ARTICLE
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol.30; No.3.| 2022

Silver Nanoparticles Synthesized from *Euphorbia tirucalli* L. Latex Extract: Characterization and Antibacterial Activity Assessment

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جزيئات الفضة النانوية المركبة من مستخلص الحليب لنبات *Euphorbia tirucalli* L التوصيف وتقييم النشاط المضاد للبكتيريا

رلى ظاهر الجايد[1]، هدى جاسم محمد التميمي[2]

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Received:	2 /6/2022	Accepted:	21 /7 /2022	Published:	30 /9 /2022

ABSTRACT

Background:

The fast production of silver nanoparticles utilizing plant latex extract from *Euphorbia tirucalli* L. is revealed in this study where the application of *Euphorbia tirucalli* latex extract in the green manufacture of Ag-NPS has been investigated as a reducing and stabilizing agent.

Euphorbia tirucalli latex silver nanoparticles (Ag-NPs) were investigated using UV-VIS Spectroscopy and produced a surface plasmonic resonance peak at 400 nm. The size of *Euphorbia tirucalli* AgNPs was determined using a scanning electron microscope (SEM), which revealed nanoparticles ranging in size from ten to sixty nm, with average of 51.6 nm. The main significance of active functional groups in the reduction and stability of *Euphorbia tirucalli* AgNPs is revealed by Fourier-transform infrared spectroscopy (FTIR).

The well diffusion assay was also used to detect antibacterial activity at four concentrations 100, 50, 25, and10 µg/ml against Gram positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* and gram negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* as harmful microorganisms and the diameters of the prevention zone appeared varying according to the concentrations that were used. This research shows that employing a low-cost, environmentally acceptable extract as a major precursor in the manufacture of Ag nanoparticles is feasible and practicable, Finally, this supply of nanoparticles might be a very important industrial initiative in the search for innovative, safe, and cost-effective antibiotic alternatives.

Materials and Methods:

This study has been conducted in the advanced plant laboratory at the College of Science for women/ University of Babylon in cooperation with the laboratories of the Ministry of Industry and Minerals and AL-Ameen center for research and advance Biotechnology, AL-Najaf province to conduct some tests for the for the period from November 2020 to March 2021.

Results:

The current AgNP synthesis method, which uses latex extract as a reducing agent, completely eliminates the use of synthetic reducing agents. Latex is very good at producing very stable and biocompatible AgNPs, which might be useful in biomedical applications

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Conclusion:

The biosynthesis of *Euphorbia tirucalli* Ag-NPs was effectively accomplished in the current work using a green technique of manufacture that involved treating silver nitrate with latex extract of *Euphorbia tirucalli* solutions. The plant latex of *Euphorbia tirucalli* was shown to be a good stabilizing reducing agent for the production of AgNPs in this approach. The process used here is very basic, straightforward, low-cost, environmentally friendly, and a superior alternative to chemical synthesis. The resulting AgNPs are very stable and have good antibacterial activity.

Key words:

Nanoparticles, *Euphorbia tirucalli*, latex, AgNPs, green synthesis.

الخلاصة

مقدمة:

تم الكشف عن الإنتاج السريع لجسيمات الفضة النانوية (AgNPs) باستخدام مستخلص اللاتكس من *Euphorbia tirucalli* L. في هذه الدراسة حيث تم التحقق من استخدام مستخلص اللاتكس *Euphorbia tirucalli* في التصنيع الأخضر لجسيمات الفضة النانوية (Ag-NPS) كعامل اختزال وعامل استقرار.

تم فحص الجسيمات النانوية الفضية *Euphorbia tirucalli* latex (Ag-NPs) باستخدام التحليل الطيفي للأشعة المرئية وفوق البنفسجية، وأنتجت ذروة رنين بلازموني سطحية عند 400 نانومتر. تم تحديد حجم *Euphorbia tirucalli* AgNPs باستخدام مجهر إلكتروني مسح (SEM)، والذي كشف عن جسيمات نانوية تتراوح في الحجم من 10 إلى 60 نانومتر، بمتوسط 51.6 نانومتر. تم الكشف عن الأهمية الرئيسية للمجموعات الوظيفية النشطة في تقليل واستقرار *Euphorbia tirucalli* AgNPs من خلال التحليل الطيفي للأشعة تحت الحمراء (FTIR).

كما تم استخدام اختبار انتشار الحفر للكشف عن النشاط المضاد للبكتيريا ضد الكائنات الحية الدقيقة الضارة إيجابية الجرام وسالبة الجرام واظهرت مناطق تثبيط بأقطار متفاوتة بحسب التراكيز المستخدمة. يوضح أن استخدام مستخلص منخفض التكلفة ومقبول بيئيًا باعتباره مقدمة رئيسية في تصنيع الجسيمات النانوية Ag أمر ممكن وعملي، وأخيرًا، قد يكون هذا العرض من الجسيمات النانوية مبادرة صناعية مهمة جدًا في البحث عن مبتكر وآمن وتكلفة– بدائل فعالة للمضادات الحيوية.

طرق العمل:

-أجريت هذه الدراسة في معمل النبات المتقدم في كلية العلوم للبنات / جامعة بابل بالتعاون مع مختبرات وزارة الصناعة والمعادن ومركز الأمين للبحوث والتكنولوجيا الحيوية المتقدمة بمحافظة النجف لإجراء بعض الاختبارات للفترة من نوفمبر 2020 إلى مارس 2021

.الاستنتاجات:

تم إنجاز التخليق الحيوي لـــ Euphorbia tirucalli Ag-NPs بشكل فعال في العمل الحالي باستخدام تقنية التصنيع الخضراء التي تضمنت معالجة نترات الفضة مع مستخلص اللاتكس من محاليل Euphorbia tirucalli. تبين أن لاتكس نبات Euphorbia tirucalli عامل اختزال جيد للاستقرار لإنتاج AgNPs في هذا النهج. العملية المستخدمة هنا أساسية جدًا ومباشرة ومنخفضة التكلفة وصديقة للبيئة وبديل ممتاز للتوليف الكيميائي. AgNPs الناتجة مستقرة للغاية ولها نشاط جيد مضاد للجراثيم.

الكلمات المفتاحية:

الجسيمات النانوية، *Euphorbia tirucalli*، الحليب النباتي، نترات الفضة النانوية، التخليق الأخضر.

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INTRODUCTION

Nanoparticles are crucial materials for basic research and a wide range of applications. Because of its unique physicochemical features and biological uses [1]. Nanoparticle research is becoming unavoidable, not only because of the applications but also because of the manufacturing processes [2]. Chemical and physical approaches have been used to study the synthesis of silver nanoparticles. Physical and chemical nanoparticle production processes may pose major environmental risks while also being technically and financially challenging [3]. thus biological approaches-based microbes or plants for use in the synthesis of silver nanoparticles as an organic (natural) reducing and stabilizing agent have been proposed as environmentally beneficial alternatives [4].

Plant materials may be more favorable than microorganisms for nanoparticle production since they do not need intra- or extracellular synthesis, purification, or microbial culture maintenance [5].

Euphorbia tirucalli L. (pencil-tree) is a tiny tree or shrub endemic to tropical locations with pencil-like branches belonging to the Euphorbiaceae family, it is considered evergreen since its stems and branches remain green all year and animals seldom feed on it [6]. Most studies explained the latex isolated from *Euphorbia tirucalli* is used as an alternative source for producing NPs in the green synthesis of NPs utilizing living plants from the Euphorbiaceae, thus, silver NPs were synthesized using latex derived from *Euphorbia tirucalli* by [7], [8].

Euphorbia tirucalli latex extracts contain Flavonoids, Alkaloids [9]. Steroids, Phenols, and Tannins [10]. Terpenic alcohol, isoeuphorol [11]. glycol [12]. The advantage of the presence of these chemicals is to reduce Ag+ ions to produce AgNPs. Furthermore, *E.tirucalli* latex has long been used to treat asthma, rheumatism, earaches, coughs, and toothaches. It is also utilized as a folk treatment for syphilis. It's used as a laxative to treat verrucae, epithelioma, sarcoma, skin tumours, and manage intestinal parasites. Therefore, this study aims to incorporate these therapeutic characteristics and functions into AgNPs by synthesizing them using *Euphorbia tirucalli* plant extracts, as a consequence maybe there is antibacterial action in combination.

Materials and Methods

- **Synthesis of *E.tirucalli* latex -AgNPs**

E. tirucalli latex was collected in sterile, clean containers and centrifuged for five minutes at 10,000 rpm. After that, Whatman No. 1 filter paper was used to filter the solution. The E. tirucalli latex-AgNPs were created using an eco-friendly process. A 500 mL beaker received 40 mL of latex, to put it briefly. Then 160 mL of water was added, and 1 minute was spent stirring. After that, 16 mL of 1 mM AgNO3 was continuously stirred into the aforementioned solution. UV-Vis spectroscopy was used to track how the surface plasmon resonance (SPR) band formed. In an incubator shaker, the churning persisted for 24 hours until the color changed. By centrifuging at 10,000 rpm for one hour, the unreacted latex was eliminated. Additionally, the solution was dried for 24 hours using an electric oven, and AgNPs were collected in a container and stored at 4°C, as explained by [13].

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- **Characterization of *E. tirucalli* latex -AgNPs**

In a Shimadzu UV-visible 1800, absorption spectral analyses were done. The surface shape and size of nanoparticles in composite films were determined using SEM. Different functional groups were detected using Fourier transforms infrared (FT-IR) spectra.

- **Anti-bacterial study**

Gram positive *Staphylococcus aureus* and *Enterococcus faecalis* and gram negative *Escherichia coli* and *Klebsiella pneumoniae* bacteria are inoculated and then activated for 18 hours at 37°C in nutritional broth, and the turbidity tube of 0.5 was used. The MacFarland technique was used to quantify turbidity. A cotton swab was used to cultivate microorganisms on sterilized nutrient agar plates. Wells were cut out with the end of the sterilized pasture pipette after 5-10 minutes. From the stock concentration of 400g/ml, 100 µl of AgNPs solution were made from each concentration (25, 50, 100, and 200 g/ml) was placed in the wells and incubated for twenty-four hours at 37° C. The length of the inhibition zone was measured in millimeters, as [14] explained in them study.

Results and Discussion

- **The Visual characterization**

The bio-reduction of silver ions to silver nanoparticles using *Euphorbia tirucalli* latex extract was discovered as a consequence of the color change. The observable changes in color from white to brown after adding 1mM of silver nitrate solution, owing to activation of surface plasmon vibrations, demonstrate the formation of AgNPs [15]. Spectral tests in the UV-Vis range verified it even more, Figure 1.

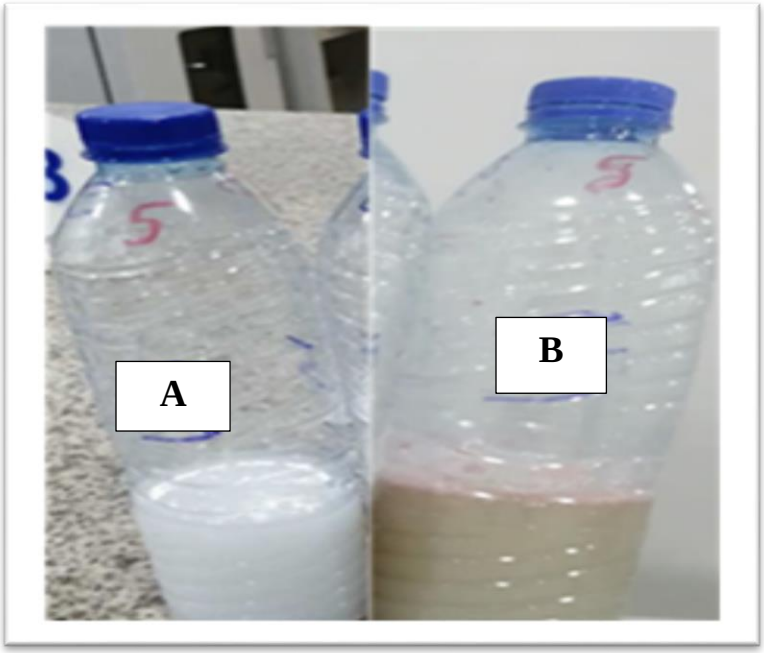

Figure 1 A: Biosynthesis of silver nanoparticles using *Euphorbia tirucalli* latex before color change B: After the color change

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UV-Vis analysis

The interaction between silver ions and the biomolecules present in the latex extract of Euphorbia tirucalli is clearly visible in the absorption spectra of silver nanoparticles created in the reaction solution. In the UV-visible spectra, a notable absorption band at 400 nm was discovered. The broad absorption band at 400 nm is related to the Surface Plasmon Resonance of silver nanoparticles (SPR). When subjected to electromagnetic radiation, silver nanoparticles vibrate, and this oscillation generates a unique peak value via [16]. The observed broadening of the peak, according to [17], indicates that the nanoparticles were polydisperse. figure 2.

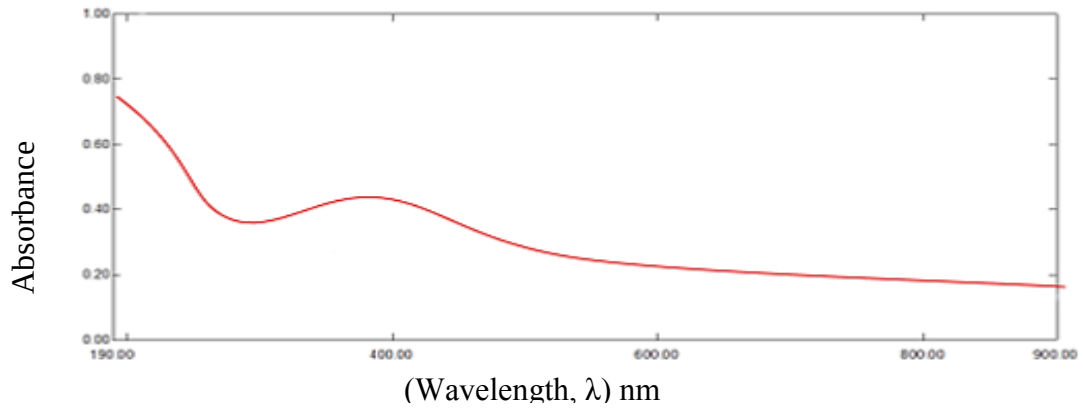

Figure 2: UV–visible absorption spectrum of AgNPs synthesized by latex of *Euphorbia tirucalli*

- **Scanning electron microscopy (SEM)**

Figure 3 show the heterogeneity with nanoscale dimensions of about 51.6 nm, and the clear agglomeration of the atoms, which can be attributed to the fact that the increase in the mixing period has an important role by giving the atoms a clear assortment and the absence of inter-clumping of the atoms, as the increase of silver nitrate to the extract leads to an increase in the rate of surface roughness, which indicates the increase in the size of the grains and the decrease in the grain boundaries, and this is consistent with the results obtained from the X-ray diffraction examination.

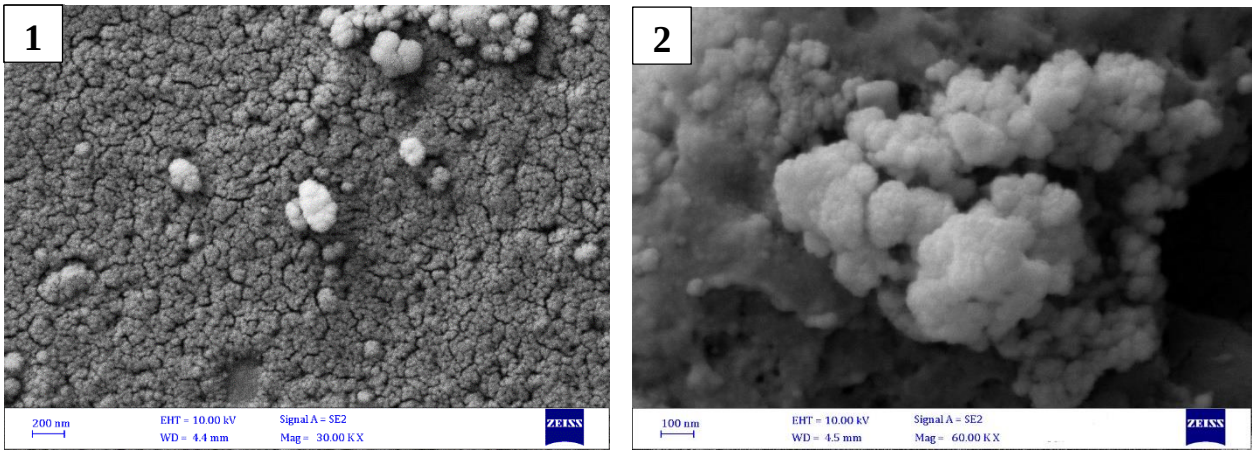

Figure 3: SEM of Silver nanoparticles prepared by latex of *Euphorbia tirucalli* (1):200nm, (2):100nm

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- **FT-IR spectroscopy**

FT-IR spectrum analysis in latex *Euphorbia tirucalli* AgNPs was used to identify the probable biomolecules involved in reducing and capping the bio reduced silver nanoparticles. FT-IR was used to investigate silver nanoparticles' reducing, capping, and stabilizing capabilities. The absorption bands on the spectrum in Figure 4 and Table 1 indicated the existence of active functional groups in the latex of *Euphorbia tirucalli*.

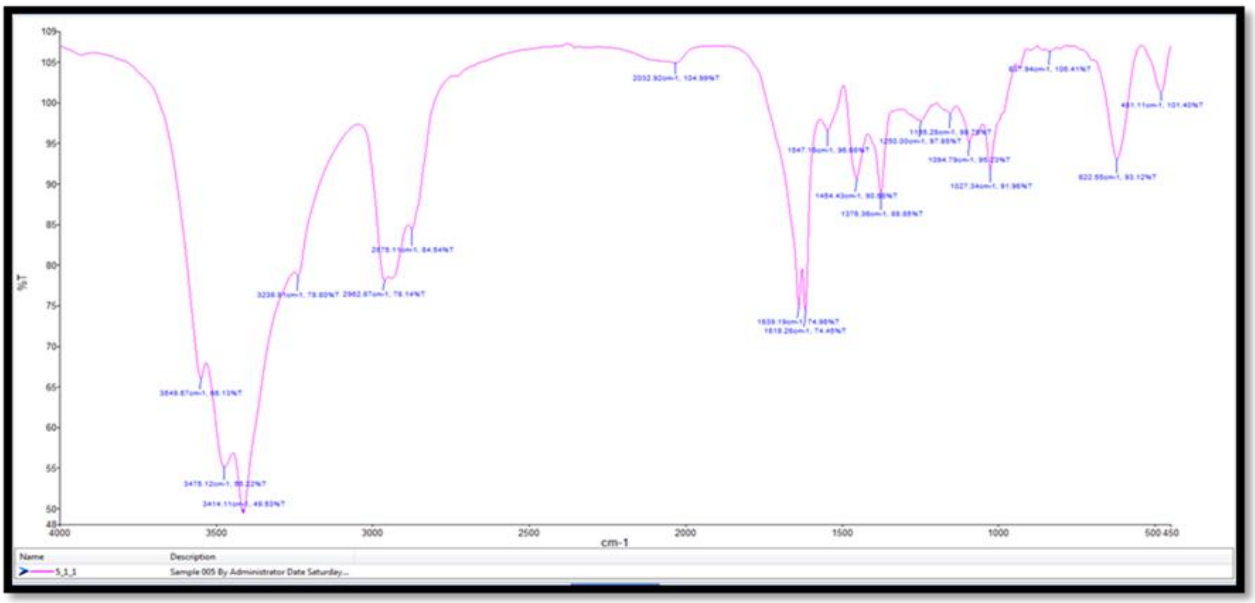

Figure 4: Fourier Transform Infrared Spectroscopy of Biosynthesized nanoparticles by *Euphorbia tirucalli*

The spectra of AgNPs made from *Euphorbia tirucalli* latex extract revealed absorption bands at (3549.67, 3475.12, 3414.11, 3238.81 cm^{-1}), which are typical of (O-H) [18], Figure 4 Table 1. Latex extract from *Euphorbia tirucalli*, 1mM $AgNO_3$ solution, and generated AgNPs, After AgNPs production, the spectra of AgNPs showed a change in transmission value and reduced in intensity to 2875.11 cm^{-1}, indicating that OH (alcohols) were responsible for the reduction of Ag^+. A (C-H) symmetrical vibration of saturated hydrocarbon has a vibrational peak of 2032.92 cm^{-1}. The fact that the peak has shrunk to 2032.92 cm^{-1} shows that hydrocarbons were involved in the conversion of Ag^+ ions to AgNPs. The presence of alkane groups (C=C) is indicated by the peaks at 1639.19 cm^{-1} and 1618.26 cm^{-1}, and the existence of nitro compound (N-O) is indicated by the peak at 1547.16 cm^{-1}. The FT-IR spectra of AgNPs display a band at 1376.36 cm^{-1}, which corresponds to the bending vibration of the phenol. The C-N stretching vibrations of the amines are responsible for the band of AgNPs detected at 1027.34 cm^{-1} [19,20] These findings point to the existence of proteins and their binding to silver nanoparticles, which might contribute to their stability, Table 1.

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Table 1: The peak values, Functional group and bond type of *Euphorbia tirucalli* latex AgPNs

peak values	Compound Class	group
3549.67	alcohol	O-H stretching
3475.12	alcohol	O-H stretching
3414.11	alcohol	O-H stretching
3238.81	alcohol	O-H stretching
2962.87	alcohol	O-H stretching
2875.11	alcohol	O-H stretching
2032.92	aldehyde	C-H stretching
1639.19	alkane	C=C stretching
1618.26	conjugated alkene	C=C stretching
1547.16	nitro compound	N-O stretching
1376.36	phenol	O-H bending
1250	aromatic ester	C-O stretching
1155.28	tertiary alcohol	C-O stretching
1094.79	aliphatic ether	C-O stretching
1027.34	amine	C-N stretching
837.94	halo compound	C-Cl stretching
622.55	halo compound	C-I stretching
481.11	Aryl disulfides (S-S stretch)	Thiols and thio-substituted compounds

- **Antibacterial activity**

The antibacterial activity of silver nanoparticles from *Euphorbia tirucalli* latex extract was tested at four concentrations (100, 50, 25, and 10 µg/ml) against the four bacterial species used in this study (gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae*) and (gram-positive bacteria *Staphylococcus aureus, Enterococcus faecalis*) by varying inhibition zone diameters. The greatest inhibition zone was seen against *E. faecalis* with an 18 mm zone of inhibition followed by a 17mm zone of inhibition against *K. pneumoniae* while the zone of inhibition against *Staph. aureus* and *E.coli* were 16 and 15 mm respectively, all with concentrations of 100µg/ml. On the other hand, the zone of inhibition with 50 µg/ml concentration appeared against both *K. pneumoniae* and *E. faecalis* at 12 mm, but the concentration of 25 µg/ml showed a zone of inhibition against *E. faecalis* only at 10mm, while the last concentration (10µg/ml) never show any zone of inhibition against all bacteria used in our study Table 2, Figure 5.

Table 2: Diameters of inhibition zone for *Euphorbia tirucalli* latex extract AgNPs against four species of bacteria

Plant species	Bacterial species	Concentration / diameter of inhibition zone (mm)			
		100 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml
Euphorbia tirucalli	*E.coli*	15	0	0	0
	K.pneumoniae	17	12	0	0
	Staph.aureus	16	0	0	0
	E.faecalis	18	12	10	0

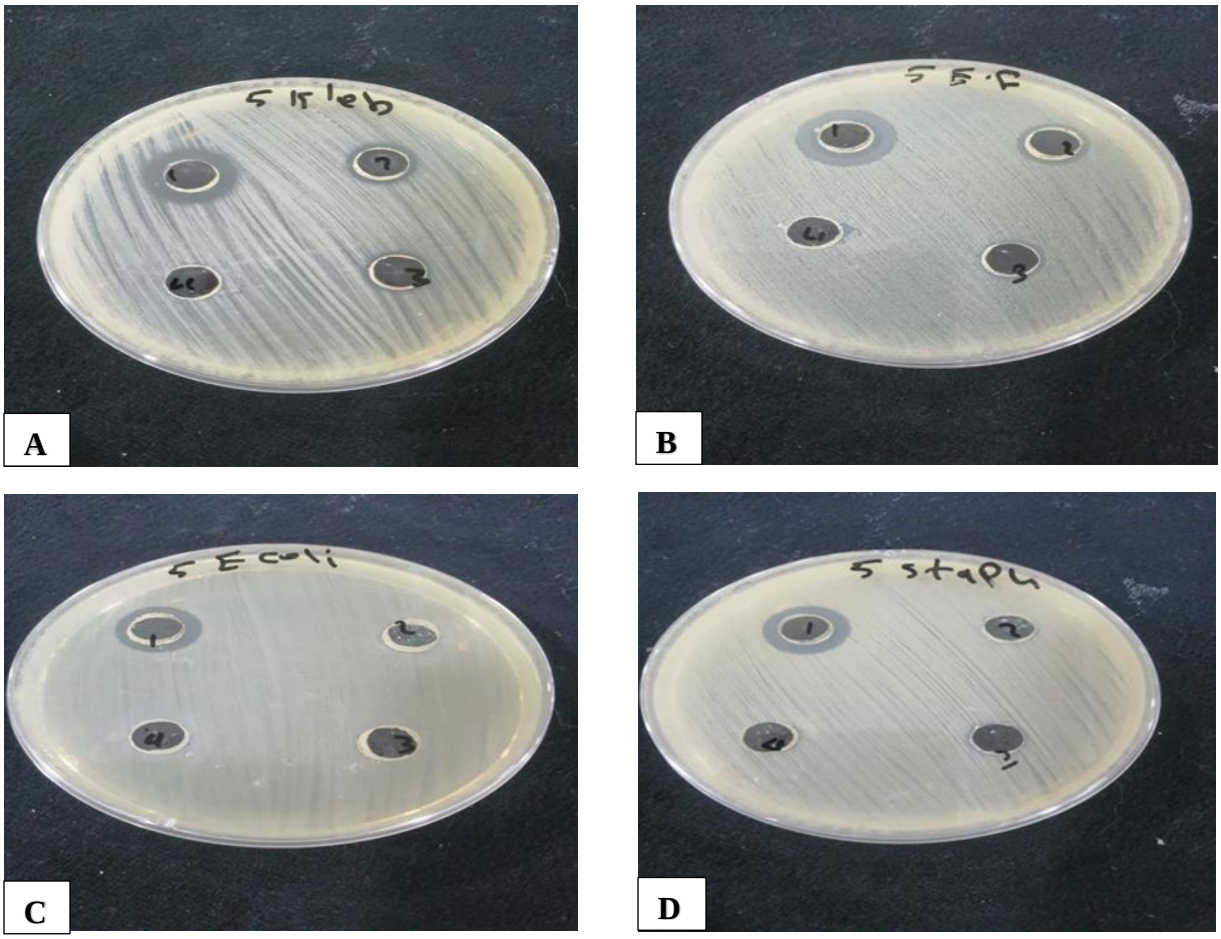

Figure 5: The Antibacterial activity of AgNPS produced from the latex of *Euphorbia tirucalli* (with four concentrations 1-100, 2-50, 3-25, 4-10) µg/ml against A- *Klebsiella pneumoniae*, B- *Enterococcus faecalis*, C- *Escherichia coli*, D- *Staphylococcus aureus*

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The size and shape of the generated nanoparticles can effect bacteria's growth and block them, such as by enzyme analysis and cell leakage, causes presence the variation in the diameters of inhibition zones [21]. These preparations have the ability to suppress pathogenic bacteria. The bactericidal effect of metal nanoparticles is due to their small size and high surface-to-volume ratio, which allows them to interact intimately with microbial membranes, rather than the release of metal ions in solution [22].

Acknowledgments:

Sample of Acknowledgments Sample of Acknowledgments Sample of Acknowledgments.

Conflict of interests.

There are non-conflicts of interest.

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