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Antibacterial Activity of Synergistic Effect of Colicin and Gold Nanoparticles Against *Pseudomonas Aerugensa*

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Abstract

Fifty of urine samples were collected from patients with urinary tract infection (UTI). The samples were collected from AL- Yarmuk hospital in Baghdad. All of the isolates were diagnosed using biochemical test and vitek. The result showed that 30 (60%) isolates identified as *E.coli* from 50 urine samples. The colicinogenic isolates were determined using cup assay methods. The results showed that 10 out of 30 isolates (33.3%) were detect as colicin producers from 30 isolate identified as *E.coli* depending on the clear zone that observed against the sensitive isolate. Colicin was extracted from the efficient isolate. Colicin activity (320 U/ml) was determined by well assay method. The protein concentration (520 µg /ml) estimated by using Bradford assay. The watery extract of Chilli papers (*Capsicum baccatum*) was extracted and used it as reducing and capping agent for gold nanoparticles synthesis. The characterization of the gold nanoparticles was done by UV-Visible Spectrophotometer, Transmission Electron Microscope (TEM), and resulting spherical nanoparticles with diameter ranging between (35-70 nm). The antibacterial activity of colicin alone and gold nanoparticles alone and combination of colicin with gold nanoparticles against ten isolates *Pseudomonas aerugensa* isolated from burn samples, using tube method. The results showed that all the three treatment had antibacterial activity but the combination of gold nanoparticles and colicin is better than used each one separately.

Keywords: Colicin, gold nanoparticles, Chilli papers, antibacterial

الفعالية الضد بكتيرية للتاثير التازري للكوليسين وجزيئات الذهب النانوية ضد بكتريا الزائفة الزنجارية *Pseudomonas aerugensa*

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الخلاصة

تم جمع خمسون عينة ادرار من مرضى مصابين بألتهاب المسالك البولية في حاويات معقمة، تم اخذ العينات من مستشفى اليرموك التعليمي في محافظة بغداد. شخصت العزلات مظهريا باستخدام الفحوصات الكيموحيوية وجهاز الفايتك ، شكلت بكتيريا القولون *E.coli* ثلاثون عزلة اي ما نسبته 60% من اصل خمسين عينة. تم التحري عن العزلات المنتجة للكوليسين باستخدام طريقة اقراص الاكار Cup assay،

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أظهرت نتائج الفحص أن 10 عزلات اي نسبة (33.33٪) كانت منتجه للكوليسين اعتمادا على المنطقة الشفافة التي لوحظت ضد السلالة الحساسة من اصل ثلاثين عزلة شخصت *E.coli* . تم استخلاص الكوليسين من العزلة الكفوئة المنتجة للكوليسين. حددت فعالية الكولسين باستخدام طريقة الحفر وكانت الفعالية (320 وحدة/ مل) وتم تقدير تركيز البروتين باستخدام طريقة برادفورد، اظهرت نتائج الفحص أن تركيز البروتين يساوي (520 ميكروغرام /مل).صنعت جزيئات الذهب النانوية باستخدام المستخلص المائي للفلفل الحار، حيث استخدم المستخلص كعامل مختزل، ثم شخصت جزيئات الذهب النانوية باستخدام الطرق المعتمدة منها المجهر الالكتروني النفاذ ومطياف الاشعة فوق البنفسجية المرئية. حيث اظهرت نتاج التشخيص تكون جزيئات نانوية بشكل كروي وقطر يتراوح بين 35 الى 70 نانوميتر. تم تحديد الفعالية الضد بكتيرية للكوليسين وجزيئات الذهب الناوية كلا على حدا وكذلك الفعالية التازرية للمزج بين الكوليسين وجزيات الذهب ضد عشر عزلات من بكتريا *Pseudomonas aerugensa* وقد بينت النتائج انه لجميع المعاملات تاثيرات تثبيطية للبكتريا ولكن المزج كان له التأثير الاكبر.

Introduction

Escherichia coli is an important genus belong to the family Enterobacteriaceae that found in human and animal intestine because its share in facilitation of digestion and fermentation of food [1-2]. There are some bacteria secret substances which as a protein in nature used as a defensive mechanism against related or another genus of bacteria, from these the colicin that secret by *Escherichia coli* and these character benefit in use the colicin as antibiotic in treated some diseases and inhibition growth of some bacteria [3]. Some researcher define the colicin as a protein substance that secreted by different genus of bacteria and characterized by have bactericidal activity against other strains, and mode of action depend on specific receptor in sensitive cell for these colicin [4-5]. There are some scientist said that the colicin it's a toxic protein produce by some strain of *E .coli* and became active against related or nearby strain [6]. The colicin is a weapon that the *E .coli* uses it in competitive war against other *E .coli* or other bacteria to get nutrient [7]. Colicins are proteins that consist from three specific domains, amino-terminal translocation (T) domain, central receptor-binding (R) domain, and carboxy-terminal cytotoxic (C) domain [8-9]. The colicin particles was proteins in nature, also some colicins are composed from protein with carbohydrate or lipids but its few, for these we find the colicin particle is similar to any protein particle composed from amino acids that form small peptide chain and these peptide bind with each other to produce three dimensional shape of protein [10]. The colicin made and secreted in few amount in the bacterial cell that have plasmid only [11], but various external factors affecting the regulatory expression of colicin have been revised extensively by Cascales *et al*., in 2007 [8].

The term of nanoparticles (NPs) usually gain for the particles that have size ranging from 1-100 nanometers (nm). The raw metals have inert properties and when decrease in the sizes of the metals to the atomic level their properties changing to the benefit form [12], the nanoparticles have unique physico-chemical and biological properties which can be used in different suitably applications[13]. Certain nanopowders possess antimicrobial properties. When these powders contact cells of *E. coli*, or other bacteria species and viruses, over 90% are killed within a few minutes [14]. Due to their antimicrobial effect, nanoparticle of silver and titanium dioxide (<100nm) are assessed as coatings for surgical masks. Zinc Oxide nano particles can decrease the antibiotic resistance and enhance the antibacterial activity of Ciprofloxacin against microorganism, by interfering with various proteins that are interacting in the antibiotic resistance or pharmacologic mechanisms of drugs [15]. The application of using the gold nanoparticles in biomedical products is being developed for drug delivery, cancer therapy, diagnostic devices, biosensing, and bacterial growth inhibition [16].

Material and methods:

Bacterial isolation and identification

Fifty of urine samples were collected from patients with urinary tract infection (UTI) the samples were collected from AL- Yarmuk hospital in Baghdad. All urine Samples were collected in sterilized containers, in the laboratory within aseptic conditions; the collected samples were streaked directly on MacConkey agar and EMB agar (Himedia/India) and incubated for 24h at 37ºC. Pink colonies were picked. Further identification tests included the morphological characteristics and biochemical tests were carried out depending on Harley and Prescott in 2002, and Brenner in 2005 [17, 18].

Extraction of crude non-bound colicin [19].

After determination of colicinogenic *E. coli* using cup assay methods **[20]** the colicin extracted from efficient isolate as following:

- The overnight culture of bacterial isolates in volume 2.5 ml of nutrient broth was used to inoculate100 ml of sterile nutrient broth supplemented with 5 % glycerol and incubated in shaker incubator for 14 hrs at 37 C.
- At cell density of about $3x10^8$ cells/ ml (14 hrs. incubation of late log phase), Mitomycin- C was added at a final concentration of 2 µg / ml, and incubate in shaker incubator for another 3 hrs.
- The culture was centrifuged at 5000 rpm for 30 min in cooling centrifuge.The supernatant was taken for assay of colicin using well methods **[**21**],** and the concentration of protein was determined **[**22**].**
- Chloroform ((10 %) was added for killing any cells may be found in the supernatant. All supernatants were cultured on Brain heart infusion agar in order to confirm the absence of *E.coli* cells.

Synthesis and characterization of Gold Nanoparticles (Au NPs).

Gold nanoparticles were synthesis by green method using chilli papers (*Capsicum baccatum*) as reducing and stabilizing agent [23]. The morphological feature of gold nanoparticles identified using UV–Vis Spectral Analysis [24] and Transmission Electron Microscope (TEM) [25].

Antibacterial activity

Antibacterial activity of colicin alone and gold nanoparticles alone and combination of (colicin + gold nanoparticles) were investigated by using an tubes method [26] against ten isolates of *Pseudomonas aerugensa* that isolated from burn samples.

a. Loopfull of every isolates of *P. aerugensa* was inoculated to nutrient broth and incubated for 24 hrs at 37°C.
b. 12 tubes used for each isolate serially from (1) to (12).
c. Each tube was contained 1ml from Muller Hinton broth.
d. Put 1ml of (stock colicin or gold nanoparticles or (0.5 ml) from gold nanoparticles and (0.5ml) from stock colicin when determine the activity of synergistically activity of mixed colicin and gold nanoparticles) only in tubes that label with number (1). Transfer 1ml from tube number (1) to tube number (2) and 1ml from (2) to number (3) and continues these processes to tube number (12).
e. Inoculated each tubes with (0.1) ml of bacteria from overnight broth.
f. The positive control was made such as in (b-c-d) without inoculated the tubes by bacteria.
g. The negative control was made such as (b-c-e) without putting (crude colicin extracts, Chilli paper extract, gold nanoparticles, mixed colicin and gold nanoparticles).
h. Incubated the tubes for 24 hrs at 37°C.

The positive result (no growth) was seen after 24 hrs by comparison with positive controls.

Results and Discussion

Isolation and identification

Thirty isolates (60%) identified as *E.coli* from 50 urine sample and others were not *E.coli*. The highest percentage of *E.coli* isolation from UTI revealed that *E.coli* was the main causative agent of UTI. the *E. coli* cause 90% of the urinary tract infection[27].

Determination efficient colicinogenic *E.coli.*

The main purpose of collection and identification of *E.coli* was for determination of the efficient isolate that able to produce colicin. There are several methods can be used for screening about colicin but in this study we used cup assay method and resulting 10 out of 30 isolates identified as *E.coli* isolate (33.3%) as colicin producers according to inhibition zone resulting from these processes one efficient isolate had been selected from ten colicin producers isolates, because it gave a higher inhibition zone (23 mm) against the sensitive isolate among producers isolate.

Characterization of Synthesis Gold Nanoparticles (Au NPs).

The first method for characterization of biosynthesis gold nanoparticles was UV–Vis spectrophotometry. The Figure-1 explained all obtained results. Three UV–Vis tests were piloted in different time intervals and observed that the color changed of the gold nanoparticles with a time

progresses. In the first (30) minutes no color change with no peak observed; After four hours the color change from yellow to red with a peak showed in wave length at (552.50 nm) and absorption (0.694), after 24 hours the color converted to a ruby red and the peak detected in wave length at(550.00 nm) and absorption value (1.490). The result showed revealed that the color change play an important role in detected the formation of nanoparticles, and this was confirmed by the appearance of the peaks in conjunction with the absorbance during the time progresses. The peaks were appears gave a spectroscopic signature to form a surface plasmon resonance (SPR) of gold nanoparticle [23]. The peaks shifted not much with time from (552.50nm) to (550.00 nm) with increase in absorbance from (0.694) to (1.490) were revealed a linked point between the more reduction reaction and formation nanoparticles [28]. This study was agreement with study reported by Kumar *et al*., in 2015 [23] that when appearance the dark color confirm the formation of nanoparticles and efficient reduction the Au^3 to Au^0, another agreement work reported by Grace and Pandian in 2007 [29] that proved the color change from yellow to wine red (ruby red) indicated the formation of gold nanoparticles.

The TEM images of prepared gold nanoparticles using chilli papers extract are shown in Figure-2, and the resulting image revealed formation mainly spherical nanoparticles with diameter ranged (35-70 nm) without any aggregates and dispersed, on one hand this is due to presence of the repulsive nature of the capping and stabilizing agent coated the surface of AuNPs [23]. On the other hand biomolecule was supporting formation of spherical shape [30].

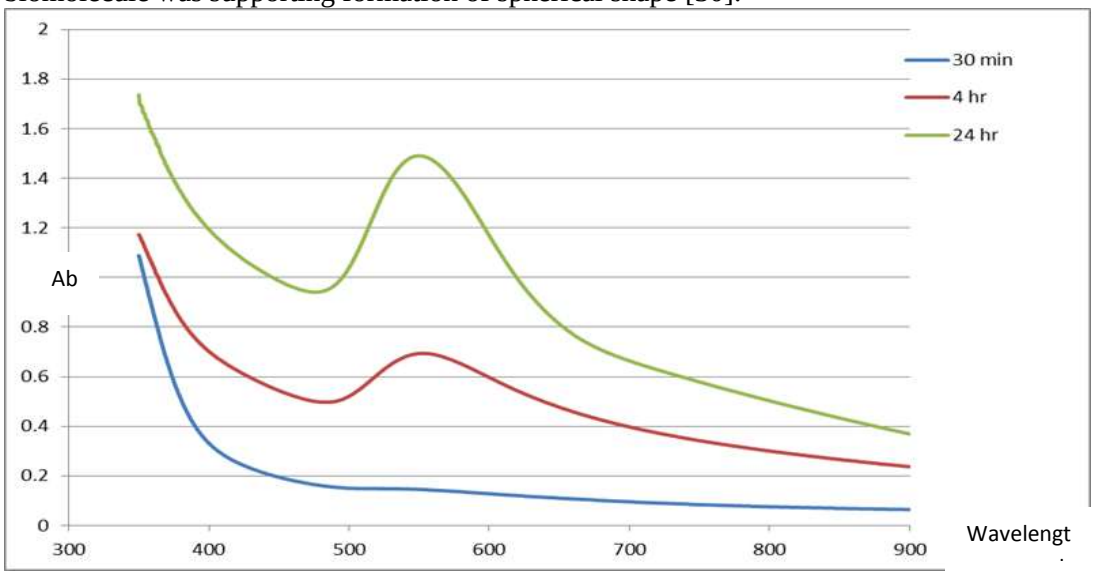

Figure 1- UV–Vis spectrophotometry of gold nanoparticles synthesis by chilli papers extract

Figure 2- Transmission electron microscopic (TEM) images of Au NPs Synthesized using chilli papers extract**.**

Determination of Antibacterial activity of colicin, gold nanoparticles, combination of (colicin + gold nanoparticles) against *Pseudomonas aerugensa* isolated from burn samples, using tube method.

The activity of colicin against *Pseudomonas aerugensa* bacteria showed some isolates of showed a resistance against colicin and this was clearly appeared in isolates (P3 and P9) as in Table-1 . While the remaining isolates affected by different colicin concentration. The highest colicin activity (32 U/ml) where appeared at colicin concentration (16.25 µg/ ml) against (P2) isolate.

The recent work revealed the crude extract of colicin extraction from producer *E.coli* showed a wide activity spectrum on other gram negative bacteria in different concentrations, this was because the colicin active against related or nearby strain [6]. The colicins produced by some isolates of *E.coli* are effective against many species of Gram negative and some Gram positive bacteria [31]. The agreement study was reported that the colicin had the ability of inhibiting the growth of one or more species and/or strains of pathogenic Enterobacteriaceae [32].

Table 1-Antimicrobial effect of crude colicin extracted from *E. coli* against *Pseudomonas aerugensa* isolated from burn samples, using tube method.

Isolates		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Dilution	Protein conc. µg/ ml	(-) Growth / (+) No growth									
1/2	260	+	+	_	+	+	+	+	+	_	+
1/4	130	+	+	_	_	+	+	+	_	_	+
1/8	65	+	+	_	_	+	+	+	_	_	+
1/16	32.5	+	+	_	_	_	_	_	_	_	_
1/32	16.25	_	+	_	_	_	_	_	_	_	_
1/64 to 1/4096	8.125	_	_	_	_	_	_	_	_	_	_
Activity U/ml		16	32	0	2	8	8	8	2	0	8

The gold nanoparticles had antibacterial activity against *Pseudomonas aerugensa*. The Table-2 revealed Gold nanoparticles give activity against *Pseudomonas* only at high concentration (1395, 697.5, and 348.75 ng/ ml), with highest activity (8 U/ml) at concentration (348.75 ng/ ml) as in isolates (P5 and P9).

Table 2- Antimicrobial effect of Gold Nanoparticle against *Pseudomonas aerugensa* isolated from burn samples, using tube method.

Isolates		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Dilution	Gold Nanoparticles conc. ng/ ml	(-) Growth / (+) No growth									
1/2	1395	+	+	+	+	+	+	+	+	+	+
1/4	697.5	+	_	_	+	+	+	+	+	+	+
1/8	348.75	_	_	_	_	+	_	_	_	+	_
1/16 to 1/4096	174.38	_	_	_	_	_	_	_	_	_	_
Activity U/ml		4	2	2	4	8	4	4	4	8	4

There are several studies proved that the green synthesis gold nanoparticles had antibacterial activity. The agreement study found antibacterial effect of green synthesis gold nanoparticles against *Pseudomonas aeruginosa* and *Staphylococcus aureus* [33]. The mechanisms that the gold nanoparticles effected on bacteria described by many studies, they mentioned that the effect of nanoparticles may be interaction with the cell wall of bacteria that lead to the formation of pores in these walls [33-35].

The activity of the combination colicin and gold nanoparticles against *pseudomonas aerugensa* showed in Table -3 and showed the significance activity compared with the activity of colicin alone or gold nanoparticles alone in same bacteria. The higher activity of combination in this bacteria it was (512 U/ml) as in isolates (P6 and P7).

Table 3- Synergistic effect of colicin and Gold Nanoparticles against *pseudomonas aerugensa* isolated from burn samples, using tube method.

Isolates			**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
Dilution	**Protein conc. µg/ ml**	**Gold Nanoparticles conc. ng/ ml**	**(-) Growth / (+) No growth**									
1/4	**130**	**697.5**	+	+	+	+	+	+	+	+	+	+
1/8	**65**	**348.75**	+	+	_	+	+	+	+	+	+	+
1/16	**32.5**	**174.38**	+	+	_	+	+	+	+	+	_	+
1/32	**16.25**	**87.19**	+	+	_	+	+	+	+	+	_	+
1/64	**8.125**	**43.59**	+	+	_	+	_	+	+	_	_	+
1/128	**4.06**	**21.81**	+	+	_	_	_	+	+	_	_	+
1/256	**2.03**	**10.91**	+	+	_	_	_	+	+	_	_	_
1/512	**1.015**	**5.45**	_	_	_	_	_	+	+	_	_	_
1/1024 to 1/8192	**0.507**	**2.73**	_	_	_	_	_	_	_	_	_	_
Activity U/ml			**256**	**256**	**4**	**64**	**32**	**512**	**512**	**32**	**8**	**128**

The colicin had antibacterial activity as well as gold nanoparticles, and when the two components were mixed the synergic potential was appears instead of using each one separately. The higher activity was observed against target bacteria in the combination of recent study due to the gold nanoparticles enhanced the activity of colicin by conjugation with each other. They are several studies proved that the antimicrobial activity were increased when using combination of gold nanoparticles with antibiotics, antibodies, and probiotic rather than used nanoparticles alone or antibiotic alone [36-37-38].The combination of gold nanoparticles with vancomycin showed potent inhibitor growth of *E.coli* [39]. The antimicrobial activity of vancomycin, amoxicillin and penicillin G increased when combination with nanoparticles [40].

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