



## Evaluation of inhibitory and plasmid curing activity of *Citrus aurantium* L fruit extracts against some bacteria isolated from urinary tract infections

<sup>1\*</sup>Ahmad Abdalrazaq Ebrahim Alnuimi, <sup>2</sup>Nawar Talal Hamid Alsaffawi

<sup>1\*,2</sup>Department of Biology, Education College for Pure Sciences, University of Mosul, Mosul, Iraq

E-mail: <sup>1\*</sup> [ahmadalnuimi4@gmail.com](mailto:ahmadalnuimi4@gmail.com), <sup>2</sup> [nawar9779@uomosul.edu.iq](mailto:nawar9779@uomosul.edu.iq),

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### Abstract

In this study, the sensitivity and resistance of six types of bacterial isolates, *Escherichia coli*, *pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Proteus mirabilis*, and *Klebsiella pneumoniae*, against 10 antibiotics were tested for 10 antibiotics, Ciprofloxacin (CF) 25 g / ml Amoxicillin (AX) 50 g / ml, Ampicillin (Am) 50 µg / ml, Erythromycin (Ery) 15 µg / ml, Gentamycin (GN) 25 g / ml, Rifampicin (RA) 50 µg / ml, Streptomycin (Sm) 25 µg / ml, Tetracycline (TE) 15 µg / ml Trimethprim (Tri) 10 µg / ml Nalidixic acid (Nal) 30 µg / ml. The bacterial isolates showed different resistance to most antibiotics. One isolate of each bacterium was selected for subsequent experiments. The aqueous and ethanol extracts of *Citrus aurantium* L fruit were prepared. The inhibitory effect of the prepared plant extracts on the selected bacteria was investigated by using the Agar diffusion method and the Turbidity method. The prepared plant extracts showed a clear growth inhibitory activity, which was estimated by measuring the inhibition zone with a millimeter of studied bacteria. The results obtained showed that the value of the growth inhibitory activity increased with increasing the concentration of the extracts. Sub minimum inhibition concentration of plant extracts was used as a curing to remove antibiotic resistance of the bacterial species under study. Varying values for the percentage loss of antibiotic resistance were obtained as a result of the efficacy of the extracts, but some extracts did not show curing efficacy. The results of the curing experiments were supported by the characterization of the plasmid DNA on the agarose gel of the curing isolates. Electrophoresis showed the disappearance of the plasmid DNA bundles from the agarose gel for most of the bacteria under study compared to the non-curing isolates.

**Keyword:** Bacteria Urinary tract infections, Plasmid DNA, *Citrus aurantium* L extracts

تقييم الفعالية التثبيطية والتحييد البلازميدي لمستخلصات ثمار النارنج لبعض البكتريا المعزولة من التهابات  
المجاري البولية

<sup>1\*</sup>، احمد عبد الرزاق إبراهيم النعيمي، <sup>2</sup>نوار طلال حامد عزيز الصفاوي

<sup>2\*,1</sup> قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق

#### الخلاصة:

في هذه الدراسة تم اختبار حساسية ومقاومة ستة انواع من العزلات الجرثومية وهي *Escherichia coli*، *pseudomonas aeruginosa*، *Staphylococcus aureus*، *Proteus vulgaris*، *Proteus mirabilis* و *Klebsiella pneumoniae* تجاه 10 مضادات حيوية وهي Ampicillin (Am) 50 µg / ml، Amoxicillin (AX) 50 g / ml، Ciprofloxacin (CF) 25 g / ml، Erythromycin (Ery) 15 µg / ml، Gentamycin (GN) 25 g / ml، Rifampicin (RA) 50 µg / ml، Nalidixic acid، Trimethprim (Tri) 10 µg / ml، Tetracycline (TE) 15 µg / ml، Streptomycin (Sm) 25 µg / ml و (Nal) 30 µg / ml. اظهرت العزلات الجرثومية مقاومة متباينة لأغلب المضادات الحيوية وتم اختيار عذلة واحدة من كل نوع من الجراثيم لأجراء التجارب اللاحقة. حضرت المستخلصات المائية والايثانولية لثمار نبات النارج *Citrus aurantium* L. fruit. تم التحري عن التأثير التثبيطي للنمو للمستخلصات النباتية المحضرة على انواع الجراثيم المختارة وذلك باستخدام طريقة الانتشار بالأكار Agar diffusion method وطريقة قياس العكارة Turbidity method. المستخلصات النباتية المحضرة اظهرت بشكل واضح فعالية مثبطة للنمو تم تقديرها بقياس منطقة التثبيط بللم للجراثيم المدروسة. ووضحت النتائج التي تم الحصول عليها ان قيمة الفعالية المثبطة للنمو تزداد بزيادة تركيز المستخلصات. التركيز تحت المثبط الادنى (Sub-MIC) Sub minimum inhibition concentration للمستخلصات النباتية استخدم كعامل محيد لإزالة مقاومة المضادات الحيوية للأنواع الجرثومية قيد الدراسة. تم الحصول على قيم متباينة لنسبة فقدان المقاومة للمضادات الحيوية كنتيجة لفعالية المستخلصات لكن لم تظهر بعض المتخلصات فعالية محيدة.

**الكلمات المفتاحية:** بكتريا المجاري البولية، الدنا البلازميدي، مستخلصات النارج.

#### Introduction:

Urinary tract infections (UTI) are the most common type of bacterial infection worldwide. During 2016, it is estimated that 150 million cases are recorded annually worldwide [1]. Infection occurs in both sexes and all ages, and females are more susceptible to infection than males due to the anatomical and physiological differences of the urogenital system in addition to the difference in lifestyle, and the incidence of infection increases in the elderly men. And when the infection occurs, different parts of the urinary system may have the infection [2]. Microbial infection, especially with the following species *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* are among the main causes of UTIs [3,4]. As it leads to the emergence of various signs and symptoms, including pain when urinating, dysuria, bloody urine, heartburn, frequent urination, nausea and vomiting [2]. As a result of the emergence of multidrug-resistant bacterial strains, the incidence of urinary tract infections has increased dramatically and this disease has become a major social and economic burden worldwide [2]. Natural herbs are highly effective in removing bacterial resistance with high efficiency, and they are available and cheap in addition to the side effects are very little or no. So, it has attracted the interest of researchers interested in exploring the treatment of UTIs with medicinal plants. There is a need for more discoveries in order to reveal the plant components responsible for treating urinary tract diseases and their mechanism of action [2]. *Citrus aurantium* belongs to the family of *Citrus aurantium* (Sour orange), and its scientific name is *Citrus aurantium*. It also follows the genus *Citrus*, which contains four economically important groups, the most important of which is the orange group of *Citrus aurantium*. [5]. Also known as Seville orange, sour orange, or bitter orange, *Citrus aurantium* is a small tree about five meters high with scented white blossoms [6, 7]. Citrus fruit originated

in East Africa, Arabia and Syria, and is cultivated in Spain, Italy and North America [6,7]. *Citrus aurantium* is a very common plant that is available and cheap in Diyala Province / Iraq [8].

**Medical part** the following parts of *Citrus aurantium* are used for medicinal purposes (flowers, fruits, roots, leaves, and essential oils) [10-9].

#### **Medicinal uses:**

Citrus fruits, peel, leaves, seeds, and essential oils are used in food, perfumes, medicinal and cosmetic applications, as well as in the food, confectionery, and perfume industry (11). They are widely used in nutritional supplements to control appetite, weight, athletic performance and energy. Citrus products are also used in the form of food such as juices and bitter orange jam ((12, 11. It is believed that Citrus has the following vital activities as it is considered an antioxidant (13,14), Anti-inflammatories (15), antimicrobials (16,17), and may be responsible for preventing cancer and degenerative diseases (14). These vital activities of aurantium are due to the presence of bioactive compounds such as phenols, flavonoids, essential oils and vitamins (18,19). *Citrus aurantium* is also used in the treatment of many diseases such as anxiety (20), lung and prostate cancer (21), digestive disorders and obesity (22,23). *Citrus aurantium* is rich in natural vitamin C and antioxidants that increase the activity of the immune system (24,25). *Citrus aurantium* is traditionally used to treat the intestines and some disorders (such as constipation, cramps, diarrhea and abdominal cramps), respiratory tract convulsions (such as cough, cold, bronchitis and tuberculosis), obesity, menstrual disorders, cardiovascular disease (angina, high blood pressure) and Stress and depression (26).

For the purpose of determining the effectiveness of the Citrus fruits on antibiotic-resistant bacteria, the study was designed to:

- ❖ study the sensitivity and resistance of isolated species to antibiotics

study the inhibitory and curing effect of *Citrus aurantium* extracts on bacteria under study and Characterization of the plasmid DNA content by using gel electrophoresis technique of bacterial species before and after treatment with Citrus fruit extracts.

#### **Research Method**

Citrus fruits were obtained from the local market in Mosul.

#### **Antibiotic resistance and sensitivity of isolated bacteria:**

The medium was prepared by adding the appropriate antibiotic to the sterilized agar nutrient medium cooled to (45-50 °C) and the final concentration ( $\mu\text{g/ml}$ ) which prepared stock solutions for antibiotics according to the method used by [27] by dissolving a certain amount of the antibiotic in the appropriate solvent, then sterilizing all the stock solutions by filtering (0.22). micrometer) and kept until use at a temperature of - 20 °C, according to [28].

#### **Preparing of plant extracts:**

#### **Preparation of aqueous extracts:**

An electric grinder was used to crush the Citrus fruits, as 40 gm of the ground fruit were added to 160 ml of sterile distilled water, so that the ratio became (4: 1) weight: volume. Then crushed the plant under study using the Grater3, Moleny-supplied Blender Crusher in an ice bath for one hour, after which using the electric magnetic stirrer stir the mixture for 60 minutes in order to detonate the plant cell walls for the purpose of soaking. Leave the mixture in the refrigerator for 24 hours, use Several layers of gauze to filter the mixture, then filter the mixture by using a Buechner funnel and using Whatmann No.1 filter paper, thus preparing the aqueous plant extract. In airtight glass bottles and in an atmosphere free of moisture, the samples were preserved by freezing for later use [29].

### **Preparation of alcoholic extracts:**

Prepared according to the method of the researcher [30]. And modified according to the method of the researcher [31]. Dissolve in 400 ml of ethyl alcohol at a concentration of 95% 40 g of the vegetable form in an ice bath, then shake the mixture well and leave in the refrigerator for 24 hours, after which it was filtered through a Buechner funnel and then placed in the Rotary Vaccume Evaporator which is provided by Electrothermal. The device is based on evaporation under vacuum pressure and a temperature of up to 40 ° C. The layer formed after evaporation from the crude extract was collected, and then it was frozen by freezing in sterile glass bottles with a tight lid until use.

### **Antimicrobial activity:**

The inhibitory efficacy of alcoholic and aqueous extracts on the growth of the studied bacteria was tested depending on the [32] where the broth medium fed with the bacteria to be tested was inoculated and after incubation for 24 hours at a temperature of 37 ° C, the suspension was diluted using physiological saline and by comparison with the standard control tube of McFarland's solution, which is equivalent to 108 cells / Then, 0.1 ml of the bacterial suspension was spread on the nutrient media using a cotton swab, then the dishes were left for half an hour at a temperature of 37 ° C in order to absorb. Using the drilling method, he studied the effect of plant extracts on the bacteria to be studied, where holes were made in the dishes containing the nutrient pellet medium using a 6 mm stainless steel borer, then 0.1 ml of each concentration of aqueous and alcoholic extracts was placed in each hole, after which the dishes were incubated for 24 hours. At a temperature of 37 ° C, the damping zones around each hole are measured in mm [33].

### **Measurement of minimal inhibition concentration (MIC) using agar dilution technique:**

The MIC of extracts was tested by the agar dilution method [34], With some modifications. Serial doubling concentrations of plant extracts were prepared, whose value ranged between (12.5-200) mg / ml, and different proportions of these extracts were added to Muller-Hinton agar medium, and after hardening of the media, the dishes were inoculated as one drop with bacterial suspensions and the dishes were incubated at a temperature of 37 ° C for 24 hours. . The lowest inhibitory concentration is then estimated after 24 hours, which represents the lowest concentration of the plant or chemical extract in which no visible bacterial growth was observed, while (Sub-MIC) represents the lowest inhibitory concentration in which the bacteria can grow, which was used in the curing experiments [35,36].

### **Plasmid curing test:**

The method of [37]. was followed, a culture of isolated bacteria under study was prepared where it was grown in the presence of the plant extract and at the required sub-MIC for 24 hours at a temperature of 37 ° C, after which several decimal dilutions were brought to the bacterial culture and 0.1 ml was taken. From the last three years, it was spread on the nutrient agar and incubated at 37 ° C for 24 hours. The plates were incubated at a temperature of 37°C for a period of 24 hours after which the colonies were transferred to media containing the antibiotics used under study separately, Colonies that fail to grow on medium of antibiotics are curing colonies. Calculates the percentage of bacterial colonies lost to antigen resistance. The physical loss of curing plasmids is inferred by the electrophoresis process on gel electrophoresis [38].

### **Isolation of plasmid DNA:**

#### **The plasmid was extracted from bacterial cells using Promega's prepackaged kit:**

5 ml of the broth medium containing a suitable antibiotic are inoculated with a young colony of isolated bacteria under study and incubated for 24 hours, the bacterial growth is transferred to 1.5 ml tubes, then a centrifugation is carried out at maximum speed for one minute and the saliva is completely discarded. Added 100 µl of Solution I, and are re-suspended sediment using a mixing device Vortex. Add 200 µl of Solution II and mix by gently inverting several times without using a mixer. Add 150 µl of Solution III and mix directly several times as in the previous step. Conduct the centrifuge for 10 minutes. Transfer 400 µl of the filtrate to a new 1.5 ml tube. Add 0.8 ml of 95% ethanol and mix by stirring the tube and leave at room temperature for 5 minutes. Centrifuge at maximum speed for 10 minutes and get rid of the odorant ethanol. The precipitates was washed with 200 µl of 70% ethanol and mixed gently, then centrifuged for 5 minutes and the ethanol was removed. The tube is placed upside down on blotting paper to get rid of the ethanol residue until the precipitate is completely dry. The precipitate representing the plasmids was dissolved with 50 µl of TE solution and preserved until use.

#### **Agarose gel preparation and electrophoresis of isolated plasmid DNA:**

For DNA migration and detection, a 1% agarose gel is prepared and to obtain this concentration, 0.5 g of agarose powder is dissolved in (50) ml of X1 TBE and 3 µl of red safe dye is added, this is done using a heat source with constant stirring until boiling and left to cool To a temperature of (60-50) C. Then the gel solution is poured into the Tray basin of the relay device after the special comb is installed to form the Wells pits at the edges of the gel, taking into account that the pouring is quietly to avoid the formation of bubbles, and that they are formed by using a pipette, then leave the gel until it solidifies.

Then the Tray is placed in an electrophoresis sump containing an appropriate amount of X1 TBE solution, after which the comb is gently lifted. Transfer samples are prepared by mixing (5) µl of DNA sample with (3) µl of loading solution. After that, the relay is switched on by passing the electrical current at a voltage difference of (5) volts / cm, and the process takes (2-1.5) hours. The gel is then imaged under ultraviolet rays using a Gel Documentintation to be able to visualize the plasmid DNA bundles.

### **Results And Discussion**

#### **Antibiotic resistance**

Bacterial species were tested for antibiotic resistance the results were as shown in the table below:

**Table 1.** the numbers and percentages of antibiotic-resistant bacterial species.

Antibiotics Bacteria	No. Species	AX (%)	Am (%)	Sm (%)	Ery (%)	GN (%)	RA (%)	Cf (%)	TE (%)	Tri (%)	Nal (%)
<i>S. aureus</i>	29	(86) 25	(82) 24	(48) 14	(82) 24	(68) 20	(48) 14	(65) 19	(51) 15	(68) 20	100
<i>E. coli</i>	32	100	100	17(53)	(90) 29	(40) 13	(46) 15	(65) 21	(87) 28	(84) 27	29 (90)
<i>K. pneumonia</i>	9	(71) 10	6 (66)	8 (88)	100	1 (11)	1 (11)	8 (88)	6 (66)	8 (88)	100
<i>Pr. vulgaris</i>	4	(100)	(100)	S	S	S	S	S	S	2 (50)	S
<i>Pr. Mirabilis</i>	4	1(25)	1(25)	1(25)	S	S	S	S	S	1(25)	S
<i>Ps. aeruginosa</i>	11	100	(90) 10	8 (72)	9 (81)	5 (45)	3 (27)	3 (27)	7 (63)	(90) 10	100

R: Resistance, S: Sensitive, AX: Amoxicillin, TE: Tetracycline, Tr: Trimethoprim, RA: Refampicin, Sm: Streptomycin, Ery: Erythromycin, Am: Ampicillin, Nal: Nalidixic acid, GN: Gentamicin; Cf: Ciprofloxacin.

From the results of Table (1) the bacteria showed a variation in their resistance to antibiotics, as most of the isolates gave multiple resistance to most of the antibiotics used, with the exception of bacteria *Protus vulgaris* that gave resistance to amoxicillin and ampicillin by 100%, and by 50% to trimethoprim. Bacterial resistance to antibiotics has become one of the major problems that threaten public health. In recent recent years, infections caused by intestinal bacteria that are multidrug-resistant have been recorded in almost all European countries for several reasons, including the indiscriminate and widespread use of antibiotics in the treatment of infections caused by bacteria, which exacerbated The crisis, as it led to the emergence of strains that have multiple resistance to most groups of antibiotics, this problem appears clearly in hospitalized patients, as it causes great economic losses in health care centers as it increases treatment costs [39]. . Bacteria have more than one type of resistance, the first of which is self-resistance, and internisc resistance occurs as a result of the possession of these bacteria possessing natural

characteristics that distinguish them from others responsible for them by genetic factors that they possess, which makes them less susceptible to antibiotics, including the impermeability of the outer wall of these bacteria, as well as their ability to spit out the antibiotics. The cell. As for the other type of resistance to these bacteria, it is the acquired resistance. Acquired resistance appears as a result of exposure of the sensitive strains of these bacteria to antibiotics and their transformation into resistant strains by several means, including, Chromosomal mutations, Pump-flow pumps, transposon elements, Effervescent plasmids and other methods of Resist plasmid. [40].

### Inhibition Activity of Plant Extracts Test

Table (2) The inhibitory effect and MIC of the *Citrus aurantium* L fruit extracts with different concentrations on the bacterial species (diameter of the inhibition zone, measured in mm).

Bacteria	Type of extract	Concentration of extract (mg/ml)					MIC
		200	100	50	25	12.5	
<i>Staph. Aureus</i>	Aqueous	23	20	19	17	15	0.125
	Alcoholic	24	23	18	16	14	
<i>E. coli</i>	Aqueous	19	12	-	-	-	1
	Alcoholic	18	16	14	13	11	
<i>Klebsiella pneumoniae</i>	Aqueous	20	17	15	12	9	0.125
	Alcoholic	18	14	12	11	9	
<i>Proteus mirabilis</i>	Aqueous	24	19	17	15	10	0.125
	Alcoholic	23	15	13	10	9	
<i>Proteus vulgaris</i>	Aqueous	21	16	8	-	-	0.5
	Alcoholic	23	18	14	12	9	
<i>Pseudomonas aeruginosa</i>	Aqueous	23	17	12	11	10	0.125
	Alcoholic	24	26	23		13	

(-) no effect

From the results of the above table, it appears that the extracts of the Citrus fruits have a clear effect on inhibiting the growth of the bacteria under study. The diameters of the inhibition circuit varied between high and weak inhibition and non-inhibition, and the concentration was given 200 mg / cm<sup>3</sup> the highest effect of either the aqueous or alcoholic extract on the bacteria under study, and the concentration value ranged. The minimum inhibitory level is between (0.125-1) mg / cm<sup>3</sup>. *Citrus aurantium* contains many primary and secondary metabolites responsible for the therapeutic effects of *Citrus aurantium* contains vitamins, minerals, phenolic compounds and terpenoids, Among the various components of narang are the flavonoids belonging to phenols which are important due to their pharmacological and physiological role and important health benefits [41,42].

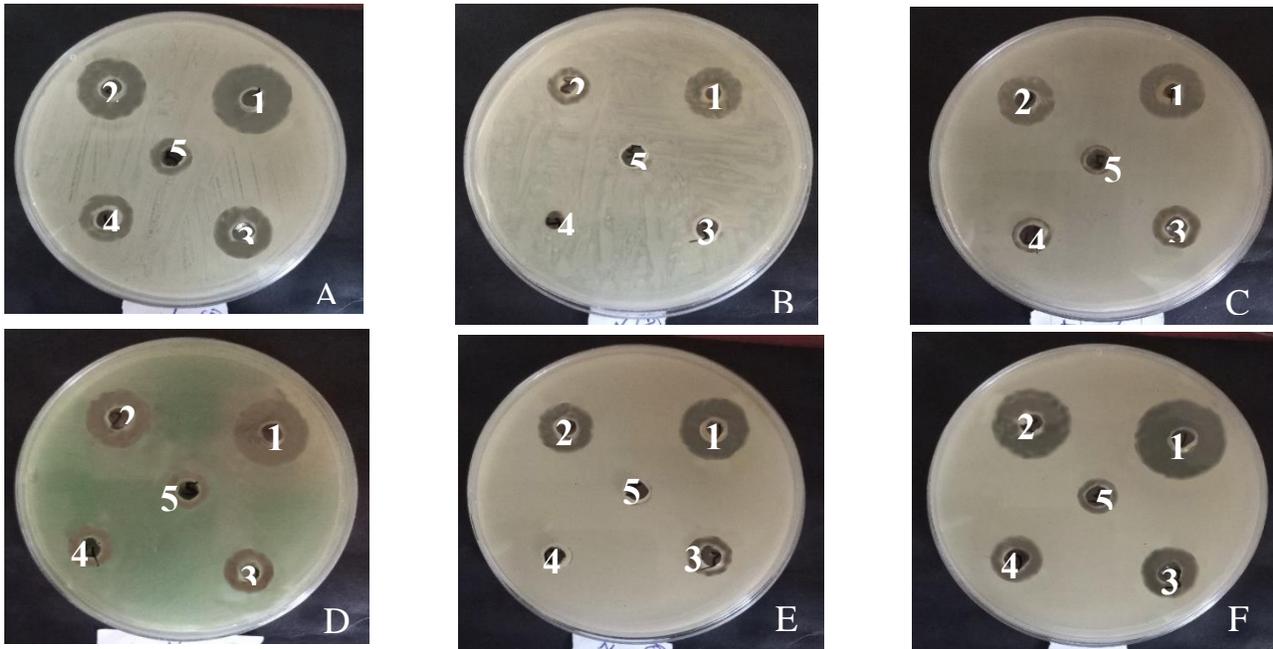


Figure (1): A group of images of the effect of the water extract of *Citrus aurantium* with different concentrations on the bacteria:

A. *Staph. Aureus*, B. *E. coli* C. *Kleb. Pneumonia*, D. *Pseudo. aeruginosa* E. *Proteus vulgaris* F. *Proteus mirabilis* Concentration 1(200 mg/cm<sup>3</sup>), 2(100mg/cm<sup>3</sup>), 3(50mg/cm<sup>3</sup>), 4(25mg/cm<sup>3</sup>) 5(12.5mg/cm<sup>3</sup>).

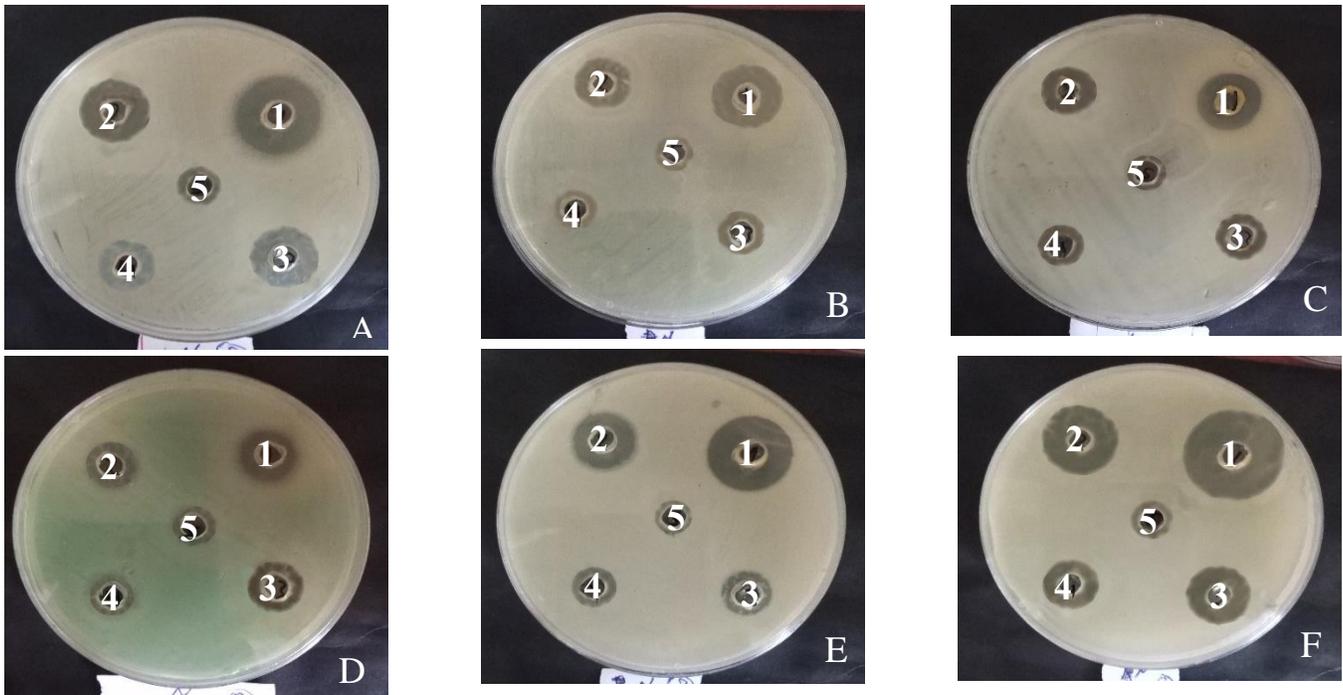


Figure (2): A group of images of the effect of the alcohol extract of *Citrus aurantium* with different concentrations on the bacteria:

A. *Staph. aureus*                      B. *E. coli*                                  C. *Kleb. Pneumonia*  
 D. *Pseudo. aeruginosa*              E. *Proteus vulgaris*                  F. *Proteus mirabilis*

Concentration 1(200 mg/cm<sup>3</sup>), 2(100mg/cm<sup>3</sup>), 3(50mg/cm<sup>3</sup>), 4(25mg/cm<sup>3</sup> 5(12.5mg/cm<sup>3</sup>).

**Curing with the aqueous and alcoholic extract of *Citrus aurantium***

Table (3): shows the removal of antibiotic resistance of the bacterial species under study using the aqueous extract of *Citrus aurantium*.

Bacteria	Antibiotic media added in µg/ml									
	AX 50	Am 50	Sm 25	Ery 15	Gn 25	RA 50	Cf 30	TE 15	Tri 10	Nal 30
<i>Staph. aureus</i> / control	R	R	R	R	R	R	R	R	R	R
<i>Staph. aureus</i> / curing by aqueous extract	R	S	S	R	S	R	R	R	R	R
<i>Staph. aureus</i> / curing by alcoholic extract	S	S	S	S	S	S	S	S	S	S
<i>E. coli</i> / control	R	R	R	R	R	R	R	R	R	R
<i>E. coli</i> / curing by aqueous extract	R	R	w	W	R	S	R	R	R	R
<i>E. coli</i> / curing by alcoholic extract	R	R	S	W	S	S	R	R	S	R
<i>Klebsiella pneumonia</i> / control	R	R	R	R	R	R	R	R	R	R
<i>Klebsiella pneumonia</i> / curing by aqueous extract	R	R	S	R	S	S	R	R	R	R
<i>Klebsiella pneumonia</i> / curing by alcoholic extract	S	S	S	S	S	S	S	S	R	S
<i>Proteus mirabilis</i> / control	R	R	R	S	S	S	S	S	R	S
<i>Proteus mirabilis</i> / curing by aqueous extract	S	S	R	---	---	---	---	---	R	---
<i>Proteus mirabilis</i> / curing by alcoholic extract	S	S	S	----	---	---	---	---	S	---
<i>Proteus vulgaris</i> / control	R	R	S	S	S	S	S	S	R	R
<i>Proteus vulgaris</i> / curing by aqueous extract	R	S	---	---	---	---	---	---	R	S
<i>Proteus vulgaris</i> / curing by alcoholic extract	S	S	S	S	S	S	S	S	S	S
<i>Pseudomonas aeruginosa</i> / control	R	R	R	R	R	R	R	R	R	R
<i>Pseudomonas aeruginosa</i> / curing by aqueous extract	S	R	S	R	R	S	R	R	S	w
<i>Pseudomonas aeruginosa</i> / curing by alcoholic extract	S	S	S	S	S	S	w	S	S	w

R: Resistance              S: sensitive              W: Weak              ---: The test not done

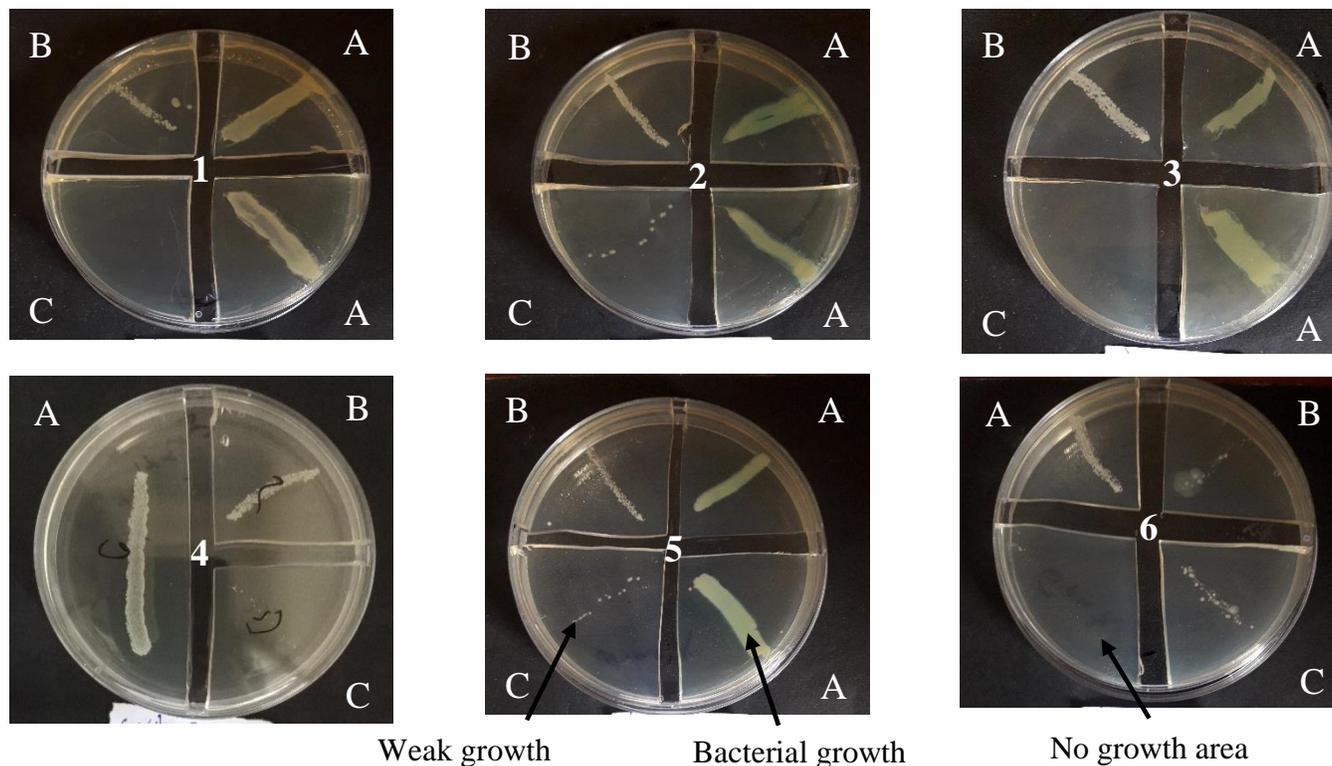


Figure (3): A group of images of the effect of the aqueous and alcohol extract of *Citrus aurantium* on bacteria growing on antibiotic media

A: before adding *Citrus aurantium* extract      B: after adding the aqueous extract of *Citrus aurantium*  
 C: after adding the alcoholic extract of *Citrus aurantium*  
 1: *E. coli* on sm                      2: *Pseudo. aeruginosa* on cf                      3: *Pseudo. aeruginosa* on TE  
 4: *Staph. aureus* On Ery              5: *Pseudo. aeruginosa* on Nal                      6: *Kleb. Pneumonia* on Ery

From the above pictures, the results of the curing are shown, the removal of antibiotic resistance, through the absence of bacterial growth or weak growth after adding the aqueous or alcoholic extract of *Citrus aurantium*. Through the above results it was found that many of the bacterial species under study have remained resistant to a number of antibiotics, including (TE, Tr) after curing and this may indicate that the gene related to resistance to these antibiotics is located on the bacterial chromosome and was not affected by this process. As for the discrepancy after curing between the bacterial species under study in terms of their resistance to antibiotics, it is because the plasmid responsible for carrying antibiotic resistance is R-plasmid, which consists of two molecules and each molecule contains genes responsible for antibiotic resistance. The results of the curing showed that the bacterial species *S. aureus*, *K. pneumonia* and *P. aeruginosa* were the most affected by the curing test for both aqueous and alcoholic extracts of *Citrus aurantium*, while the bacterial species *E. coli* and *Pr. vulgaris* and *Pr. Mirabils* are the other affected by the curing using water and alcoholic extracts of *Citrus aurantium*, but at a lower level than the bacterial species *S. aureus*, *K. pneumonia* and *P. aeruginosa*, and it may be due to what these aqueous and alcoholic extracts contain of effective compounds, including tannins, phenols, alkaloids, flavonoids, and others [43].

### Effect of aqueous and alcoholic extracts as curing agent of growth of isolated bacteria:

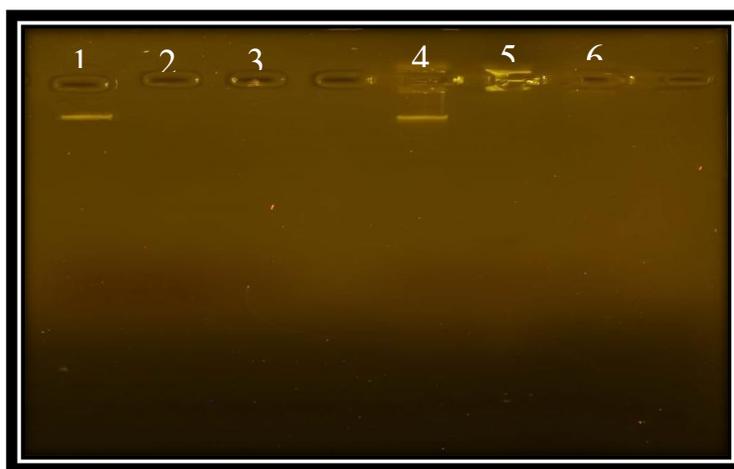
The method of testing the turbidity mentioned in the materials and working methods was used to study the effect of the extracts used under study on the growth of bacterial species before and after curing.

Table (4) The optical density values at 600 nm of the treated and untreated bacterial cultures with aqueous and alcoholic extracts at a concentration of 200 mg / ml

Bacteria	Control sample before curing of the extracts	Control sample after curing of the aqueous extract	Control sample after curing of the alcoholic extract
<i>Staph. aureus</i>	1.276	0.640	0.817
<i>E. coli</i>	1.524	0.722	0.817
<i>Kleb. pneumonia</i>	2.590	1.006	0.750
<i>Proteus vulgaris</i>	2.540	0.650	0.700
<i>Proteus mirabilis</i>	1.68	0.656	0.660
<i>Pseudo. aeruginosa</i>	3.062	0.606	0.740

The above table notes that the curing agent (aqueous and alcoholic extracts) caused a clear decrease in the optical density values of the bacterial colonies after the neutralization process. There are many factors that affect the numbers of bacteria, including temperature and pH, which affect the activity of enzymes, proteins, and cell membranes of the bacteria. Any change in these two factors will be reflected in the growth and preparation of the bacteria (44). Also, plant extracts may contribute with the materials they contain. Effective in influencing the growth and viability of bacteria (45).

### Characterization of Plasmid DNA Content by Gel Electrophoresis



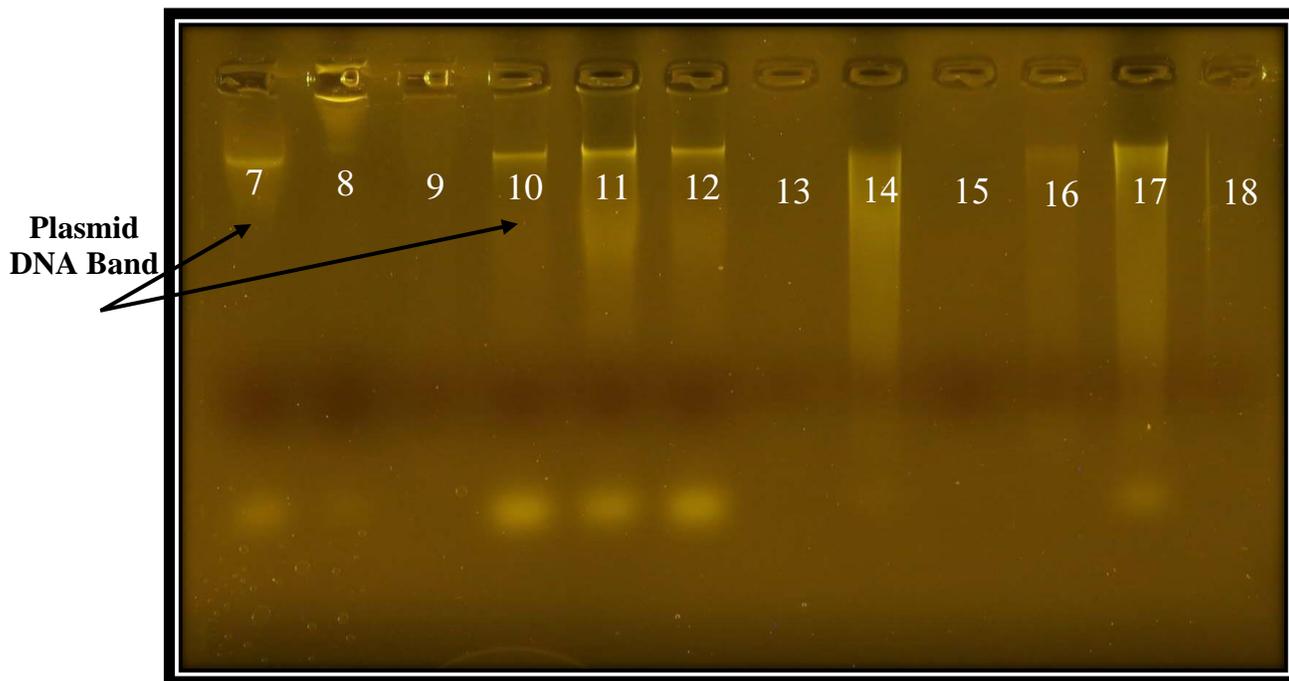


Figure (4) Electroporation of plasmid DNA content of bacterial species under study before and after curing with *Citrus aurantium* extracts

#### Column

1. *S. aureus* bacteria before curing with the extracts.
2. *S. aureus* bacteria after curing with water extract.
3. *S. aureus* bacteria after curing with alcohol extract.
4. *E. coli* bacteria before curing with the extracts.
5. *E. coli* bacteria after curing with water extract.
6. *E. coli* bacteria after curing with alcohol extract.
7. *Klebsiella pneumoniae* before curing with the extracts of
8. *Klebsiella pneumoniae* bacteria after curing with water extract.
9. *Klebsiella pneumoniae* after curing with alcohol extract.
10. *Ps. aeruginosa* bacteria before curing with the extracts of
11. *Ps. aeruginosa* bacteria after curing with water extract.
12. *Ps. aeruginosa* bacteria after curing with alcohol extract.
13. *Pr. vulgaris* before curing with the extracts.
14. *Pr. vulgaris* bacteria after curing with water extract.
15. *Pr. vulgaris* bacteria after curing with alcohol extract.
16. *Pr. mirabilis* bacteria before neutralizing the extracts.
17. *Pr. mirabilis* bacteria after curing with water extract.
18. *Pr. mirabilis* bacteria after curing with alcohol extract.

The apparent contrast between the bacterial species after curing in terms of their resistance to antibiotics is due to the fact that the plasmid that carries antibiotic resistance is of the R-plasmid, as it is in the form of two molecules and each molecule carries genes responsible for resistance. From the above results of curing, we find that the rates of curing efficiency of plant extracts on the bacteria under study showed a variation in the rates of curing and loss of resistance to antibiotics. The plasmid DNA of the parent cell and the distribution of these plasmids during the process of cell division leads to the production of nuclear cells with few plasmids and at other times lacking plasmids. Consequently, the bacteria missing the plasmids are transformed from resistance to antibiotics to sensitive [46].

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