

## Inhibitory Effect of Cobalt Oxide Nanoparticles with Biosynthetic Licorice Against Bacterial Species Isolated

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### Abstract

Conclusion 120 management samples were collected for samples from patients of Samarra Hospital and some clinics in Salah Al-Din Governorate / Samarra for the period from January 2023 until the end of June 2024, were diagnosed based on a set of phenotypic characteristics of the developing isolates and biochemical tests, distributed over 20 samples. The results of the diagnostic tests were that the bacteria coli Escherichia, which are 35 isolates, i.e. (43.75)%, while the bacteria Klebsiella spp appeared by 15 samples, i.e. (18.75) %, and Staphylococcus aureus bacteria appeared by 15 samples and by (18.75) %. As for the bacteria Staphylococcus.epidermidis, 10 samples appeared by (12.5)%, while the yeast Candida albicans appeared 5 samples by (6.5)% The bacterial species that have been isolated are among the most common types that cause urinary tract infection (UTI), as the bacteria that are the most present of the species that have been isolated are the bacteria coli Escherichia, followed by Klebsiella spp bacteria, Staphylococcus aureus, Staphylococcus.epidermidis and Candida albicans yeast respectively, and it was found that the plant extract with the nanomaterial cobalt oxide With licorice glabra glycyrrhiza prepared by the second method, Green synthesis nanoparticles by plant extract, is more efficient than the nanomaterial prepared by the first method, Artificial roads nanoparticle in inhibiting bacterial isolates, the proportion of E. coli bacteria was (32) mm, while S. aureus bacteria were (28) mm. And bacteria Klebsiella 31)) Extract nano-oxide cobalt with licorice, by the second green method T2)) was one of the best types of work and the effect of its inhibition is high on the bacterial species used, where it was observed to contain plant extracts The following phytochemical analysis ingredients: alkaloids, glycosides, phenols, saponins, flavonoids and tanginations. Successful use of plant extract Nanocomposites cobalt and glabra glycyrrhiza in the treatment of infections caused by urinary tract infection caused by E. coli bacteria with cobalt oxide extract with licorice. Then tests were carried out for cobalt oxide nanosolutions prepared by the second method, tests were AFM, UV, FTIR, the nanomaterials were of a smaller size of 5–12nm called quantum dot, and no agglomeration occurred, it was found that the MTT, cytotoxicity assay for Nanocomposites cobalt solutions used to inhibit bacterial species showed that the substance is non-toxic to the solution for the treatment of infections caused by Escherichia coli bacteria and Staphylococcus aureus.

**Keywords:** *Cobalt Oxide Nanoparticles, Biosynthetic Licorice, Bacterial Species*

## 1. INTRODUCTION

Urinary tract infections are one of the most important health problems suffered by many people around the world, urinary tract infection (UTIs) is infected with all age groups, including males, females and the elderly, urinary tract infections (UTIs) are bacterial diseases that affect the urinary system, kidneys, bladder and prostate, urinary tract infections are common all over the world, and have direct and indirect social and economic effects. It is thought that urinary tract infections may affect approximately 160 million individuals worldwide each year. Urinary tract infections cause nearly one million emergency room infections and more than 100,000 hospitalizations in the United States each year, bacteria being the leading and most common cause of urinary tract infections, although viruses, fungi and parasites may also play a role in infection [1]. Gram-negative bacteria cause 90% of urinary tract infections, while Gram-positive bacteria cause about 10%. *E. coli* bacteria are the most common cause of urinary tract infections, accounting for 65-90% of cases, types of enterococci, *Klebsiella pneumoniae*, types of *Citrobacter*, *Pseudomonas aeruginosa*, and *Staphylococcus* are also causing urinary tract infections, frequent urination, pain when urinating, back pain, dysuria and stomach pain are all indicators of a urinary tract infection or inflammation, the presence of bacteria in the urinary system is sometimes associated with the absence of symptoms[2] and the degree of complexity is associated with Injury depending on the presence of a structural or functional abnormality Nanobiotechnology is a new discipline and one of the important and cutting-edge sciences and the use of nanoparticles (NPs) ranging in size from 1 to 100 nm in scientific studies is an important step in the development of science and medicine,. Nanoparticles (NPs) have been widely studied due to the unique physical and chemical important properties, such as antibacterial capabilities, catalytic activity, optical properties, electrical properties, magnetic properties, Nano biotechnology is a new and important topic in nanoscience. It combines nanotechnology intelligence, biosynthesis and the design of cutting-edge molecules capable of working. Due to its biocompatibility, biodegradability, non-toxicity, adhesion, antibacterial activity, as well as its natural origin, low cost and antibacterial activity and especially against the negative and positive bacteria of the Gram tincture.

## 2. MATERIALS AND METHODS

### 2.1 Collection of the Samples

120 samples of urinary tract infection infections were collected for patients with urinary tract infection (UTI) from Samarra Hospital. Where the eyes were taken under the supervision of the specialist doctor, where it was found that 90 samples showed infections and were transferred to the hospital laboratory and were initially planted on the middle of MacConkey agar and the center of Mannitol Salt Agar and incubated at 37 ° C for 48-24 hours.

### 2.2 Diagnosis of Bacteria Isolates

#### 2.2.1 Diagnosis by Classical Methods

Bacterial isolates were diagnosed based on their culture, microscopy and biochemical tests and according to what was mentioned in the following:

#### 2.2.2 Microscopic diagnosis

Swabs were prepared from bacterial colonies growing on the medium of MacConki Acar, which showed a metallic green luster on the medium of EMB (green metallic sheen) and the medium of solid salt mannitol and dyed with Gram dye and examined under optical microscopy.

### **2.2.3 Cultivating qualities**

The culture characteristics of the isolates developed on the growing media were recorded (shape, size, color, edges and heights of colonies on the growing media).

### **2.3 Examination of the effect of the prepared scares on the growth of bacteria:**

The method of diffusion (Agar-well diffusion, method) was used, where the surface of the medium of Mueller-Hinton Media was inoculated by 0.1 ml of the stuck-containing bacteria that was disseminated by the sterile culture diffuser and then holes were made in the middle by a sterile cork piercer with a diameter of 5 mm and 6 pits per dish. Where the prepared scare of the plant extract was placed by 0.1 ml in each hole with the use of one of these pits as a control pit to which 0.1 ml of sterile distilled water was added, the dishes were incubated at a temperature of 37 ° C for 24 hours to observe the growth inhibition areas around these pits and the diameters of the growth inhibition areas were evaluated in mm [3] .

### **2.4 The green method of preparing cobalt oxide CoO**

#### **2.4.1 A preparation of licorice extract *Glabra glycyrrhiza***

The weight of 20 g of locally grown licorice was taken and dissolved in 200 ml of distilled water and then placed on the magnetic stirrer mixer for an hour at a temperature of 30 ° C, then the temperature was gradually raised to 70 ° C with the spiral movement using the magnetic electrode until it dissolves and then filtered using cotton inside a glass funnel and the process was repeated a second time after completing the volume of water to 200 ml and transferred to the magnetic mixer and a sample of the solution was taken with a test tube to compare the color until it settles for an hour or more depending on the solution by following the precipitate if it is present until it disappears and the color by looking at the light passing through the tube [4]

#### **2.4.2 Nano tests for prepared solutions:**

The following tests were done for all solutions and the tests were carried out in the laboratories of biotechnology / Al-Nahrain University, the description of the prepared nanoparticles:

1. AFM is a test used to study surfaces at the Nano scale level, which gives a three-dimensional image of the Nano properties of the Nano abstract, using surface analysis, the study of nanomaterial's to measure the properties of the material such as strength and hardness.
2. FT-IR (Fourier transform infrared spectroscopy) infrared furometer is a test for examining and identifying effective chemical bonds [5]
3. UV (UV-visible analysis) UV visible spectrophotometer is a transmittance and absorption coefficient test and determines the energy gap of strong charges [6]

## **3.RESULTS and DISCUSSION**

### **3.1 Sample collection and diagnosis:**

120 samples were collected for samples from patients of Samarra Hospital and some clinics in Salah Al-Din Governorate / Samarra, about 20 samples were neglected, due to incomplete data, in addition

to other interventions such as urinary tract infection, where work was done on 100 samples distributed to two age groups from 15 years to 30 years and from 30 to 50 years for the period from January 2023 until the end of June 2024. The swabs were planted on the middle of the maconki and the middle of the blood acar, then incubated for 24 hours at room temperature, and the isolates were diagnosed depending on a set of phenotypic characteristics of the developing isolates and biochemical tests, distributed over 20 samples, i.e. 20% of the urinary tract infection isolates in which no bacterial growth occurred (Nigative UTI), while the 80%, i.e. 80%, occurred in which there was a bacterial growth (Positive UTI), i.e. the presence of infection Studies indicate that urinary tract infection is one of the diseases with simple, complex and common societal effects. and widespread among all age groups has confirmed many researches [7]

Urinary tract infection (UTI) is a global problem of importance in most countries, where it was found that different age groups are infected with urinary tract infection at different periods of life and in the proportions mentioned that the detection of infection early helped in limiting the disease from progressing in addition to the possibility of treatment and providing primary health care [8] Studies indicate that urinary tract infections (UTI) are the most common type of infection, as the incidence ranges on Length of life between 50-60 in adults [9] Research has confirmed that urinary tract infections (UTI) are a problem of global importance in many countries, with infection rates found to be high in more developed societies with health care priorities for urinary tract infections.

(- Healthcare HAUTIs) associated UTIs) especially for the injured, research into the pathogens, early detection of the causes of the UTI, investigation of appropriate treatment for the cause of injury and improvement thereby mitigating the damage caused [10] about the progression of the disease Activate Window, the results of diagnostic tests showed that the bacteria coli Escherichia, which is 35 isolates, i.e. (43.75)%, while the bacteria Klebsiella spp appeared by 15 samples, i.e. (18.75)%, and Staphylococcus aureus bacteria appeared by 15 samples and by (18.75) %, while the bacteria *Staphylococcus.epidermidis* appeared 10 samples by (12.5)%, while the yeast *Candida albicans* appeared 5 samples by (6.5)%. The bacterial species that have been isolated are among the most common types that cause urinary tract infection. UTI), where the most common bacteria of the isolated species are coli Escherichia, followed by *Klebsiella* spp, *Staphylococcus aureus*, *Staphylococcus.epidermidis* and *Candida albicans* yeast.

Respectively, the reason for the presence of these bacterial types increases with age, as many studies have confirmed that urinary tract infection abounds in adults more than young people, and there are various factors for this change, including therapeutic catheterization, in addition to various infections, kidney stones, weak immunity, or a urological surgery procedure, either *Staphylococcus.epidermidis* bacteria return to their presence on the skin and may lead to weak immunity, thus creating the opportunity to move to the urethra and multiply in the bladder and then cause inflammation. The results coincided with the findings of the study [11] which indicated a high incidence rate in adults over the age of 30 years, urinary tract infection is increasing, as the prevalence of urinary tract infection in men reached approximately 20% compared to about 12% in the general population and between 30-40% of men will suffer from urinary tract infection at least once in their lives in the [12] included 108 samples, the percentage of urinary tract infection for the age groups of 30-40 years was about 60%, while 40% did not show urinary tract infection, for age groups less than 30 years or appeared in a very small percentage and caused by lack of health awareness either bacteria,

*Staphylococcus aureus* One of the reasons for the presence of these bacteria is their natural presence in the human body or is a natural flora, so the occurrence of any defect causes its transformation into inflammation that the presence of these types *Escherichia coli* and *Staphylococcus aureus* and *Klebsiella* and are found on the skin specifically in wet places and enter the tissues through scratches and wounds in addition to the invasion of these types of urinary tract where these types work with their virulence factors and enzymes to affect the physiological function of the urinary system causing inflammation[13].

*Candida albicans* are more common in women, while men are affected by prostatitis, all the negative intestinal types of Cram dye, which are found within the normal flora of the intestine. It is often one of the types resulting from fecal infections, and this type of infection is very common in such places[14] as indicated by a study to increase the incidence of urinary tract infection in general with age [15]

**Table (1) Proportions and isolated numbers of urinary tract infection (UTI)**

No	Type of Bacterial	Number of isolates	Percentage %
1	<i>Escherichia coli</i>	35	43.75
2	<i>Klebsiella pneumoniae</i>	15	18.75
3	<i>Staphylococcus aureus</i>	15	18.75
4	<i>Staphylococcus epidermidis</i>	10	12.5
5	<i>Candida albicans</i>	5	6.30
Total		80	100

Table (1) shows the effect of different scarification of the nanomaterial cobalt nitrate with licorice, where the first and second methods showed the extent of the effect of scare on the inhibition of bacterial species as shown where *E.coli* gave to fear (100,75,50,25) in the first method (T1)) as a result of inhibition respectively (26,23,19,11) and gave in the second method (T2)) (32,25,24,21) As for *S.aureus* bacteria, they gave in the first method (T1) respectively also (23,20,17,14) and gave in the second method (T2) (28,28,24,22) while *k.peneomoniam* bacteria gave results in the first method (T1) respectively (18,14,11,10) The second method (T2) was (31,30,27,23) and gave *S.epedermids* bacteria in the first method (T1) respectively to scare the same result (15,13,10,7) The second method (T2) had a result (31,29,28,27) The result of *C.albicans* yeast was in the first method (T1) respectively (22,20,13,9) The second method had a result of (32,31,30,28) Through the previous results, the inhibitory effect of the studied nanoscale extract on bacterial species isolated from urinary tract infection,[16] and this effect varied according to the concentrations used, and the concentration of 100 mg / ml in the first method T1 was the most effective compared to other concentrations of the bacterial type *E.coli*, which had an effect with different ranges on the growth of the germ, while the second method T2 green effect was greater and this effect shows the effectiveness of the nanoextract on the

bacterial type. , followed by a concentration of 75%, then a concentration of 50%, and a concentration of 25%, respectively, it is noted that the concentration of 100% was the highest effect on the inhibition of bacterial species [17] The researcher [18] pointed to the inhibitory effect of both plant extract licorice towards bacterial. Our result agreed with the researcher [19], who pointed to the inhibitory activity of G Glycyrrhiza extract The reason for the sensitivity of the isolation can be attributed to the absence of its outer phospholipid membrane, which facilitates the entry of oil compounds present in plant extracts may cause an increase in permeability with the penetration of ions from vital internal cellular components that eventually lead to damage to the enzyme system of bacteria Arabkhani et al. 2022)). that there is a clear variation in the level of inhibition between plant extracts compared to antibiotics used, as the levels of antibody effect exceeded the inhibitory action of the plant extracts mentioned [20] As for the bacteria *S.aureus*, the second green T2 method had a higher effect than the first chemical method T1 as shown in Table (1), where the highest inhibition rate towards this type was reached at a concentration of 100% attributable to the inhibitory activity on bacterial species due to the disruption of membrane lipids and thus the leakage of cell contents to the outside and its death, and cobalt nitrate can generate hydrogen peroxide, which leads to cell death As for the first method, T1, the inhibition ratio at the same concentration was lower than the second green method, and the effectiveness of the nanoscale is shown by the green method [21]

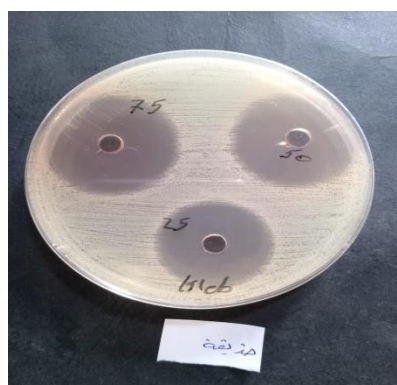
As for the bacteria *k.pneonia* between Table (1) that the second method T2 green higher impact than the first chemical method T1 where the highest inhibition rate towards this type at a concentration of 100% and note that this concentration is the best concentration is inhibited and that the bacterial species are affected by it, that the work of the nanoextract with this effectiveness indicates its success and the strength of its effect in bacterial species, it has also been proven that licorice nanobacterial effect by weakening the membrane Bacterial whatever its condition in relation to the dye of Cram leading to its degradation [22] As for the bacteria *S.epedermids*, the result in Table (1) is that the second green T2 method is also more effective than the first chemical method T1, where the highest inhibition rate towards this type was reached at a concentration of 100%, and note that this concentration is the best concentration in which inhibition is carried out, and the Perera et al 2020 study indicates that the degree of effectiveness depends on the size, so the nanomaterial gives inhibition and Mohsen results. [23] *C.albicans* show the effect of plant nanoextracts used in the study on the growth of Candida The results showed that the inhibitory efficacy of 100 mg / ml of the nanoextract showed high inhibitory efficacy as shown in Table (1) that the nanoextract in the second method showed that its effect is high and clear inhibitory effect on Candida albicans. In another study, the alcoholic extract of licorice plant has an inhibitory effect on fungi [24] Previous studies showed that the effectiveness of licorice is due to the active compounds found in the plant, as the licorice plant consists of coumarin compounds and isoflavones and contains more than 300 types of polyphenolic compounds, triterpene saponins and various types of flavonoids [25] Licorice has also been shown to contain polysaccharides, simple sugars, pectins, amino acids and minerals such as mercury, lead and cadmium, and various sources have confirmed that licorice roots have antibacterial, antiviral, anti-fungal, anti-inflammatory and anti-cancer efficacy [26].

**Table (2) Effect of different scavenging of nanomaterial by the first and second methods on inhibition of the growth of cobalt nitrate bacteria.**

Method	concentration	Diameter of retarding zone mm				
		<i>C.albicans</i>	<i>S.epedermids</i>	<i>k.peneonia</i>	<i>S.aureus</i>	<i>E.coli</i>
Control	H2O	0	0	0	0	0
T1	100	22	15	18	23	26
	75	20	13	14	20	23
	50	13	10	11	17	19
	25	9	7	10	14	11
Control	H2O	0	0	0	0	0
T2 glabra Glycyrrhiza	100	32	31	31	28	32
	75	31	29	30	28	25
	50	30	28	27	24	24
	25	28	27	23	22	21

**T1: Cobalt nitrate + sodium hydroxide (first method)**

**T2: Cobalt nitrate + licorice (method 2)**





**Figure (1)** Effect of different fear of nanomaterial on bacterial species Cobalt oxide

#### 4.CONCLUSIONS

*Escherichia coli*, *S. aureus*, and *Klebsiella* spp. were among the most commonly isolated pathogens in patients with urinary tract infections (UTI). Nanoparticles synthesized by green synthesis using plant extract were more effective than nanoparticles synthesized by chemical methods in inhibiting bacterial isolates. Nanoplant extracts contain chemical compounds that act as reducing and stabilizing agents for nanoparticle synthesis.

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