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Evaluation of Antimicrobial Activity of Plants Extract Against Bacterial Pathogens Isolated from Urinary Tract Infection among Males Patients

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ABSTRACT

Background: Urinary tract infection (UTI) is a considerable problem affecting the health of people each year. It is caused by various Gram-positive (G+ve) and Gram-negative (G-ve) pathogens. It is an important illness in the world affecting all age groups across their life span.
Objectives: To identify the most common aerobic bacteria that cause UTIs and their antibiotic susceptibility and antimicrobial activity of plant extracts of the males' patients.
Materials and methods: The study involved 35 midstream urine samples from the male students (University of Baghdad, Baghdad, Iraq) with suspicious symptoms of UTI, during the period from January-March 2018. Each urine sample was cultured first on Mannitol Salt Agar and MacConkey agar plates to differentiate between G+ve and -ve bacteria. The isolated bacteria were subjected to certain antibiotics and 100% plant extract oils.
Results: Out of 35 urine samples, there were 62 isolates. There were 34 (54.8%) G-ve and 28 (45.2%) G+ve pathogens. *Staph. epidermidis* (n=17, 27.4%) was the most common isolated pathogen, while *P. aeruginosa* (n=5, 8.1%) was the least isolated organism. Vancomycin was the most sensitive antibiotic for the *Staph. epidermidis* (52.9%) and *Staph. epidermidis* (45.5%). While oregano oil was more sensitive to *Staph. epidermidis* and *Staph. aureus* in 64.7% and 63.6% respectively. Imipenem was the most effective antibiotic for the three common G-ve isolates *E. coli*, *K. Pneumoniae*, and *Enterobacter spp* in 83.3%, 90%, and 85.7% respectively. Oregano oil was the most susceptible plant extract for *E. coli* (91.7%).
Conclusion: Plants oils are potentially a good source of antimicrobial agents. Besides, the plant extract oils are cheaper than antibiotics. Therefore, they could be used in UTI medicine.

Keywords: Antibacterial; Plant extract; Pathogenic bacteria; UTI.

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INTRODUCTION

Urinary tract infections (UTIs) are caused by both Gram-negative (G-ve) and Gram-posative (G+ve) microorganisms as well as by certain fungi. *E. coli* is the most common causative pathogen for both complicated and uncomplicated UTIs. For the agents involved in uncomplicated UTIs, uropathogenic *E.Coli* is followed in prevalence by *K. Pneumoniae*, *Staph. saprophyticus*, Enterococcus faecalis, *group B Streptococcus (GBS)* , *Proteus mirabilis* , *P. aeruginosa*, *Staph. aureus*, and *Candida spp* . [1]. *E. coli* and *Staph. aureus* types are two opportunistic pathogens, they are the most severe risk to life [2]. The common G-ve species *E. coli* is found in the intestine of humans. According to the World Health Organization (WHO), more than 80% of the world documented are increasing resistant rates of *Staph. aureus* and *E. coli* to most types of antibiotics [3]. Since several plant antimicrobials contain different functional groups in their structure, their antimicrobial activity is attributed to multiple mechanisms [4]. Treatment of the UTIs depends on the responsible bacteria because all bacteria possess their susceptibility towards a variety of antibiotics [5]. Studies have estimated 16S rRNA gene sequencing for clinical microbiology, the utility of that permit identification of specific microbial taxa [6]. A promising source of antibacterial compounds is a variety of secondary metabo-

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lites of medicinal plant products that have been therapeutic [7]. Their antimicrobial activity is attributed to multiple mechanisms, they contain different functional groups unlike antibiotics and relatively smaller resistance of the bacteria to the plant antimicrobials [8]. The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in the plants. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or all parts of the plant [9]. The antimicrobial property of essential oils from plants was used empirically for centuries, but only recently has studied scientifically. An increasing body of research is being compiled on the antimicrobial activity of various plant oil extracts [10].

The present study has designed to identify the most common aerobic bacteria that cause UTIs and detect the antibiotic susceptibility and antimicrobial activity of the plant extracts against resistant antibiotics from male patients.

MATERIALS AND METHODS

Collection of Urine Sample

This prospective study was conducted at the health center belongs to the University of Baghdad, Baghdad city, Iraq. The current study was covered the period from January-March 2018. Male patients with clinical suspicious symptoms of UTI (dysuria, loin pain, fever, frequent urination, and feeling the need to urinate despite having an empty bladder) [1] were enrolled in the study. Any student who refuses to participate in the study or takes antibiotics in the last 7 days for UTI or other infectious conditions was excluded from the study. The study approved by the Ethical Committee of the University. Informed consent was taken from each participant.

Cultivation and Isolation Bacteria

Each urine sample was cultured first on Mannitol Salt Agar, MacConkey agar and Blood agar plates to differentiated between Gve+ve and G-ve bacteria, then cultured by using another medium such as Nutrient agar and Blood agar by utilizing a sterile standard loop (1ml) then incubated at 37^{o}C for 24 hours [9].

Biochemical Analysis

According to the methods described by Bergeys manual, the morphology and biochemical tests were conducted of determinative bacteriology [10].

Antimicrobial Susceptibility Testing

The following antibiotic discs; ampicillin (10μg), imipenem (30μg), ceftazidime (30μg), ciprofloxacin (5μg), chloramphenicol (30μg) were used for G-ve bacteria, while methicillin (5μg), gentamycin (5μg), chloramphenicol (30μg), penicillin G (10μg), and vancomycin (30μg) were used for G+ve bacteria. The bacteria isolates were regarded as sensitive or resistant according to Clinical Laboratory Standards Institute (CLSI) criteria [11].

Plant Material

The plant oils Cloves, Oregano, and Cinnamon were obtained from Hemani company in the local market city of Baghdad city and 100% concentrations of oil were prepared according to MacFaddin method [12].

Well Diffusion Method

The plates agar were cultured with both G-ve and G+ve bacteria. Wells were cutting into the pour plates with 5 mm sterile cork borer were loaded with 100 μl of the way of supernatant. The plates were incubated at 37^{o}C for 18-24 hours. The hindrance was identified by the zone of clearing around the supernatant well. As per the outcomes above, one isolate of *Staph. aureus* resistance was chosen and utilized as a part of the ensuing examinations [13].

DNA Extraction

Genomic DNA was extracted from the detected bacterial isolates according to the protocol of Wizard Genomic DNA Purification Kit, Promega. Quantus Fluorometer was used to detect the concentration of extracted DNA.

Primers Selection

The set of primers 27F (AGAGTTTGATCTTGGCTCAG) and 1492R (TACGGTTACCTTGTTACGACTT) were used for amplification of 16s rRNA for identification of bacteria at the gene level [14].

Sanger Sequencing

After PCR protocol products were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation-Korea. A consensus sequence of the 16s rRNA gene was generated from forward and reverse sequence data by using genius software. DNA sequencing data were analyzed using BLAST with the NR database of NCBI GenBank.

Isolation and Identification of Bacteria

Isolation and Identification of G+ve Pathogens

The macroscopic examination of isolates on Mannitol salt ager can ferment mannitol and turn the color of the medium from red to yellow were classified as a presumptive *Staph. aureus* and *Staph. epidermidis* isolates as shown in Figure 1. The G+ve cocci appeared as single-cell pairs by the microscopic examination of the bacteria.

The isolates on Blood agar were showed yellow-gray colonies with 3-4 mm in diameter on the zones of $\beta-$ hemolysis. This description is mentioned in a previous study [15].

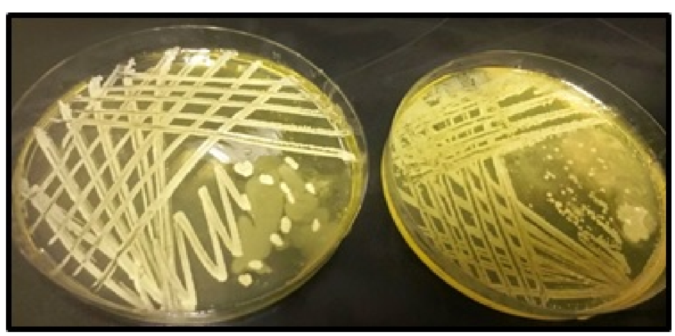

Figure 1. A presumptive of *Staph. aureus* on mannitol salt agar.

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Biochemical Characteristics

As mentioned by Harith and Eman's study [16], the current results of biochemical tests referred that the isolates were positive to coagulase, catalase, citrate, and ferment of mannitol, while oxidase and motility tests were negative.

Isolation and Identification of G-ve pathogenes

The macroscopic examination of presumptive *E. coli*, *P. aeruginosa*, *Klebsiella spp*, and Enterobacter pp isolates gave on the blood agar large flat colonies that produced hemolysis zones with odor and pale colonies non-fermenting on MacConkey agar as shown in Figure 2.

In this study, the genomic DNA of the bacterial isolate was successfully extracted and 16S rDNA was amplified by PCR using specific primers that give a distinct amplicon pattern with a size of 1500 bp when analyzed in gel electrophoresis as shown in Figure 3.

Statistical Analysis

The data were entered and analyzed through using the software IBM SPSS version 22. The results were presented in tables as frequencies and percentages.

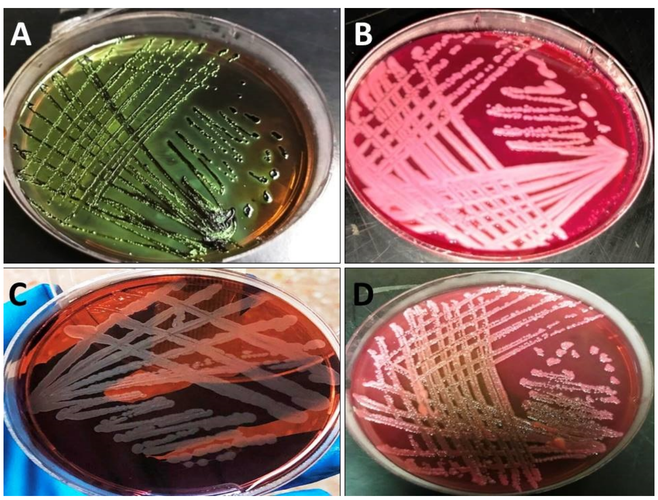

Figure 2. Isolated bacteria from the urine of the patients with UTI on MacConkey agar mediums at 37^{o}C for 24 hrs. A: *E. coli*, B: *K. Pneumoniae*, C: *P. aeruginosa*, and D: *Enterobacter spp.*

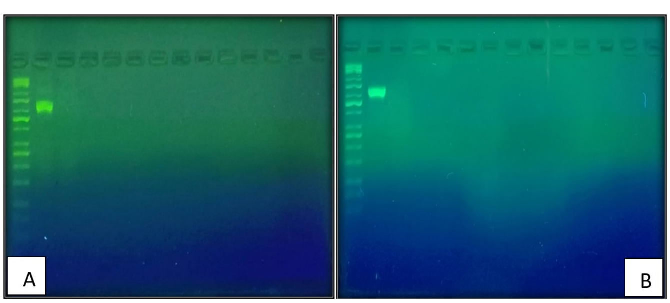

Figure 3. Agarose gel 1% electrophoresis (100v/mAmp for 90 min) of amplified 16s rRNA (1500pb) gene of A: *Staph. aureus* DNA B: *E. coli.*

RESULTS

The age ranged was from 18-23 years with a mean of 20.43 years ± 1.703. Out of 35 specimens, 27 (77.1%) were yielded 2 bacteria and the remaining were 8 isolated single microorganism per specimen. Therefore, the total number of isolated pathogens were 62. The majority of the isolated microorganism was G-ve 34 (54.8%), and 28 (45.2%) were G+ve pathogens as shown in Table 1. The most common isolated bacteria was *Staph. epidermidis* (n = 17, 27.4%), followed by *E.Coli* (n = 12, 19.4%), and the least was *P. aeruginosa* (n = 5, 8.1%) as shown in Table 1.

Staph. epidermidis and *Staph. aureus* were susceptible to methicillin in 17.6 % and 9.1 %, to the chloramphenicol 41.2 %, and 18.2 %, and to vancomycin 52.9 %, and 45.5 % respectively. On the other hand, both strains were mostly sensitive to oregano oil in 64.7 %, and 63.6 % respectively as shown in Table 2.

There were various levels of susceptibilities to different antibiotics among the studied G-ve isolates. Imipenem was the most sensitive antibiotic against the 3 most common G ve isolates *E. coli* (83.3%), *K. Pneumoniae* (90%), and *Enterobacter spp.* (85.7%). Chloramphenicol was the most susceptible antibiotic against *P. aeruginosa* (60%). While Ciprofloxacin was the least sensitive antibiotic for the isolated pathogens (25% was the maximum sensitivity to *E. coli*). On the other hand, plant extracts were more susceptible to the isolated microorganisms in comparison to the used antibiotics. Cloves' susceptibilities against the isolated organisms were ranged from 42.9% to the *Enterobacter spp* to 80% to the *P. aerug-*

Table 1. Frequency of 62 isolated bacteria from the urine of 35 male patients.

Bacteria	Frequency	Percent
G+ve		
Staph. epidermidis	17	27.4
Staph. aureus	11	17.7
Total	28	45.2
G-ve		
E.Coli	12	19.4
Klebsiella spp.	10	16.1
Enterobacter spp	7	11.3
P. aeruginosa	5	8.1
Total	34	54.8

Table 2. Antimicrobials susceptibility of 28 isolated gram-positive bacteria.

Antimicrobials	*Staph. epidermidis* Number = 17	*Staph. aureus* Number = 11
	Number (%)	Number (%)
Methicillin	3 (17.6)	1 (9.1)
Gentamycin	6 (35.3)	3 (27.3)
Chloramphenicol	7 (41.2)	2 (18.2)
Penicillin G	8 (47.1)	3 (27.3)
Vancomycin	9 (52.9)	5 (45.5)
Cloves	10 (58.8)	7 (63.6)
Oregano	11(64.7)	7 (63.6)
Cinnamon	6 (35.3)	5 (45.5)

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Table 3. Antimicrobials susceptibility of 34 isolated gram-negative bacteria.

Antimicrobials	*E. coli* Number=12	*K. Pneumoniae* Number=10	*Enterobacter spp* Number=7	*P. aeruginosa* Number=5
	Number/%	Number/%	Number/%	Number/%
Ampicillin	1 (8.3%)	0 (0)	2 (28.6%)	2 (40%)
Ceftazidim	5 (41.7%)	3 (30%)	0 (0)	2 (40%)
Ciprofloxacin	3 (25%)	0 (0)	0 (0)	0 (0)
Chloramphenicol	6 (50%)	5 (50%)	3 (42.9%)	3 (60%)
Imipenem	10 (83.3%)	9 (90%)	6 (85.7%)	0 (0)
Cloves	9 (75%)	6 (60%)	3 (42.9%)	4 (80%)
Oregano	11 (91.7%)	5 (50%)	2 (28.6%)	1 (20%)
Cinnamon	8 (66.7%)	4 (40%)	3 (42.9%)	4 (80%)

inosa. Oregano was the most sensitive antibiotic against *E. coli* (91.7%) as shown in Table 3.

DISCUSSION

The role of the plant extract in UTIs patients has been unwell studied relative to other antibiotic agents in particular imipenem. However, initial studies have been indicated a possible role in some bacterial diseases including UTI. Our results were revealed that the most common bacteria isolated from urine samples was *Staph. epidermidis* (27.4%) followed by *E. coli* (19.4%), the finding differs from Magliano et al study from Milano-Italy [17] which have reported that *E. coli* are accounted for 67.6% of the total isolates. Other studies have also revealed that *E. coli* is the most common isolated pathogen [18, 19]. The study by Ibrahim et al. from Duhok [20] has shown that *E. coli* is the most common bacteria in pediatric patients diagnosed with UTI. The bacteria are increasing sensitivity to the antibiotic such as gentamycin, amikacin, and norfloxacin. Recurrent uses of antibiotics might lead to making the pathogens are resistant to antibiotics [20].

The results by Tankhiwale et al. study [21] have concluded that 87 from the total isolates (217) were G-ve bacilli. 48.3% of them were extended-spectrum beta-lactamases producers (ESBL). *E. coli*, *K. pnuemoniae* , and Acinetobacter were ESBL producing microorganisms. The study has also found that multidrug resistance has a statistically significant difference (P-Value<0.05) between the ESBL producing pathogens (90.5%) and non ESBL producing isolates (68.9%) [21]. Therefore, periodic surveillance of ESBL producer pathogens and their antibiotic susceptibility testing is necessary to avoid treatment failure in subjects with UTI. Ouno et al. study has reported that the most common causative agent in the UTI was *E. coli.* Chloramphenicol and ciprofloxacin are effective for all isolated pathogens and could be considered for empirical therapy [22].

The plant extracts essential oils have a wide spectrum of biological activities that hold promise in medicine and agriculture, because of its low toxicity, non-persistence in the environment, biodegradability, and affordability [23]. Tibyangye et al. study [24] have illustrated that the essential oil derived from O. suave was active against all isolated pathogens (*E. coli*, *K. Pneumoniae*, *Staph. aureus*, E. feacalis, M. morganii, Citrobacter species, Enterobacter species, and*P. aeruginosa*) except Acinetobacter species. SHARMA et al. from India have used two essential oils (clove and eucalyptus), clove oil was effective for all the four pathogens (*S. indicum*, *E. coli*, *Staph. aureus*, and B. subtilis) used in the study. B. subtilis was the most susceptible bacterial strain to clove oil. Eucalyptus oil was effective for the *S. indicum* and *E. coli* but was much less in comparison to clove oil. *Staph. aureus*, and B. subtilis were found completely insensitive to eucalyptus oil [25]. Our results were promising in using the plant extract in clinical practice as they were revealed that Oregano oil was the most effective plant extract used for *E. coli* (91.7%). Moreover, the plant extract in Iraq has low cost in comparison to antibiotics. Therefore, we recommend using Oregano oil as empirical therapy for patients with UTI.

Oregano was used antibacterial, anti-inflammatory in traditional medicine, its antimicrobial activity against *E. coli*, Pseudomonas sp., and Salmomella sp., has been confirmed in previous studies [26]. A combination of the oregano oil and quinolone antibiotics (tosufloxacin, levofloxacin, ciprofloxacin) was completely eradicated in all stationary phase of the *E. coli* [27]. The study has been concluded that their results were facilitated the development of more effective therapies for persistent UTIs [27].

This was a preliminary study and had limitations included a small sample size, no control group, a limited number of antibiotics used, and no comparison between the plants and antimicrobials' susceptibilities to the isolated pathogens.

In conclusion, the study was shown that G-ve was more prevalent than G+ve pathogens. *Staph. epidermidis* and *E. coli* were the predominantly isolated organisms from subjects with UTI. We could use the plants in the treatment of UTI because they were more effective for the most isolated pathogens and cheaper than the antibiotics.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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