



Phytochemical Screening, Antibacterial and Cytotoxic Activities of *Petroselinum Crispum* Leaves Grown in Oman

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Abstract

Petroselinum crispum belongs to the Apiaceae family, is a well-known spice and vegetable. The essential oil obtained from the fruit has also strong action on the central nervous system. Present study is designed to evaluate the phytochemical screening, antibacterial and cytotoxic activity of the leaves of *Petroselinum crispum* collected from Oman. Results of phytochemical screening indicate the presence of primary and secondary metabolites. Antimicrobial activity was measured using disc diffusion method against *S.aureus*, *E.coli*, *P.aeruginosa* and *K. pneumonia*. Brine shrimp test was used to estimate cytotoxic activity. In antibacterial assay, *Petroselinum crispum* leaves extracts showed very strong results, inhibition zones ranged from 7 – 20 mm. So this plant can be used as a good source of potential antimicrobial agent. Hydro alcoholic and Petroleum ether extracts showed the highest antibacterial activity. Furthermore, Ethyl acetate and Hydro alcoholic extracts have almost killed 90% of the shrimp larvae at higher concentration of 1000 µg/ml. LC50 values for the two extracts was 51.95 and 88.15 µg/ml, respectively. Non polar fractions like chloroform extract have displayed low cytotoxic activities as compare to polar extracts.

Keywords : Antibacterial, Apiaceae, Brine shrimp, Cytotoxicity, Parsley, *Petroselinum crispum*.

1. Introduction

Petroselinum crispum belongs to the Apiaceae family. It is a well-known spice and vegetable. Its herb and root are widely known for their effects on digestion, stomach, kidney, blood, and liver [1]. Parsley has been claimed in Arab Traditional Medicine to possess variety of properties including

laxative, diuretic and antiurolithiatic.

The leaves are used as hot application against inflammatory condition, mastitis and haematomata [2]. Parsley, widely used as a salad ingredient or as a healthy garnish, capable of masking foul odors, as it has a spicy scent. Parsley tea was given to the troops in the trenches suffering from dysentery. Various parts of the plant have been used for tumors of different organs including the stomach [3]. The two major phenolic compounds extracted from parsley flakes were identified as apiin and malonyl-apiin [4]. Myristicin in parsley oil was found as a dominant compound (32.75%) that exhibited a moderate antioxidant activity. Apiol was the second dominant compound (17.54%), but it might be the major contributor to the antioxidant

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activity of parsley oil [5]. Callus cultures and cell suspensions produced aldehydes (nonanal and decanal) that were also detected in parsley plant. The callus cultures and cell suspensions also produced limonene, acetophenone, and benzotiazol; these were not observed in the plants [6]. The aroma volatiles of desert parsley were analysed using routine procedures, and 45 constituents were positively identified, Major constituents of the sample were 4-methoxy-6- (prop-2-enyl) benzo-1,3-dioxolan (myristicin) [7]. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) leaves and stems were determined on methanol and water extracts. Methanol-derived leaf extracts exhibited significantly greater radical-scavenging activity towards both lipid and water-soluble radicals. Bacterial cell damage, resulting in significant greater growth inhibition of *B. subtilis* and *E. coli* [8]. Diabetic rats treated with parsley demonstrated significantly lower levels of blood glucose, alanine transaminase and alkaline phosphatase [9]. The present study was conducted with the objectives (i) Preliminary Phytochemical Screening (ii) to evaluate anti-bacterial activity and (iii) to estimate the cytotoxicity potential of organic extracts from leaves of *Petroselinum crispum*.

2. Materials and Methods

2.1. Materials

All solvents were of analytical grade. Gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were obtained from microbiology department, College of Arts and Sciences, Nizwa University. Filter paper discs of diameter 6mm were obtained from Whatmann Company, catalogue number: 8174900. Nutrient agar and Plastic Petri dishes were purchased from Sharlau Chemie Company. Brine shrimp eggs (ARTEMIA CYSTS) were purchased from GOAQUA, Taiwan. Sea salt was obtained from Al-Qurum, Muscat. All solvent were evaporated at low pressure using Yomato Rotary evaporator model RE810. Agar plates were incubated in Genelac incubator Model no:

MINO/75F.

2.2. Sample Collection

Petroselinum crispum leaves were collected from Muscat, Oman in February 2012. After collection, the leaves were dried in shade for two weeks and weighed, it was then ground using kitchen blender to get powder.

2.3. Preparation of Organic Extracts

Petroselinum crispum leaves powder (150 g) was soaked in ethanol. The solvent was then decanted out and filtered under vacuum using Buchner apparatus to give clear solution. The ethanol was evaporated at low pressure using rotary evaporator to obtain crude extract. This crude extract was re-dissolved in ethanol: distilled water 1:1 mixture and re-extracted successively with different solvents. All solvents were then removed using rotary evaporator to give corresponding crude extracts.

2.4. Phytochemical Screening

The significance of this parameter is to examine the presence or absence of phytoconstituents in different extract. For phytochemical screening, the 5g of dried and powdered drug materials were extracted in a Soxhlet apparatus with Petroleum ether, chloroform, methanol and water successively. The extracts were dried and weighed. The presence or absences of different phytoconstituents were detected as per method described by Peach and Tracy [10].

2.5. Antimicrobial Test

Antibacterial activity was tested by the disc diffusion method [11]. The assay employed strains of gram positive bacteria, *S. aureus*, and gram negative bacteria *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa*. Extracts were diluted in four concentrations, 1000, 500, 250 and 125 µg/ml. Amoxicillin was used as a positive control drug.

2.6. Cytotoxicity test

Brine shrimp (*Artemia salina* Leach) larvae were used as indicator organisms for cytotoxicity assay. The Brine Shrimp Test (BST) was conducted as described by

McLaughlin and his coworkers [12]. The BST used four concentrations, 12.5, 125, 250, 500, and 1000 µg/ml for each extract. LC₅₀ values and 95% confidence intervals of each extract were generated by Finney probit analysis of shrimp percent mortality data using a computer program [13].

3. Results and Discussion

3.1. Phytochemical screening

Phytochemical screening indicates the presence of primary and secondary metabolites in petroleum ether, chloroform, alcohol and water soluble parts in the drug. Alkaloid, carbohydrate, phenolic compound, tannins, flavonoids, Proteins and amino acids and saponins were detected.

3.2. Antibacterial Activity

Table 2 shows results of antibacterial activities of different extracts of *Petroselinum crispum*. Hydro alcohol and petroleum ether extracts of *Petroselinum crispum* have shown (inhibition ranging from 20 mm to 7 mm) good antibacterial activity against all four micro-organisms: *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at different concentrations as compared to the standard drug Augmentin. Chloroform extracts showed very less activity. Peter et al reported in one of the studies that methanol-derived leaf extracts exhibited

significant activity. Bacterial cell damage, resulting in significant greater growth inhibition of *B. subtilis* and *E. coli*. This reported study supports the present work which has been conducted in Oman. Medicinal potential of Parsley and its exploitation can certainly support achieving indigenous medical treatment in Oman.

3.3. Cytotoxic Activity

The results for brine shrimp lethality test of different extracts of *Petroselinum crispum* are presented in table 3.

It is evident from the table that all the extracts are active against the brine shrimp larvae. Chloroform, Ethyl acetate and Hydro alcoholic extracts have killed the shrimp larvae at higher concentration of 1000 mcg/ml (mean percent mortality is 90%). The LC₅₀ values for the extracts deduced from the probit analysis results are shown in Table 4. As can be seen from the table that ethyl acetate and hydro alcoholic extracts showed very good activity against the test organism. Chloroform extract showed moderate activity.

LC₅₀ value of Ethyl acetate and Hydro alcoholic extracts is very less i.e. 51.9 and 88.1 µg/ml; it means that these two extracts are highly active and showing very good cytotoxic activity. Chloroform extract is showing activity but less as compared to the polar extracts, its LC₅₀ value is 224.2 µg/ml.

Table 1. Detection of Phytoconstituents in leaves *Petroselinum crispum*.

S. No.	Constituents	Result
1	Alkaloids	+
2	Carbohydrate	+
3	Phenolic compound and tannins	+
4	Flavonoides	+
5	Proteins and Amino Acids	+
6	Saponins	+
7	Resin	-
8	Lipids/ fats	-
9	Anthraquinone	-

+ = present, - = absent

Table 2. Antibacterial activities of extracts against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae*.

Name of extract	Concentration (µg/ml)	Inhibition Zone (mm)			
		<i>E-coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Hydro alcoholic	1000	12	20	19	15
	500	10	17	16	13
	250	17	12	14	10
	125	0	8	8	7
Chloroform	1000	10	11	12	0
	500	9	9	8	0
	250	7	0	0	0
	125	0	0	0	0
Petroleum ether	1000	15	12	14	11
	500	13	9	11	10
	250	9	8	9	0
	125	7	7	7	0
Amoxicillin	30	25	24	27	22

Christian and his coworkers also reported cytotoxic activity of Parsley in one of his study [14]. In Oman, most of the medicinal plants are scientifically not evaluated. So this present study was designed to get some preliminary pharmacological data. These findings are useful for further research to identify, isolate and characterize the specific compound which is responsible for the higher cytotoxic activity.

4. Conclusion

Antibacterial activity of *Petroselinum crispum* leaves extracts conducted and very

good results were obtained. So this plant can be used as a good source of potential antimicrobial agent. In addition, cytotoxic assay of *Petroselinum crispum* leaves extracts of ethyl acetate and hydro alcoholic extracts showed very good cytotoxic activity against brine shrimp larvae. Chloroform extract showed lesser activity as compare to the polar extracts. The brine shrimp lethality test (BST) has been found to be a quick, versatile method for evaluating general bioactivity of plant extracts.

Table 3. Mean mortality of brine shrimp larvae when exposed to Chloroform, Ethyl acetate Hydro-alcoholic extracts from *Petroselinum crispum* leaves. (n = 10 larvae per treatment).

Concentration mcg/ml	Mortality (%)		
	Chloroform	Ethyl acetate	Hydro alcoholic
12.5	30	30	40
125	50	40	50
250	30	60	60
500	50	80	80
1000	90	90	90

Table 4. Probit analyses of mortality (LC_{50}) of chloroform, Ethyl acetate and hydro alcoholic extracts of *Petroselinum crispum* leaves against brine shrimp larvae (n = 10).

Extract	LC_{50} (95% CI) (ppm)
Chloroform	224.227 (119.139 – 429.070)
Ethyl acetate	51.959 (0.352 - 169.778)
Hydro alcohol	88.159 (11.341 – 233.672)

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