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Isolation And Identification Multidrug Resistance Bacteria From Urinary Tract Infectious And Effect Of *Capsicum Annuum* oil.

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ABSTRACT

This study was done to assess the proliferation bacterial and susceptibility resistance antibiotic pattern of (UTIs) pathogens. For identification of mid stream urine samples 40 causative microbial agents from 10-50 years clinical sample with clinical symptoms to be UTI were collected and appropriate biochemical tests. These samples were collected from Teaching Laboratories Center in Baghdad. The antimicrobial sensitivity test was carried out by Well Diffusion Assay technique using Muller- Hinton agar. Overall female only the most isolates were *Escherichia coli* with average rate of 50% followed by *Enterobacter* spp. (12%). Whereas, *Klebsiella*, *Pseudomonas Aeruginosa* and *Enterococcus faecalis* showed frequency rate of 9% for each. (Family Solanaceae) species *Capsicum frutescent* L. Usually used most of the spice, (red peppers) is also increased benefits with multiple healths human. In the study, yield extract included constituents phytochemical of (n-hexane, chloroform, ethyl acetate, acetone and methanol) extracts of dried fruit. Antibacterial activity in vitro of extracts was determined by WADA method gram positive bacteria (*Staphylococcus Aureus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas Aeruginosa*, *Klebsiella pneumonia* and *Enterococcus faecalis*). MIC evaluated by two fold serial broth tube dilution method ranged between 0.312 to 5 mg/mL

Keywords: Antibacterial, Minimum inhibitory concentration, pathogenic bacteria

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INTRODUCTION

Family of the Gram-negative bacteria (Enterobacteriaceae) is causes urinary tract infections (UTIs) most found hospital- and healthcare, bloodstream infections and various intra-abdominal infections [1]. Most commonly observed isolates *E. coli* productions are and rarely observed among other bacteria [2]. The antibiotics group betalactam have been widely used treatment of serious infections caused by gram negative bacteria, these antibiotic groups occurred quickly worldwide but resistance against other. Into uncomplicated and complicated UTIs are classified infections on pre-and post-treatment evaluation which have effects, extent of estimation of the urinary tract duration of antimicrobial therapy [3,4]. Family Solanaceae the genus *Capsicum* has five species *Capsicum annum* and *Capsicum frutescent* that are commonly recognized as domesticated ,agricultural crop and one of the popular vegetables also for the combination of color and taste nutritional values of its fruit . A micro plate method application and Development to evaluate efficacy of essential oils against *Penicillium italicum* Warmer, *Colletotrichum musea* and *Penicillium digitatum* [5]. Chili importance in human life and possesses dietary significance but also displays pharmacological such as (antimicrobial, antioxidant, anti-inflammatory and anti-carcinogenic) [6]. Anti-microbial have been investigated properties Extracts from *Capsicum annum* fruit, different *C. annum* varieties extracts Crude have inhibited growth of many species of Gve^+ and Gve^- bacteria [7].

Need to discover alternative (antimicrobial drugs) there is a for infectious diseases treatment screening on extracts plant various for the isolation compounds having antimicrobial activity.

Many reported that plants medicinally production different number of secondary metabolites with using against pathogens [8]. In this study the purpose identify the most common aerobic bacteria that clinical UTIs and to testing the ability to antibiotic susceptibility of isolates in this area of University of Baghdad. The UTIs included (ureter, bladder, kidney and urethra) is normally sterile space, with mucosa epithelium. The mechanism against UTI defense is constant flow ante grade from the (kidneys to the bladder) with complete emptying of the bladder to the urethra. The urine has specific anti microbial characteristics, itself also consistence low urine (pH, glycoprotein and poly morpho nuclear cells) which inhibits bacterial attachment to the bladder mucosal wall [9].

Investigate the ability of red peppers. Previously determined to possess the greatest antimicrobial activity against antibiotic resistance isolated bacteria from UTI.

MATERIAL

Isolation Bacteria:

All sample collected were Midstream urine in sterile containers from patient suffering (signs and symptoms) of UTI from Of Baghdad University students in Iraq suffered from urinary tract infections between the years 2016- 2017. Isolated bacteria were culture on different media (Nutrient, Mannitol salt, Blood agar and Mac Conkey agar plates) then incubated at (37°C for 24 hours). Then identified by Gram's stain and standard biochemical tests [10].

Antimicrobial susceptibility testing:

The method of (disc diffusion method) Antibiotic AST was performed for each isolate utilizing. This was performed on MHA with the following antibiotic discs (Ampicillin 10µg, Amikacin 30µg, Ceftazidim 30µg, Ciprofloxacin 5µg, Chloramphenicol 30µg, Gentamicin 10µg, Oxacillin 1µg, Tetracycline 30µg, Vancomycin 30µg, and Erythromycin 15µg). After incubation (24 hrs at 35°C) Sensitivity was read. The isolates bacteria were regarded as comparing with according to CLSI criteria [11].

Experimental Procedures:

Crude aqueous can preparation from *Capsicum annum* L. The fruits of *Capsicum annum* collected from market Baghdad/Iraq were dried at 37 °C at room temperature. The dried fruits were powdered and kept in clean beaker for use. About (10 gm) of the powder was with (100 ml) DW. Was heated the mixture

for a continuously at many hours in a (Soxlet at 400C) after that filtered in a (double layer) and reduced to half its volume by an evaporator rotary. Evaporated in the (hot air oven) the sample was completely and the solid residue was weighed [12].

Liquid dilution Method for detection of MIC:

The dilution broth technique described previously [13] was deceased where the extract ration red peppers was prepared to the highly concentration stock (200 mg/ml) in diethyl sulfoxides and (two-fold) dilution to a working between concentration ranging from (2, 2.5, 3) mg/ml.

Well Diffusion Method:

Well diffusion method (WDA) of [14] was used. The medium used was sterile (MHA) medium. Was loaded 100µl in one well of extract at each concentration (Test). After that incubated at 37° C for 24 hrs and the diameter of inhibition zone was measured.

Chromatographic analysis of essential oils composition:-

Essential Oil was analysis by using GC-MS to recognize the chemical composition of Capsicum annuumessential oils. The series II Gas Chromatograph interfaced Mass Selective was used HP Chemstation software (version b.02.05) Detector and controlled. Capillary column (30.0 cm x 25 mm x 0.25 mm) chromatographic separation was achieved using. The stationary phase column comprised of 5:95% diphenyl: dimethylpolysiloxane blend. The condition oven temperature of 35°C for 3 min operating GC, to 280°C at the rate of 10oC/min, and then kept constant at 280oC (25 min). The injector and detector temperatures were set at 270oC and the carrier gas was helium flowing at a rate of 1.2 ml/min [15].

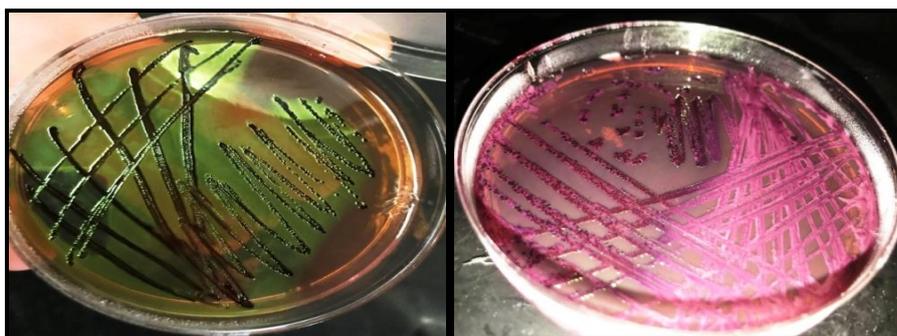
RESULT AND DISCUSSION

All the collected samples are from urine of patients. The age of patients are ranged between (10 to 50) years and the patient group comprises females only (Table 1).

Table 1: Bacterial isolates from urine of UTI women.

Bacterial Isolates	No. (%)
E. coli	14 (34.1)
Klebsiella spp	9 (21.9)
S. Aureus	8 (19.5)
Enterobacter sp	5 (14.6)
Pseudomonas spp	4 (9.7)
Total	40(100)

Pathogensisolated in these women were E. coli (34.1%), Klebsiella spp. (34.1%), P. Aeruginosa 7 (9.7%), S. Aureus (19.5%) and Enterobacter sp (14.6%). The results of final identification on the basis of biochemical analysis. (Figure 1)



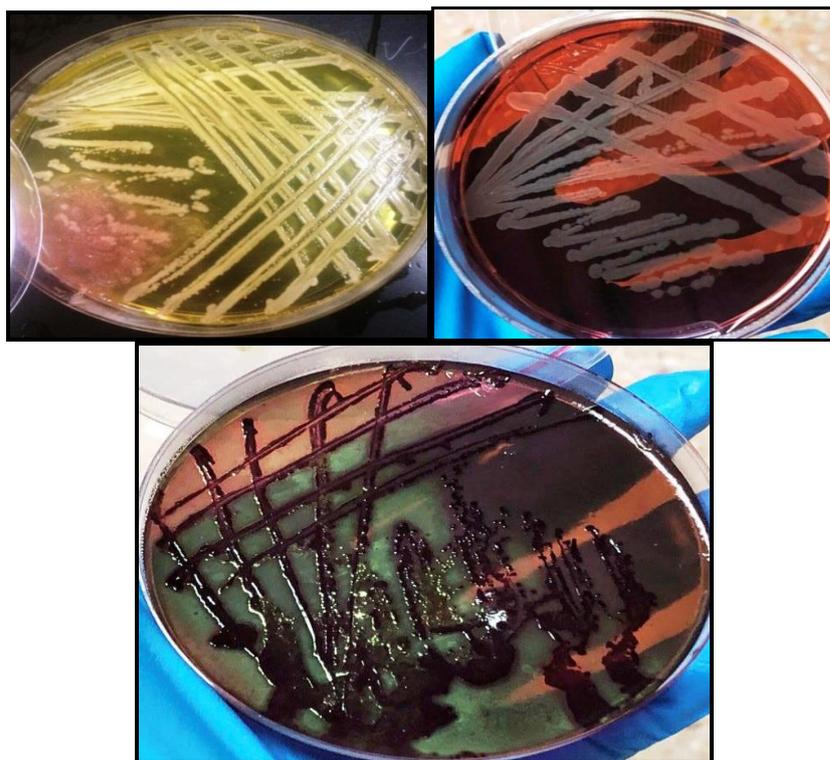


Figure 1: Bacterial isolation from UTI infection A: E.coli B: K. pneumonia C: S. Aureus D: P. aeruginosa E: Enter occurs faecalis after 24hrs at 27°C.

The most common type UTI the clinical disease are E.Coli .A high incidence of UTI in females than males [16].In vitro studies numerous have shown the E. coli and Klebsiella spp agree with [17].As the most common causes of UTI. Result shows found to be (E. coli, P.aeruginosa, Enterococcus faecalis and K. pneumonia) resistant to Ampicillin, amoxicillin, Ceftazidim, Erythromycin and Tetracycline between (92.7-96.5 %) but moderately resistant to other between (70.5-67.2 %).On the other hand S. Aureus isolates were highly resistant to Oxacillin, Gentamicin, Ciprofloxacin and Chloramphenicol (94.4%), while sensitive Vancomycin and Ceftazidim (76.5%).Agree result with [18] show the E. coli isolates β -lactam resistance is probably due to the production of (TEM β -lactamases), which may be the chromosome or on a plasmid genetically localized on.

The different varieties of Red peppers extracts effective were in zone inaction the test cultures tested in the study. Though all Capsicum extracts showed an against both bacterial isolates. The MIC and MBC values of the four varieties of Capsicum annum against bacteria strain.

The MIC a range between (4.0 – 5.0) mg/ml. The least MIC value from C. annum. The lowest MIC for the MDR isolates of bacteria was observed in 4.7 mg/ml respectively. The negative controls containing the extracts in

The antimicrobial activity of the oil extract:-

The antimicrobial activity of oil was extracted from the fruits of (Capsicum annum) was determined using a well diffusion assay. The results , table [2] show a good antimicrobial activity of oil extract against (Gve+ and Gve- bacteria), this may be justified due to the presence of (capsanthin compound) is considered as one of the important carotenoids of red pepper (account for 70-80% of total capsanthins) [19].

; This compound was known as the active compound in the red pepper, which has antimicrobial effects, antioxidant effects [16], anti-tumor and antimutagenic effects [17]. The results table (2), show that the MIC values of the oil extract were (3) μ g/ml. against bacteria which are Escherichia coli, Pseudomonas Aeruginosa and Klebsiella pneumonia(Figure 2) .The alcoholic presence of groups (-OH) in the structure of the studied oil extract the activity increase of the plant extracts to the microbial growth inhibitor. So the alcoholic

compounds and their derivatives are considered as antiseptic agents [18], which are changing the protein nature (Denaturation) and increase cell membranes permeability of [19].

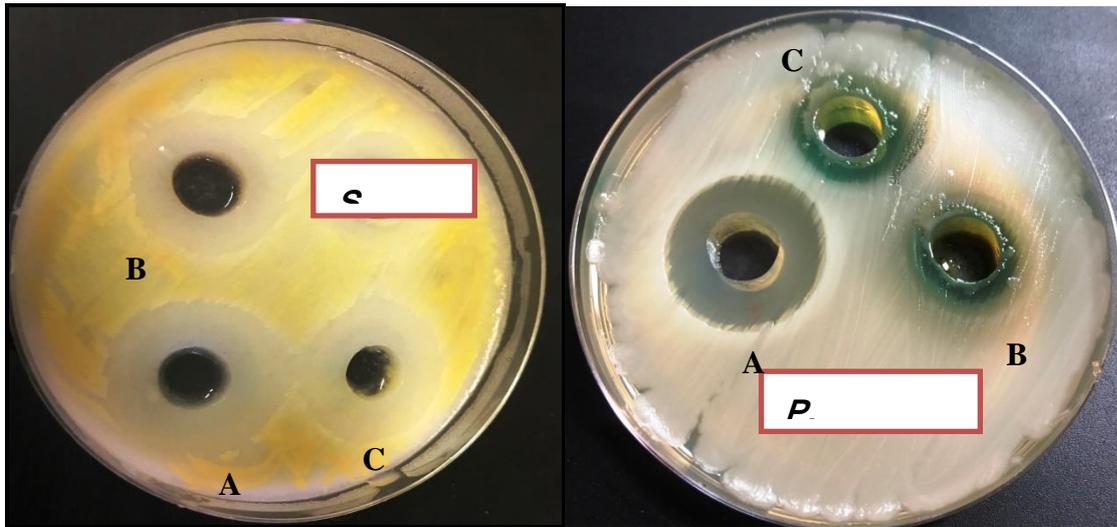


Figure2: Antibacterial activity of MIC (2, 2.5, 3) mg/ml Capsicum annum oil

Table2: The antimicrobial activity of the oil extract

Bacterial strains	Inhibition zone(mm)
Escherichia coli	11
Klebsiella pneumonia	9
Staphylococcus Aureus	10
Pseudomonas Aeruginosa	8
Enterococcus faecalis	7

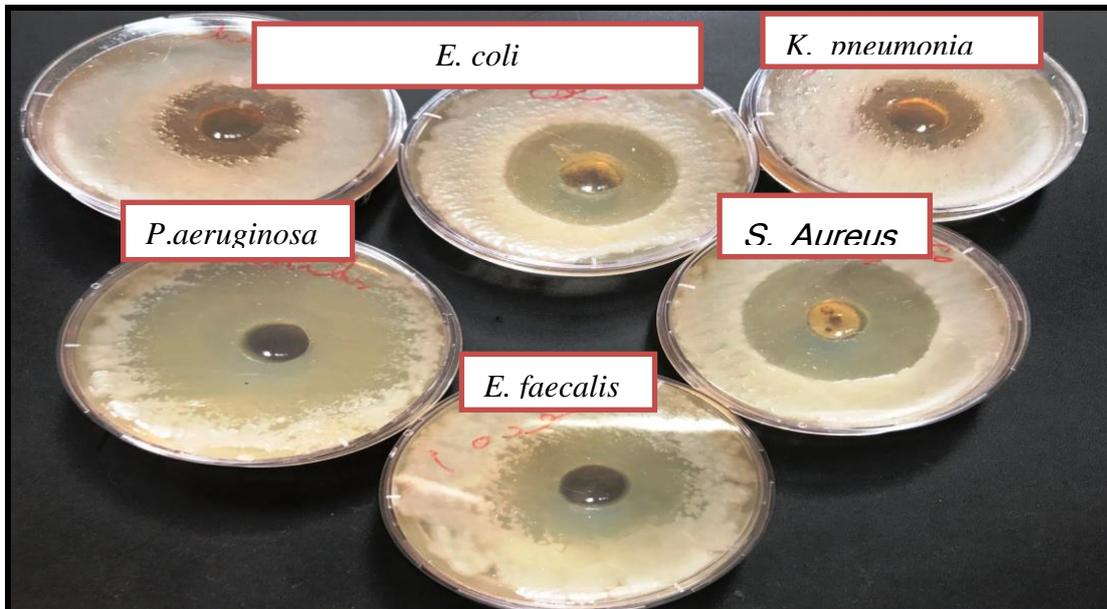


Figure 3: Inhibitory effect by WDA against gram positive and gram negative bacteria on Muller Hinton Agar at 37°C for 24 hrs.

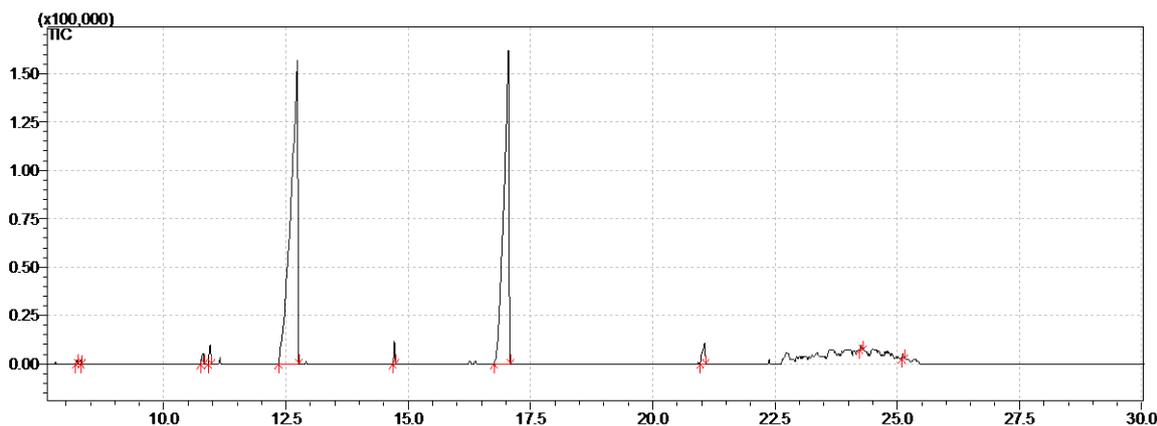


Figure 4: GC chromatogram OF Capsicum annul L. OIL

Table 3: Composition of oil extracts of Capsicum annum L.

NO	COMPOUNDS	RT MIN
1	Cyclobutane ,ethyle	2.133
2	Cyclopropane,propyl	2.133
3	Propane,2- cyclopropyl	2.133
4	3,4Dimethyldihydrofuran-2,5-dione	2.133
5	Oixirane,1-methylethyl	2.133
6	Dimthylphosphinic azide	8.225
7	2,1-Benzisoxazole	8.225
8	1-Butanamine,4-methylthio	8.225
9	2-Butynenitrile,4,4,4-trifluoro	8.225
10	Imidazo1,5a pyramiding	8.225
11	6-Azabicyclo3,2,0haptan-7-one	8.325
12	1,3-Pentadiene	8.325
13	Cyclobutane, methylene	8.325
14	4-penten-1-	8.325
15	Ethanedione,di-2-furanyl	10.808
16	1,4-phenylene bis2-furoate	10.808
17	1,2Benzenediol,o-2furoyl-o-butoxycarbonyl	10.808
18	4-pyridazinamine	10.808
19	Furan-2-carbonyl chloride,tetrahydro	10.950
20	Botanic acid ,2-propenyl ester	10.950
21	Benzenepropanoyl bromide	12.733
22	Beta-Miocene	14.717
23	Diethyl phthalate	17.050
24	Ethanethioic acid	21.058
25	Octane,1-iodo	24.258
26	1,5-heptadine-3,4-diol	25.117

CONCLUSION

The present investigation reveals mainly on the rural people health and treatment as they are deprived from the modern facilities and they are not aware of general health care. Recent discoveries reported that UTI pathogens are gradually becoming drug resistant and for that reason herbals which considered to be the best useful remedies. The herbals are the best alternative and like the supernatural blessing to the very poor people of the villages but as they do not know which plants are essential for the treatment of UTI, for that reason they have to go to the local traditional healers for the treatment of this disease. If proper documentation, cultivation procedure and dosages administration of these medicinal plants are done and focused in a very simple way to the village people it may yield a better for their own treatment. As there was no past scientific report regarding antimicrobial and phytochemical. In the present study we have investigated the composition of essential oils obtained from fruits of hot pepper cvs. Using GC-MS. Among the identified constituents predominant were aldehyde, terpenoids, hydrocarbons, fatty acids and their esters.

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