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Effect of Jatropha Seed Extract–Mediated Green Synthesis of Silver Nanoparticle on *Staphylococcus aureus* Isolated from Clinical Mastitis

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Abstract

THE OBJECTIVE of this work was to synthesize AgNPs using environmentally friendly processes, characterize these structures, and assess their antibacterial efficacy against *Staphylococcus aureus* isolated from mastitic cow's milk that was obtained from various dairy farms in Dakahlia governorate, Egypt. Totally, one hundred mastitic milk samples were cultured on Paired Parker agar media, based on morphological, cultural, biochemical characters and PCR amplification of species- specific gene. Additionally, silver nanoparticles (JCAgNPs) were synthesized using extract from *Jatropha curcas*, and their evaluation was done through, transmission electron microscopy (TEM), and UV-visible spectroscopy analysis, zeta potential and the Fourier Transform Infrared Spectroscopy (FTIR analysis). The generation of nanoparticles confirmed through colour changed from light yellow to brown and UV-visible spectrophotometer confirmed its production at 300 - 700 nm. The Transmission Electron Microscopy (TEM) analysis revealed that the silver nanoparticles were spherical shape with a size range of approximately 11–20 nm and have a zeta potential of 18.6 Mv. Antibacterial potential of JCAgNPs alone was assessed against the two *S. aureus* isolates by disk diffusion method. Furthermore, the selected isolates were then tested for the effect of JCAgNPs combined with antimicrobials: vancomycin (VA), trimethoprim / sulphamethoxazole (SXT), cefotaxime (CTX) amoxicillin/clavulanic acid (AMC).Obviously, the antibacterial activities of the selected antimicrobials were increased in the presence of JCAgNPs against *S. aureus* strains. JCAgNPs increased the activity of the selected antimicrobial agents by which could be applied as a novel therapeutic method against *Staphylococcus* mastitis infections.

Keywords: *Staphylococcus aureus,* Mastitic milk; *Jatropha curcas* Seed extract, Antibiotic susceptibility; JCAgNPs.

Introduction

Globally, one the most important food-borne pathogens is *Staphylococcus aureus (S. aureus)*. It is a bacitracin-resistant and catalase-positive Gram-positive bacteria. Methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) are two types of *S. aureus* that are highly potent and versatile pathogens that are relevant in both nosocomial and community-associated infections [1]. Methicillin-resistant *S. aureus* (MRSA) was the first multidrug-resistant (MDR) bacterium in human medicine [2]. Since MRSA is resistant to all β-lactam antibiotics, there are very few safe and effective treatment options available. Moreover, these strains frequently have a bunch of resistance-determinants against other antibiotic groups (such as tetracyclines, aminoglycosides, macrolides, and fluoroquinolones), which explains how they first gained the title of "superbug" [3]. MRSA-containing foods are thought to be essential sources of antibiotic resistance genes. [4].

A cutting-edge, interdisciplinary field combining material science, chemistry and biology is called nanotechnology [5]. Nanomedicine is a rapidly developing, promising field and active areas of modern research. The technology is used to construct, synthesize, and modify particles with sizes ranging from 1 to 100 nm and it maximizes the use of inert metals like as platinum, gold, and silver to create metallic nanoparticles that have great

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therapeutic promise for a range of biological uses. Of all the metal nanoparticles, silver nanoparticles (AgNPs) have been studied the most. [6]. Applications for AgNPs include antimicrobial, antiviral, and antifungal properties **[7].** According to Ferdous and Nemmar [8], AgNPs are being used as fascinating tools to handle a variety of newly emerging therapeutic issues. Even with their beneficial qualities, AgNPs production can be an expensive and risky process. This is due to the usage of several chemicals and the possibility of the creation of hazardous byproducts [9, 10]. To get over these problems, it was rely on the environmentally friendly synthesis of AgNPs.

The basic idea of green techniques is to use harmless proteins to synthesise nanoparticles by reducing ions of metal in an aqueous solution. The majority of biomolecules, including proteins, enzymes, and DNA, are very costly, quickly break down, and susceptible to contamination. However, a variety of extracts of plant are readily available, relatively inexpensive, and stable under the most of environmental factors (such as temperature, pH, and salinity) [11, 12].

A member of the Euphorbiaceae family, *Jatropha curcas* (*J. Curcas*) is a physic nut plant that grows in tropical and subtropical regions such as Southeast Asia, Africa, India and Central and South America [13]. Jatropha is a multipurpose plant that has long been used to treat a variety of human and veterinary conditions due to its potent biological and immunological properties [14]. Numerous Jatropha species have been demonstrated in the past to have antimicrobial and antifungal activity [15]. Therefore, this study aimed to to isolate and identify S. aureus from One- hundred mastitic cow milk samples collected from dairy farms in Dakahlia Governorate, Egypt. In addition, Biological (green) synthesis of ecofriendly silver nanoparticles from the Jatropha curcas seeds extract and to estimate of the green – synthesized silver nanoparticles antimicrobial activity against clinical *S. aureus* isolates either alone or combined with antimicrobial agents.

Material and Methods

Sample collection

One- hundred mastitic milk samples were collected from Dakahlia Governorate dairy farms in Egypt between December 2021 and August 2022. Samples were collected aseptically into sterile Falcon tubes, labelled and transported in ice box to the laboratory of Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Mansoura University for bacteriological examination.

Sample preparation, isolation and purification procedures

After centrifuging the milk samples, 0.1 mL of the sediment was directly streaked onto the surface of Baird Parker agar media (HiMedia Pvt. Ltd.) supplemented with 1% potassium tellurite (Oxoid) and 5% egg yolk. The mixture was then incubated for an overnight at 37°C. Typical jet-black colonies with a transparent halo zone were subcultured for purification on Tryptone Soya Agar (TSA; Oxoid, UK) medium after being taken from the plates. Purified isolates were kept in a 30% glycerol solution at -80 °C for farther examination.

Morphological and biochemical identification

Gram staining of the retrieved isolates and the standard biochemical tests for *S. aureus* were conducted in accordance with [16].

Molecular characterization of S. aureus

DNA extraction

To prepare the whole cell lysate for each isolate, three to five colonies were suspended from an overnight culture in 200 µL of nuclease-free water, vortexed, boiled for five minutes, and centrifuged at 13,000 g for one minute. In order to facilitate additional molecular characterization, the supernatant was collected, utilized as a DNA template for PCR, and stored at -20 °C [17].

Polymerase chain Reaction (PCR)

Species-specific (*S. aureus*-specific thermonuclease (*nuc* gene) was used to confirm *S. aureus* by PCR using the following primer sets: the *nuc*-F: (GCGATTGATGGTGATACGGTT) and *nuc*-R: (AGCCAAGCCTTGACGAACTAA AGC) [18]. The PCR reaction and cycle conditions were carried out in accordance with [19] as earlier description. A final volume of 25µL was achieved by adding 12.5µL of master mix (Biolab Inc., New England), 1µL of forward and approximately 10 pmol of reverse primer, 5.5µL of nuclease-free water, and nearly 5µL of DNA template. The initial denaturation was carried out for five minutes at 94°C, followed by 35 cycles of 30 s at 94°C, one minute at 60°C, and one minute at 62°C. The final extension was carried out for ten minutes at 72 °C. The PCR products observed by electrophoresis on 1% agarose gel stained in ethidium bromide solution in tris borate buffer (TBE) and photographed.

Biological synthesis of silver nanoparticles

Extraction of Jatropha curcas

The seeds of *Jatropha curcas* were locally obtained. About 50 g of seeds were crushed using an ordinary coffee grinder and ground kernel were

cooked with 500 mL triply distilled deionized water for 2 h. All the aqueous solutions were prepared using triply distilled deionized water [20].

Silver nanoparticle biosynthesis

The precursor for the synthesis of silver nanoparticles was silver nitrate. Sigma-Aldrich provided silver nitrate analytical grade ($AgNO_3$). In a standard reaction procedure, 20 mL of 10^{-3} M aqueous silver nitrate solution and 5 mL of seed extract were combined. The mixture was heated to 80°C for 15 minutes, at which point the resultant solution turned reddish in color. Thus, indicating that silver nanoparticles are formed. *J. curcas* seed extracts serve as the reducing and stabilizing agents in this procedure. Plant extract was gradually eroded and chemically degraded to produce silver nanoparticles. The higher concentration of silver nitrate, the more intensity of color. The pellet that was used for further research was created by synthesizing AgNPs from the plant extracts and centrifuging it for 10 minutes at 5000 rpm (Hettich EBA20S Portable Centrifuge) [21].

Characterization of biosynthesized silver nanoparticles

The biosynthesized silver nanoparticles were examined using gold sputtering for size and shape analysis, transmission electron microscopy (TEM, JOEL JSM 6380 LA), and UV-visible spectroscopy analysis was used to confirm Surface Plasmon Resonance (SPR). The zeta potential of the colloidal solution of nanoparticles was determined by measuring the distribution of the nanoparticles with the Zetasizer nano instrument (Malvern) using an X-ray diffractometer (Rigaku Miniflex 600, Rigaku Co., Tokyo, Japan) and Bruker Alpha to record the IR and X-ray diffraction profile. The Fourier Transform Infrared Spectroscopy (FT-IR) analysis of the extract from *Jatropha* seeds (JS) and the generated seed cake nanoparticles (JCAgNPs) was also carried out.

Minimum inhibitory concentration of JCAgNPs

By using the broth microdilution method, the minimum inhibitory concentration (MIC) of JCAgNPs was determined. JCAgNPs were diluted at two fold serial dilution and were cultivated in 200 µl of Luria Broth LB. The broth was inoculated with 1×10^4 CFU from an overnight culture of *S. aureus*. The inoculated tubes were then incubated at 37°C for a maximum of 24 hours [21].

Antibacterial activity of JCAgNPs, antibiotic and/or conjugate against S. aureus isolates

According to Tyagi and Malik [22], the antimicrobial susceptibility of two chosen *S. aureus* isolates was assessed using the disc diffusion method on Mueller-Hinton agar media. Three different types of sterile paper discs (6 mm) were used in this study: (1) antibiotic discs alone (AMC, SXT, VA, and CTX) with a 5µg concentration; (2) sterile paper discs saturated with 10µl of JCAgNPs (3) Conjugate: antibiotic discs loaded with 10µl of JCAgNPs to see the combined activity. Using a bent glass rod, approximately 100 µL of an inoculum suspension containing 10^5 CFU/mL was applied to the Mueller-Hinton agar plate surface. After allowing the inoculated plate to dry for a few minutes, the disks were placed using sterile forceps. After 18 to 24 hours of incubation at 37°C, the zones of inhibition surrounding the disks were measured with a pair of callipers to the nearest millimetres.

Results

Isolation and identification of S. aureus isolates

Thirteen *S. aureus* isolates were recovered based on morphological, cultural, biochemical and molecular characters out of one hundred mastitic milk samples (Fig. 1A). *S. aureus* isolates were identified as Gram-positive cocci grouped in clusters (Fig. 1B). *S. aureus* produced black jet colonies on Baird Parker media surrounded by halo zone and appeared as golden yellow colonies on TSA. Biochemically, the presumptive isolates were positive for catalase and coagulase tests. The obtained isolates were successfully amplified at 270 bp using a species-specific *nuc* gene (Fig. 2).

Synthesis, optical and structural characterization of biosynthesized JCAgNPs

After three hours of storage in a cool dark environment, the $AgNO_3$ solution's physical appearance changed to a dark solution after the addition of extract from *Jatropha curcas* seeds, indicating the synthesis and chemical reduction reaction of AgNPs. The reaction mixture's colour changed from light yellow to brown to indicate the formation of nanoparticles (Fig. 3A), UV-visible spectrophotometer confirmed its production at 300 and 700 nm (Fig. 3B). The single SPR at nearly 390 nm that was found in the current study corresponds to silver nanoparticles.

The Transmission Electron Microscopy (TEM) analysis revealed that the silver nanoparticles were in a monodispersed aggregated form, with a size range of approximately 11–20 nm and the particles had a spherical shape (Fig. 4A). Three elements were found in the JCAgNPs' EDX spectrum: oxygen, chlorine, and silver, with percentage weights of 18.13, 7.95 and 70.39%, respectively. The low concentration of chloride indicated that the silver nanoparticles were not highly contaminated, confirming the formation of AgNPs (Fig. 4B).

Obviously, JCAgNPs have a zeta potential of 18.6 Mv (Fig. 5A). This indicated that negatively charged groups make up the majority of the capping molecules on the surface of JCAgNPs and are also responsible for the moderate stability of the nanoparticles. The XRD diffraction patterns of JCAgNPs were captured (Fig. 5B), displaying three distinct diffraction peaks in the 2 Theta (θ) range at 38.225, 64.658 and 77.558°, which correspond to the 110, 210, and 211, respectively. The obtained nanoparticles' crystalline structure was verified by the peaks. The functional groups enveloping the JCAgNPs were identified by Fourier-transform infrared analysis (Figure 6A & B). Spectra peaks of JCAgNPs at 525, 1041, 1307, 1647, 2921, and 3338 cm^{-1} were observed in Figure 6A. But according to Figure 6B, the *J. curcas* leaf extract's spectra peaks were at 525, 1041, 1307, 1646, 2924, and 3274 cm^{-1}.

Antibacterial activities of biosynthesized JCAgNPs towards the clinical S. aureus isolates

The observed Minimal inhibitory concentration value for biologically synthesized JCAgNPs was 20µg/L. JCAgNPs were tested alone for their antimicrobial activity against two selected clinical isolates of *S. aureus* (S1 and S2) isolated from cases of clinical mastitis by disc diffusion method. For *S. aureus* S1, JCAgNPs produced inhibition zone diameter of 10 mm in a dose dependent manner. On the other hand, for S2 the inhibition zone were scored as 12mm against JCAgNPs and the inhibition zone increase in a dose dependent manner (Table 1).

Antibacterial activities of dual effect of biosynthesized JCAgNPs

S. aureus S1 is tested against amoxacilline/claviolinic acid and displayed a 16.7mm diameter inhibition zone, while wider inhibition zone (17.6) was produced when amoxicillin/ clavulanic acid used in combination with JCAgNPs. Similarly, sulfathiazole/trimethoprim produced 25 mm inhibition zone with S1, and larger inhibition zone (32.6) was produced when it combined with JCAgNPs, similarly vancomycin displayed a 19 mm inhibition zone diameter (22.2) against *S. aureus* and larger inhibition zone was obtained when it combined with JCAgNPs. Similarly, Cefotaxime produced a zone diameter of 19.2 mm and wider zone of inhibition (22.5) was obtained when it combined with JCAgNPs (Table 2).

Similarly, *S. aureus* S2 is tested against amoxacilline/claviolinic acid and displayed a 22mm diameter inhibition zone, while wider inhibition zone (31mm) was formed when amoxicillin/ clavulanic acid used in combination with JCAgNPs. sulfathiazole/ trimethoprim produced 29.1 mm inhibition zone with S2, and wider inhibition zone (33.6)was produced when it combined with JCAgNPs. Vancomycin displayed a 31.8 mm inhibition zone against *S. aureus* and wider inhibition zone (33.5) was produced when it combined with JCAgNPs. Cefotaxime produced a zone diameter of 17.9 mm and wider zone of inhibition (21.1) was produced when it combined with JCAgNPs (Table 2).

Discussion

S. aureus drug resistance has been getting higher due to bacterial evolution and antibiotic abuse. MRSA clinical antibacterial treatment has become more difficult in recent years. Growing data has shown that *S. aureus* resistance mechanisms are extremely complex, particularly for MRSA, which has multiple antibiotic resistances. Thus, it is crucial to comprehend MRSA's drug resistance as soon as possible and to clarify its molecular mechanism of resistance in order to effectively treat *S. aureus* infections [22].

As a result, researchers have concentrated their efforts on the investigation of new antimicrobial drugs against different kinds of microbial pathogens, including *S. aureus*. With comparatively low toxicity, low price and high bioavailability, natural products like plants are demonstrating promising antimicrobial activity [23, 24]. Plant extracts, among other natural products, are useful in the green biosynthesis of AgNPs. Hence, in the present study, we aimed to synthesize NPs from *J. curcas* and to evaluate its antimicrobial potential against clinical *S. aureus* isolates.

In the current study, 13 *S. aureus* isolates from 100 milk samples collected from mastitic cows from Dakahlia dairy farms based on biochemical and molecular characters [26, 27]. Our results are in agreement with a previous study [28] which concluded that out of the 60 *staphylococcal* isolates, 32 *S. aureus* were identified using PCR-amplification of the *nuc* gene, which was used as a baseline test.

Jatropha species have a wide range of biological activities and are a great source of active biomolecules including terpenes, and plant metabolites [29]. In addition, it contains diterpenoids, flavonoids, and steroids [30]. These substances are important because they help biologically reduce and stabilize silver ions, which are then successfully converted into silver nanoparticles [31]. Other *Jatropha* species, mainly Jatropha *gossypifolia* and *Jatropha curcas*, have reportedly been utilized for the synthesis of AgNPs [32]. In this study, the chemical reduction reaction and JCAgNP synthesis were carried out using extract from the seeds of *Jatropha curcas*. It also reported as a secure technique for making silver nanoparticles (J-AgNPs)

by utilizing extract from *Jatropha integerrima* leaves as an antimicrobial and reducing agent [33].

In the present investigation, various techniques were employed to evaluate the synthesis of JCAgNP which appear as spherical structure which indicated by the UV absorption at 390 nm during characterization, and TEM analysis subsequently verified this. Furthermore, an equivalent trend seen to those documented previously [34]. Many studies about the metallic nature of the biologically synthesized biofilm-AgNPs are made possible by the use of EDX. [35], the Edx analysis results reveals a major signal at 3 keV, indicating that the synthesis of AgNPs was successful. Furthermore, the EDX spectrum reveals the following elements: aluminium (Al) with 0.27%, phosphorus (P) with 0.65%, oxygen (O) with 9.11%, chlorine (Cl) with 17.68%, carbon (C) with 17.88%, and silver (Ag) with 54.42%. Furthermore, Aqueous leaf extract from *Jatropha curcas* was found to be capable of producing stable silver nanoparticles (Zeta potential: 23.4 mV) with an absorption band at 430 nm [36]. Demissie and Lele [37] reported that the JCAgNPs is stable and does not show any considerable aggregation.

X-ray diffraction (XRD) has also been utilized to verify that the particles nature were crystals. The XRD results observed in this investigation clearly confirmed that the nanoparticles are crystalline in nature and are created when the phytochemical components of *Jatropha* seed extracts reduce Ag^+ ions. In addition to the typical peaks, some extra, unidentified peaks were also observed close to the distinctive peaks of the nanoparticles. These peaks might be the result of extract proteins or other bioorganic compounds [37]. Measurements using FTIR spectroscopy are performed to pinpoint the biomolecules that attach themselves to the silver surface. When the plant extract's spectra were compared to those of JCAgNPs, a small change in the absorption bands' magnitudes was observed. It has been verified by the presence of a 3338 cm-1 peak in both JCAgNPs and *jatropha* extract that the primary amines contribute to the reduction of the metal salts into nanoparticles [38]. Aromatic and aliphatic amines' C-N stretching vibrations (1307 cm^{-1}), and phenolic and alcoholic groups' C-O stretching vibrations (1041 cm^{-1}), C-N stretching vibrations of aliphatic and aromatic amines (1307 cm^{-1}), C-O stretching vibration C-H stretching of methylene groups of proteins (2921cm^{-1}) was seen both on the surface of nanoparticles as well as in the extract [39,40] which attests to the phytochemicals from plants being capped on the nanoparticles' surface and stabilizing them. Additionally, the amide I band is characterized by the band at 1647 cm^{-1} [41]. The amide band I is the stretch mode of the carbonyl group attached to the amide linkage.

Since silver nanoparticles can cause DNA single strand breaks and alter gene expression, they may influence bacterial resistance to antibiotics [42]. The current study found that increasing the concentration of JCAgNPs against *S. aureus* isolates increased the inhibition zones. This result was in agreement with Alizadeh et al. [43] who found that treating bacteria with different amounts of silver nanoparticles significantly changed the number of bacteria that were reduced at concentrations of 100 and 150 µg/mL.

These compounds work together to provide the principal interaction and affinity for acting and accumulating in cell walls and membranes, whereas antibiotics concentrate on a single target in bacterial cells. AgNPs, on the other hand, attack multiple cellular structures. AgNPs can alter and disrupt membrane proteins and cellular barriers, they allow antibiotics to act at the membrane or intracellular level, enabling the concentration of antibiotics, the weakening of bacteria, and even the avoidance of resistance mechanisms that already exist [44],

Rai et al. [45] reported that antibiotics like penicillin G, amoxicillin, erythromycin, and vancomycin are bound to the silver nanoparticles; this combination strengthens the antibacterial action against both gram-negative *(E. coli)* bacteria and gram-positive *(S. aureus)*. Ag^+ works within microbial cells through a variety of antimicrobial mechanisms [46]. Ag^+ blocks the microbial cytochrome's electron transport chain [47]. According to Huang et al. [48], Ag^+ combines with microbial DNA and RNA to produce harm. In gram-positive bacteria, Ag+ inhibits the synthesis of the cell wall and the 30S ribosomal subunit, preventing protein translation. Ag^+ ions also contribute to the formation of ROS, which can be lethal to eukaryotic host cells as well as bacteria [43, 49, and 50].

In this investigation, the biosynthesized JCAgNPs combined with the selected antibiotics were estimated. Both *S. aureus* isolates produce a synergistic effect of antibiotics (amoxicillin/clavulanic acid (AMC), cefotaxime (CTX), vancomycin (VA) and trimethoprim / sulphamethoxazole (SXT) when combined with 10 µg/ L of JCAgNPs. Our results showed an agreement with Khatoon et al. [44] who reported the synergistic antibacterial effects of AgNPs combined with antibiotics when a transitioning from vancomycin-resistant to susceptible in *E. coli*. Furthermore, Vazquez-Muñoz et al. [51] reported that the growth of *S. aureus, E. coli and S. typhimurium* could be inhibited by up to 50% by the additive and synergistic effect of conjugated treatment with chloramphenicol + AgNPs. These same strains were able to have their growth inhibited at a rate that

approached 95% when treated with AgNPs plus kanamycin concurrently.

Conclusions

The synthesis of silver nanoparticles (JCAgNPs) through a cost-effective and environmentally friendly biological method with a plant photochemical cap and an aqueous extract of *Jatropa curcas* seed extract has the potential to be a potent antibacterial agent. JCAgNPs demonstrated a strong antibacterial effect, making them suitable for use as an antimicrobial agent against *S. aureus* strains in biomedical applications. The use of JCAgNPs as an antibacterial is supported by these data; nonetheless, suitable methods for their application need to be investigated and developed without contributing to make the situation worse. But since antibacterial properties can be associated to the nanoparticles total surface area of the future work will concentrate on optimizing parameters for the synthesis of relatively small nanoparticles, which would further enhance the antibacterial activity. Further molecular research should focus on the function of biosynthesized JCAgNPs in various infections.

Acknowledgement

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study was ethically approved by Research

Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University,Egypt (Code No: vmms.24.04.1).

TABLE 1. Antibacterial activities of different concentrations of biosynthesized JCAgNPs towards S. aureus (S1 & S2) isolates by disc diffusion method

JCAgNPs Concentration	Inhibition zone (mm)	
	S1	S2
10 µg /L	10	12
20 µg /L	11	15
40 µg /L	12	20
80 µg /L	15	22
100 µg /L	20	23

TABLE 2. Antibacterial activities of dual effect of biosynthesized JCAgNPs with different concentrations and four selected antibiotics

Antibiotic Name	Code	Discs	Inhibition zone (mm)	
			S1	S2
Amoxicillin/clavulanic acid	AMC	AMC only	16.7	22
		AMC + JCAgNPs	17.6	31
Cefotaxime	CTX	CTX only	19.2	17.9
		CTX + JCAgNPs	22.5	21.1
Vancomycin	VA	VA only	19	31.8
		VA + JCAgNPs	22.2	33.5
Sulphamethoxazole /Trimethoprim	SXT	SXT only	25	29.1
		SXT + JCAgNPs	32.6	33.6

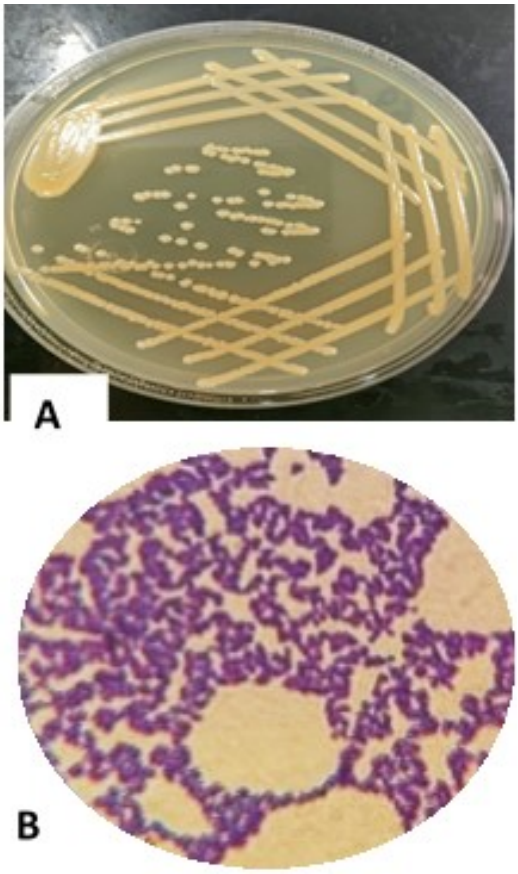

Fig.1. Morphological identification of Staphylococcus aureus. A) S. aureus colonies on Tryptone Soya Agar (TSA) culture showing the yellow – golden colonies and B) Gram stain of S. aureus showing grapes like Gram positive cocci.

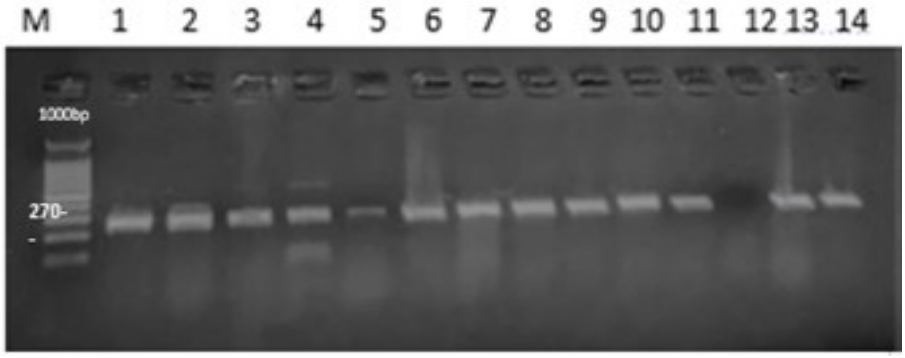

Fig. 2. Amplification of nuc gene at (270 bp) from S. aureus. M, 100bp
- ladder; lane 1: S aureus (S1 strain), Lane 2, S aureus (S2 Strain), lane 12, negative control, lanes 4-14 amplified nuc gene of the rest S aureus samples.

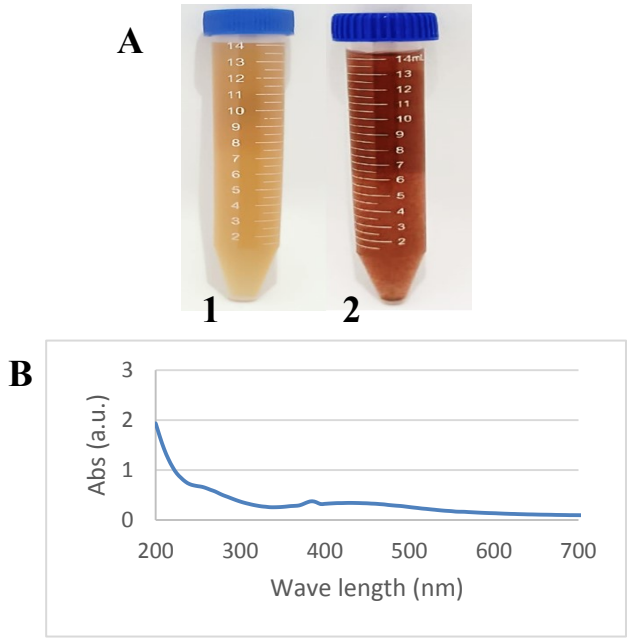

Fig. 3. A) Colour change after AgNPs synthesis: 1) extract; 2) colour changed after adding AgNO3 as a result to JCAgNPs formation, B) UV spectroscopy.

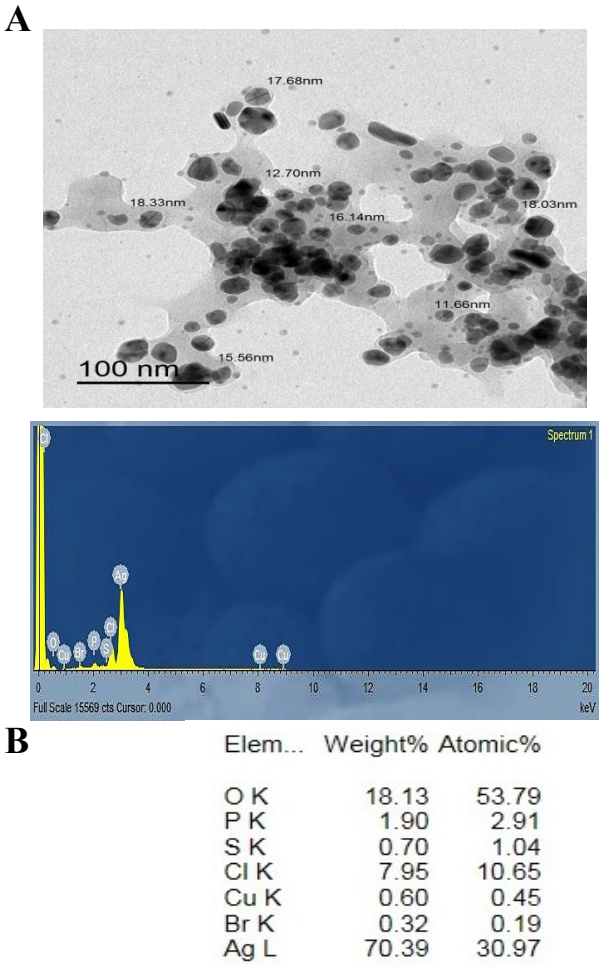

Fig. 4. Structural characterization of JCAgNPs A) TEM micrograph showing size of JCAgNPs particles and B) EDX spectrum of JCAgNPs.

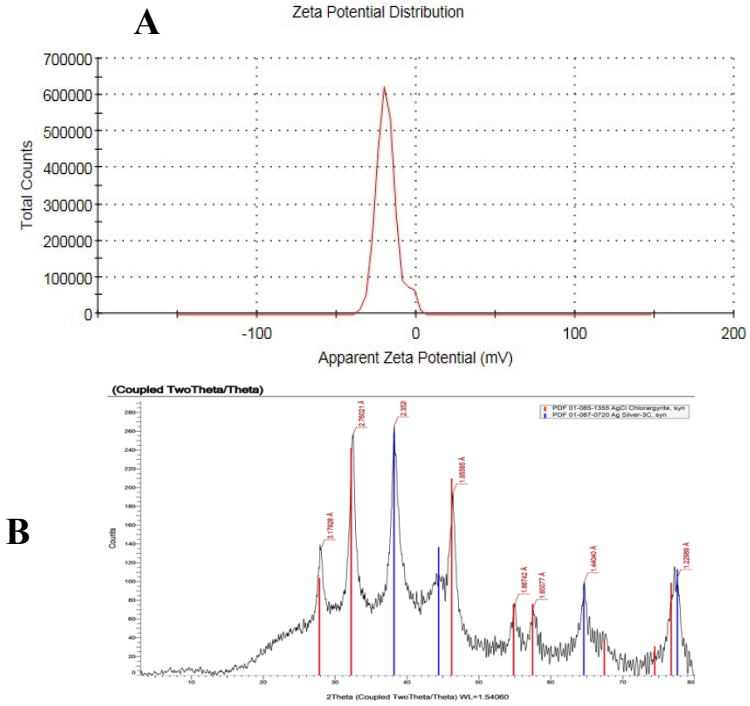

Fig. 5. Characterization A) Zeta potential; B) XRD patterns of JCAgNPs synthesized by treating Jatropha seed extract with aqueous 10^{-3}(M) $AgNO_3$ solution.

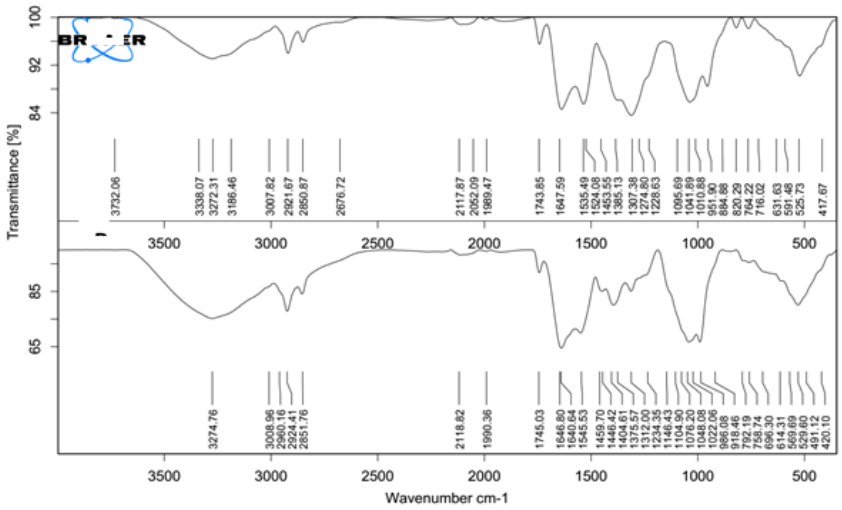

Fig. 6. FT-IR patterns of A) JCAgNPs and B) Jatropha seed extract

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تأثي جسيمات الفضه النانويه المخلقه من مستخلص بذور الجاتروفا على المكورات العنقودية الذهبية المعزولة من التهاب الضرع

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الملخص

الهدف من هذه الدراسه هو تصنيع AgNPs باستخدام تقنيات صديقة للبيئة، وتوصيف هذه المركبات، وتقييم فعاليتها المضادة للبكتيريا ضد المكورات العنقودية الذهبية (*S. aureus*) المعزولة من حليب الابقارالذي تم الحصول عليه من مزارع الألبان المختلفة في محافظة الدقهلية، مصر. بشكل إجمالي، تمت زراعة مائة عينة من حليب مجمع من حالات التهاب ضرع اكلينيكي على أوساط بكتيريه خاصه، بناءً على الخصائص المورفولوجية والكيميائية للميكروب وتفاعل البلمره المتسلسل للجينات المحددة للأنواع. بالإضافة إلى ذلك، تم تصنيع جسيمات الفضة النانوية (JCAgNPs) باستخدام مستخلص من نبات الجاتروفا كوركاس، وتم تقييمها من خلال المجهر الإلكتروني النافذ (TEM)، والتحليل الطيفي للأشعة فوق البنفسجية المرئية، وإمكانات زيتا، وتحليل FTIR. تم تأكيد من تكون الجسيمات النانوية من اللون المتغير من الأصفر الفاتح إلى البني وأكد مقياس الطيف الضوئي المرئي بالأشعة فوق البنفسجية إنتاجها عند 300 - 700 نانومتر. كشف تحليل المجهر الإلكتروني أن الجسيمات النانوية الفضية كانت ذات شكل كروي يتراوح حجمها حوالي 11-20 نانومتر ولها إمكانات زيتا تبلغ 18.6 ميجا فولت. تم تقييم القدرة المضادة للبكتيريا لـ JCAgNPs وحدها ضد عزلتي *S. aureus* بواسطة طريقة الانتشار القرصي. علاوة على ذلك، تم بعد ذلك اختبار العزلات المختارة لمعرفة تأثير JCAgNPs مع مضادات الميكروبات: فانكومايسين (VA)، تريميثوبريم / سلفاميثوكسازول (SXT)، سيفوتاكسيم (CTX)، أموكسيسيلين / حمض الكلافولانيك (AMC). من الواضح أن النشاط المضاد للبكتريا قد زاد في وجود جسيمات JCAgNPs ضد سلالات المكورات العنقودية الذهبية. زادت JCAgNPs من نشاط العوامل المضادة للميكروبات المختارة والتي يمكن من خلالها تطبيقها كطريقة علاجية جديدة لعدوى التهاب الضرع.

الكلمات داله: المكورات العنقودية الذهبية، التهاب الضرع ، مستخلص بذور الجاتروفا كركاس، حساسية للمضادات الحيوية، JCAgNPs