Phytochemical screening of *Petroselinum crispum* (Mill.) Fuss and ***in vitro*** evaluation of its antimicrobial activity against some uropathogens

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Petroleum ether, methanol and aqueous extracts from the leaves and stems of *Petroselinum crispum* (Apiaceae) were screened phytochemically and evaluated for their antimicrobial activity. The extracts were tested against five standard bacteria ,one Gram positive bacteria (*Staphylococcus aureus*) , four Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae ,Proteus Mirabilis and Pseudomonas aeruginosa*) and one standard fungi (*Candida albicans*) using disc diffusion method .

The petroleum ether extract of the leaves and stems of *P.crispum* showed antimicrobial activity against standard tested microorganisms varied between very active and active whereas the aqueous extract showed lowest activity against all tested microorganisms . The most active extracts (petroleum ether and methanol) were tested against 30 clinical isolates (Bacteria and *C.albicans*) collected randomly from the Military and AlRibat Hospitals. The petroleum ether extract and methanol extracts of the leaves and stems of *P.crispum* showed active against all tested microorganisms . For the different *P. crispum* extracts, the minimum inhibitory concentrations (MICs) against standard and clinical isolates were determined using disc diffusion method and compared to antibacterial activity of two reference drugs and one antifungal drug.

The phytochemical screening of *P.crispum* leaves and stems petroleum- ether extract, revealed the presence of coumarins and flavonoids in low concentration ,sterols in high concentration and absence of saponins ,alkaloids ,anthraquinones ,tannins ,triterpenes and cyanogenic glycosides. The methanol extract showed the presence of moderate concentration of saponin, coumarin ,flavonoids,triterpenes sterols, high concentration of tannins , alkaloids , absence of anthraquinones and cyanogenic glycosides

Keywords: *P.crispum*, phytochemical screening, antimicrobial activity

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1. Introduction:

Urinary tract infections (UTIs) are the leading cause of Gram-negative bacteremia in patients of all ages and are associated with a high risk of morbidity and mortality, especially in the elderly, and account for significant health care costs (Orenstein and Wong 1999 ; Stamm and Norrby,2001 ; Al-Jiffri *et al*., 2011) . Women are more likely than men to get UTIs because of their urinary tract's design (Shaaban *et al*., 2012). Most of UTIs are caused by Gram-negative bacteria like *Escherichia coli, Proteus Mirabilis, Proteus vulgaris Klebsiella sp, Pseudomonas aeruginosa, Acinetobacter, Cerruti,* and *Morganella morganii* (Foxman, 2010; George and Manges, 2010). Also UTIs are caused by Gram positive bacteria including *Enterococcus sp, Staphylococcus sp,* and *Streptococcus agalacticae* (Tangho and Mcaninch,2004).

Plants remain the most common source of antimicrobial agents .Their usage as traditional health remedies is the most popular for 80% of the world population in Asia, Latin America and Africa and is reported to have minimal side effects (Doughari, 2006). These are coupled with ample inherited information in the field of medicinal plants and traditional herbal users which originally were unique blends of indigenous cultures of various nations (Khalid *et al*., 1986; Ahmed *et al*., 2013 ; Dahab *et al*., 2013; Hassan *et al*., 2014; Mohammed *et al*., 2015; Ebrahim and Almagboul , 2015 ; Elrufai *et al*., 2015).

Petroselinum crispum (Mill.) Fuss **:** Common English name is parsley while in Arabic language it is called Bakdonis or Maqdounis (Majeed and Muhamood, 1988). *P. crispum* belongs to the Apiaceae family. Although native to Europe and western Asia, the herb is now cultivated and consumed throughout the world (Bailey and Bailey ,1976). The petroleum ether ,methanol and aqueous extracts of leaves and stems of *P. crispum* yielded 1.877,27.268 and 12.574gm respectively after evaporation and dryness . *P.crispum* has been used in folk medicine as a remedy for many different ailments, the herb has gained also a wide reputation as a powerful diuretic (Tyler, 1993; Bisset, 1994; Anderson *et al*., 1996; Duke, 1987). *P. crispum* had been claimed in Arab Traditional Medicine to possess variety of properties including laxative, diuretic and anti urolithiatic, the leaves are used as hot application against inflammatory condition, mastitis and haematomata (Al-Howiriny *et al* .,2003).

El Astal *et al*. (2003) found *E. coli* was more affected by the ethanolic extract of *P.crispum* . But it did not elicit pronounced effect on the tested Gram –positive organisms. Al-Hadi *et al*.

(2013) found that petroleum ether extracts showed the highest antibacterial activity and phytochemical screening indicate the presence of primary and secondary metabolites.

Dostalova *et al*. (2014) showed antimicrobial activity of aqueous extracts of *P. crispum* that had higher potential to inhibit bacterial growth. Al Marwa *et al*. (2013) detected the presence of primary and secondary metabolites in petroleum ether ,chloroform ,alcohol and water-soluble parts of the drugs. Phytochemical screening of *P.crispum* has revealed the presence of several classes of flavonoids (Fejes *et al*.,2000). Several bioactive flavonoids have been isolated from *P.crispum* leaves and were known to exhibit antimicrobialactivities (Wong and Kitts,2006).

2. Materials and methods:

2.1. Plant Material

The leaves and stems of *P.crispum* were purchased from Khartoum Central Market (May 2015) and identified by taxonomists of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) .The plant was washed with water then dried in room temperature for at least 7 days . All parts of the plant were crushed to fine powder and stored at room temperature in the dark**.**

2.2. Method of extraction

Extraction was carried out according to the method described by (Sukhdev *et al.* ,2008).Hundred grams of the plant sample was coarsely powdered using mortar and pestle and successively extracted with petroleum ether and methanol using Soxhlet extractor apparatus . Extraction was carried out for about four hours for petroleum and eight hours for methanol till the color of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus and the yield percentages were calculated as followed: Weight of obtained extract / weight of plant sample X100. Hundred grams of the plant sample was soaked in 500 ml hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered, freezed and dried using freezdrier to obtain powder and yield percentage was calculated.

2.3. Test Microorganisms

All reference microorganisms were obtained from the Department of Microbiology (MAPTMRI). Five strains of bacteria *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 , *Klebsiella pneumoniae* ATCC 53657 , *Proteus Mirabilis* ATCC 6380 ,

Pseudomonas aeruginosa ATCC 27853, and one fungi *Candida albicans* ATCC 7596. Clinical isolates were collected from growth cultures of UTIs patients, from Military and Al Ribat Hospitals .The clinical isolates were confirmed and identified using standard biochemical tests .

2.3.1. Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8C.F.U/ ml (Turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspensions were swabbed uniformly on the surface of Mueller Hinton agar (MHA) and the inoculums was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No. 1, 6mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24h in the inverted position. The antimicrobial activity tests were duplicated and mean values of results were measured.

The zone of inhibition of antibacterial activity results were expressed in terms of <9 mm zone was considered as inactive; 9-12 mm as partially active; while 13-18 mm as active and >18 mm as very active (Mukhtar and Ghori ,2012). Instead of nutrient agar, Sabouraud Dextrose Agar was used. The inoculated media was incubated at 25°C for 2 days for *Candida albicans* .

2.3.2. Antibacterial and antifungal activity of reference drugs against the standard and isolate organisms

For comparisons we used two antibacterial drugs (Ciprofloxacin 30 µg and Gentamicin 10 µg), and one antifungal drug (Fluconazole 50 mg) . Four concentrations (40, 20,10,5 µg/ml) of antifungal suspensions were prepared. Sterilized filter paper (Whatman No. 6mm in diameter) was soaked with 20µl of each concentration of the reference drug using automatic microtitre pipette , and allowed to diffuse at room temperature then incubated at 37°C for 24 hours .After incubation the resultant growth inhibition zones were measured

2.3.3. Determination of Minimum Inhibitory Concentrations (MICs*)*

MICs is the lowest concentration of antimicrobial agent that completely inhibits the growth. *P. crispum* extracts were prepared in the series of decreasing concentrations in the following

order 50, 25 , 12.5 and 6.25 mg / ml. The antimicrobial activity tests were duplicated and mean values of results were measured for MICs.

3. Results and discussion

3.1. Results

The average of the diameters of growth inhibition zones produced by *P.crispum* leaves and stems petroleum ether; methanol and aqueous extracts against standard organisms are shown in Table 1. On the other hand, the antimicrobial activities of standard chemotherapeutic agents against the standard strains of certain bacterial and fungal species are shown in Tables 2 and 3.Then the most active petroleum ether and methanol extracts were tested against 30 clinical isolates .The results shown in Table 4 and the antimicrobial activities of standard chemotherapeutic agents against the tested clinical isolates of certain bacterial and fungal species are shown in Tables 5 and 6.

The minimum inhibitory concentrations of (MICs) were determined *against* standard organisms and tested clinical isolates Tables 7and 8.

The phytochemical screening of petroleum ether and methanol extracts of P.crispum was detected for their secondary metabolites (Table 9).

3.1.1. Screening for antimicrobial activity of *P.crispum petroleum* ether, methanol and aqueous extracts

Results of Table 1 show that, the leaves and stems of *P.crispum* exhibited inhibitory activity against all tested organisms. The petroleum ether extract of the leaves and stems of *P.crispum* showed very active (21 mm) against *S.aureus* , active (16,-17 mm) against *E.coli* ,*P.Mirabilis* ,*P .aeruginosa* and partially active (13-15 mm) against *K.pneumoniae* and *C.albicans* .Methanol extract of the leaves and stems of *P.crispum* showed active (17 mm) for *S.aureus* and *K.pneumoniae* ,partially active (14,12 mm) for *P.Mirabilis* ,*P .aeruginosa* , *C.albicans* and *E.coli* whereas aqueous extract of the leaves and stems of *P.crispum* showed low activity for *P.Mirabilis* , *S.aureus* , *K.pneumoniae* , *P .aeruginosa* and inactive against *C.albicans*. The petroleum ether and methanol extracts which were the most active against the standard organisms were further tested against thirty (30) clinical isolates. The obtained results are resumed in Table4 and showed that the petroleum ether and methanol extracts of

the leaves and stems of *P.crispum* exhibited active against all tested organisms. The minimum inhibitory concentration (MICs) was determined for most active petroleum ether and methanol extracts of the leaves and stems of *P. crispum* against standard organisms and tested clinical isolates (Tables 7, 8) .

The minimum inhibitory concentration for petroleum ether extract of the leaves and stems of *P.crispum* was 12.5 mg/ml against *S.aureus* ,*E.coli* ,*P .Mirabilis* , *P.aeruginosa* and < 6.25mg/ml for *K.pneumoniae*, and *C.albicans* .Methanol extract was <6.25 mg/ml against all organisms. MICs of petroleum ether and methanol extracts of the leaves and stems of *P.crispum* against all the clinical isolates were < 6.25 mg/ml .

Table 1. Antimicrobial activity of different extracts of the leaves and stems of *P.crispum* against standard microorganisms

Part Used	Solvents	*S.aureus*	*E.coli*	*K.pneumoniae*	*P.mirabilis*	*P.aeruginosa*	*C.albicans*
Leaves and stems	**Petroleum ether**	21	16	13	17	17	15
	Methanol	17	12	17	14	14	14
	Aqueous	11	10	11	12	10	8

Key: *S: Staphylococcus arueus , E: Escherichia coli , K: Klebsiella pneumoniae , P: Proteus mirabils ,P: Pseudomonas aeruginosa ,C: Candia albicans*
The antimicrobial activity tests were duplicated and mean values of results were measured.

Table 2. Antibacterial activity of standard antibacterial drugs against standard bacteria

Antibiotic	Cons µg/mL	*S. aureus*	*E. coli*	*K. pneumoniae*	*P .Mirabilis*	*P. aeruginosa*
Ciprofloxacin	30	22	16	23	20	20
Gentamicin	10	21	15	22	18	20

Table 3 . Antifungal activity of standard antifungal drug against standard *C.albicans*

Drug	Concentrations used µg/ml	MDIZ(mm)
Fluconazole		*C.albicans*
	40	(-)
	20	(-)
	10	(-)
	5	(-)

Table 4. Antimicrobial activity of the leaves and stems of *P. crispum* against different clinical isolates

Clinical Isolates	Number of isolates	Activity MDIZ(mm)	
		Petroleum ether	Methanol
S.aureus	*5*	16	12
E.coli	*5*	15	12
K. pneumonia	*5*	15	11
P .mirabbilis	*5*	16	13
P. a eruginosa	*5*	14	12
C.albicans	*5*	14	13

Table 5. Antibacterial activity of standard antibacterial drugs against different clinical isolates **:**

Clinical Isolates	Number of isolates	Activity MDIZ(mm)	
		Ciprofloxacin 30µg/mL	Gentamicin 10µg/ml
S.aureus	5	18	13
E.coli	5	9	16
K. pneumonia	5	18	16
P .mirabbilis	5	20	13
P. aeruginosa	5	30	16

Table 6. Antifungal activity of standard antifungal drug against clinical isolates of *C.albicans*

Drug	**Concentrations µg/mL**	**Activity MDIZ(mm)**				
		Candida albicans1	***Candida albican2***	***Candida albicans3***	***Candida albicans4***	***Candida Albicans5***
	40	20	10	10	10	9
	20	14	8	9	9	8
Fluconazole	**10**	(-)	(-)	(-)	(-)	(-)
	5	(-)	(-)	(-)	(-)	(-)

Table 7. The Minimum Inhibition Concentrations (MICs) of *P.crispum* leaves and stems organic extracts against standard microorganisms

Microorganisms	**Petroleum ether (mg/mL)**				**Methanol (mg/mL)**				**Aqueous (mg/mL)**			
	50	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**
S.aureus	15	14	11	-	13	11	12	12	-	-	-	-
E.coli	15	14	11	-	23	15	12	14	-	-	-	-
K.pneumoniae	17	14	10	-	18	15	14	11	-	-	-	-
Pr .mirabilis	15	14	11	8	10	9	11	14	-	-	-	-
Ps.aeruginosa	14	12	11	-	13	11	12	12	-	-	-	-
C.albicans	17	14	13	11	12	10	10	15	-	-	-	-

Table 8. The Minimum Inhibition Concentrations (MICs) of *P.crispum* leaves and stems organic extracts against different clinical isolates

Clinical isolates	Number of isolates	Petroleum ether (mg/mL)				Methanol (mg/mL)			
		50	25	12.5	6.25	50	25	12.5	6.25
S.aureus	*5*	14	13	11	8	11	12	12	14
E.coli	*5*	14	13	11	8	11	11	10	10
K.pneumoniae	*5*	14	13	11	9	12	11	10	6
P .Mirabilis	*5*	14	13	11	9	12	11	12	11
P.aeruginosa	*5*	14	14	11	8	11	10	10	9
C.albicans	*5*	15	14	12	9	11	12	9	9

Table 9. Phytochemical screening of the leaves and stems of *P.crispum* petroleum ether and methanol extracts

Test	Extracts	
	Petroleum ether	Methanol
Saponins	-	++
Coumarins	+	++
Alkaloids	-	+
Anthraquinones	-	-
Tannins	-	+++
Flavonoids	+	++
Sterols	+++	++
Triterpenes	-	++
Cyanogenic glycosides	-	-

+ Trace, ++ Moderate , +++ High, -Negative

3.2. Discussion

The petroleum ether ,methanol and aqueous extracts of the leaves and stems of *P.crispum* were screened for their antimicrobial activity against six standard microorganisms .One Gram positive *Staphylococcus aureus* ,four Gram-negative bacteria (*Escherichia coli ,Klebsiella pneumoniae , Proteus Mirabilis ,Pseudomonas aeruginosa*) and one fungi namely *Candida albicans* using the disc diffusion method.

The *P.crispum* of the leaves and stems extract petroleum- ether showed very active (21mm) against *S.aureus*, active (16-17mm) against *E.coli, P.mirabilis and P .aeruginosa* .It also showed partially active (13-15mm) against *K.pneumoniae* and *C.albicans*. Therefore *S.aureus* being a Gram positive microorganism was found to be more susceptible than all Gram-negative bacteria and this could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack of natural sieve effect against large molecules due to small pores in their cell wall envelope. This finding is similar to AL Hadi *et al*. (2013) who found that the petroleum ether extract showed activity against *S.aureus* , *K.pneumoniae*, ,*E.coli* and *P .aeruginosa* .

The methanol extract of the leaves and stems of *P.crispum* showed active (12mm) against *S.aureus* and *K.pneumoniae* , partially active (12mm) against *E.coli*, *P.Mirabilis* ,*P .aeruginosa* and *C.albicans*(14mm) ,in contrast ElAstal *et al* . (2013) who found that *E.coli* was more affected by the ethanol extract of *P.crispum* and inactive against *S. aureus* .

The aqueous extract of the leaves and stems of *P.crispum* showed low activity against *S.aureus* ,*K.pneumoniae* , *E.coli* , *P .aeruginosa* , *P.Mirabilis* and inactive against *C.albicans*, unlike Dostalova *et al*. (2014) who reported that the aqueous extract of *P.crispum* a higher potential to inhibit bacterial growth of *E.coli* .Therefore the non-polar petroleum ether was the most active extract , followed by the polar methanol and the aqueous extract was the least active.

The preliminary phytochemical screening of the petroleum ether extract of leaves and stems of *P.crispum* revealed the presence of coumarin and flavonoids in low concentrations ,sterols in high concentration and absence of saponin, alkaloids, anthraquinones, tannins, triterpenes and cyanogenic glycosides .The methanol extract showed the presence of moderate concentration of saponin ,coumarin , flavonoids, triterpenes, sterols, high concentration of tannins and devoid of alkaloids, anthraquinones and cyanogenic glycosides (Table 8) .The activity of *P.crispum* can be due to the presence of secondary metabolites which were detected by phytochemical screening .Similar results were obtained by Al Marwa*et al*. (2013), Fejes*et al* . (2000), Ojala *et al*. (2000) and Wong and Kitts (2006).

P.crispum can be used as a good source of potential antimicrobial agent against some uropathogens*in vitro*.

4. Conclusions:

Petroleum ether, methanol and aqueous extracts of the leaves and stems of *P.crispum* showed variable activity against all microorganisms tested .Petroleum ether extract showed the highest activity, followed by the methanol extract and the aqueous extract showed poor activity .

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