European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 09, 2020

DIAGNOSIS ALKALOIDIC COMPOUNDS on CITRULLUS COLOCYNTHIS FRUIT and COMPARE its EFFECT WITH SOME ANTIBIOTICS on ISOLATED BACTERIA GROWTH FROM URINARY TRACT INFECTION PATIENTS

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***Abstract**:*
Identification of alkaloidic substances of on Citrullus colocynthis Fruit done by using High Performance Lquid Chromotography(HPLC) technique,the results appeared the fruit containe many compounds as:Citrullo,Cucurbitacin,Colocyanthin and Colocynthitin by 17.68,39.84,29.67 and12.80%.Isolation and Identification of bacteria done at College for women laboratories/Tikrit University, As it was collected (200) median diuresis samples from the reviewers and sleepers in Salah Al-Din General Hospital / Tikrit city / Iraq with urinary tract infections and it included males and females for ages between (15-60) years, The results showed (160) growth samples when cultivated, at a rate of (80%), as they gave two types of growth, negative for the gram stain (65.6) and positive for the gram stain (34.4). The infection rate was higher in females (66.87%) while males (33.13).
The susceptibility of the isolated bacterial species to resistance to 6 antibiotics was tested for different groups, in which the bacterial isolates differed in their sensitivity and resistance to the studied antibiotics, The effectiveness of aqueous and alcoholic extracts was also tested at concentrations of 25%, 75% and 100%, where the alcoholic extract was more efficient compared to the aqueous extract, and the concentration of 100% was the most effective concentration on the bacterial isolates..

Keywords: Citrullus colocynthis +plant extract+antibiotics+bacteria.

INTRODUCTION

Urinary Tract Infections (UTIs) Are considered Inflammatory response as a result of colonization of microbial pathogens of the sterile urinary tract, it is one of the most common bacterial infections(Thoini,2015), and second only to respiratory tract infections ((RTI)(Aabenhus *et al* .,2017), affects males and females of all age groups (Kireçci et al., 2015), however females are more likely to be infected with one in five being infecte affected during her lifetime (Ahmad *et al*., 2015;. Infections occur in any part of the urinary system (Abdullah, 2017). In uninfected people, the urine is free of bacteria but it contains fluids, salts, and waste products. Flora into the urinary tract (Kathryn, 2017). Bacterial infection is caused by bacteria(Nicolle,2014) Although there are other causes, fungal, viral and parasiticHowever, Gram-negative bacteria recorded the highest percentage of infection events compared to

Gram-positive bacteria(Foxman,2013),and Urinary tract infections may or may not be asymptomatic(Bhaskaran,2019) (AL-Samarraie,et al,2019).The intestinal family is the most common pathogen for urinary tract infections and the most isolated are *E. coli*, followed by *Klebsiella* Spp, *Proteus* Spp, in addition to the genus *Pseudomonas* Spp, *Enterobacter* Spp., *Staphylococcus* Spp. and *Streptococcus* Spp. (Verle *et al*.,2015).

Antibiotics have contributed to the treatment of urinary tract infections, but the widespread use of antibiotics has led to the emergence of resistant disease strains and the harmful effects of chemotherapy (Kalal and Nagaraj, 2016) (Sohani, 2019), Therefore, research has focused on limiting the use of antibiotics and providing natural therapeutic alternatives from medicinal plants because they are available, safe and inexpensive, thus limiting the development of new resistant strains (Pulipati *et al*., 2017;Frieri *et al*., 2017).

The medicinal and physiological effect in medicinal plants is attributed to the active ingredients with nutritional value, and these components include alkaloids of importance in the field of pharmaceutical preparations based on natural products (Bunsupa *et al*., 2017), also phenols or flavonoids have toxic and fatal effects for both positive and Gram negative bacteria (Dzoyem et al., 2018), and glycosides play an important role in treating urinary tract infections (Shah et al., 2009), as well as saponins have anti-inflammatory, anti-neoplastic, anti-bacterial and viral effects (Oleszek and Oleszek, 2020),and saponins have anti-inflammatory, tumor, and antibacterial and viral effects and tannins which have several benefits for humans through their antimicrobial activity (Hassanpour *et al*., 2011) desides sterols are valuable in treating several diseases, including the treatment of bacterial infections (Tarkowská and Strand ., 2016)) and turbines have antibacterial activity (Ezzat *et al*., 2012).

C.colocynthis is a plant of the Cucurbitaceae family (Mohammad and Kameswara, 2014). Its seeds contain several effective compounds, including Elatercin, Saponins, hemtriacontane and 6 fixed oils that act as antibacterial agents (Hussain, 2014). Studies have shown that the effect of the alcoholic extract is more than the aqueous extract, and the highest concentration of the extract is the most effective (Majeed and Al Shatti, 2002). Al-Mousawi (2006) and Marzouk and others (2011) confirmed that the C.colocynthis fruit extract has a high inhibitory effect on Gram-negative and positive bacteria. (Degola *et al*., 2019) also pointed out that these substances can be used in pharmaceutical treatments as anti-bacterial, fungal, and parasitic infections and anti-inflammatory. Therefore, this study was conducted to isolate and diagnose bacterial types from patients with urinary tract infection and the effect of some antibiotics and *C. colocynthis* seed extract on it.

MATERIALS & METHODS

* Preparing plants extracts: fruits with seeds of *C. colocynthis* plant were washed by tap water and soaked in 2% of sodium hypochloride solution for 15-20 minut and washed with sterilized water and dried at room temperature,100gm of each plant milled and used for extraction in 1000ml of hot water and the extract dried by using water path at 600C inorder obtain 8.2gm dried extraction then different concentration 25,50,75,100% prepared from the dried extraction in addition to control 0% (Al-Janabi et al,015). The alcoholic extract was prepared in the same way as the aqueous extract and with the same weights except for the replacement of distilled water with ethyl alcohol at a concentration of 76%.

*** Diagnosis of active compounds in the plant:** Effective compounds of the powder (pulp + seeds) of C. colocynthis were diagnosed for in the laboratories of the Ministry of Science and Technology - Baghdad, as 1.0 g of *C. colocynthis* dried powder was taken and dissolved in sulfuric acid concentration 3% for two hours and the extract was filtered by filter paper with a

diameter of 2.5 mμ. The remaining fraction was alkalized by ammonium hydroxide NH4OH (Ph9.5) and placed in the columns. The alkaloids were removed by (CH2CL2 (6ml / g) and then the extract was evaporated for the purpose of drying with nitrogen vapor and the remaining portion obtained was dissolved in 1ml CH3OH in order additional analyzes by means of HPLC (High Performance Liquid Chromotography) technology according to the ideal separation of the original standard, then the concentrations were determined by comparison between the area of the standard model with the studied sample area under the conditions of the same separation, and the standard solutions (Table 2) and (Fig.1) were separated. and to identify the area of the standard model package (peck area), the retention time, and the height of the beams. Then the holding time, area and height of the beams resulting from the sample injection were measured. After that, the packages obtained were compared with the packages of the standard solution the result was under the same conditions. The process was repeated on all samples of the plant that were diagnosed and under the same working conditions (Nishizawa, 1999). The concentration of compounds was calculated in the model and in the following equation: -

$$\text{Compound Conc.}\mu g/mL = \frac{\text{peak area of plant compound}}{\text{peak area of standard pattern}} \times \text{standard pattern conc.} \times \text{delution factor}$$

Table 1.Compounds,Retention time, Pick area and the concentration of standard C. colocynthis.

Compounds	Retention time	Pick area	Concentration(µg/ml)
Citrullo	2.21	164813	25
Cucurbitacin	3.30	170878	25
Colocynthin	5.05	147844	25
Colocynthitin	6.22	224198	25

*Antibiotics: In the current study, antibiotic tablets were used for the purpose of testing the sensitivity and resistance of the isolated bacterial species to antibiotics, which included Penicillin(P), Nalidixic acid(NA), Imipenen(IMP), Ampicillin(AM), Cefixime(CFM)and Gentamicin(CN).

*Preparation of Culture Media**:** 1- Blood agar 2- Agar MacConke 3 - Nutrient Agar 4- Nutrient broth
5- Eosin- methylene blue agar 6- Mannitol Salt Agar 7- Muller-Hinton 8- Simmon's Citrate agar 9- Kligler Iron agar 10- Peptone Water 11- MR_VP media 12- Brain-heart agar

* Collection of sample : This study was conducted in the city of Tikrit / Salah al-Din/Iraq. General Hospital for the period from the beginning of October of 2019 until mid-December of the year 2020. 200 urine samples were collected randomly from patients and reviewers suffering from infections urinary tract at Salah al-Din General Hospital, these samples were taken from both sexes and of all ages, and patients who took antibiotics for treatment were excluded. Samples were collected in sterile plastic bottles with the patient being instructed to collect urine from the middle flow of the urine and washing the genital area well with soap and water, taking into account not to touch the bottle to the patient's skin to avoid contamination of the sample with the natural flora that is endemic to the skin in the contact area (Vandepitte et al., 2003).

*** Urine culture:** A drop was taken from each urine sample using the Loop bacterium and implanted on three culture media, which are haemocytes, maconkey and saline mannitol

medium. The cultivated dishes were incubated at 37 ° C for a period of 18-24 hours, and then they were transferred colonies growing into new media for the sake of purification and obtaining individual colonies, the pure colonies were conserved using nutrient agglomerates at 4^0C slant until subsequent diagnostic tests are performed (Abdulazeez et al .,2020) (Leboffe and Pierce, 2011).

*** Identification of Isolated Under Study :** The isolates under study were diagnosed according to the following tests
1- morphological 2- Microscopical

*** Antibiotic susceptibility Test.**
** Test the Inhibitory Efficacy of Plant Extracts:* The Agar diffusion method was used by Wells to test the sensitivity of bacteria to plant extracts at concentrations of 25, 75, 100 mg / ml in the hole of the nutrient medium,while mixture extract include only 100 concentrate and the method included the work of three equal dimensions hols in the solid Muller-Hinton medium, with a cork porer, then planting 1 ml of the bacterial suspension on the medium, then leave the dishes in the refrigerator for one hour, then put the extracting solutions 0.2 ml per hole, then it was incubated in the incubator at a temperature of 37^0C for 24 hours, and the results were read by measuring the diameter of the inhibition zone (Al-Numaan, 1998).
** Statistical analysis:* The data were analyzed using chi-square statistical analysis.

RESULTS & DISCUSSION

The results of the analysis using HPLC technology showed that the pulp and seed extract of the fruit of the of C. colocynthis plant contained several effective alkaloid compounds, including: Citrullo, Cucurbitacin, Colocynthin and Colocynthitin, with concentrations of 17.68, 39.84, 29.67 and 12.80%. (Table 2) and (Figure 2). Numerous studies have indicated the diversity of active substances and their importance in inhibiting bacteria and other micro-organisms, including a study conducted by the researcher (Ahmed, 2013), which included conducting the chemical detection of active substances in the fruits and seeds of *C. colocynthis*, As the results of the chemical test revealed that the fruits contain tannins, flupatinates, saponins, flavonoids, cyclosides, terpenes, phenols, resins, carbohydrates, alkaloids and glycosidic steroids. As for the seeds, they contained tannins, saponins, flavonoids, terpenes, phenols, resins, amino acids, carbohydrates, alkaloids, in addition to steroidal cyclosides. Some of these active substances have also been isolated, such as soaps, tannins, and volatile oils their percentages in fruits were 92.10, 6.17 and 04.2% respectively, while their percentages in seeds were 77.3, 39.7 and 7.18 respectively.

Table 2.Compounds,Retention time,Pick area,Dilution factor and compounds concentration (µg/ml)and (%) of studied *C. colocynthis* plant.

Componds	Compound Pick area	Standard Pick area	Standard concentration	Dulation factor	Compond conc. (µg/ml)	Compond conc.(%)
Citrullo	100292	164813	25	20	760.64	17.68
Cucurbitacin	234328	170878	25	30	1714.15	39.84
Colocynthin	150986	147844	25	30	1276.59	29.67
Colocynthitin	98793	224198	25	30	550.81	12.80

Culture result	No.	(%)
Positive	160	80.0
Negative	40	20.0
Totale	200	100.0

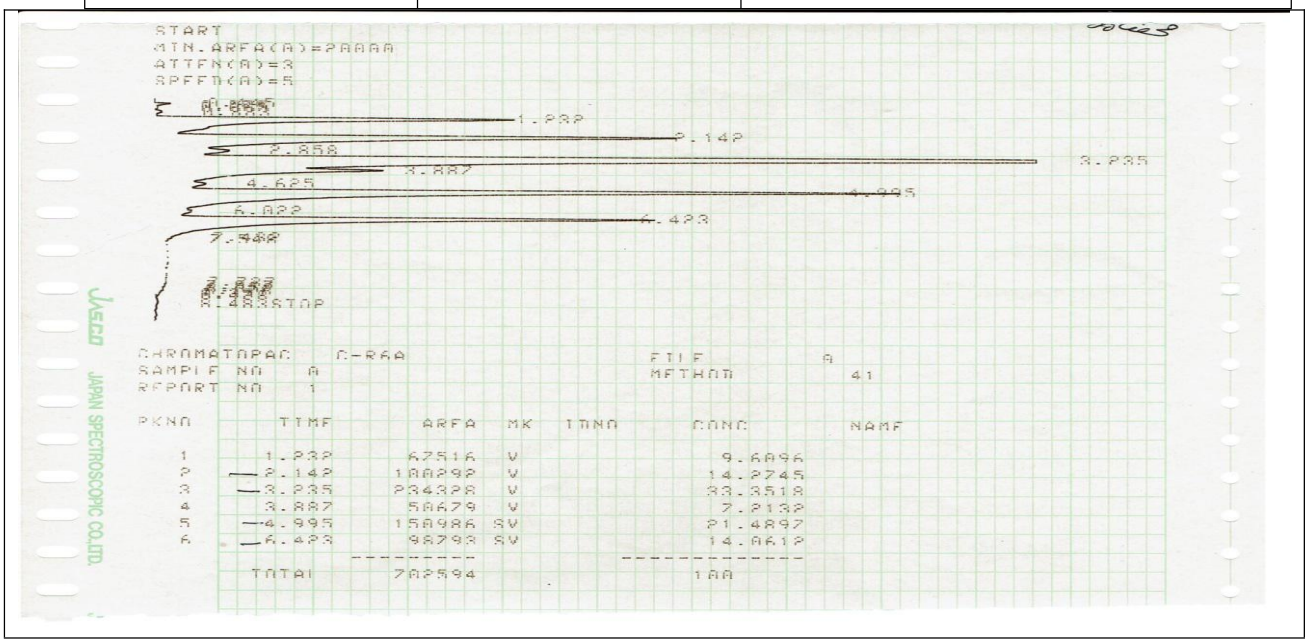


Figure 3. Chromatogram HPLC analysis of studied of *L. sativum* plant.

*Samples culturing: The results showed that (160) samples gave growth when cultivated, at a rate of (80.0%) of the total samples, while the remaining 40 samples, at a rate of (20.0%), did not give any bacterial growth, as shown in Table (3). , Some specimens not growing may be due to a fungal pathogen or viral or anaerobic bacteria that cannot be isolated by the usual transplantation methods that were used in this study and which require special culture media and special conditions for development or as a result of the patient's use of inappropriate antibiotics that led to the disappearance of pathological bacteria (Al Douri, 2011). The result of growth is high compared to what the researcher (Salem, 2019) obtained 150 positive growth samples with a percentage of (61.7) out of a total of 246 samples while (Al-Hamdani, 2019) obtained 105 positive growth samples and a percentage (70%) of a total of 150 samples and (periodic , 2011) which had a growth rate (71.05%)

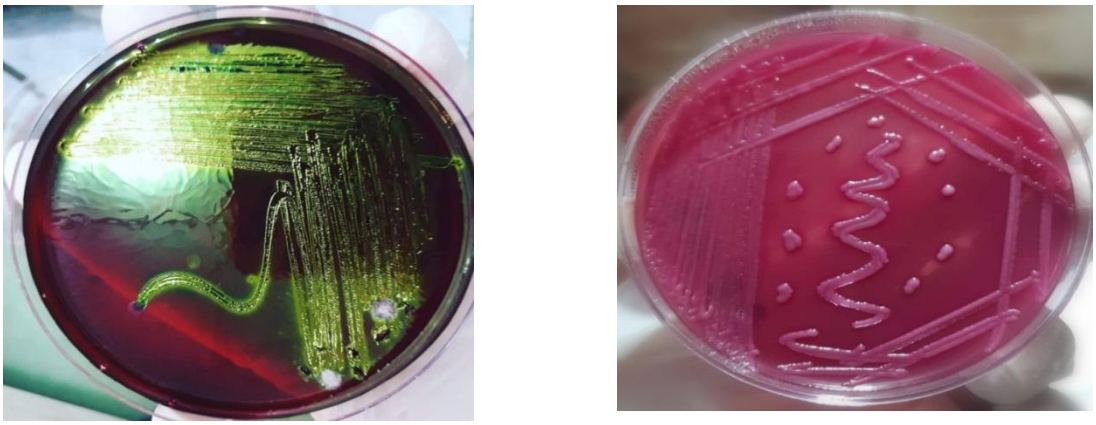
Table(3) The total number of samples taken from patients with urinary tract infections

The samples that showed growth in the culture media gave two types of growth, positive to Gram positive by 34.4% and negative to Gram negative by 65.6%, as shown in Table (4). (57%) are negatively pigmented bacteria and (43%) positively pigmented bacteria, and (Ibrahim, 2010) in Tikrit, where (57.5%) bacteria were negative for the Gram stain and (39.5%) bacteria were positive for the Gram stain. Therefore, the Gram negative bacteria had the highest percentage of bacteria positive for the Gram stain. This is consistent with the current study.

Table(4).Number and Precentage of Gram Posative and Negative Isolated Bacteria.

Isolated kinds	No.	(%)
Positive	55	34.4
Negative	105	65.6
Totale	160	100.0

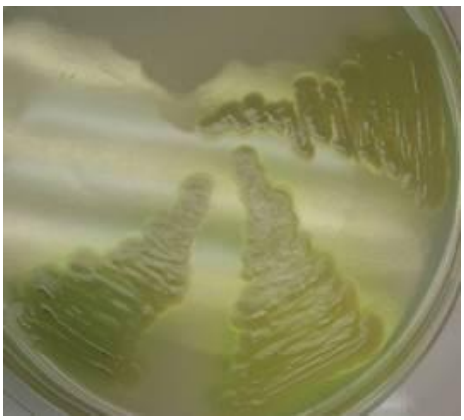
*Phenotypic and microscopic diagnosis of urinary tract infections bacterial isolates: The results of the phenotypic and microscopic tests (using the Gram stain) that were performed on the growing bacteria showed that all the Gram-negative bacteria are bacillary and grow on the McConkey medium, which does not allow the Cram-positive bacteria to grow on it. *E.coli* colonies appeared pink on McConkey medium as a result of lactose fermentation (Figure A2), *E.coli* colonies are dry, convex, medium-sized and regular, and *E. coli* colonies when grown on EMB medium showed a bright metallic green color (Figure B 2). These bacteria are one of the natural plants found in the intestine of humans and animals and one of the most common causative species. For urinary tract infections (Ahmad et al., 2015).



B A

Figure 2. *E. coli* colonies

As for *K. pneumonia* colonies appeared as irregular mucous due to their possession of the capsular layer on its surface and were larger than those of *E. coli* (Figure 3) also colonies of Ps. aeruginos a appeared large and irregular with a rough and wrinkled surface and were distinguished by a bluish green color on the nutrient media (Figure. 4) due to their production of Pyocyanin as well as Fluorceines. Both are characteristic and diagnostic of this bacterium, while it appeared on McConkey's medium pale color due to not fermenting lactose sugar, it is a Beta-hemolytic (Brooks et al., 2014). It is bacillus shaped either singly or in pairs or chains, and its farms have a musty smell.

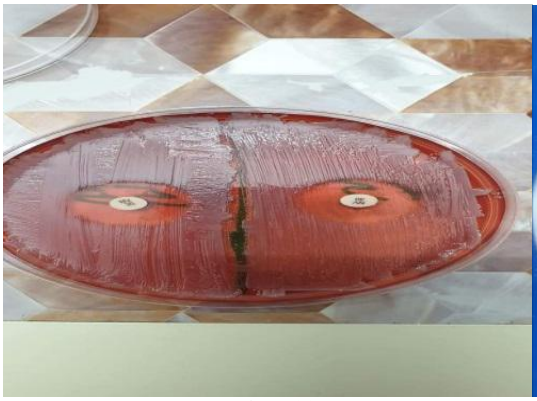


Figure(4)

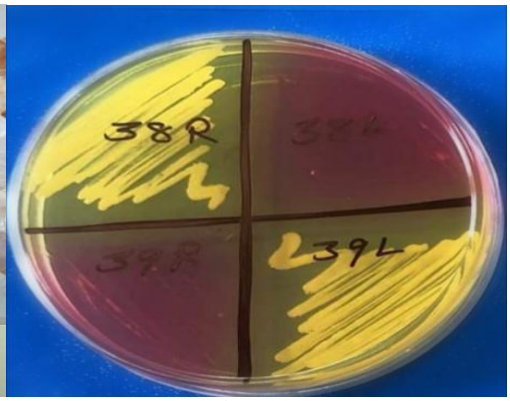
Figure(3)

As for the colonies of *Ser.marcescens*, they are convex and pale in color on the center of the McConkey acar, due to its non-fermentation of lactose (Ryan et al., 2004). It has a smell similar to the smell of rotting fish or chips. As for colonies of *E. cloacae*, they were marked

by a pinkish-red color on the center of McConkey's agarite, while they appeared pale in color on the medium of the nutrients, also, Acinetobacter haemolyticus gave distinct colonies of creamy white color on the center of the haemocyst, with a mucous texture (when the capsule was present) and smooth surface as they appeared on the EMB medium in blue or bluish gray color (Doughari et al., 2011),while the gram-positive bacteria were distinguished by their spherical shape, where the s*taphylococcus* appeared in a spherical form and in the form of irregular clusters and was characterized by small, somewhat convex colonies, gave a white color when they grew on the blood cells (Shittu et al., 2004). The saline mannitol medium is a selective and differential medium for this genus and is a medium that does not allow gram-negative bacteria to grow on it, When the colonies growing on this medium are circular, convex and regular with smooth and shiny edges, and the color of the center changes from pink to golden yellow, evidence that the developing bacteria is *Staph.auerus*. (Figure 5) The reason for the yellow coloration is the formation of acid as a result of fermentation of mannitol sugar and the change of pH of the neutral medium to acidic, this is what distinguishes this bacterium from other species of this genus that cannot ferment mannitol sugar in addition to its sensitivity to anti-novobiosin (Figure 6) and a blood analyzer on the center of the blood cells, and this is consistent with what Varrone and his group (2014) reported. (Figure7),Whereas, *Staph.epidermidis* appeared in small, rounded, smooth, opaque and white colonies due to its non-fermentation of mannitol sugar (Figure. 8) in addition to its insensitivity to the anti-novobiosin, which distinguishes it from Staph.aureus, which is not hemolytic in the middle of the blood cells

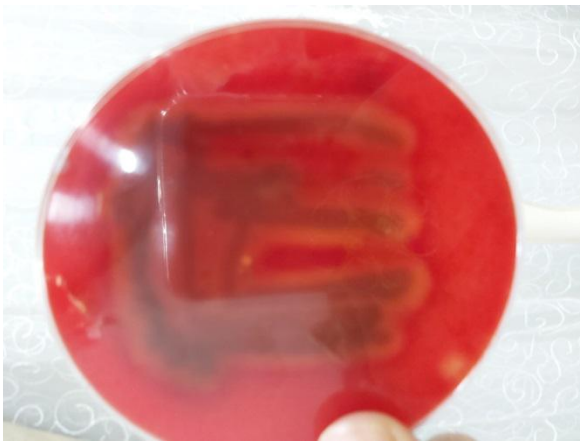


Figure(5)



Figure(6)

Figure(8)



Figure(7)

***Resistance of bacterial isolates to antibiotics**:The results of (Table 5) indicate that the bacterial isolates differed in their resistance and sensitivity to the studied antibiotics. The

isolate *Acin haemolitycu* recorded the highest percentage of resistance to(IPM)which reached 100%, while the isolates were *Pseu.aeuroginosa. Serr. marcescens* is 100% sensitive to the same antigen, Pseu isolation was recorded. aeroginosa isolate showed 100% resistance to (NA) antibiotic, while Serr. Marcescen sensitivity reached 100%, and the resistance of the isolates to anti-AM varied, as the isolate showed aeuoginosa Pseu. Resistance rate was 100%, and the isolates showed *Staph-aureus*, aeuroginosa Pseu, cloacea. Enter and Serr. Marcescens is 100% resistant to antibitic(P).

The resistance of the bacterial isolates to the anti-CN and CFM antagonists varied, but with less than the previous antibiotics. The reason why isolates are resistant to antibiotics is due to the possession of these preservative bacteria. There are also many mechanisms that make them resistant to antibiotics, and the most important of these mechanisms is their production of lactamase-, as studies have shown that they possess many types of these enzymes that break down a wide range of lactam-antagonists. (Weller *et al*., 1997) In addition, some isolates changed their sensitivity to antibiotics, and this is due to the bacteria possessing several mechanisms to resist antibiotics in addition to neutralizing the effectiveness of the antagonist by producing specific enzymes that lead to blocking the action of the antagonist, which is modulating it to the target site on which the antagonist works, changing the permeability of bacterial cells to prevent Antagonist entry or via the effux system that bacteria possess (Levinson and Jawetz, 2004) The resistance of these bacteria may also be the result of a change in the composition of certain enzymes or the loss of their function and thus lead to negative changes in the site on which the antibiotic works, or the resistance results from enzymes that remove the toxicity of the antibiotic (Kadir et al., 2010), as for the resistance of the bacteria to the anti-lactam- β It comes from the bacterial production of lactamase-that degrade the lactam-loop (Magdy, 2013). This study agreed with (Al-Mazrouei, 2017) who obtained a resistance rate (100%) for anti.penicillin and (75%) for anti-Ampicillin, while it differed from it in the ratios of anti-Gentamicin and Nalidixic acid that were (10%) and (20%) respectively, it also agreed with (Alsamarai and Ali, 2016) where they found the percentage of resistance high for both Nalidixic Acid and Cefixime, as it was (82.9%), while it differed from it in the ratio of Imipenem resistance which was (14.6%), which is low compared to what was recorded in the current study. While it was close to the percentages of the two researchers (Al-Jubouri, 2019) and (Jamil, 2017) and in Tikrit, which were (66.7%) and (75%) respectively, while Al-Jebouri et al., 2013) found that Staph-aureus Sensitive (100%) to Nalidixic, and the percentage of resistance is low to Gentamicin, as it was (33.3%), which does not agree with the results of the current study.

Table 5. Sensitivity of bacterial species isolated from urinary tract infections to antibiotics

Antibiotc	Response	*Staph. aureus*		*Staph. epidrmidis*		*E.coli*		*K.pnumonia*		*Pseu. aeuroginosa*		*Enter. cloacea*		*Acin. haemolitycu*		*Serr. marcescens*	
		No.	%	No.	%	No.	%	No.	%	No.	%	N	%	No.	%	No.	%
IPM	R	27	77.1	5	25	4	8.3	26	89.7	0	0.0	1	20	3	100.	0	0.0
	S	8	22.9	15	75	44	91.7	3	10.3	11	100	4	80	0	0.0	9	100
NA	R	29	82.9	1	5.0	25	52.1	19	65.5	11	100	2	40	1	33.3	0	0.0
	S	6	17.1	19	95	23	47.9	10	34.5	0	0.0	3	60	2	66.7	9	100
AM	R	27	77.1	12	60	28	58.3	26	89.7	11	100	3	60	2	66.7	2	22.2
	S	8	22.9	8	40	20	41.7	3	10.3	0	0.0	2	40	1	33.3	7	77.8
P	R	35	100.	12	60	39	81.3	26	89.7	11	100	5	100	1	33.3	9	100
	S	0	0	8	40	9	18.7	3	10.3	0	0.0	0	0.0	2	66.7	0	0.0
CN	R	18	51.4	17	85	18	37.5	26	89.7	6	54.5	2	40	1	33.3	6	66.7
	S	17	48.6	3	15	30	62.5	3	10.3	5	45.5	3	60	2	66.7	3	33.3
CFM	R	29	82.9	13	65	30	37.5	23	79.3	6	54.5	1	20	2	66.7	6	66.7
	S	6	17.1	7	35	18	62.5	6	20.7	5	45.5	4	80	1	33.3	3	33.3

* Effect of aqueous and alcoholic extracts on bacterial isolates: The effect of the concentrations of the aqueous and alcoholic extract varied in their effect on the growth of the studied bacterial isolates, and the sensitivity of the isolates to both the aqueous and alcoholic extract varied. (Table 6), the effect of 100% concentration was significant for both extracts, and the highest inhibition diameter was for Staph.epidermids when treated with alcoholic extract, with a concentration of 100%, While the isolate Pseu.aeruginosa showed little sensitivity to aqueous extract with an inhibition diameter of 8 mm, and Entero cloacea showed high sensitivity to the mixture of both extracts, while the sensitivity of the isolate Pseu aeroginosa to the mixture of extracts was minimal with an inhibition diameter of 10 mm.

The results may be attributed to the containment of the extract in the higher concentration to the amount of active substances more than the remaining concentrations. As for the mechanism of the effect of the extracts, it is due to inhibiting the formation of the cell wall of the microorganism or to inhibiting the synthesis of some essential proteins in the wall and some extracts impede the regularity of permeability through the formation of complexes with walls. Cells, inhibiting some enzymes that have an important metabolic role in growth and reproduction, and disrupting cell membranes or changing their function (Cowan, 1999), also (Ambi et al., 2007) and Najafi et al., 2010) concluded that *C. colocynthis* extracts contain the active substances referred to above. Al-Khafaji (2006) indicated that the inhibitory activity of bitter melon fruit extract is due to its containment of most of the active compounds that have a clear effect on the growth of microorganisms. The superiority of the alcoholic extract in its effect on the growth of bacterial isolates may be due to its ability to extract the largest amount of active substances from the plant powder, as there are active substances that not only dissolve in water but also dissolve in alcohol, which leads to the extraction of the highest amount of active substances found in the plant (Shahadah and his group, 2011), found that the extraction of active substances such as acene acts as a natural antagonist that inhibits bacterial growth by destroying the SH group required for cell replication (Slusarenko et al., 2008).The researcher also indicated that the inhibitory activity is due to the fact that it contains the enzyme Urease, which has great efficacy in the hydrolysis of urea to the compounds of ammonia and carbamate in bacteria and a number of microorganisms. It was also able (El-Safar, 2015) to investigate the protease enzyme in the fruits of *C. colocynthis* where it was It is found in high concentrations and very effective on bacteria and other microorganisms.

Table 6.Effect of Aques,Alcholic and the Mixture of of *C. colocynthis* on Isolated Bacteria Growth(mm).

Extreacts / Isolated bacteria	Aqueous				Alcholic				Mixture
	(%)Concentration								
	25	75	100	Mean	25	75	100	Mean	100
E. coli	8	12	14	11.3 cde	10	15	15	15.0b	12E
Staph. aureus	8	12	18	12.7 abc	10	14	14	14.7b	16B
Staph. epidermidis	10	14	18	14.0 a	14	16	16	17.3a	14Cd
K. pneumonia	4	10	16	10.0 ef	8	15	15	13.7bc	15Bc
S. marcescens	6	10	16	10.7 de	8	12	12	13.3c	13De
P. aeruginosa	R	4	12	8.0 f	R	8	8	10.0d	10F
Acinetobacter. Haemo.	10	12	14	12.0 bcd	12	14	14	14.0bc	12E
Entero. Cloacea	8	14	18	13.3 ab	10	16	16	15.3b	18A
Conc.Mean	7.71 C	11.0 B	15.8 A		10.3 c	13.8 B	13.8 B		

***The same laters means no significant difference amng them**

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