Original Article

Estimateof Biological Activity of Parsleyextract on the Isolated Pathogenic Bacteria in Baghdad City

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Abstract

This research investigates the most common urinary tract infection bacteria and their susceptibility to antibiotic disc and parsley (Aquas and methanol) extracts. The urinary tract microbial were isolated from patients of urinary tract infection (UTI) pregnant women patients from Baghdad Hospital. The pathogenic organisms were collected, cultured and identified. 50 microbial isolate wasset as, *E. coli 56* (46.66%), *Staphylococcus aureus19* (15.8%). *Proteus mirabilis* 6 (5%) and *Klebsiella pneumonia* 3 (2.5%). The pathogenic bacterial showed various zone of sensitivity to different antibiotics. Within the parsley Antimicrobial activity ranged from 3 to 20 mm. Antibacterial activity of parsley extract (5-22) mm on UTI isolated pregnant women and methanol extracts was between (3-15) mm. Therefore, Parsley extract had a pharmacological to treat all of 50 isolates that were identify from patients. In conclusion, the findings suggested that different extractsmay content as a broad-spectrum bactericidal factor, to control the emergent pathogens any way of their drug resistance mechanismsto treat urinary tract infections.

Key word: *parsley, Petroselinumcrispum, UTI infection, water, methanol, inhibition zone.*

Introduction

Urinary infections, are the most common form of infection in community practice. Annually, Among the 150 million of people worldwide have been invistigated with urinary tract infection [(1).] Managing urinary tract infection requires investigation,possible site for a responsible infection and pathogen that can include both the upper or the lower urine tract system[(2).] Lower urinary tract infections (cystitis) are characterized by a spectrum of dysuria, urgency, recurrence andSuprapubic pain [(3).]

The causes of urinary tract infection, by (sex and age)beforehand use of antibiotics and pollution inside or out of the hospital not to remind that they differ from environment to other. The microorganisms that at most cause urinary tract infection are G-negative intestinal microbial, essentially*Escherichia coli*, which are a lot offstudied microorganisms, follow by another G-negative bacteria like*Klebsiella*, *Enterobactersp,Acinetobactersp,Proteus sp,* The *Pseudomonas S.*[(4).] This problem was increased bacterial resistance. So in the last decade studies on natural remedies for the development of alternative drugs, whether natural or synthetic plant extracts have also been a valuable source of natural products to maintain health Human, and therefore in Iraq, increased plant compounds used for medical purposes as well as the world WHO Health Plants Ceremony is the best source for a variety of medications [(5).]

Medicinal plants has become diffuse and has enriched massive different biological effect and the combination of native, EuropeanandAfrican cultures [(6).] A number of researchers around the world have investigated the antiactivity properties of extracts plant[(7).]Parsley is a medicinal plant containing many proven medicinal properties including antioxidants,

anti-diabetic, analgesic, cramps, immunity, anticoagulant, ulcer, laxative, estrogen, diuretic, antihypertensive for fungus[8]. The beneficial effects of zucumonumcrispum on the digestive system claimed in ethnic medicine from various states, have been demonstrated by the mechanisms of spasm, analgesia, gastro protective, anti-secret and laxative in modern scientific investigations [9]Many researchers indicates that in several plants there are multi compound such as peptides unsaturated with long chain alkaloidal (aldehydes, phenols, and ethanol, chloroform, water, butanol and methanol are soluble compounds)[10]. Furthermore, the activity, of plant crispum on urinary tract inflammation, has been demonstrated through diuretic activity. The antiseptic property, can be (urinary tract) due to antimicrobial activity.Therefore, The purpose of this study areisolation, identification of microbial urinary tract infection with testing the sensitivity of the microbial organism to some antibiotic disc and biological activities of (parsley extract plants) of medicinal uses in Iraq.

Material and Methods:-

Sample collection:- A total 50 urine samples were collected from pregnant women infected Urinary Tract Infections from Baghdad hospital. The samples were collected an aseptically in sterile container from period April-August, 2019.

Diagnosis bacteria:- All isolated with selective and differential media were identified depend on morphological, Gram stain and classical method biochemical analysis, used for identification of bacteria including: IMVIC test.

Preparation plant:- For this work, plants parsley dried and ground to powdered of plant materials were used for extraction with different solvents (methanol and watery) were obtained from market in Baghdad City. The plants were cleaned with tap water then were process with (distilled water). The plant dried in room temperature, grounded to powdered, and stored in room temperature at 25°C until uses

Extract preparation:-

A total of 100 gm. of the leave parsley powder was steeped in (200 ml) of different solvent (ethanol, and watery) till 2 days, and filtered out oflayer muslin cloth and centrifuged at 3000×g for 15 minutes. The ethanol and methanol extracts were concentration with a Rotator evaporator at 40°C. After that, the extract was kept aseptically in sterile vials at 4°C until use. [11].

Antibacterial Activity of extracts by diffusion method:-

Antibacterial activity of parsley extract (watery and methanol) were evaluated by determine inhibition zone (mm) diameter and compared with standard antibiotic disc, disc diffusion method was used by sterile filter paper disc (diameter : 6 mm) which impregnated with extract in a known volume (20 µl) and appropriate concentration of the extract(500, 1000, 1500 µg/ml) and placed on a plate of inoculated nutrient agar , so used discs containing gentamicin (10 µg/disc) as positive controls and discs containing sterile water (20 µg/disc) as a negative control in the study. The incubation period (1 day), evaluated the antimicrobial activity of every concentration by measuring inhibition zone with (mm). All methods were performed in deplicate and the values ofmeanwere taken.

Susceptibility for antibiotic disc:

The resistance of microbial isolates to different antibiotics was estimated by using the diffusion diskmethod on Mueller-Hinton agar plates (Bauer *et al*.,1966)[12], The antibiotics disc includethe: amoxicillin (25 µg), Trimethoprim (10µg), Penicillin (10 U), Ofloxacin (5 µg), gentamicin (10 µg), Nitrofurantoin (100 µg). These antibiotic disks were submitted on culture agar plates and incubated at 37 C for 24 hrs. Then determine and measured Inhibition zone (mm) and recorded as sensitive and Resistance according to (CLSI) guidelines (CLSI, 2010)[13].

Statistical Analysis

Statistically was carried out using statistically software (SPSS version 10). The comparisons between groups were done using P <0.05 was considered as statistical significant.

Results

Petroselinumcrispumis a herbal plant it's used in traditional medicine for the treatment of UTI. For there, its precise role has not been investigated through a clinical study. Thus, our study is the first clinical trial study trying to explore the role of (Petroselinumcrispum) parsley in the treatment of pregnant women at urinary tract infection. Urinary tract infection, are caused by many microorganisms including gram positive like *Staphylococcus* and gram negative such as *Ecoli* and *pseudomonas and protues*. A total of 50 bacterial isolate were collected from clinical sample of UTI patients from Baghdad hospital isolates as 43 positive culture while 7 as negative culture were isolated from 50 UTI sample.

Distribution of bacterial population in pregnant women with UTI was explained in the (table e 1.) which include four strain of organism. *Escherichia coli* (*E. coli*) was the most common organism isolated accounting for (n= 17, 34%) and the second highest organism was *Klebsiella* (n=11; 22%) followed by *Staphylococcus* (n=7; 14%) and *Proteus* (n=7; 16%).

Table 1: Distribution of bacterial population from urine sample according to (Number and percentage).

Organism isolated	Number (N0.)	Percentage (%)
Escherichia coli	17	34
Proteus mirabilis	7	16
Klebsiella pneumonia	11	22
Staphylococcus aureus	7	14
No growth	7	14
Total	50	34

Table 2shownAll thepathogenic isolates, represented differences in different biochemical characteristics, all the bacterialisolates were a negative result for motility excepted *Escherichia coli and Proteus mirabilis*as well as (+ve) positive (catalase and citrate) so urease (+ve) positive for all except *E. coli*, while (-ve) negative oxidase for all bacterial isolate. The results observed that there is an agreement with the previous studies. Gram-negative non-sporulatingrods that are oxidase negative and catalase-positive.

Table 2: Result of biochemical test of bacterial isolates.

Bacterial isolates			Biochemical test		
	Citrate	Urease	Oxidase	Catalase	Motility
Escherichia coli	+	_	_	+	+
Klebsiellapneumoniae	+	+	_	+	_
Staphylococcus aureus	+	+	_	+	_
Proteus mirabilis	+	+	_	+	+

The table (3),observed that *E. coli* were found sensitive to Nitrofurantoin, Gentamicin, Ofloxacin (I.Z = 16, 20, 18) mm respectively. While this bacteria was resistance to the other antibiotics. *Staphylococcus aureus* were found sensitive toAmoxicillin, Gentamicin, Ofloxacin with inhibition zoon (5, 10, 5) mm respectively. While this bacteria was resistance to the other antibiotics. While the*Klebsiellapneumoniae* were found sensitive toGentamicin, Ofloxacin with inhibition zoon (22, 18) mm respectively. While this bacteria was resistance to the other antibiotics. And*Proteus mirabilis* were found sensitive toTrimethoprim,Gentamicin with inhibition zoon (7, 10) mm respectively. While this bacteria was resistance to the other antibiotics.

Table 3: Antimicrobial susceptibility test against isolated pathogens.

Antibiotics	Organism typeName of Organism			
	Klebsiella	E. coli	S.aureus	Proteus irabilis
Amoxicillin	-	-	-	-
Trimethoprim	-	16	-	7
Gentamicin	22	20	10	10
Ofloxacin	18	18	5	-
Nitrofurantoin	-	-	-	-
Pencilline	-	-	-	-

The result in table (4)there was, a significant deference among all concentrations of watery extract toward each bacterial isolates, in the watery extract the results, showed appearance that aqueous extracts of Parsleywas strong activity at high concentrations (1500µg/ ml) against all isolates while the weakest activity at low concentrations (500µg/ ml) on *E coli, K. pneumoniae*, *Staphylococcus aureus and Proteus mirabilis,*theinhibition zones were increased with the increasing of concentrations of the extract treatment. The best concentration was at 100% against *E coli, K. pneumoniae*, *Staphylococcus aureus and Proteus*

*mirabilis*with inhibition zones of (22, 19.5, 12, 13) mm respectivelywhen compare with positive control.

Table 4: The effects of watery extract of parsley on growth of bacterial isolates.

Bacteria Spp	Concentrations of watery extracts			Positive control
	500	1000	1500	Gentamycin
Escherichia coli	12	15	22	20
Klebsiella pneumonia	11	16	19.5	22
Staphylococcus aureus	6	8	12	10
Proteus mirabilis	7	8	13	10

Table (5)showed that, a significant deference among all concentration, of Methanol extract toward each bacterial isolates.In the Methanolextract the results, showed that methanol extracts of parsleystrong activity at high concentrations (1500µg/ ml) against *E coli, K. pneumoniae*, *Staphylococcus aureus and Proteus mirabilis*with inhibition zones of (15, 11.5, 9.5, 8) mm respectively.Whilst the inhibition zone in both concentration (500, 1000µg/ ml) f o r *E coli,* was (5,10 mm) followed by *K. pneumonia* in (500, 1000µg/ ml) was (6mm, 9mm) inhibition zoon. And w e a k e r activity against was *Staphylococcus aureus (6mm, 8mm) and Proteus mirabilis was* (3mm, 6mm).

Table 5: The effects of methanol extracts of parsleystems on the growth of bacterial isolates

Bacteria Spp	Concentrations of Methanol extracts			Positive control
	500	1000	1500	Gentamycin
Escherichia coli	5	10	15	20
Klebsiella pneumonia	6	9	11.5	22
Staphylococcus aureus	6	8	9.5	10
Proteus mirabilis	3	6	8	10

Discussion

-In this paper (50) isolates.Include*E. coli* was most predominant,uropathogen with (34%), followed by (22%) *Klebsiellasp, Staphylococcus aureus* (14%), *Staphylococcus sp*. and *Proteusmirabil*(14. %) These result agree with Rana et al [(14)] who found that *E. coli* 43% followed by *Klebsiella pneumonia*14.1%, *Pseudomonas aeruginosa* and *Proteus mirabilis* 9.4 %, *Staphylococcus aureus* 7.8%, *Morganellamorganii*

6.2%.

Result on antibiotics susceptibility is different, by clinical isolates has become a major factor in drug choice and success of treatment. Similarly other studies, was found gram negative bacteria isolate of UTI were multi drug resistant to Ampicillin, Amoxicillin, Ceftizoxime, Cefepime, Tetracyclin. and Nitrofuration[15].

Result of this study present that (*Petroselinumcrispum*) Parsley extracts had effect on both G positive (+ve) and G negative (-ve) bacteria isolated from pregnant women suffering of urinary tract infection. Except *staph. aureus* which showed no activity at concentrations (500µg/ ml), because the extracts of *parsley* are broad spectrum in their activities, The methanol extract weaker activity against*Staph.aureus*(500µg/ ml) (I.Z = 7 mm and*protuesmerabeles*(500µg/ ml) (I.Z =5) mm, while highly activity against *E.coli , k. pneumonia.*. The results of this study were agree with the results conducted byresearches[16-17]they areshowed ethanolic extracts are inhibited the different species of G positive and G negative bacteria with inhibition of 8 from 11 bacteria species. Therefore parsley leave extract could be use as one method because most of plant are safe with little side effectand a wide range of antibiotic resistant microorganisms parsley plants have been used for the treatment in traditional medicine of urinary tract disease[18].

Conclusions

Our results in the current research conclude the aqueous and methanol extracts of parsley showed a significant antibacterial activity but the watery extract is more effective than methanol extract. Therefore it can be used against urine bacterial infections. All isolates have sensitivity to Gentamycin, so Gentamycin possess higher efficacy whilst Ofloxacin has lower efficacy except *Staph aureus.*

Recommendation In future study identification the chemical nature componrnt of parsleyand study effect of each component on oral pathogenic bacterial growth and other organisms.

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Ethical Clearance: None

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article.

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References

(1) Akram, M, Mohammed S, and Asad U K. "Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India." Annals of Clinical Microbiology and Antimicrobials. 2007; 6 (1): 4.

(2) Najar M , SaldanhaC, and BandayK. "Approach to urinary tract infections." Indian Journal ofNephrology,2009; 19(4): 129.

(3) Mandell G, et al. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia, PA: Churchill-Livingstone Elsevier: 2010, p. 4028

(4) Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010; 7:653–660.

(5) Marjorie Murphy Cowan. Plant Products as Antimicrobial Agents. Clinical Microbiology. Review. 1999; 12(4): 564–582.

(6) Oliveira C, Junqueira R, Stehmann J, Brandão M. Potential of medicinal plants as a source of new antimalarial drugs: species listed in the bibliography Ethno Brazil. Braz J Med Biol Res.2003;5:23–31.

(7) More G, Tshikalange TE, Lall N, Botha F, Meyer JJ. Antimicrobial activity of medicinal plants against oral microorganisms. J Ethnopharmacol. 2008;119:473–477.

(8) Mallappa KS,Mohd S A, and Uma RS.Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action.Evidence-Based Complementary and Alternative Medicine.2016;1-21.

(9) Mohamed T, ShaabanHanan A,Ghozlan A, and Marwa M. El Maghraby. Susceptibility of Bacteria Infecting Urinary Tract to Some Antibiotics and Essential Oil. Journal of Applied Pharmaceutical Science. (2012); 2 (4): 90-98.

(10) Khalida K. Inhibitory Effect of Parsley (*PetroselinumCrispum*) Juice against Some Urinary Pathogens in Vitro. The Iraqi Postgraduate Medical Journal. 2012; 11(3):336-342.

(11) Ashwak J, Yashub K,Jane CB, Harison M.Studies on Antifungal Properties of Some Plant Extracts (Garlic, Fenugreek, Ginger) Against of Clinical Isolate Candida Species. International Journal of Scientific Engineering and Technology Research Volume.2013; 2 (19):2180-2185.

(12) Bauer A, Kirby W, Sherris J, and Turk M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology.1966; 45:493-49.

(13) Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 20th. Informational Supplement. Approved standard M07-A8. Clinical and Laboratory Standards Institute:2010.

(14) Rana M A, Sawsan M A , Khabat A A , Wesam Al . Antibacterial activity of parsley and celery aqueous extract on the isolated bacteria from children UTI in Erbil city. International Journal of Advanced Research 2014; 2 (9): 895-903.

(15) Sheffield JS.,and Cunningham F G. Urinary tract infection in women. Obstetrics and Gynecology.2005; 106 (51):1085-92.

(16) Seyyednejad SM, Maleki S, Mirzaei D N, and Motamedi H. Antibacterial activity of Prunusmahaleb and Parsley (Petroselinumcrispum) against some pathogen. Asian Journal of Biological Sciences. 2008; 1(1): 51-55.

(17) Adwan G, Abu-Shanab B. and Adwan, K. In vitro Interaction of Certain Antimicrobial Agents in Combination with Plant Extracts against Multidrug-resistant Pseudomonas aeruginosaStrains. Middle-East Journal of Scientific Research. 2009; 4 (3): 158-162.

(18) Naema NF, Dawood B, and Hassan S. A study of some Iraqi Medicinal plants for their spasmolytic and antibacterial activities. Journal of Basrah Researches Science.2010; 36(6):67-73.