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Antibacterial effect of Jaft extract and molecular study of *cnf-1* genes in Uropathogenic *Escherichia coli*

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Abstract

Background: *Escherichia coli* is one of the more causative and frequent pathogens of urinary tract infection.

Objectives: The study conducted to evaluate the potential of Inner Stratum of Oak fruit (Jaft) extract in uropathogenic *E.coli*. Determine the effectiveness of an antibiotic against uropathogenic *E.coli* and detecting the genes codes of cytotoxic necrotizing factor1 (cnf-1) by PCR technique.

Methods: Four clinical samples of *E.coli* were gained from patients with urinary tract infections. Diagnosis of isolates was draw on conventional bacteriological methods and final identification was involved by vitek2 system. Four concentration (100%, 75%, 50%, 25% mg/ml) from ethanolic extract of Jaft was prepared and used agar wells diffusion method to execution antibacterial activity. Kirby -Bauer method was utilized to assess six of an antibiotics against isolates. Polymerase chain reaction technique was used in investigation of cytotoxic necrotizing factor 1 (*cnf-1*) genes in *E.coli*.

Results: The results of antibiotics sensitivity test showed that all four isolates of *E.coli* were highly sensitive (100%) to Meropenem, Amikacin and resistance (100%) to Trimethoprim, whearse (75%) of isolates were resistance to Ceftriaxone, Ceftazidim, and Levofloxacin. The results of evaluation of plant extract appear that Jaft extract inhibited *E.coli* in all concentrations and the maximum of inhibition zones were in concentration (50% mg/ml) and diameters of inhibition zones were (20 mm in three isolates and 27mm in last one). Cytotoxic necrotizing factor1 (*cnf-1*) genes were found out by molecular technique in four isolates, the results revealed that all isolates (100%) of *E.coli* were carried this gene.

Conclusion: We found in this study that UTI is caused by multi drug resistance (MDR) *E.coli* also found that the antibacterial effect of ethanolic extract of Jaft was strong against uropathogenic *E.coli* and *cnf-1* genes present in all isolates ,this indicate Cytotoxic necrotizing factor 1 play an important role in infectious disease such as UTI .

Keywords: *Escherichia coli*; PCR; Jaft extract; Kirby -Bauer method; *Cnf-1* genes

1. Introduction

Urinary tract infection is one of the repeated nosocomial disease and the prevalent bacterial infection in community **[1].** UTIs consider the second bacterial infections of human after the respiratory tract infection, UTI obtained from bacteria (Gram negative- bacteria), virus and also fungi have been involved; Uropathogenic *E.coli* (UPEC) is the remarkable

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essential causative factor responsible for approximately 80-90 % of all UTI in patients groups **[2]**. Antibiotic resistance in the management of uncomplicated and complicated community -acquired UTIs is an earnest medical matter ; the emergence of resistant bacteria was from widespread use of antibiotics[3]. Herbal medicines are known as a protection system against pathogenic bacteria .Inner Stratum of Oak (Jaft) are from herbal medicines, Oak is present in many regions of the world is containing many major component such as alkaloid, tannin, flavonoid and aromatic component. In many studies antimicrobial evaluation of different extract of Oak indicates activity against bacteria [4]. In *E.coli* several ferocity genes causing disease, these ferocity factors are capsul, adhesins, cytotoxic necrotizing factors1 (cnf1) and others [5]. Genes of ferocity factors were located on chromosomes or plasmids bacteria. By employing molecular technique, bacterial genome has been sequenced completely [6]. The objective of the current study were

- to evaluate the sensivisity of *E.coli* towards variance antibiotics,
- evaluate the activity of Jaft extract against *E.coli*, and
- detect (*cnf-1*) genes among clinical isolates of *E. coli* and study its roles in UTI

2. Material and methods

2.1. Bacterial strains isolation

Four isolates of *E.coli* were proned in this research. Isolates were collected from midstream patient's urine of all the ages and both sexes in the March 2024 (from microbiology laboratory of Tikrit teaching hospital, Tikrit, Iraq). The specimens were inoculated on blood agar, Eosin Methylene Blue and macConkey agar plate. All plates were incubated at 37 ^{0}C for 18-24 hrs.

2.2. Identification of isolates

Isolates were recognized as described in [7]. In addition, the isolates were identified by vitek 2 system.

2.3. Antibiotic sensitivity test

In this study, we use six common antibacterial agents tables (1). Disks paper of antibacterial agents in this test were placed on a plate of Muller-Hinton agar that is inoculated to for a bacterial lawn. The plates were incubated to allow growth of the bacteria and time for the agent to diffuse into the agar. If the bacteria is sensitive to it, a clear zone will occur around the disk where growth of bacteria has been inhibited [8].

Table 1 Antibiotics used in current study

No.	Name of antibiotic	Code	Concentration of antibiotic
1	Ceftriaxone	CRO	10 µg
2	Ceftazidim	CAZ	30 µg
3	Trimethoprim	TM	5 µg
4	Meropenem	MEM	10 µg
5	Levofloxacin	LEV	5 µg
6	Amikacin	AK	10 µg

2.4. Preparation of ethanolic extract of Inner Stratum of Oak fruit (Jaft)

Fourty gram of Jaft powder solved in (160) ml of ethanol 95 % for prepare ethanolic extract and were forwented in room temperature for 24 hours, by Whatman filter paper, the solvents were filtered and then the extract was stabled in petri dish at room temperature for drying[9].

2.5. Evaluation of antibacterial activity

Four concentration (100%, 75% , 50% , 25% mg/ml) from ethanolic extracts of Inner Stratum of Oak fruit (Jaft) were prepared.

Well diffusion method was followed [10]. First prepared Muller Hinton agar plates,the broth cultures of selected bacteria were incubated at 37 ^{0}C for 18 hours to obtain a uniformly culture. Four wells of 8 mm in diameter in each plates were cut out using sterile well cutter. In to each of the wells by using a micropipette, 200 µl of the plant extract was added and for 30 mins allowed to diffuse at room temperature. These plates were then incubated at 37 ^{0}C for 18 hours. The diameter of the inhibition zones is measured in millimeters, then antibacterial activity was determined.

2.6. Extraction of DNA and PCR

2.6.1. DNA extraction

DNA was extracted from the four isolates of *E.coli* using a specialized promega kit.

2.6.2. Measuring of DNA concentration and purity

The concentration and purification of extracted DNA were measured by a Nano-drop spectrophotometer.

2.6.3. Primer sequences of cnf1 genes

Table 2 Primer sequences

Virulence factor	Target gene	Primer sequence	Size of amplicon (bp)	References
Cytotoxic necrotizing factor	*Cnf-1*	F:AAG ATG GAG TTT CCT ATG CAG GAG R:CAT TCA GAG TCC TGC CCT CAT TAT T	498 bp	[11]

2.6.4. Detection of cnf-1 genes by PCR

PCR amplification of extracted DNA was done in a volume of 24.5 µl containing the following as described in table (3):

Table 3 Content of the reaction of PCR

No.	Content of reaction	Volume
1	Templet DNA	5 µl
2	Primers	0-5 µl
3	Master mix	12.5 µl
4	Nuclease free water	6.5 µ l
	Total volume	24.5 µl

2.6.5. The PCR thermocycler conditions: Described in Table (4).

Table 4 PCR program conditions for detecting *cnf1* genes

<table>
<tr><th>phase</th><th>Tm ^{0}C</th><th>Time</th><th>Cycle number</th></tr>
<tr><td>Initial Denaturation</td><td>95 C</td><td>5 min</td><td>1</td></tr>
<tr><td>Denaturation</td><td>95 C</td><td>40 sec</td><td rowspan="3">35</td></tr>
<tr><td>Annealing</td><td>58 C</td><td>1 min</td></tr>
<tr><td>Extension</td><td>72 C</td><td>40 sec</td></tr>
<tr><td>Final extention</td><td>72 C</td><td>7 min</td><td>1</td></tr>
</table>

Output of PCR were detached on 1.5 % agarose gel with electrophoresis and underneath UV light was imagened.

3. Results and discussion

Four samples of *E.coli* showed on EMB agar green metallic colonies, while inoculating samples on MacConkey agar showed pink colored, non-mucoid colonies and grayish white and gamma-hymolytic colonies on blood agar. Gram staining revealed gram negative rod bacteria. Colonies of *E.coli* also identified using IMViC tests , oxidase test , TSIA test.

In this study, we used disc diffusion method for testing the susceptibility of *E.coli* to antibiotic, the isolates of *E.coli* were tested for six variance antibiotics and the results showed that all *E.coli* isolates were completely sensitive (100%) to Meropenem, and amikacin figure (1) and resistant (100%) to trimethoprim, wherease, (75%) of isolates were resistance to Ceftriaxone, Ceftazidim, and Levofloxacin (table 5 and figure 2). [12] In their studies indicated the prevalence of antimicrobial resistance among urinary isolates in India and *E.coli* was more isolates showed resistant to commonly used antibiotic to treat UTI. [13] mention in their studies a significant number of the UTI were caused by multi drug resistant *E.coli* and UTI treatment by antimicrobial agents needs to be powerful promotes by susceptibility testing to avoid advance extension of antimicrobial agent resistance in patients.

Table 5 Results of *E.coli* antibiotic sensitivity test (Inhibition zones)

Antibiotic disk	Number of sensitive (%) isolates	Number of intermediate (%) isolates	Number of resistant (%) isolates
CRO	1(25%)	-	3 (75%)
MEM	4(100%)	-	-
LEV	1(25%)		3(75%)
CAZ	-	1 (25%)	3(75%)
AK	4(100%)	-	-
TM	-	-	4(100%)

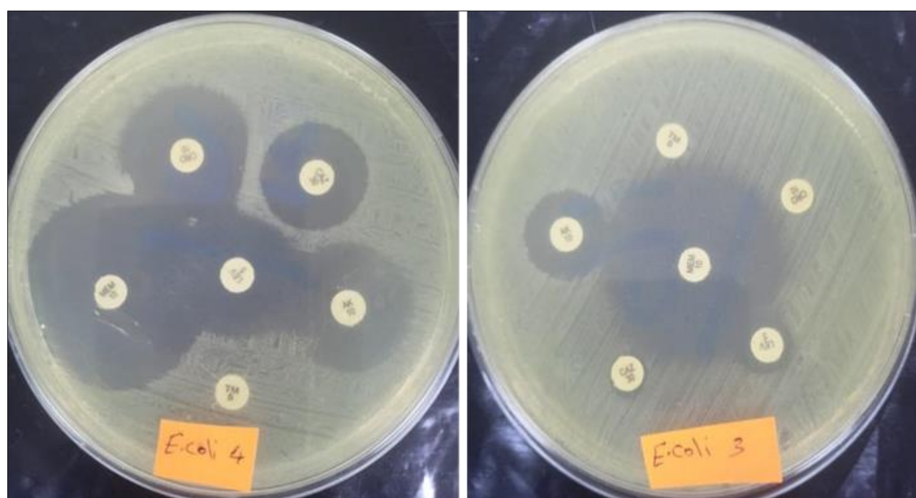

Figure 1 and 2 Susceptibility test of *E.coli* isolates using disc diffusion method

Well diffusion agar method was employed for the tested antibacterial activity of ethanolic extract of Jaft against *E.coli* isolates and the results were shown in table (6). According to the obtained results, ethanol extract of Jaft indicate activity against all the *E.coli* isolates and in all concentrations see figure (3), the maximum inhibition zone is 27 mm in con. %50 mg/ml. In some studies Oak extract showed activity against some microorganisms such as *Candida albican, S.aureus, Micricoccus leutus, E.coli, Bacillus subtilis* and *Bordetella branchiceus* this extract contain the potential component like nitosan, amidan, oil, gersit and tannin, antimicrobial activity of Oak extract involve complex mechanisim like inhibition of nucleic acid, cell wall, metabolism and cell membrane. In Iran, people use Jaft to treatment of microbial infection from antient times because its antifungal and antibacterial effective [14].

Table 6 Antibacterial activity of Inner Stratum of Oak fruit (Jaft) ethanolic extract against *E.coli*

Isolates	100%mg/ml	75% mg/ml	50% mg/ml	25% mg/ml
E.coli 1	13 mm	17mm	20 mm	16 mm
E.coli 2	17 mm	19 mm	20 mm	19 mm
E.coli 3	14 mm	10 mm	20 mm	19 mm
E.coli 4	16 mm	22 mm	27 mm	25 mm

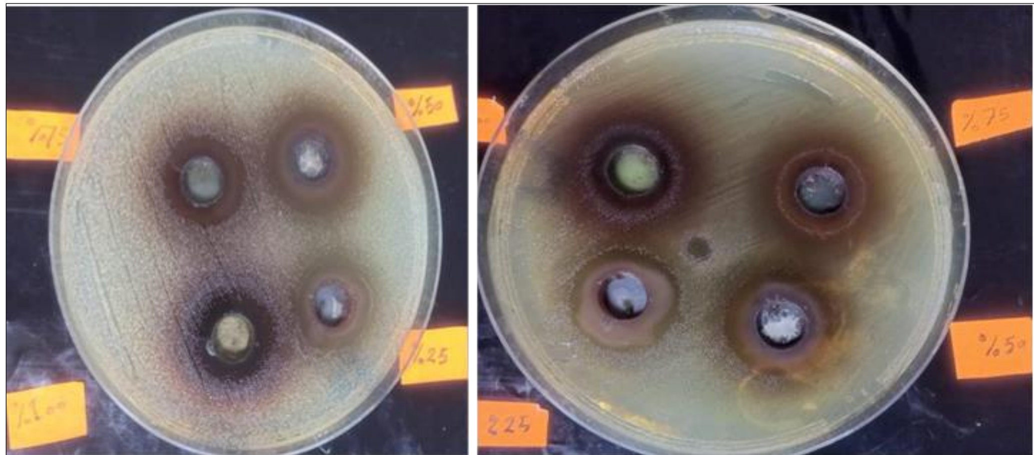

Figure 3 Inhibition zones of ethanolic Jaft extract against *E.coli* isolates

PCR is the suitable approach for diagnosis and discovering of virulency genes in microorganisms. The present results established that all isolates (100%) of *E.coli* were carried of *cnf-1* genes (see fig 4).[1] In Addis Ababa, Ethiopia, found in their studies among UTI patients the *cnf-1* gene is present in 58(29 %) isolates of *E.coli*, obtained in Iran 36.5% ; in Pakistan 20% ; Romania 13% ; Tunisia 3% and Poland 12.1%. However, when the mutation obtain at the level of the gene, this give negative PCR result. Therefore negative PCR doesn't mean no present of ferocity gene in bacteria. cytotoxic necrotizing factor (cnf-1) is toxicants substances produce by Uropathogenic *E.coli*, cnf -1 expansion incorborate of bacteria by shedding of bladder cell.With an extensive complement of virulency factors, the strains of *E.coli* are more effective pathogens [15].

[16] recorded in their studies that the isolates of *E.coli* was a multidrug resistance and the *cnf-1* gene was present just in uropathogenic *E.coli* in percentage 10% . CNF is a form of toxins created by UPEC helps *E.coli* in settlement and diminish epithelial turnover, CNF1 can stimulate the in vitro transference of epithelial to mesenchymal tissue, which may increase the exposure of cancer also may produce multinucleation in cultured cells and necrosis. The predominance of virulence factor genes difference between the results this study and the results of other studies may be expected to variance in sample proportion. The severity of UTIs yields by *E.coli* is due to the announcement of an extensive scale of toxins [17]. Studies the role of these factors would be essential in understanding the urinary infections, which in turn may generate the evolvement of universal vaccines to evade such infections.

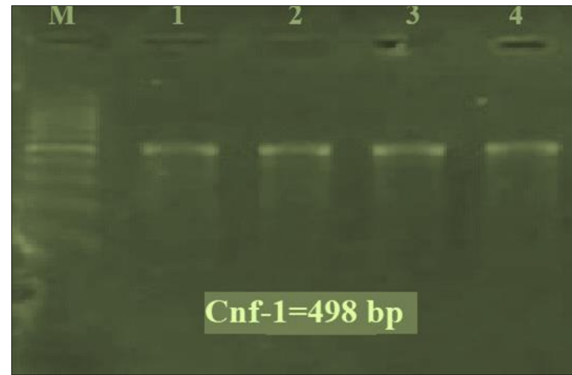

Figure 4 Electrophoresis of amplified *cnf-1* genes (498bp) of *E.coli* by PCR, isolates 1-4 were positive results for of *Cnf-1* gene. Lanes: M, 100 bp ladder marker

4. Conclusion

It concluded that the antibacterial effect of ethanolic extract of Jaft was strong against uropathogenic *E.coli* and It was supposed that in the near future the Jaft extract may become a new substances to control inflammatory disease and oxidative stress pathogenesis. We also concluded in this study that UTI is caused by multi drug resistance (MDR) *E.coli*, in current years UTIs caused by *E.coli* has increased due to the irrational and increasing use of antibiotics.Cytotoxic necrotizing factor 1 play an important role in infectious disease such as UTI. Knowing the virulence factors of pathogens and chosen the best antibiotic for therapy of *E. coli* is important for prevention,correct management and control of UTIs

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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