Ag NPs, where the main interaction between negatively charged bacterial and the positive charge ions is an electrostatic attraction [23-24]. occur between antibacterial samples and the test bacteria electrostatic adsorption, chemical interaction (chemical adsorption), and hydrophobic interaction (physical interaction) [25]. AgNPs interact with the internal parts of the bacteria, that will effect on the vital functions [26] (Figure 1), the aim of the work, to studying the effect of the green synthesis to prepare Ag NPs using natural sources, such leaves, peels, seeds Extract and to esteem their antibacterial properties as well as cytotoxic effects.

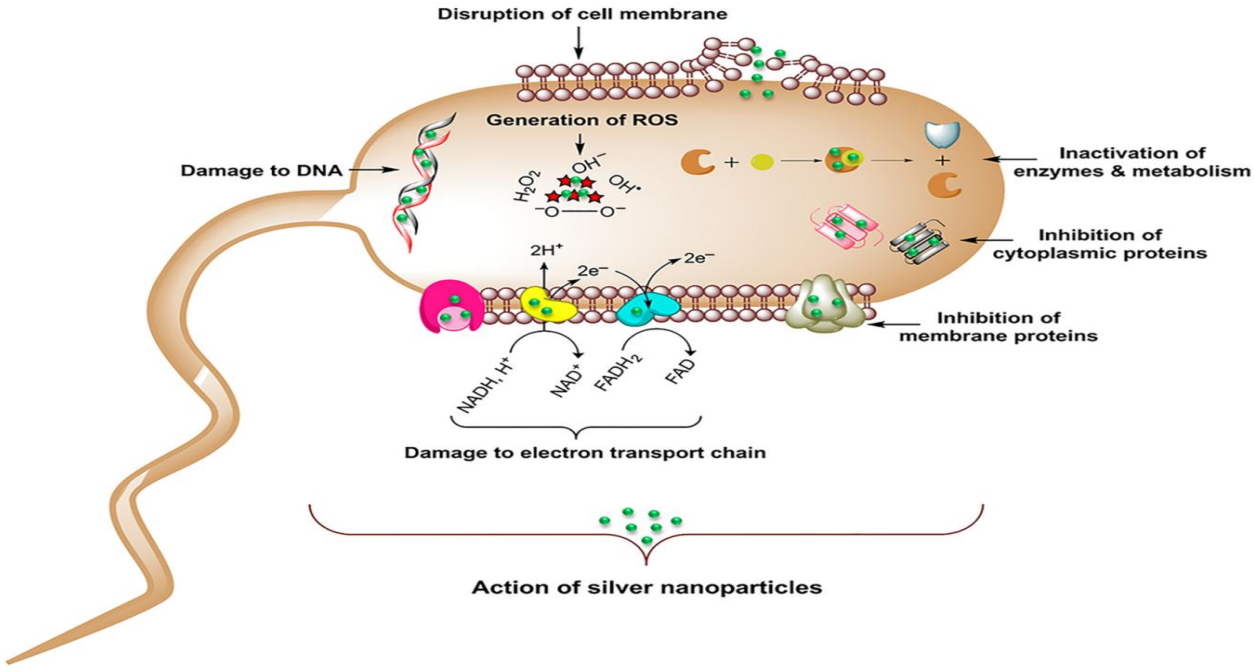


Fig. 1: Pathways of cellular damaging by AgNPs[26].

Action Mechanisms

AgNPs interaction with bacteria in three major mechanisms: first penetration the membranes of the cell causing killing the bacteria, second disruption the internal parts of the bacterial cell including DNA and proteins, third release of Ag ions interfering with cellular processes of the bacteria [27].

Factors Affecting upon Activity of AgNPs

appeared studies the effect of AgNPs properties, such as particle size, shape and morphology, on the inhibition effectivity against bacteria. The small size of AgNPs were the higher effective on the different microorganisms types [28].

Experimental parte

1-Selection of Plant

Fresh Citrus Limon were chosen randomly. The selected fruits and leaves green colour, and without any sign of infection, washed with running water and then with deionized water, to removing the dust, and then dry. Use equal weights (50 g) of lemon leaves, peels, and seeds. To obtain the plant extract, the selected samples were placed in an electric blender to cut them into small pieces, then 10 ml of deionized water was added and left for 24 hours. Finally, filter paper was used to remove any suspended particles from the plant extract of the three different parts of the lemon tree, and then the extract samples storing at (4°C – 8°C) to be utilized in the following step.

2-Synthesis of AgNPs

Green synthesis of AgNPs an environmentally friendly manner involved addition of 5 ml of the manufacture plant extract into 20 ml (0.004 M) of silver nitrate $AgNO_3$ and stirred continuously at room temperature in dark, for some period. After that synthesis of AgNPs was observed at different intervals of time, with depend on the varying sources of plant extracts. To study the structural properties. The samples are prepared as films by chemical spry pyrolysis method under constant conditions. The structural properties investigating by X-ray diffraction and atomic force microscopy.

3-Test bacteria under study

Different types of pathogenic bacteria used in the test under study were obtained from the laboratories of the biology Sciences at the College of Education for Pure Sciences, Tikrit University, and identified to the species level. The diagnosis was confirmed using the system Vitek 2compact system, that included three types of Gram-positive, *Staphylococcus aureus, Staphylococcus hominis, and Enterococcus faecali*s, also test three types of Gram-negative, *Klebsiella pneumoniae, Escherichia coli, and Enterobacter cloacae*

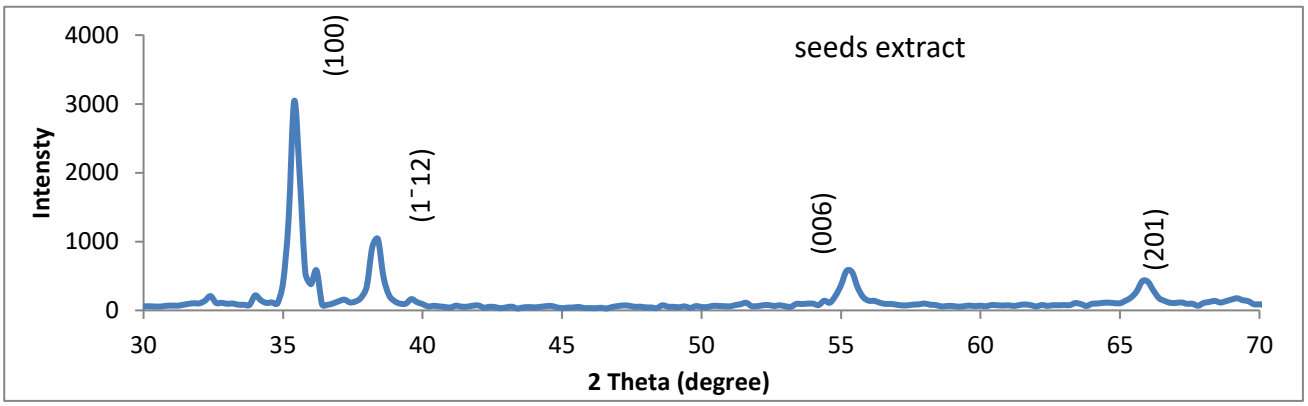
4-Biological Activity

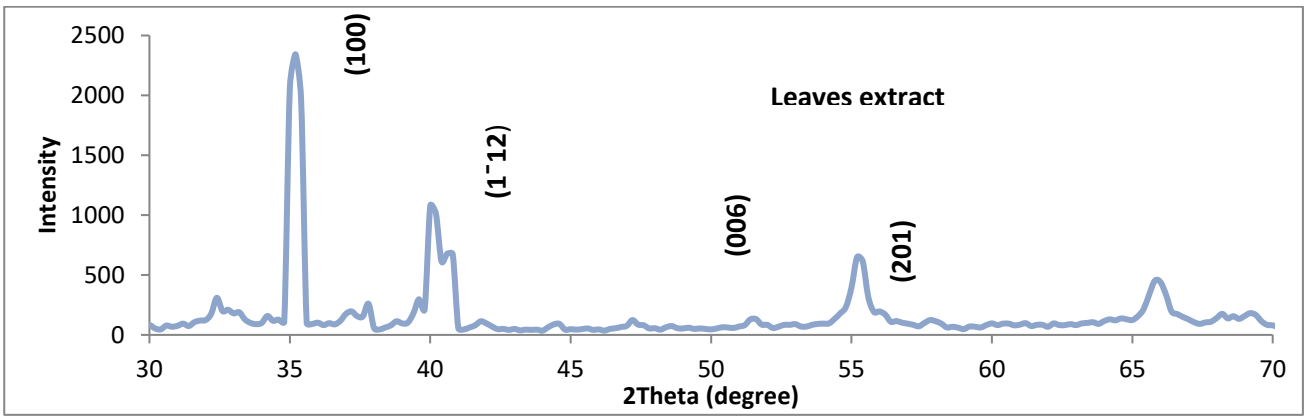
To study the biological activity of these samples, agar diffusion method was used to test a sensitivity of bacteria to these substances. The method involved making holes with a cork piercer, 6 mm in diameter, on a Mueller-Hinton agar medium injected by the test bacteria under study, aged 18-24 hours, and with a cell count of (108*1.5) cells/cm3, compared to a McFarland tube of 0.5. 0.1 ml of the substance was added to each hole (1), then left agars in a refrigerator for 2-4 hours to allow the extract solution to diffuse. The ager incubated at 37.5°C

for 24 hours, and growth-free zone of a tested bacteria was measured. The results were then recorded.

Result and Discussion

The X-ray diffraction patterns of the samples are emphasizing the crystalline nature of samples. appear in figure 2. The diffraction pattern for many peaks at different diffraction angle (2θ) at (35.0228°, 40.3309°,55.2410°), (35.0105°,40.3011,55.2342) and (35.0152,40.3101,55.2104) correspond to orientations (100), (1¯12) and (006) respectively. It is apparent from figures all samples are preferentially orientated along the preferred orientation (100). The crystallite size calculated using Scherrer equation, appear from the results change in the crystallite size, that because the extract influence, and found changed depending on the type of extract, was (12.6,15.4,17.2) when use (Seeds, Leaves, peels extract) respectively, as shown in table 1.





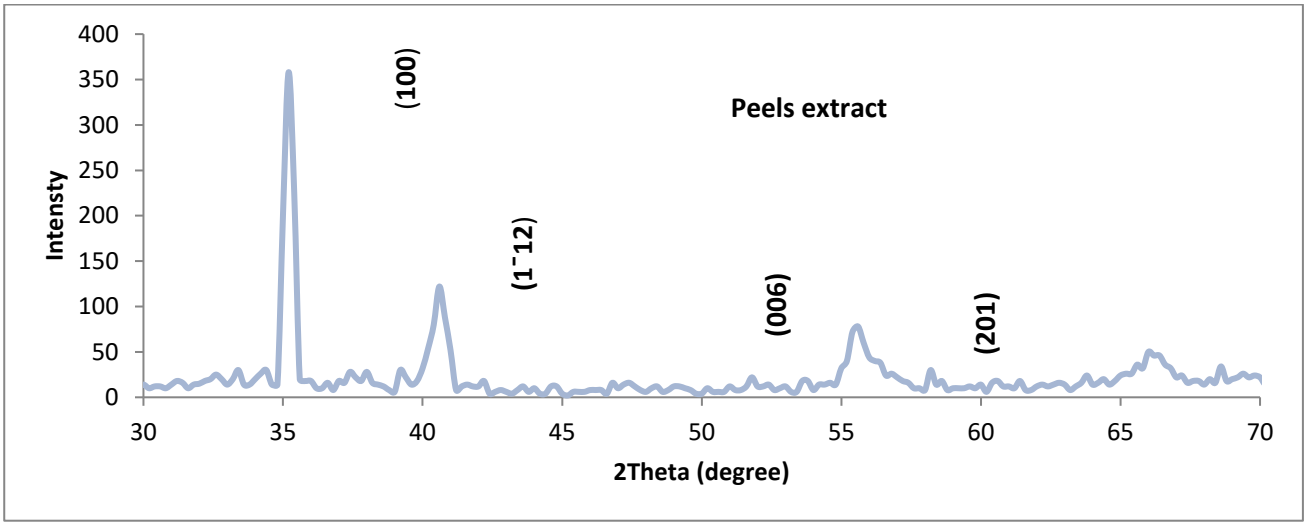


Fig 2: X-ray diffraction of silver nanoparticle.

Table 1: appear the effect of extract on the Crystallite size (nm) of AgNPs

Type of Limon extract use to prepared AgNPs	Millers coefficients (hkl)	2theta (degree)	Crystallite size of AgNPs (nm)
Leaves extract	(100) (1¯12) (006)	35.0105 40.3011 55.2342	15.4
Peels extract	(100) (1¯12) (006)	35.0152 40.3101 55.2104	17.2
Seeds extract	(100) (1¯12) (006)	35.0228 40.3309 55.2410	12.6

Dislocation density has been calculated from the mathematical formula $\delta = 1 / D^2$ (lines/m^2) [29-30]. Table 2 show the effect of extract type on the dislocation density, that will effect on the antibacterial activity.

Table 2: appear the effect of extract on the Dislocation density.

Type of Limon extract use to prepared AgNPs	Dislocation density (lines/m^2)
Leaves extract	0.004216
Peels extract	0.003380
Seeds extract	0.006298

Surface Roughness

Figure 3 show AFM images in 2D and 3D for AgNPs films, appear in images the effect of extract type on the surface morphology and the particle size distribution. Appear from figure (3) the surface roughness of AgNPs samples change when use different extract types, that

refer to the effect of extract type on the preparation interaction of AgNPs, that will effect on the structural properties of samples.

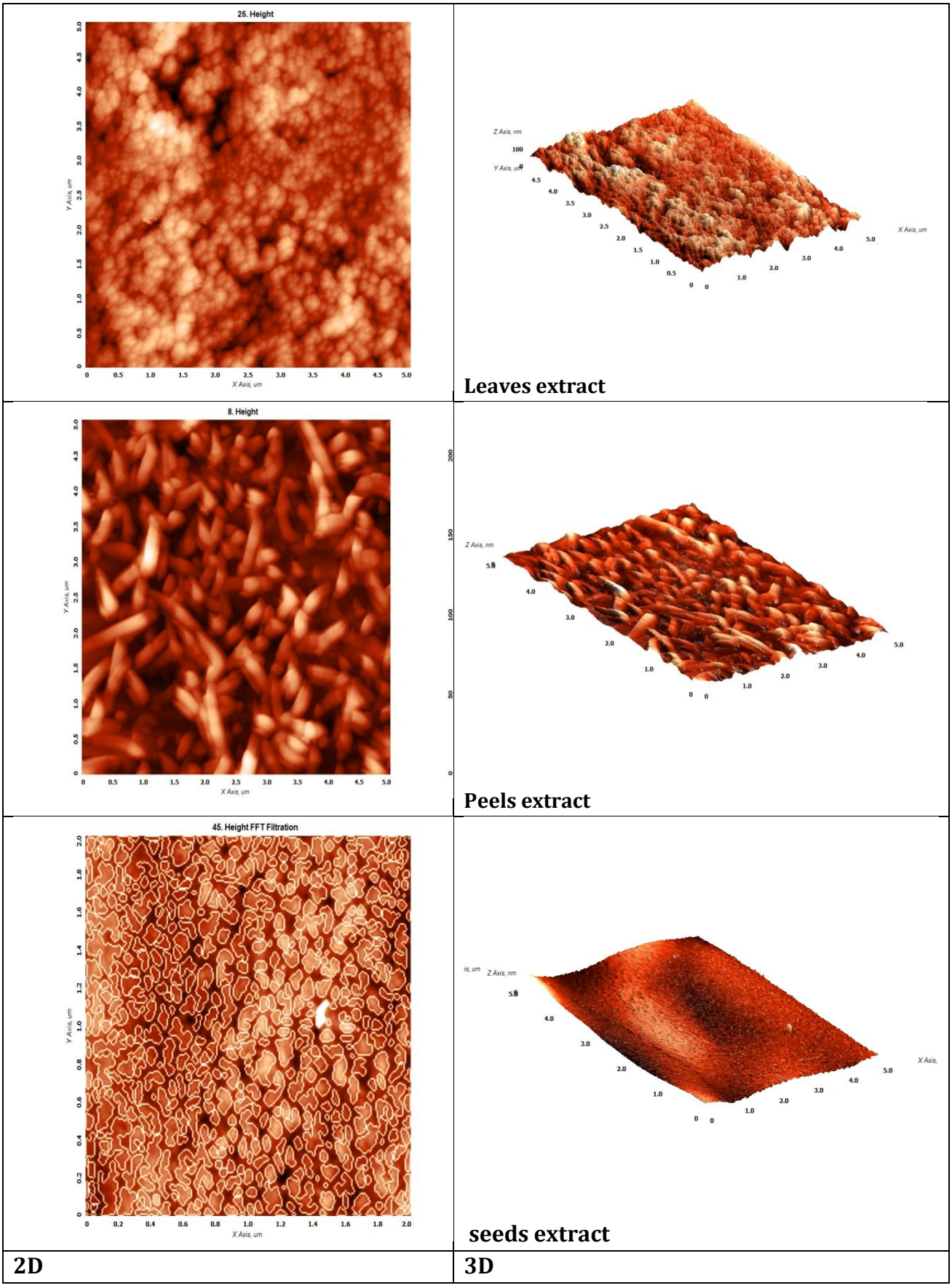
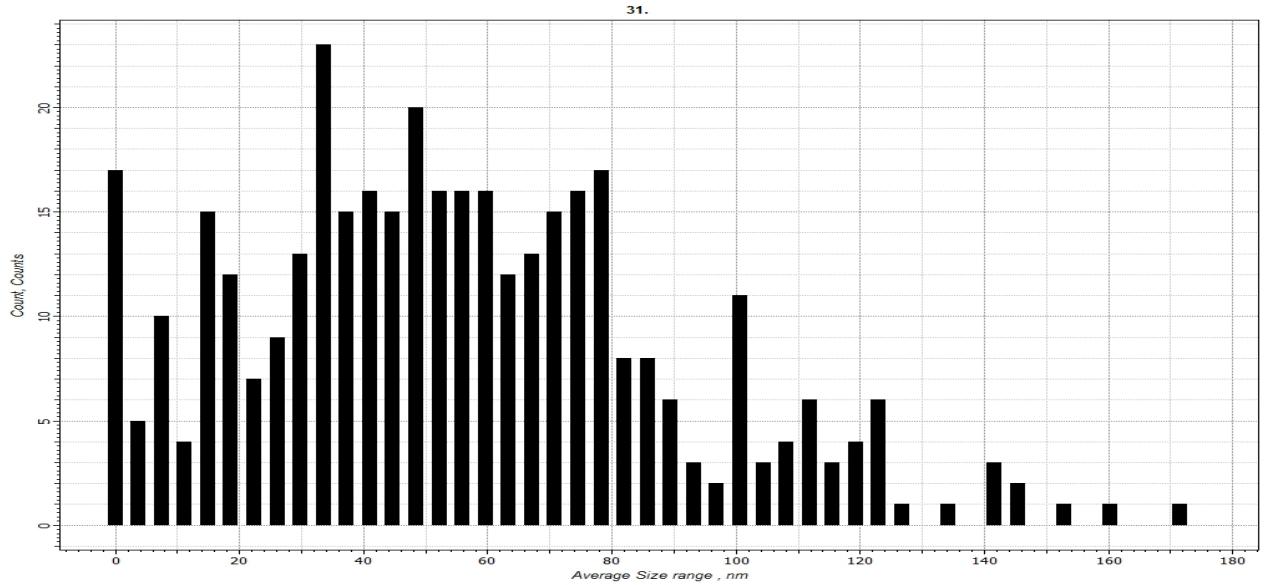
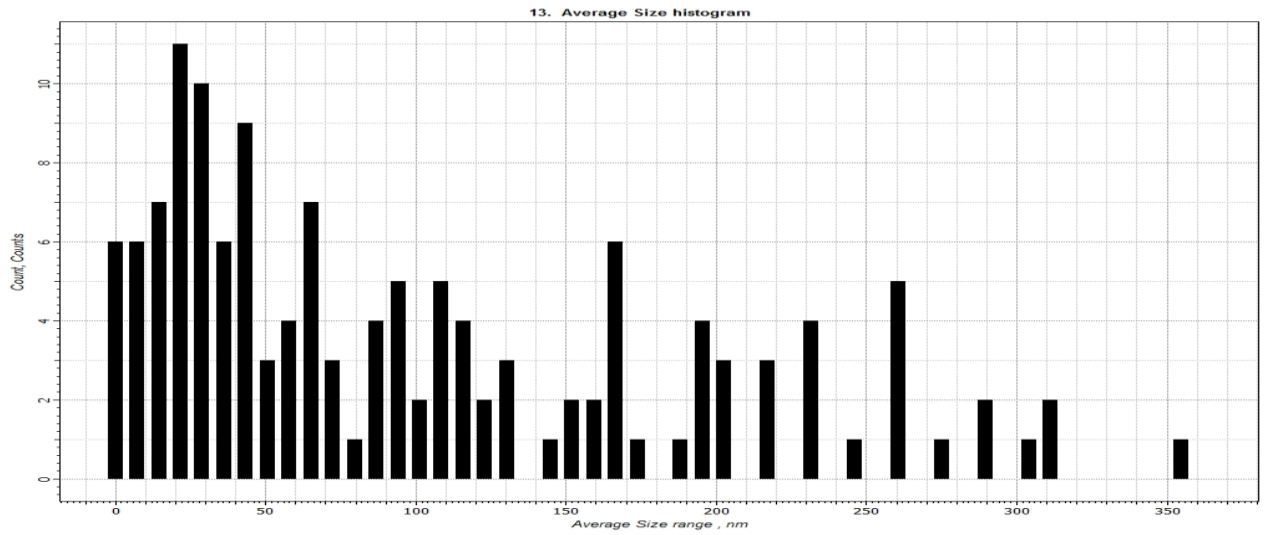


Fig 3: AFM analysis of AgNPs 2D and 3D images depict the surface topology and size distribution of the AgNPs.

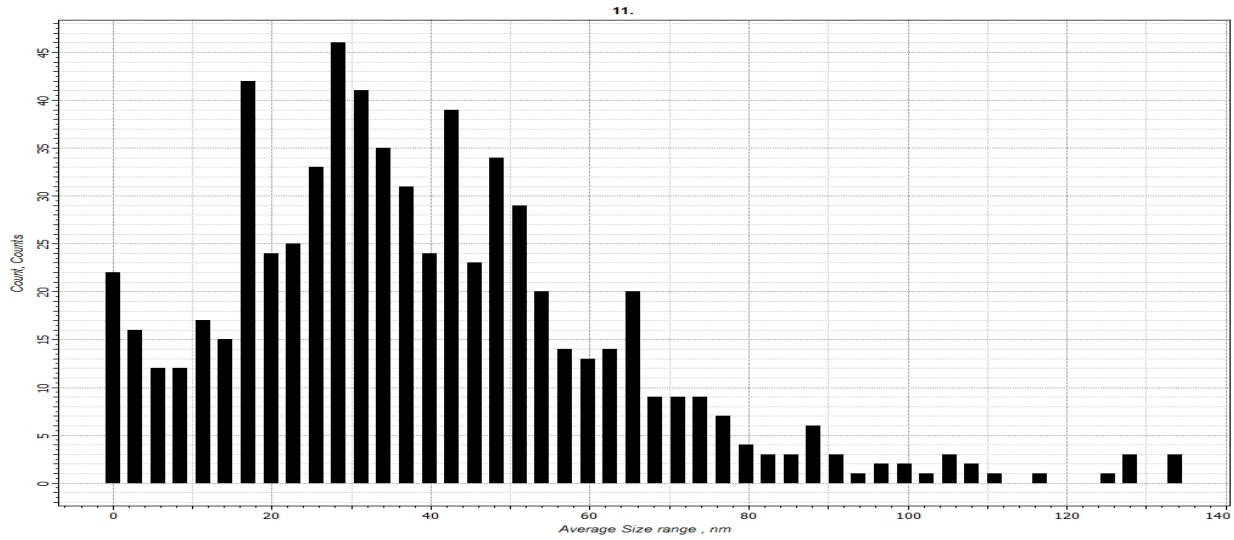
Figure (4) explain the histogram analysis of average particle size, the histogram analysis of AgNPs sample shown the effect of the extract type on the particle size.



Leaves extract



Peels extract



seeds extract

Fig (4). Show the histogram analysis of average particle size.

Optical Properties

In the figures (5,6) appear the relation between absorption, transmittance with wavelength. Figure (5) show the absorption curve of the prepared samples and show the effect of extract type on the curve. Appear from figure (5) the effect of crystallite size on the absorption, higher absorption when used seeds extract to preparing Ag Nps. From Figure (6) appear the effect preparation condition on the transmittance. The optical properties support the structural properties, the absorption increase with decreasing the crystal size, grain size, that because increase in the aspect ratio, that Enhance the influence with light and effect on the absorption and transmittance.

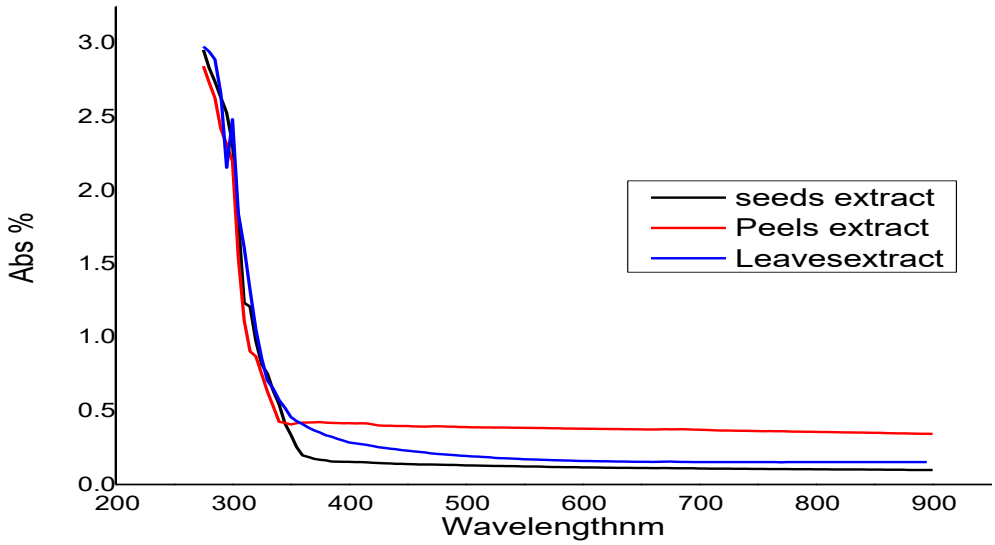


Fig (5). Show the influence of extract type on the absorption of AgNps samples.

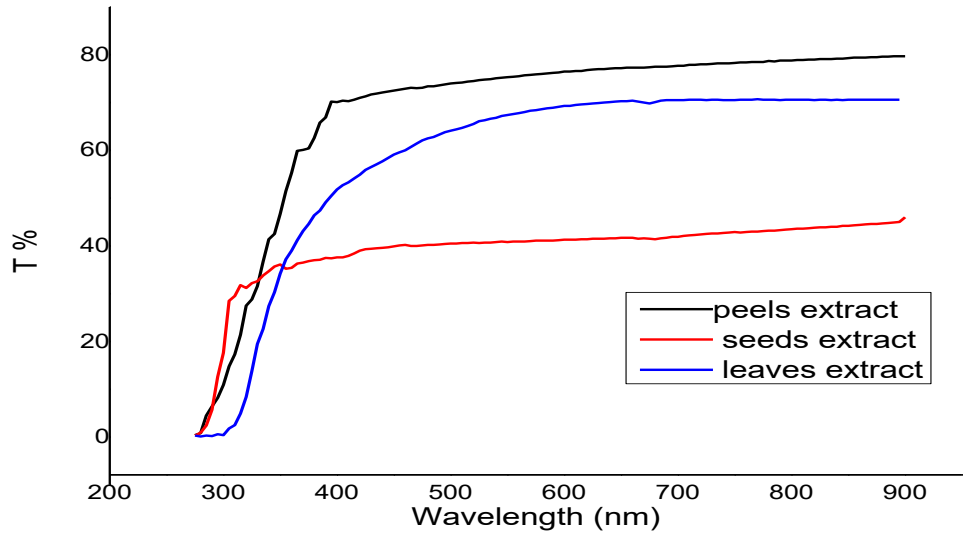


Fig (5). Show the effect of extract type on the transmittance of AgNps samples.

Antimicrobial Activity

The inhibition zone of Ag Nps depending on the addition type of extract appear that in figure (7) and table (3). The antibacterial activity of samples against Gram-positive bacteria showed enhancement in activity (change in the inhibition zone) due to synergistic effect of silver and essential oil components. From figure (7) and Table (3) showed inhibitory ability to all kinds of bacteria under test, and the highest killing diameter was 45 mm against *Staph.hominis* and the lowest inhibition diameter was 25 mm for all types of bacteria when use leaves extract.

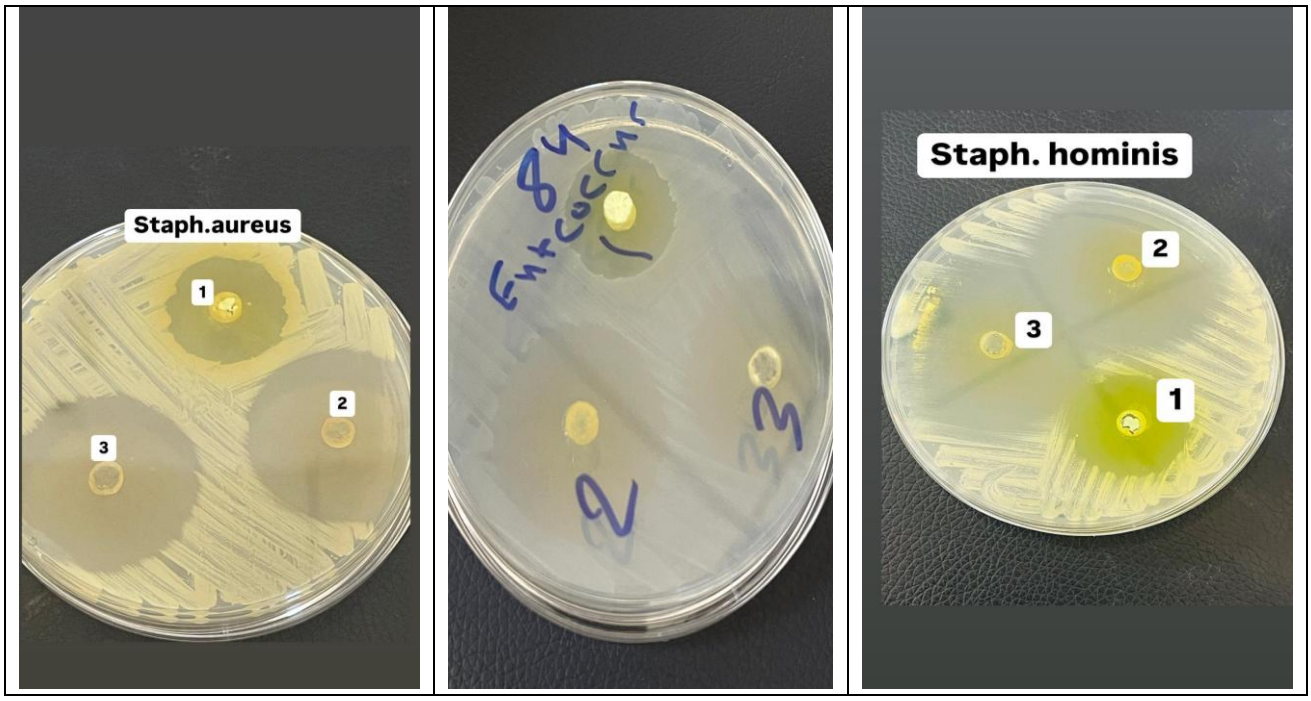


Fig (7) showed the inhibition zone for prepared AgNPs against Gram-positive bacteria.

Showed the results good antibacterial activity, which was more inhibition in the *Staph.hominis* than in *Staph.aureus* and *E.faecalis* respectively, appear from results the antibacterial activity dependence on the type of extract

Table (3) explain the relation between the inhibition zone Gram-positive bacteria and AgNPs when use deferent type of extract to prepare it.

Type of Limon extract use to prepared AgNPs	The inhibition zone for deferent of type of bacterial		
	E.faecalis	*Staph.hominis*	*Staph.aureus*
Leaves extract	25	25	25
Peels extract	34	45	38
Seeds extract	33	45	35

The results in figure (8) and Table (4), showed inhibitory activity against Gram-negative bacteria, the highest inhibition diameter was 40 mm against Enterobacter cloacae, and the lowest inhibition diameter was 14 mm against E. coli. The second extract (Peels extract) in addition appear inhibitory activity opposed to every one of the kinds of bacteria under test, and the highest inhibition diameter was 45 mm against Staph. hominis, and the lowest inhibition diameter was 16 mm against E. coli. The third extract (Seeds extract) also appeared

inhibitory activity opposed to every one of the kinds of bacteria under test, and the highest inhibition diameter was 45 mm against Staph. hominis, and the lowest inhibition diameter was 20 mm against E. coli.

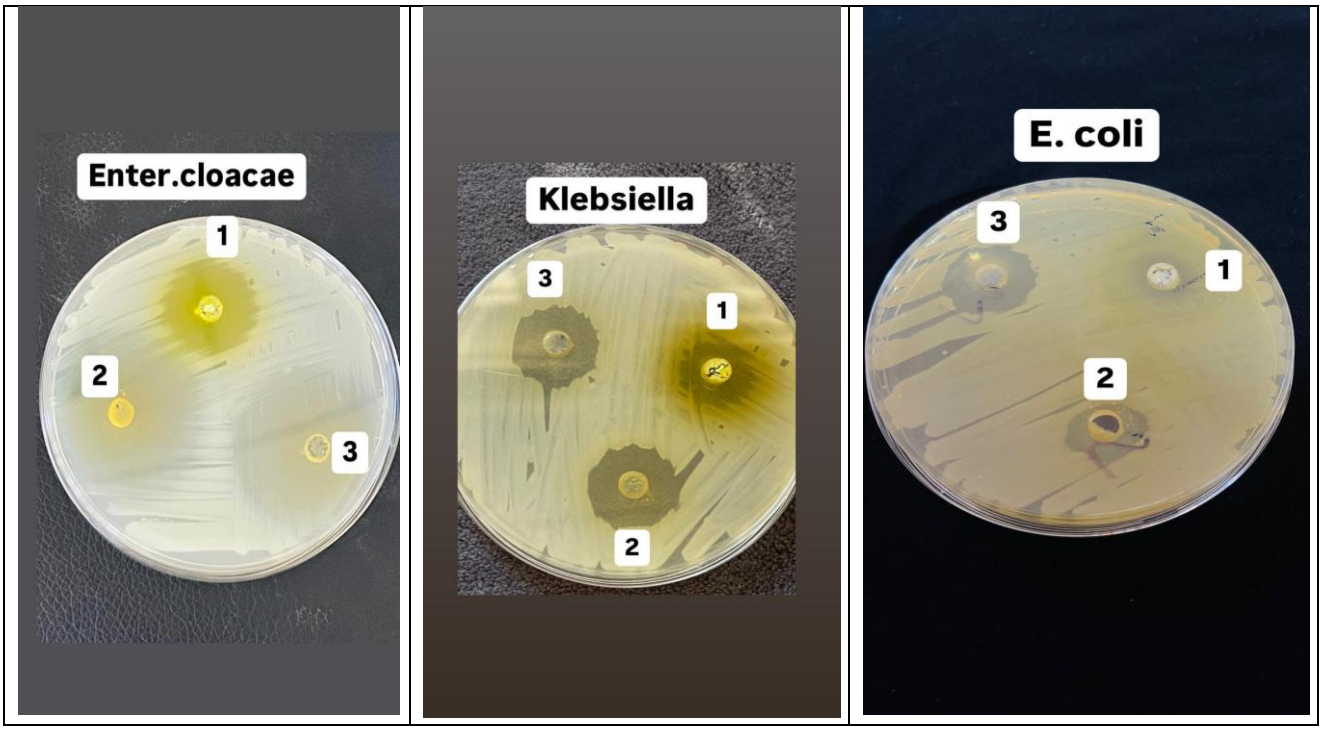


Fig (8) showed the inhibition zone for prepared AgNPs against Gram- negative bacteria.

Appear from the samples image a highest inhibition zone was against Entero.cloacae compared with *K.pneumoniae* and *E.coli* respectively, appear from results the antibacterial activity dependence on the type of extract.

Table (4) explain the relation between the inhibition zone of Gram-negative bacteria and AgNPs when use deferent type of extract to prepare it.

Type of Limon extract use to prepared AgNPs	The inhibition zone for deferent of type of bacterial		
	Entero.cloacae	*E.coli*	*K.pneumoniae*
Leaves extract	33	14	20
Peels extract	40	16	27
Seeds extract	40	20	23

Conclusion

Using plant extracts to prepare nanomaterials is eco-friendly, safety, simple. the orientation (100) was the preferred orientation for all samples. Appear from (AFM) the surface roughness of AgNPs samples change when use unlike kinds of extract, that indicated to the effect of extract type on the preparation interaction of AgNPs, that will effect on the structural properties of samples. The extract type effect on the crystallite size, grain size, optical properties, that refer to the increase in the surface area. Higher absorption when used seeds extract to preparing Ag Nps. The optical properties support the structural properties, the absorption increase with decreasing the crystal size, grain size, that because increase the aspect ratio, that Enhances the response to the light and effect on the absorption and transmittance. A significant improvement in antibacterial activity of samples and the effect of

extract type on the crystallite size and grain size, for that the inhibition zone will increase when decrease the crystallite size. AgNPs active against positive and negative gram bacteria, best inhibition zone 45 mm against Staph.hominis, when less inhibition zone 14 mm against *E.coli.* The inhibition activity of every prepared sample is river to the interaction between prepared samples and sample test, which causes killing the bacteria.

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تحضير جسيمات الفضة النانوية باستخدام الطريقة الخضراء ودراسة تأثير المستخلص على الحجم البلوري و النشاط المضاد للبكتريا

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جزيئات الفضة النانوية، التخليق الأخضر، النشاط المضاد للبكتيريا، ونكهة الليمون

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الايميل
الموبايل:

الخلاصة:
في هذا البحث، تم تحضير جسيمات الفضة النانوية (AgNPs) باستخدام مستخلص الليمون الحامض. تم استخدام طريقة جديد للتخليق الأخضر لجسيمات الفضة النانوية باستخدام أجزاء مختلفة من الليمون. تم استخدام حيود الأشعة السينية لدراسة الخصائص الهيكلية للعينات وحساب حجم البلورة ثم دراسة تأثير الأجزاء المختلفة من نبات الليمون الحامض . يظهر من النتائج تغير في حجم البلورة، وذلك بسبب تأثير المستخلص، ووجد أنه تغير اعتمادًا على نوع المستخلص (12.6، 15.4، 17.2 نانومتر) عند استخدام (مستخلص البذور والأوراق والقشور) على التوالي، كما تم تحليل النتائج باستخدام مجهر القوة الذرية (AFM)، وتظهر النتائج تحسنًا في الخصائص الهيكلية باستخدام المستخلص النباتي. كما تم التحقيق في الخصائص البصرية (الامتصاص والنفاذية)، و يظهر أن الخصائص البصرية للعينات المحضرة تعتمد على نوع المستخلص، أعلى امتصاص عند استخدام مستخلص البذور لتحضير جسيمات الفضة النانوية. أظهرت النتائج نشاطًا مضادًا للبكتيريا ممتازًا لجسيمات النانو الفضية ضد سلالات البكتيريا: المكورات العنقودية الذهبية موجبة الجرام، والزائفة الزنجارية سالبة الجرام، والإشريكية القولونية سالبة الجرام. تعتمد حساسية البكتيريا المختبرة لجسيمات النانو الفضية على حجم بلورات جسيمات النانو الفضية وكثافة الخلية. أظهر الحجم البلوري الصغير لجسيمات النانو الفضية نشاطًا مضادًا للبكتيريا عاليًا، بينما أظهر الحجم البلوري الأكبر لجسيمات النانو الفضية نشاطًا مضادًا للبكتيريا أقل. لتقييم النشاط المضاد للبكتيريا لجسيمات النانو الفضية ضد أنواع البكتيريا موجبة الجرام (E. faecalis و Staph. hominis و Staph. aureus)، ثبت أن مستخلص الليمون الحامض حسّن أداء الجسيمات النانوية ووجد أن أعلى قطر تثبيط كان 45 مم ضد Staph. hominis، بينما وجد أعلى نطاق تثبيط 40 مم ضد أنواع البكتيريا سالبة الجرام (Entero.cloacae و E. coli و K. pneumoniae). ضد Staph.hominis، حيث كانت منطقة التثبيط أقل بمقدار 14 ملم ضد E.coli