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Antibacterial Activity of Synergistic Effect of colicin and Gold Nanoparticles against *Klebsiella pneumonia*

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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 Klebsiella pneumonia isolated from urine samples, using tube method.

Keywords: *Colicin, gold nanoparticles, Chilli papers, antibacterial*

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 byCascales*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

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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].

MATERIALS AND METHOD

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 onHarleyand Prescott in 2002,andBrenner in 2005 [17,18].

Extraction of crude non-bound colicin [19].

Afterdetermination of colicinogenic*E. coli*using cup assay methods **[20]** the colicin extracted from efficient isolateas 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*Klebsiella pneumonia* that isolated from burn samples.

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 selectedfrom 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 that when appearance the dark color confirm the formation of nanoparticles and efficient reduction the to.

Determination of Antibacterial activity of colicin, gold nanoparticles, combination of (colicin + gold nanoparticles) against *Klebsiella pneumonia* isolated from urine samples, using tube method

The activity of colicin against 10 isolates of ***Klebsiella pneumonia***is shown in table (1), and showed the different concentration of colicin has antibacterial activity for different strains of *Klebsiella pneumonia*. The highest colicin activity appears at concentration (16.25 µg/ ml) with activity 32U/ml against two strains (K1and K3). 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. The table (2) showed the affected of *Klebsiella pneumonia* by gold nanoparticles, and you will see the higher concentration of gold nanoparticles(1395 ng/ ml) inhibit growth all strains, and the higher activity of gold nanoparticles was (1024 U/ml) at concentration (2.73 ng/ ml) as in isolates (K1and K2). The table (3) represents the activity of the combination against *Klebsielapneumoniae* and shows that most of the isolates affected by different concentration of combination.

Table 1. Antimicrobial effect of crude colicin extracted from *E. coli* against *Klebsiella pneumonia* isolated from urine samples, using tube method.

Isolates		**K1**	**K2**	**K3**	**K4**	**K5**	**K6**	**K7**	**K8**	**K9**	**K10**
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 1/1024	8.125 to 0.507	_	_	_	_	_	_	_	_	_	_
Activity U/ml		32	2	32	16	16	16	8	4	4	8

Table 2. Antimicrobial effect of Gold Nanoparticles Against *Klebsiella pneumonia* isolated from urine samples, using tube method.

Isolates		K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Dilution	GoldNanoparticles conc. ng/ ml	(-) Growth / (+) No growth									
1/2	1395	+	+	+	+	+	+	+	+	+	+
1/4	697.5	+	_	+	+	+	+	+	+	+	+
1/8	348.75	+	_	_	_	_	_	_	_	+	_
1/16	174.38	+	_	_	_	_	_	_	_	+	_
1/32	87.19	+	_	_	_	_	_	_	_	+	_
1/64	43.59	+	_	_	_	_	_	_	_	+	_
1/128	21.81	+	_	_	_	_	_	_	_	+	_
1/256	10.91	+	_	_	_	_	_	_	_	+	_
1/512	5.45	+	_	_	_	_	_	_	_	+	_
1/1024	2.73	+	_	_	_	_	_	_	_	+	_
1/2048 to 1/4048	1.36 to 0.68	_	_	_	_	_	_	_	_	_	_
Activity U/ml		1024	2	4	4	4	4	4	4	1024	4

Table 3. Synergistic effect of colicin and Gold Nanoparticles against *Klebsielapneumoniae* isolated from urine samples, using tube method.

Isolates			K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Dilution	Protein conc. µg/ ml	GoldNanoparticles 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	0.507	2.73	+	_	_	_	_	_	_	_	+	_
1/2048	0.253	1.36	+	_	_	_	_	_	_	_	_	_
1/4096 to 1/8192	0.126 to 0.063	0.68 to 0.34	_	_	_	_	_	_	_	_	_	_
Activity U/ml			2048	4	256	64	256	64	32	128	1024	64

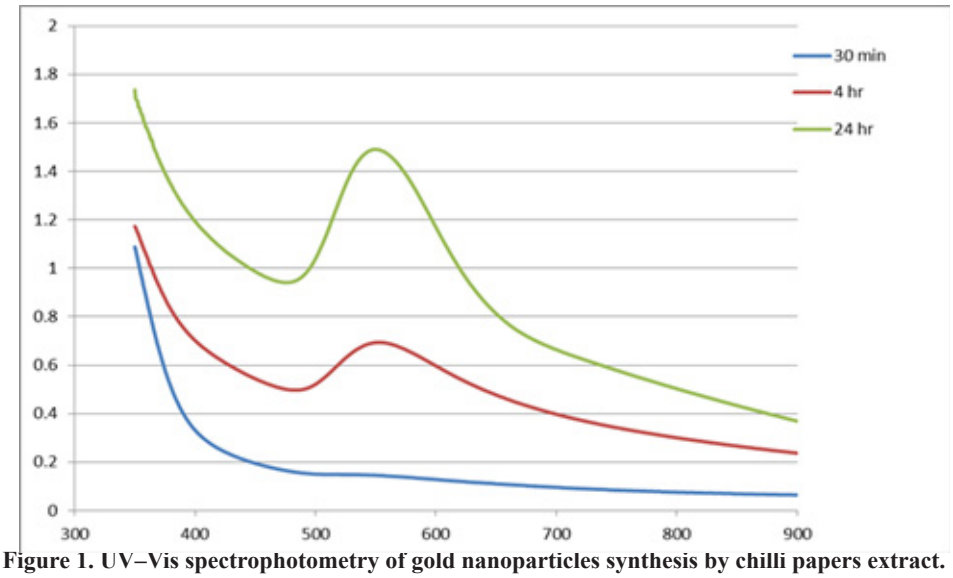

Figure 1. UV–Vis spectrophotometry of gold nanoparticles synthesis by chilli papers extract.

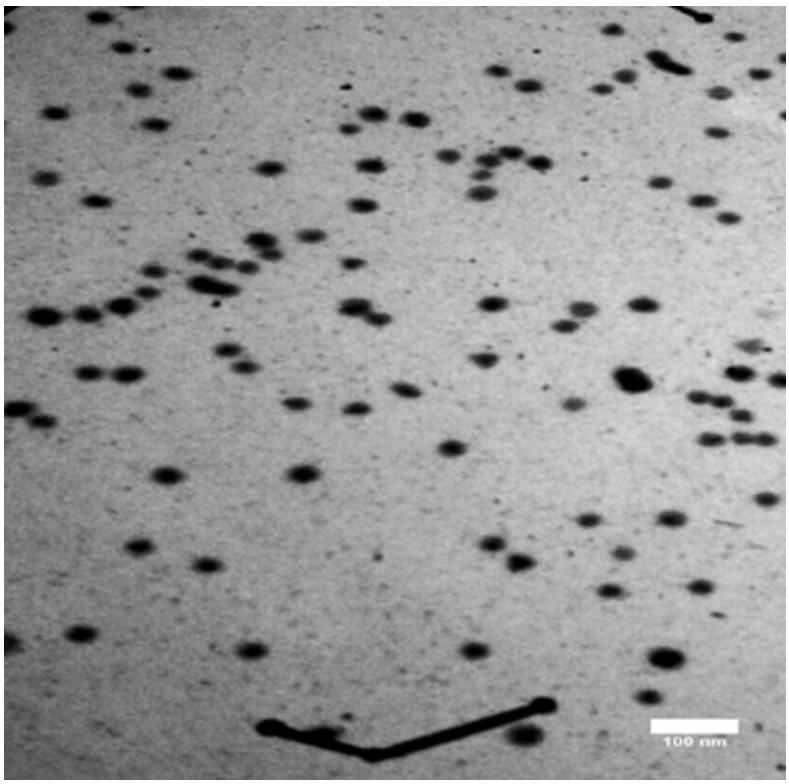

Figure 2. Transmission electron microscopic (TEM) images of Au NPs Synthesized using chilli papers extract.

CONCLUSION

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 Klebsiella pneumonia isolated from urine samples, using tube method.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Biology, College of Science, University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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