Inhibitory Effect of Parsley (Petroselinum Crispum) Juice Against Some Urinary Pathogens in Vitro

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ABSTRACT:

BACKGROUND:

Information's on the potency of many medical plants against microorganisms are scanty, and in the current wave of antimicrobial resistance against chemotherapeutic drugs, there is a need to search for plants that could be resistance-free and affordable.

OBJECTIVE:

The objective of this study was to investigate the antibacterial effects of Parsley *(Petroselinum crispum)* against uro-pathogens and to compare with the effect of some drugs used for the treatment of urinary tract infection caused by different Gram positive and Gram negative bacteria.

MATERIALS & METHODS:

A total of thirty eight Gram positive and Gram negative bacteria(*Escherichia coli ,Proteus sp.,Enterobacter spp.,Pseudomonas aeruginosa, Klebsiella sp. , Staphylococcus aureus*) isolated from urine samples of different child patients between first of June 2011 to first of July 2011 admitted to Children Mel fare Teaching Hospital in Baghdad were tested against different dilutions of Parsley (100%,1:1,1:5,1:10:1:15,1:20) to examine the inhibitory activity *in vitro* ,in addition antibiotic susceptibility test was done.

RESULTS:

All isolates showed sensitivity to concentrated parsley 100% concentration except *Pseudomonas aeruginosa* isolates, the highest effect observed on *Proteus sp*. and *Staphylococcus aureus* isolates, while inhibitory effect stopped after 1:1 dilution against all isolates, while in antibiotics therapy (29) isolates were resistant to Co-Trimoxazole and Cefotaxime and (30)isolates showed resistance to Tri-imetheprim+clavulanic acid.

CONCLUSION:

It seems that Parsley has potent antibacterial activity against some uropathogens *in vitro*.

KEY WORDS: uropathogens, Parsley, antimicrobial activity, antibiotic susceptibility, in-vitro.

INTROUDACTION:

Urinary tract infection (UTI) is one of the most common causes of hospitalization and referral to outpatient settings in children. It is estimated that at least 3% of girls and 1% of boys experience one episode of UTI before the 11th years of age [(1)].About 30-50% of these patients will have another episode within three months to two years, particularly in girls[(2,3)]. Upper UTI (pyelonephritis) is a major cause of hypertension, renal insufficiency and end-stage renal failure in children. Early treatment of UTI with an effective antibiotic is essential for prevention from long-term consequences. Delay in treatment increases the risk of scar formation in kidneys [(4)]. So in almost all children with UTI, antimicrobial therapy is initiated empirically before the result of urine culture is available. Sensitivity of bacterial uropathogens to antibiotics shows a great geographical and historical variability due to different antibiotic treatments.

Antimicrobial activities of various species and their derivatives have been reported by many works [(5, 6)].The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made contributions to human health and well being [(7)]. Many studies indicates that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds [(8,9)].

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These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses [10,11].

The chief uses of parsley are as a diuretic, emmenagogue, antispasmodic, carminative, and expectorant; of these, all but the expectorant property have received experimental support. Parsley tea and expressed juice are used medicinally. Parsley is an effective laxative, hypotensive, uterine tonic, and antimicrobial agent.

Parsley contains several active components, including apiole and myristicin, plus a large quantity of protein. However, persons on a low-salt diet should be aware parsley contains above-average quantities of sodium. The toxicity of whole parsley has probably been grossly exaggerated. While it is true large doses of concentrated parsley extracts or of pure apiole and myristicin have produced toxicity, the whole plant appears to be safe to use. Most herbalists recommend pregnant women avoid the use of parsley because of its uterine tonic or stimulating property; there are reports parsley can be used to induce abortion. Parsley herb and root have approval status by the German Commission E for the urinary tract and kidney stones. [12].

Parsley appears to increase diuresis by inhibiting the Na+/K+-ATPase pump in the kidney, thereby enhancing sodium and water excretion while increasing potassium re absorption[13].Parsley is a rich source of bioactive phytochemicals such as carotenoids, which are known to exert various positive biological effects[14].Parsley is antimicrobial[15].Parsley seed extract can reduce blood pressure (hypotensive), possibly due to its diuretic effects[16].

The aim of this study was to determine the antibacterial activity of Parsley against uropathogens isolated from children and compare the findings with sensitivity pattern of 7 antibiotics used for the treatment of urinary tract infection.

MATERIALS AND METHODS:

Collection and Identification of Plant Material: Plant materials, parsley were obtained from the local market in Baghdad, Iraq in 2011. The taxonomic identity of the plant was confirmed.

Plant extraction: The sample were washed with distal water and then squeezed to obtain pure juice, filtered by filter paper and serial of dilutions were made by sterile distilled water (D.W.) (100%,1:1,1:5,1:10:1:15,1:20).

Sample collection: A total of thirty eight bacterial isolates were isolated from urine samples. The Gram-negative species were (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter spp., Proteus sp., Klebsiella sp.)* and the gram positive specie (*Staphylococcus aureus*). Species were collected from patient's urine with urinary tract infection. They were identified by using standard biochemical tests.

Antibacterial activity: By using well assay, all isolates were cultured in Muller-Hinton agar plate (Oxide) each plate were cultured with overnight growth from nutrient broth by using sterile swab so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork porer of diameter 5.0 mm was used to make five wells in each agar plates. Six dilution were made by sterile distilled water (100%, 1:1, 1:5, 1:10:1:15, 1:20).A 50µL volume of each dilution was applied by micropipette in each well into Muller-Hinton Agar plate. The plates were allowed to stand for 1h or more at refrigerator for diffusion to takes place to avoid evaporation since the study were done on July(high temperature) and then incubated at 37ºC for 24hrs to examine inhibitory effect. The zone of inhibition was recorded in mm [17].

Sensitivity to antimicrobial agents: All isolates were tested against 7 different antimicrobial agents by using the Kirby-Bauer standardized single disc method as mentioned in **table2** [18]. (Commercial discs from Bioanalyse, Turkey).

RESULTS:

The antibacterial activities of Parsley juice showed significant variations as shown in **Table 1**.

Table 1: Antibacterial activities of parsley juice

No.	isolate	100%	1:1	1:5	1:10	1:15	1:20
		Zone of inhibition(mm)					
1	E.coli 1	11	7	[1]R	R	R	R
2	E.coli2	8	R	R	R	R	R
3	E.coli 3	R	R	R	R	R	R
4	E.coli 4	12	10	R	R	R	R
5	E.coli 5	R	R	R	R	R	R
6	E.coli 6	10	R	R	R	R	R
7	Enterobacter spp1	8	R	R	R	R	R
8	Enterobacter spp 2	R	R	R	R	R	R
9	Enterobacter spp 3	R	R	R	R	R	R
10	Enterobacter spp 4	R	R	R	R	R	R
11	Enterobacter spp 5	8	5	R	R	R	R
12	Enterobacter spp 6	9	5	R	R	R	R
13	Enterobacter spp 7	9	6	R	R	R	R
14	Enterobacter spp 8	10	7	R	R	R	R
15	Enterobacter spp 9	R	R	R	R	R	R
16	Enterobacter spp10	R	R	R	R	R	R
17	Enteracter spp 11	8	R	R	R	R	R
18	Enteracter spp 12	R	R	R	R	R	R
19	Klebsiella sp1	6	R	R	R	R	R
20	Klebsiella sp2	R	R	R	R	R	R
21	Proteus sp.1	10	6	R	R	R	R
22	Proteus sp.2	R	R	R	R	R	R
23	Proteus sp.3	8	6	R	R	R	R
24	Proteus sp.4	8	6	R	R	R	R
25	Proteus sp.5	9	7	R	R	R	R
26	P.aeruginosa1	R	R	R	R	R	R
27	P.aeruginosa2	R	R	R	R	R	R
28	P.aeruginosa3	R	R	R	R	R	R
29	P.aeruginosa4	R	R	R	R	R	R
30	P.aeruginosa5	R	R	R	R	R	R
31	P.aeruginosa6	R	R	R	R	R	R
32	S.aureus1	13	10	R	R	R	R
33	S.aureus2	8	R	R	R	R	R
34	S.aureus3	R	R	R	R	R	R
35	S.aureus4	8	R	R	R	R	R
36	S.aureus5	9	R	R	R	R	R
37	S.aureus6	10	7	R	R	R	R
38	S.aureus7	8	7	R	R	R	R

[1]: Resistance

Most bacterial isolates showed inhibition zone to Parsley juice with different diameters and the inhibitory effect lasted up to 1:1 dilution, while there were no effect by each of 1:5, 1:10, 1:15 and 1:20.*P.aeruginosa* was the only isolates that showed resistant to all different Parsley juice dilutions**.**

On the other hand the isolates showed sensitivity to Amikacin, Ciprofloxacin, while *E.coli* isolates were resistant to Co-Trimoxazole and *Proteus sp.* isolates were all sensitive to Ciprofloxacin as shown in **Table 2.**

Table 2: Sensitivity to different antimicrobial agents.

No.	isolate	AK (10 mcg)	CIP (5mcg)	COT (25mcg)	CTR (30mcg)	N.A. (30mcg)	NiT (300mcg)	Tim
1	E.coli 1	R	R	R	S	R	R	S
2	E.coli2	S	R	R	R	R	S	R
3	E.coli 3	S	R	R	R	S	R	R
4	E.coli 4	S	S	R	R	R	S	R
5	E.coli 5	S	S	R	R	S	R	R
6	E.coli 6	S	R	R	R	S	R	R
7	Enteracter spp 1	S	S	R	S	R	R	R
8	Enteracter spp 2	S	S	R	R	S	R	R
9	Enteracter spp 3	S	R	R	R	S	R	R
10	Enteracter spp 4	S	S	R	R	R	S	R
11	Enteracter spp 5	R	R	R	S	R	S	R
12	Enteracter spp 6	S	R	R	R	S	S	R
13	Enteracter spp 7	R	S	S	R	S	R	R
14	Enteracter spp 8	S	S	R	R	R	S	R
15	Enteracter spp 9	S	S	R	S	R	S	R
16	Enteracter spp 10	S	S	R	R	R	S	R
17	Enteracter spp 11	S	S	R	R	S	R	R
18	Enteracter spp 12	S	S	R	R	R	S	R
19	Klebsiella sp1	S	R	R	R	S	S	R
20	Klebsiella sp2	S	R	S	R	R	R	S
21	Proteus sp.1	S	R	R	R	S	R	S
22	Proteus sp.2	S	S	R	S	R	R	S
23	Proteus sp.3	S	R	R	R	S	R	S
24	Proteus sp.4	S	S	R	R	S	R	R
25	Proteus sp.5	S	S	S	R	S	S	R
26	P.aeruginosa1	S	R	R	S	R	R	S
27	P.aeruginosa2	R	R	R	S	R	R	S
28	P.aeruginosa3	R	S	R	R	R	S	R
29	P.aeruginosa4	S	R	R	S	R	R	R
30	P.aeruginosa5	S	S	R	R	R	S	S
31	P.aeruginosa6	S	R	R	S	R	R	R
32	S.aureus1	R	S	S	R	R	S	R
33	S.aureus2	R	S	R	R	R	S	R
34	S.aureus3	R	S	S	R	R	S	R
35	S.aureus4	R	S	S	R	R	S	R
36	S.aureus5	R	S	S	R	R	S	R
37	S.aureus6	R	S	S	R	R	S	R
38	S.aureus7	R	S	S	R	R	S	R

R**:** Resistance, S: Sensitive
AK/Amikacin(10mcg)**,**CIP/Ciprofloxacin(5mcg),COT/Co-Trimoxazole(25mcg)**,**
CTR/Cefotaxime(30mcg)**,**N.A./Nalidixicacid(30mcg)**,**NiT/Nitrofurantoin(300mcg)**,**Tim/Tri-imetheprim(1.25 mcg) +clavulanic acid(10 mcg).

DISCUSSION:

The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases [19]. Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments [20,22].This study showed that Parsley juice had effect on both gram positive and gram negative bacteria isolated from patients suffering from urinary tract infection. *Staph. aureus* isolates showed inhibition zone varied from 8-13 mm in diameter which agree with the findings of Arora and Kaur, Okemo *et.al*, Digraki *et.al* and Madamombe. that *Staph. aureus* is susceptible to a lot of plant extracts reported by several researchers [23,26].However, Gram positive bacteria were found to be more susceptible than Gram negative bacteria. This could be due to the

fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope [27, 28].

Microbial drug resistance is an inescapable consequence of the use of antimicrobial agents. The rate at which resistance occurs among microbial populations is often driven by the overuse and abuse of antimicrobial agents in many clinical settings[29].Differences in antibiotics susceptibility by microorganisms has become a major factor in drug choice and success of treatment. Great concerns have been raised regarding emerging antimicrobial resistance among bacteria that may result in unpredictable antimicrobial susceptibility and failure of therapy [30,31].The present study revealed that *E.* coli isolates were resistant to cotrimoxazol which come along with studies in recent years found that *E.* coli resistance to cotrimoxazol has increased and varies from 21% to 76.7% in different studies.[32,37] That may be related to inappropriate prescription of cotrimoxazol in our country. From other oral agents fluoroquinolones (FQs) such as ciprofloxacin have the highest activity against *E.Coli* in most studies. [38,40].So in the past few years the usage of cotrimoxazol was decreased whereas use of FQs has increased dramatically. [41].However the safety of ciprofloxacin in children is under study because of potential cartilage damage induced by this antibiotic. [42]

So knowledge of the sensitivity pattern of common uropathogens according to local epidemiological studies is necessary for selection of an appropriate antibiotic for empirical treatment. One study recommended that the policies for treatment of UTI in children should be re-evaluated every five years according to local resistance rates [43].The antimicrobial drugs are likely to become less effective not only for treating of UTI, but also for treating of other life threatening infections. However, finding alternative such Parsley juice which can be eaten raw or as a juice may be used as a prophylaxis against some gram positive and gram negative bacteria but there is a need for more studies to find active substance in Parsley juice and more *in-vitro* and *in-vivo* studies.

CONCLUSION:

It seems that Parsley has potent antibacterial activity against some uropathogens *in vitro*.

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