SYNERGISTIC EFFECT OF ANTIBIOTIC WITH GREEN SYNTHESIZED SILVER NANO PARTICLES AGIANST UROPATHOGENIC *E.COLI* BIOFILM

[1]Muna H. M. [1]N. S. Alshaikhly [3]M. H. M Al-khafaji
Researcher Assist. Prof. Assist. Prof.
[1] Dept. of Biology, College of Women Science, University of Baghdad
[3] Dept. of Biology, College of Science, University of Baghdad
drmarwahameed81@gmail.com

ABSTRACT

The bacterial ability to form biofilm had been complicated the infection healing process, due to antibiotic resistance. Urinary tract infection is an optimal example of such infections that caused by bacterial biofilms. So the current study aimed to find an alternative method to inhibit the uropathogenic bacteria using nanotechnology. Uropathogenic *Escherichia coli* (UPEC) was isolated from 110 clinical samples. These bacterial isolated were tested for their ability to form biofilm by two methods: Microtiter plate and congo red agar. Antibiotic susceptibility was detected to determine the multidrug-resistant isolates. An ecofriendly green method was depended for the synthesis of silver nanoparticles (AgNPs) using alcoholic extract of *Lepidium meyenii* yellow root. Silver nanoparticles were characterized by several techniques, and it was detected with a diameter of 44.89 nm. Anti-biofilm activity of AgNPs alone and with antibiotic was detected and the Scanning electron microscopy observations clearly indicated that 7.1825 mg/ml AgNPs prevented the biofilm formation. This is the first worldwide evidence-based research study about the biosynthesis of silver nanoparticles from alcoholic yellow root extract of plant *Lepidium meyenii* and use it against UPEC biofilm.

Keywords: antibiofilm, silver nanoparticles, optimization, synergistic, maca roots.

التأثير التآزري للمضادات الحيوية مع جزيئات الفضة النانوية المحضرة بالتصنيع الأخضر ضد الغشاء الحيوي للممرضة البولية الأشريشيا القولونية.

منى حميد مطشر[1] ندى صباح الشيخلي[2] مروى حميد الخفاجي[3]
باحث استاذ مساعد استاذ مساعد
قسم علوم الحياة كلية العلوم للبنات جامعة بغداد [2,1]، قسم علوم الحياة كلية العلوم جامعة بغداد، العراق [3]

المستخلص

أدت القدرة البكتيرية على تكوين الأغشية الحيوية إلى تعقيد عملية الشفاء من العدوى ، بسبب مقاومة المضادات الحيوية. عدوى المسالك البولية هي خير مثال على هذه الالتهابات التي تسببها الأغشية الحيوية البكتيرية. لذلك هدفت الدراسة الحالية إلى إيجاد طريقة بديلة لتثبيط البكتيريا المسببة للأمراض البولية باستخدام تقنية النانو. تم عزل الممرضة البولية الأشريشيا القولونية من 110 عينة سريرية. تم اختبار قدرة هذه البكتيريا على تكوين الغشاء الحيوي بطريقتين: اطباق المعايرة الدقيقة وأجار الكونغو الأحمر. تم الكشف عن حساسية المضادات الحيوية وذلك لتحديد العزلات متعددة المقاومة للأدوية. تم اعتماد طريقة خضراء صديقة للبيئة لتخليق دقائق الفضة النانوية باستخدام المستخلص الكحولي من الجذر الأصفر لنبات Lepidium meyenii. تميزت دقائق الفضة النانوية بعدة تقنيات، وقد تم الكشف عنها بقطر 44.89 نانومتر. تم التحري عن فاعلية الدقائق النانوية المضادة للغشاء الحيوي بمفردها ومع المضاد الحياتي وأظهرت ملاحظات الفحص بالمجهري الإلكتروني الماسح أن 7.1825 مجم / مل AgNPs حالت دون تكوين الأغشية الحيوية. هذه أول دراسة بحثية عالمية قائمة على الأدلة حول التخليق الحيوي لدقائق الفضة النانوية من المستخلص الكحولي للجذر الأصفر لنبات Lepidium meyenii واستخدامها ضد الأغشية الحيوية الممرضة البولية الأشريشيا القولونية.

الكلمات المفتاحية: مضاد للغشاء الحيوي، دقائق الفضة النانوية، تحسين، تآزري، جذور الماكا.

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INTRODUCTION

The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of multi-drug-resistant bacteria. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Now days, about 70 % of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and they can only treat with experimental and potentially toxic drugs (7). In recent years, there has been considerable interest in the problems posed by the biofilm mode of bacterial growth. A biofilm is a population of cells growing on a surface and enclosed within an exo-polymer matrix that can restrict the diffusion of substances and bind antimicrobials (28). This will provide effective resistance of biofilm cells against large molecules such as antimicrobial proteins lysozyme and complement (14). The ability of bacteria to form biofilms helps them to survive hostile conditions within host and is considered to be responsible for chronic or persistent infections (29). Chronic infection associated with the use of medical devices such as catheters may be established because of the ability of bacteria to adhere to inanimate surfaces. There is now widespread recognition of biofilms to human infection. The armament of therapeutic agents available to treat bacterial infections today is restricted to antibiotics developed specially to kill or to stop the growth of individual bacteria (19). In modern clinical microbiology, establishment of bacterial biofilms considered a pathogenicity trait during chronic infections (34). Nanotechnology may provide the answer to penetrate such biofilms and reduce biofilm formation. Silver nanotechnology can prevent the formation of life-threatening biofilms on medical devices. Silver is one of the oldest known antimicrobials. Antimicrobial silver is now used extensively to combat organisms in wounds and burns. Various sources are available for biosynthesis of Ag-NPs such as; ultrasonic, violet radiation and lithography. But, these methods are not considered eco-friendly . Hence, plant extract synthesis is considered an alternative method and to be a need of an hour, because it possesses several benefits compared to chemical methods (4). Biosynthesized Ag-NPs are considered cost-effective, eco-friendly, safe and alternative tools for biological control (33). Although the literature reports some studies related to the antibacterial activity of AgNPs, there are studies concerning the effect of these particles against adhered cells and biofilms of Uropathogenic *E. coli* (6). Therefore, the aim of the present study was to evaluate the antibiofilm potential of AgNPs against biofilms of UBEC by Congo red and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Collection of samples: A total of one hundred and ten Clinical samples were collected during the period extended from August-2020 to January- 2021. Clinical samples and swaps of urine were collected from Iraqi patient in Iraqi hospitals (AL-Emamein kadhimein medical city, al-Yarmouk teaching hospital and al-Shaheed al-Hakeem general hospital) in Baghdad city.

Bacterial Isolation and identification

The collected clinical samples were cultured on selective and differential media to isolate *Escherichia coli*. Clinical samples were inoculated directly on blood agar as well as MacConkey agar, and incubated at 37˚C for 24 hours. Later, the grown colonies were further investigated by biochemical tests, Vitek system, HiCrome UTI and Api 20E System.

Antibiotic susceptibility testing

Antibiotic sensitivity test was performed to all the clinical *Escherichia coli* isolates using modified Kirby-Bauer method for susceptibility to the following 16 different antibiotics: Ampicillin (AMP)(10), Piperacillin(TZP)(110), Cefazolin(KZ)(30), Cefoxitin(FX)(30), Ceftazidime(CAZ)(30), Ceftriaxone(CRO)(30), Cefepime(FEP)(30), Ertapenem(ETP)(10), Imipenem(IPM)(10), Amikacin(AK)(30), Gentamicin(GM)(10), Ciprofloxacin(CIP)(5), Levofloxacin(LVX)(5), Tigecycline(TGC)(15), Nitrofurantoin(F)(300), and Trimethoprim(SXT)(25). Antibiotic discs used were procured by HiMedia (Mumbai, India).Was determined using disc diffusion method according to the guidelines recommended by the National Committee for

Clinical Laboratory Standards (NCCLs-2020) and confirmed by VITEK2 compact system.

Detection of biofilm production

Biofilm formation by clinical isolates of *E. coli* was tested using two assays:

I- Congo red agar

This medium was used to determine bacterial ability to produce the slime layer. Isolates were considered as strongly positive when there was a presence of black colonies with a dry crystalline consistency. Congo red agar was made by dissolving brain heart infusion broth (37gm) with sucrose (50gm) and agar-agar (15gm) in 900 ml of distilled water, autoclaving it, and then cooling it to 55-60°C before adding congo red solution (which was made by dissolving 0.8 gram of congo red dye in 100 ml of distilled water, then sterilizing it by filtration). The prepared medium was poured into sterile Petri dishes (13).

II- Microtiter plate method

Microtiter plate method is considered as the gold standard technique to detect the biofilm**.** Micro ELISA auto reader at wavelength 490 nm was used to obtain the optical density (OD) of stained adherent biofilm. The experiment was performed in triplicate and repeated three times and control wells were achieved with media without bacteria inoculation. Strains were categorized as no biofilm producer; weak biofilm producer; moderate biofilm producer and strong biofilm producer (12).

Biosynthesis of Silver nanoparticles

Synthesis of Silver Nanoparticles (Ag NPs) using yellow root extracts of plant *Lepidium meyenii* as following:.

As 0.5 g of commercial *Lepidium meyenii* yellow root extracts powder was dissolved by 50 ml D.W and equal volume of rectified spirit. This solution was then kept undisturbed incubator overnight. After centrifugation the tea color supernatant was used as the plant extract for the synthesis of Silver nanoparticles whiles the residue was discarded (5). 0.5 g of Silver nitrate (AgNo3) was dissolved in 50 ml of D.W, and then 4 mL of this solution was taken to which 2 mL of the prepared yellow root extracts solution was added. This mixture was kept undisturbed incubator overnight. The change of the solution color from tea color to a metallic shiny golden color is an indicator for silver nanoparticles formation (Figure1), after centrifugation, the supernatant discarding while the precipitate was kept in a hot-air oven at 250 C till all the water evaporated. The obtained product was then cooled and used for further studies.

Figure 1. Synthesis of Silver Nanoparticles (Ag NPs) using yellow root extracts of plant *Lepidium meyenii*

Characterization of AgNPs

After obtain pure and quite stable dried powder of biosynthesis silver nanoparticles, it was characterized by several examinations conducted in the laboratories of the University of Technology/ Nanotechnology and Advance Materials Research Centre. That include (Colour change, UV-Vis spectra analysis,

Atomic force microscopy (AFM) analysis, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM), Zeta potential Measurements, Particles size analysis and X-Ray diffraction (XRD).

Optimization parameters for the biosynthesis of silver nanoparticles

Reaction parameters which have potential impact on the quality of the nanoparticles like solution concentration of $AgNO_3$(1 mM, 2 mM, 3 mM) was tested and (7, 24, 48 hr) incubation periods.

Anti-biofilm effects of the combination silver nanoparticles (AgNPs) and Levofloxacin: The anti-biofilm formation efficacy of AgNPs silver nanoparticles and levofloxacin was examined using the protocol described by (16). The anti-biofilm activity of AgNPs was tested by using serial two-fold dilutions with concentrations ranging from7.1825 µg/ml to 1000µg.

RESULTS AND DISCUSSION

Bacterial isolation and identification

The collected samples where cultured on selective and differential culture media, after 24 hours of incubation at 37^{o}C. The majority of the bacterial isolates were small dry flattened pink colonies, lactose fermenters with a percentage of (80%), while non-lactose fermenters was (15%), and no growth was (5%).A further investigation for *E.coli* isolates was done by sub-cultured on HiCrom UTI agar that appeared as dark pink to reddish colonies as results shown in (Figure 2):

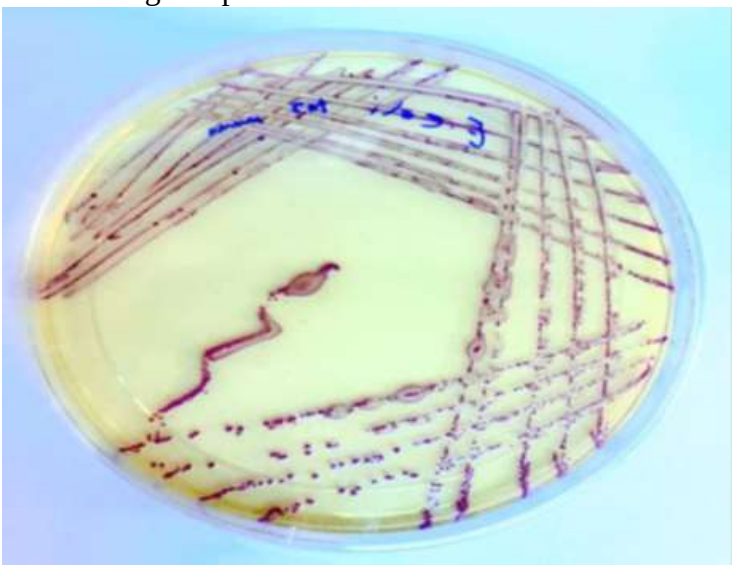

Figure 2. HiCrom UTI agar cultured with uropathogenic *E. coli* isolate after incubation for 24 hrs at 37°C

Antibiotic susceptibility

The pattern of antibiotic susceptibility of all uropathogenic *E.coli* (71) isolates in this study to 16 different antibiotics (Ampicillin, Piperacillin, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Tigecycline, Nitrofurantoin, and Trimethoprim) was determined using disc diffusion method according to the guidelines recommended by the National Committee for Clinical Laboratory Standards (NCCLs-2020) and confirmed by VITEK2 compact system. Where the results showed that all uropathogenic *E.coli* isolates resistant to (Ampicillin, Cefazolin, Ceftriaxone, Cefepime, Trimethoprim, Ceftazidime)100%, While resistant to Nitrofurantoin was 66%, Cefoxitin was 40%, Gentamicin was 15%, at the same time 100% of isolates were sensitive to Piperacillin, Ertapenem, Imipenem, Amikacin, Ciprofloxacin, Levofloxacin, Tigecycline, as results shown in (Figure 3):

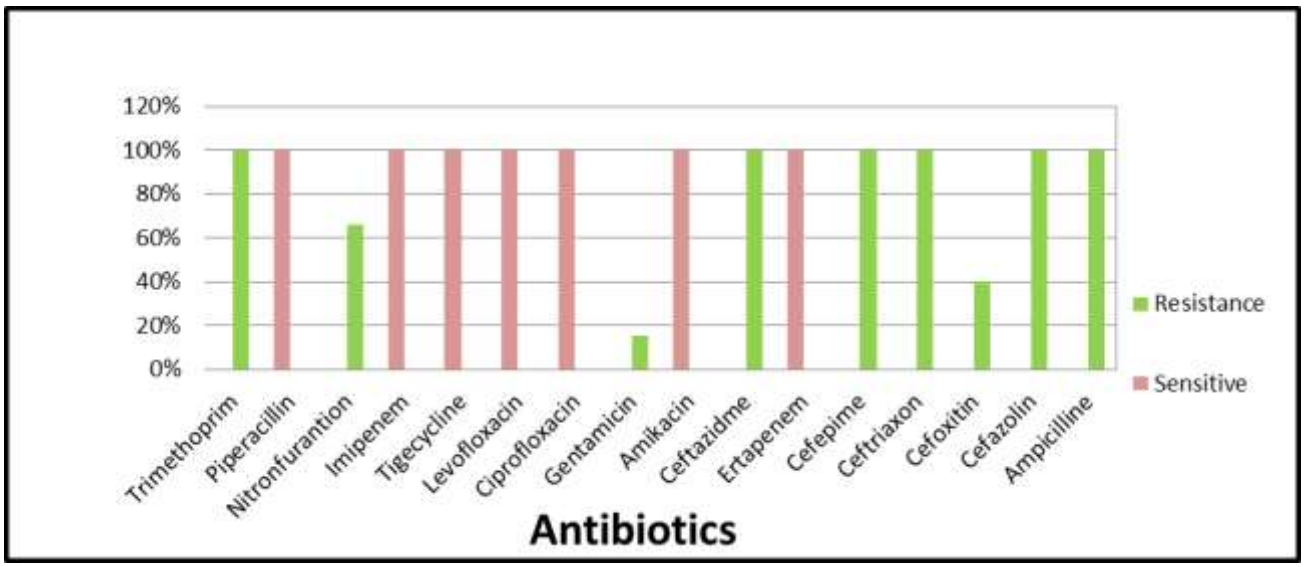

Figure 2. The percentage of Antibiotic susceptibility for uropathogenic *E.coli* isolates in this study

That the results of antimicrobial resistant tests showed a high resistant of *E.coli* isolates to the Ampicillin, and Trimethoprim. That was also reported by Vranic and Uzunovic (35) , as well as the study showed the high resistant of *E.coli* isolates to Cefazolin, Ceftriaxone, Cefepime, Ceftazidime,100%.That compatible with results of Raeispour and Ranjbar (26) and with Kader and Kumar (18). Other Study in north of Iraq revealed that *E.coli* isolates were resistance to Cefazolin, Ceftriaxone, Cefepime, Ceftazidime, Trimethoprim 100% while Nitrofurantoin resistance showed as 66%.

Detection of biofilm production by UPEC

Congo Red Agar method: Slime layer production by clinical *E.coli* isolates Under the optimized conditions was investigated by using Congo Red Agar method, where the results showed that 38/71 clinical isolates (53.52%) were biofilm producers and 33/71 (46.48%) non biofilm producers.

Microtiter plate method

The microtiter plate method is a widely used technique to detect biofilm formation, it is used to detect the ability of uropathogenic *E. coli* isolates to form biofilm, as a rapid screening method that sensitive enough as a quantitate method for biofilm screening .The results showed that 43/71 (60.56%) clinical isolates of uropathogenic *E. coli* were biofilm producers, as: 30/43(69.77) strains were highly positive biofilm producer, 9/43(20.93) moderate positive biofilm producer, 4/43(9.30) weakly positive biofilm producer, and 28/71(39.44) non biofilm producers as results shown in figure (4).

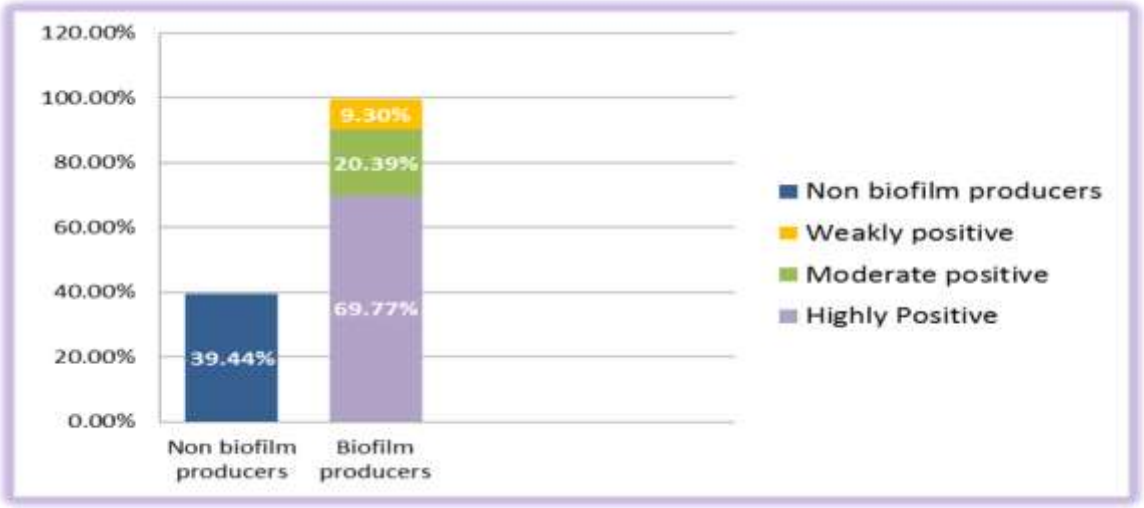

Figure 4. Prevalence of Uropathogenic *E. coli* isolates as biofilm producers by Microtiter plate method

The optical density (OD) of stained adherent biofilm was measured using a Micro ELISA auto reader at 630 nm. The experiment was carried out in duplicate and three times (16). Bacterial isolates of *E. coli* were classified as in the Table(1):

Table 1. Interpretation of results for biofilm production.

Average OD value	Biofilm production
≤ OD /ODc < ~ ≤ 2x ODc	Non / weak
2*OD c < OD t ≤ 4*OD c	Moderate
4*OD c < OD t	Strong

***Optical density cut-off value (ODc) = average OD of negative control**

In the present study, we can see that the mean value of biofilm formation amount represented by this optical density method at (490nm) for UPEC isolates particularly matched with the results mention early by Congo Red Agar method and antibiotic resistance of bacterial isolates , that may explain the role of biofilm production in resistance to antibiotics. TCP method is the ideal method for detection of bacterial biofilm formation by uropathogens (24).

Biosynthesis of silver nanoparticles

In this study, *L.meyenii* yellow root alcoholic extract was screened for production of AgNPs considering their potential for reduction of Ag ions. The primary sight for AgNPs formation as results shown the gradually changing of reaction mixture color from (pale yellow to dark brown) after addition of $AgNO_3$ solution and stirring at room temperature as clear evidence noticed by nicked eye of confirmed Ag ion reduction and the formation of Ag NPs (figure 4 a,b,c):

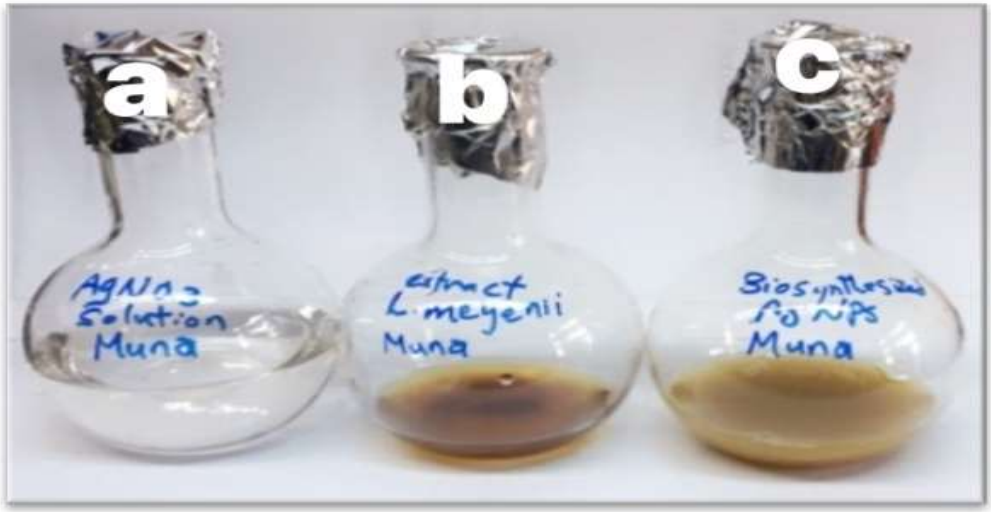

Figure 4. Purified silver nanoparticles obtained in this study via biosynthesis using plant alcoholic extract (a):$AgNO_3$ solution, (b): alcoholic extract of *L. meyenii*, (c): biosynthesis AgNPs

Characterization of biosynthesis silver nanoparticles AgNPs

The AgNPs was characterized by using several techniques according to many studies in Iraq (2, 8 , 20) and global studies (10, 16, 24) and others which showed the importance of these techniques in characterizing the AgNPs in a highly accurate and scientific manner. The UV-Vis absorption spectrum of Ag NPs was obtained from UV–Vis analysis. Various metabolites from plant extract introduced to solution make the plasmon band broad because they may be read in this spectrophotometric range, too. Surface plasmon resonance (SPR) of silver occurs at 360 nm. This peak increased with time up to 360 min. The number of peaks increases by increasing diversity of particles shapes. The control solution (without silver nitrate solution) shows no evidence of absorption in the range 300 to 900 nm. UV–visible spectra of synthesized AgNPs were recorded in the range of 320–500 nm (22). The presence of the broad resonance indicates the aggregation of the silver nanoparticles in the solution. The presence of an absorbance peak at about 360 nm clearly indicates the formation of AgNPs in the solution due to surface plasmon resonance (SPR) electrons present on the nanoparticle surface. The intensity of the SPR band increased with reaction time, indicating the synthesis of the AgNPs (8) as in figure (5):

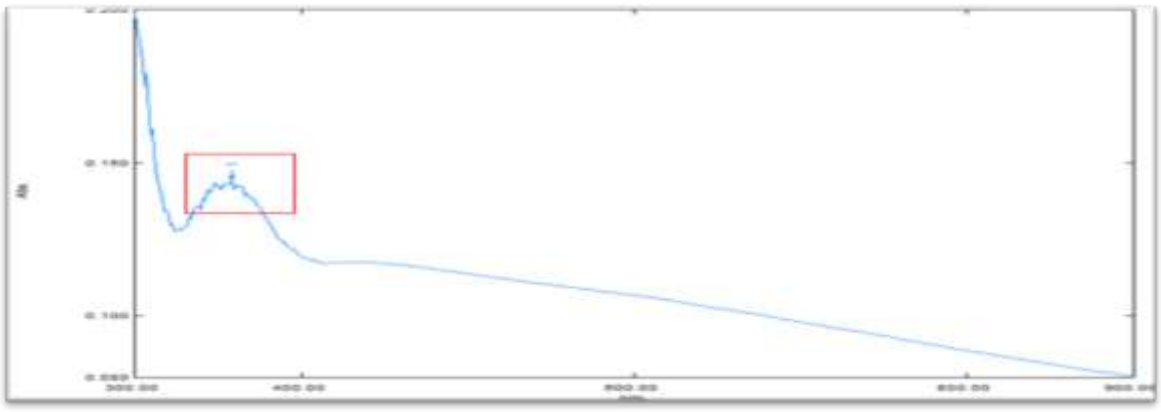

Figure 5. UV–Vis spectrophotometry Scheme of silver nanoparticles biosynthesized by yellow root *L.meyenii* alcoholic extract

The reaction mixture was characterized by Atomic force microscope AFM. And the results indicated that AgNPs formed by *L. meyenii* was in a nano size (44.99 nm). Also results demonstrated that AgNPs exhibit different shapes and the truncated triangular silver Nano plates with a lattice plane as the basal plane displayed the strongest biocide action, compared with spherical nanoparticles. The result of AFM confirmed by the Fourier-transform infrared spectroscopy (FTIR) analysis for the biosynthesized silver nanoparticles from the alcoholic plant extract comparing with FTIR analysis of alcoholic yellow root crude extract of plant *L. meyenii* are shown in Figure (6 A&B):

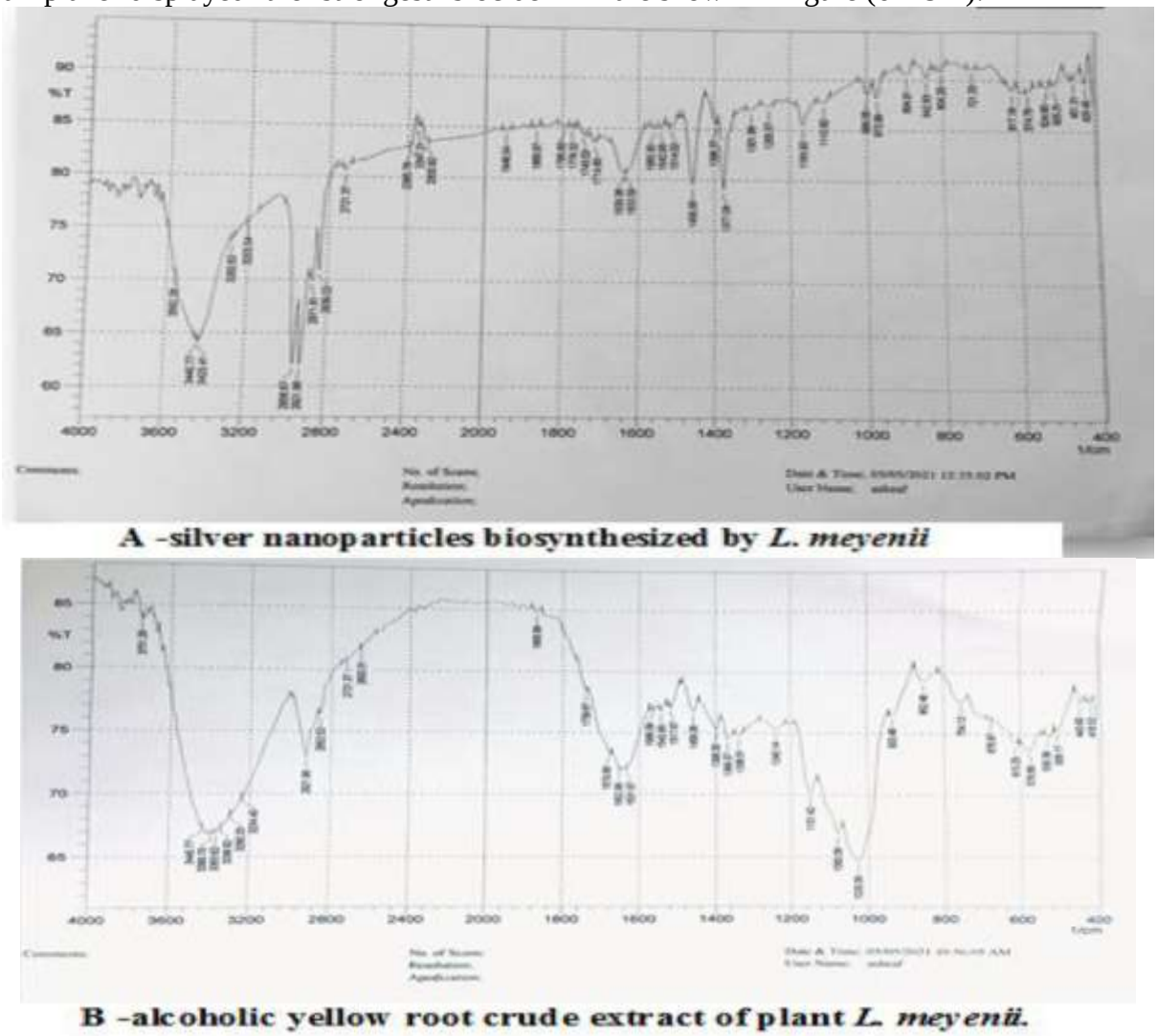

Figure 6. The FTIR analysis of: A -silver nanoparticles biosynthesized by *L. meyenii* and B - alcoholic yellow root crude extract of plant *L. meyenii*

In which the results illustrated responsible metabolites for reduction of Ag ions were detected using FTIR spectrum. Instinct absorption bands at 3440.77 and 3440.77 cm^{-1} appear in the presence of phenols and alcohols with free OH group. This band is superimposed by NH stretching peak. The bands at 2921.96 and 2956.67 cm^{-1} represent the presence of alkanes in lipids. The region of 2347.21 cm^{-1} indicates the presence of symmetric stretching of COO^{-} just in the biosynthesized solution that agrees with (32). FTIR was performed to determine the possible functional groups of biomolecules involved in the reduction of silver ions and stabilization of the biosynthesized AgNPs from *L. meyenii* reacted with Ag (III). After being characterized by color change, UV-vis, spectroscopy FTIR and AFM analyses; the biosynthesized AgNPs were examined under the Scanning Electron Microscope (SEM) to detect the predict size and morphology of them and the result confirmed the formation of AgNPs as the SEM image study showed formation of nano sized AgNPs in Figure (7):

Figure 7. Biosynthesized AgNPs by *L.meyenii* under SEM with truncated triangular shape and diameter ranging from (30-60 nm**).**

The morphology of the nanoparticles was truncated triangular and within the size range of 30–60 nm that also verifies the AFM results, bulks of the particles was truncated triangular in shape. Dynamic light scattering (DLS) measures the hydrodynamic size and the ligand shell of the formed nanoparticles. DLS of AgNPs is 53.0 nm and homogenous distribution of the formed nanoparticles with (polydispersity index: 0.285). The zeta potential is a measure of nanoparticles stability through measuring of the surface charge potential in aqueous suspensions. Zeta potential values of AgNPs were measured to be −235.5±0.7 mV. The produced nanoparticles had a negative charge on their surface, which indicates a high stability. The results of particle size analysis of biosynthesized silver nanoparticles shown in the (figure 8).

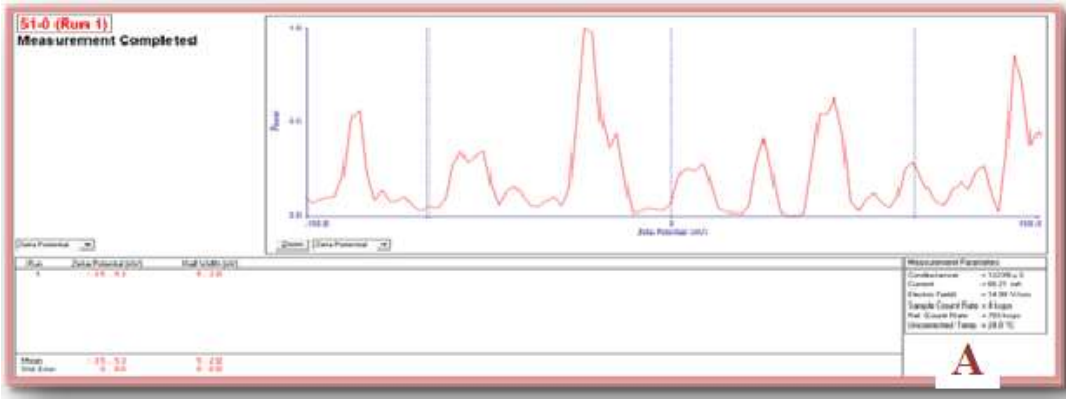

Figure 8. The Zeta Potential and Dynamic Light Scattering for silver nanoparticle, where: (A and B) size and PDI analysis, (C) ζ-potential of the biosynthesized SNPs

And the particle size distribution curves of silver nanoparticles confirm the results of particles size analysis completely. The hydrodynamic size includes the hydration layer on the surface of AgNPs; thus, this size is generally larger than the size measured from scanning electron microscopy (SEM) images. Additionally, the phytochemicals in the alcoholic yellow root extract may contribute to the hydrodynamic size. The size distribution graph shows the average size of the synthesized AgNPs to be approximately 90 nm, and the ζ- potential was observed to be −36.7 mV. A nanoparticle size value below 53.0 nm and PDI values around 0.3 are adequate for uptake by cells. Different phytochemicals present in extracts are mainly responsible for the various particle sizes. == The crystallinity of polymers phases of biosynthesized silver nanoparticles was identification by XRD analysis as in the figure (9):

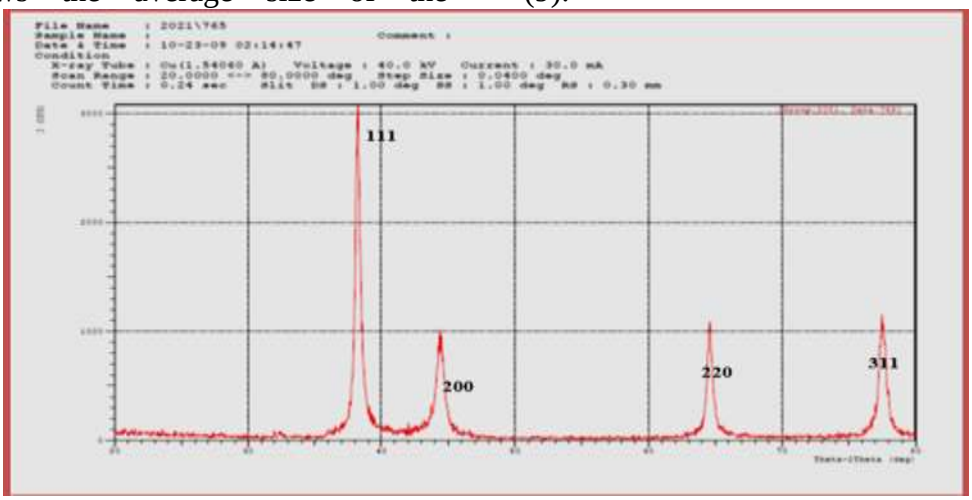

Figure 9. XRD peaks of biosynthesized silver nano particles

XRD pattern revealed distinct peaks at *20* values, which can be attributed to 111, 200, 220 and 311 crystalline planes of Ag NPs. These peaks are associated with the face-centered cubic lattice. Other peaks at 2θ values in Ag NPs pattern can be ascribed to the residues of the organic content of the plant extract. These peaks reveal the crystallization of some plant metabolite moieties on the surface of the Ag NPs, which is in agreement with Shanmuganathan et al.'s results (30). The presences of carnosic acid and flavonoids, which contain carboxylate group, have been detected in *Lepidium* species extracts. Interactions between these metabolites and silver ions cause the bioreduction of silver nitrate and synthesis of Ag NPs (11).

The optimum condition to produce AgNPs by *L. meyenii:* The parameters that examined in spectrum to evaluate the optimal condition to produce AgNPs in this study were solution concentration of AgNO3 and incubation period , Taking into account that pH-7.0 has been proven during the experiment is done . Furthermore, previous reports (31, 35), showed that bacterial survival in AgNPs solution was affected by the incubation time, and a study by Al-Khafaji et al. (2) showed the efficacy of solution concentration of AgNo3. In addition to previous studies that conducted the pH of solution that capping of AgNps as additional optimization parameter as the biosynthesis of silver nano particles stable in the solution for the capping of AgNps at pH-7.0 but not at acidic pH which can be attributed to the stability of capping proteins (21) .

The influence of different AgNo3 concentration on the production of AgNPs by *L. meyenii:* The influence of AgNo3 concentration on the production of Ag nanoparticles was evaluated by varying the concentration of AgNo3. There was a gradual increase in the production of AgNo3 nanoparticles up to 1.0 mm AgNO3 concentration. And according to AFM results that indicated the optimum production of Biosynthesis of AgNPs by the *L. meyenii* alcoholic extract at different concentrations of AgNo3 was 2 mM as indicated in table (2), revealed that optimum size (44.89nm).

Table 2. Optimum concentrations of $AgNO_3$ for production of AgNPs by *L. meyenii* alcoholic yellow root extract

$AgNO_3$ Concentrations	Size of silver nanoparticles in nm by AFM
1 Mm	83.61
2 mM	44.89
3 mM	103.0

The influence of different incubation period on the production of AgNPs by *L. meyenii*

Time is one of the important parameters which directly affect nanoparticles biosynthesis. The reduction reaction for the production of nanoparticles started when reducing agents were instantly applied to the silver nitrate solution, which is demonstrated by the color transition from white to brown. Nevertheless, it is shown that particle size decreases with increasing time, and at a given time it stabilizes. The mixture in this study was subjected to various time intervals for AgNPs synthesis (6, 12, and 24hrs.) as incubation periods for biosynthesis of AgNPs by the *L. meyenii* alcoholic as results shown in table (3) which revealed that optimum size (44.89nm) was at incubation period for 24 hr. that agree with the findings by (17).

Table 3. Optimum incubation periods for production of AgNPs by *L. meyenii* alcoholic yellow root extract.

Incubation period (hours)	Size of silver nanoparticles in nm by AFM
6	No formation
12	73.91
24	44.89

The anti-biofilm effects of the combination silver nanoparticles (AgNPs) and Levofloxacin: The biosynthesis silver nanoparticles activity of destroying the slim layer formed by the MDR UBEC isolates that isolated during this study was were examined under the SEM to detect the anti-biofilm inhibitory activity compared with the control, where the result confirmed the inhibitory activity of biosynthesis AgNPs as shown in (Figure 13 A&B). The obtained data had shown that the combination of biosynthesis silver nanoparticles + Levofloxacin had wide spectrum of antibacterial activity against MDR UBEC isolates that used in this study and that was in agreement with many study. The best antibiofilm inhibitory action was obtained from the synergistic effects of Levofloxacin

antibiotic and the biosynthesis silver nanoparticles were the result shown the antibiofilm inhibitory activity of the combination (biosynthesis silver nanoparticles + Levofloxacin) in all highly biofilm producers MDR uropathogenic *E. coli* isolates in this study with significant difference when comparing it with control ($P \leq 0.05$) as shown in table (4):

Table 4. Synergistic antibiofilm effect of biosynthesized AgNPs MIC (7.1825µg/ml) with Levofloxacin antibiotic against highly biofilm producers MDR uropathogenic *E.coli* isolates

Bacterial isolates	Levofloxacin antibiotic concentrations							
	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml	7.1825 µg/ml
Control(+)	0.157	0.163	0.178	0.188	0.194	0.199	0.210	0.215
Control(-)	0.195	0.199	0.206	0.212	0.217	0.229	0.260	0.278
HE1	0.139	0.153	0.162	0.171	0.179	0.183	0.195	0.209
HE2	0.101	0.108	0.116	0.136	0.158	0.167	0.197	0.203
HE3	0.099	0.101	0.109	0.116	0.131	0.134	0.165	0.186
HE4	0.088	0.091	0.106	0.116	0.148	0.171	0.176	0.192
HE5	0.090	0.098	0.100	0.111	0.128	0.132	0.144	0.156
HE6	0.100	0.133	0.150	0.155	0.167	0.188	0.201	0.208
HE7	0.088	0.090	0.099	0.110	0.132	0.158	0.179	0.180
HE8	0.110	0.105	0.114	0.136	0.157	0.160	0.193	0.202
HE9	0.087	0.100	0.110	0.128	0.133	0.144	0.151	0.169
HE10	0.087	0.109	0.122	0.148	0.153	0.160	0.171	0.189
LDS	0.092 *	0.084 *	0.097*	0.081*	0.085 *	0.077 *	0.075 *	0.082 *

*** (P≤0.05).**
HE = Highly biofilm producers MDR UPEC bacteria

The majority of UPEC strains are strong biofilm formers and show higher tolerance towards frontline antibiotics in biofilm form. Levofloxacin successfully inhibited biofilms at a concentration of 32µg/mL on UPEC biofilms as mentioned by Rafique et al (27). That agrees with this study result. Silver nanomaterials have attracted significant interest from chemists, biochemists, physicists and medical experts by reflecting the extraordinary functional properties and increasingly numerous applications in biomedical technology. The mechanisms of antimicrobial action of the most nanostructured silver were only partially elucidated, revealing multiple actions. It has been proposed that ionic silver interacts inside the cell with multiple target sites, as phosphorus and sulfur compounds, whose alterations lead to eventually chemical modifications of proteins and nucleic acids and metabolic disruption due to the interruption of respiratory electron transport chain. It has been suggested that once sufficient ionic silver has undergone uptake by bacterial cell, its survival is improbable. AgNPs can present enhanced antimicrobial response since they can release silver ion from the oxidative process on the metallic surface, but also present other mechanisms of action. The efficiency of silver AgNPs as bactericides can be increased by combining them to other organic antimicrobial agents, as polymers and antibiotics, with several records of synergism (25). The conjugated silver nanoparticles with Levofloxacin have synergy and additive behavior against the tested bacteria. Levofloxacin, AgNPs and its conjugation have antibacterial activity which was confirmed by MIC result. As we know lower MIC values meaning higher antibacterial activity, so as a result the conjugated silver nanoparticles with antibiotics (AgNPs-Levo) has lower MIC value than AgNPs or Levofloxacin both of them alone that confirmed that AgNPs-Levo have greater antibacterial activity. AgNPs-Levo confirmed that this conjugation is beneficial due to its dual behavior such as synergistic and additive when compared with AgNPs and Levofloxacin alone. A variety of organisms, including plants and bacteria, fungi, seaweeds, and microalgae, are involved in the biological synthesis of nanoparticles (1). This enhanced antibacterial activity of AgNPs-Levo due to the hydrophobic nature of AgNPs, the release of Ag+ and mode of action of AgNPs and Levofloxacin against the bacteria. The higher

activity was observed against target bacteria in the combination of recent study due to the silver nanoparticles enhanced the activity of Levofloxacin antibiotics by conjugation with each other. Polymeric nanoparticles are highly attractive as drug delivery vehicles due to their high structural integrity, stability during storage, ease of preparation and functionalization, and controlled release capability. There are several studies proved that the antimicrobial activity were increased when using combination of silver nanoparticles with antibiotics, antibodies, and probiotic rather than used nanoparticles alone or antibiotic alone. The synergy and additive behavior of AgNPs-Levo occurs due to the mode of action of AgNPs and antibiotic. This conjugation is always beneficial because bacteria will not develop resistant against antibiotics (10).

CONCLUSIONS

Development of resistance to human pathogens is a challenge in field of pharmaceuticals and biomedicine. Antibiotic resistance profiles lead to fear about the emergence and reemergence of MDR pathogens. Development or modification in antimicrobial compounds to improve bactericidal potential is an area of priority in this modern era. Nanotechnology provides a good platform to modify and develop nanostructures having promising applications in various fields. Therefore, an alcoholic yellow root extract of plant *L. meyenii* was found to be a good producer of AgNps which remained untouched for nanoparticles production apart from being rich sources of secondary metabolites. These AgNps were proved to be powerful weapons against strong biofilm producers MDR uropathogenic *E. coil* and increase the activity of the antibiotic Levofloxacin when combined with AgNPs of MIC (7.1825 µg/ml).

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