

Diuretic and Antimicrobial Activity of Methanolic Extract of *Petroselinum crispum* Leaves

P.DEVI^{*1}, R.MEERA², N.CHITHAMBARANATHAN³, B.KAMESWARI⁴, R.BADMANABAN⁵

¹Department of Pharmacognosy, ²Department of Pharmaceutical Chemistry,
³Department of Pharmacology, ⁴Department of Biochemistry,
K.M.COLLEGE OF PHARMACY, UTHANGUDI, MADURAI -625107, Tamilnadu, India.

⁵Department of Pharmacognosy, Shrisarvajanik Pharmacy College, Mehsana, North Gujarat, India.

***E.mail: devipmpharma@yahoo.co.in**
Phone no: 09865377764.

Abstract: Methanolic extract of *Petroselinum crispum* leaves were tested for diuretic and antimicrobial activity. The diuretic activity was tested in rats at 500 and 1000 mg/Kg, orally and compared with furosemide (20mg/Kg) as the standard. The methanol extract was found to possess significant dose dependent diuretic activity. Methanol extract of leaves of *Petroselinum crispum* was investigated for their in vitro antimicrobial properties by agar disc diffusion method against Gram positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* and Gram negative bacteria *Escherichia coli*, *Pseudomonas aureginosa* and *Salmonella typhi* and antifungal activity against *Aspergillus niger* and *Candida albicans*. All tested microorganisms showed dose dependent susceptibility towards the methanol extracts. The antibacterial and antifungal activity of the extract and standard drugs were statistically significant. Based on the current findings it can be concluded that the plant possess potent antimicrobial and diuretic activity.

Keywords: Antimicrobial activity, Diuretic activity, Methanol extracts, *Petroselinum crispum* leaves .

Introduction

Petroselinum crispum (Apiaceae) is an aromatic biennial herb. Leaves are oblong, dentate margin, flowers are yellowish green in compound umbels. Fruits are crescent shaped. The plant is used in uterine troubles, diuretic, dysmennoria, antiscorbutic, antipyretic, and carminative. Leaves and roots contain imperatorin, isopimpinellin, psoralen. Fruit contains myristicin, apiole, apigenin-7- apioglucoside. Seeds contain flavone glycoside^{1,2,3,4}. However no work has been done so far on the diuretic and antimicrobial activity of the plant. Keeping this in view the present study was undertaken in our laboratory to investigate the diuretic and antimicrobial activity of methanol extract of *Petroselinum crispum* leaves.

Materials and Methods

The plant materials were collected in the month of April from Madurai district of Tamilnadu. It was

taxonomically identified by taxonomist and the voucher specimens have been preserved in our laboratory.

Preparation of extract

The aerial parts of the of *Petroselinum crispum* was dried under shade for 30 days. The completely dried plant material was ground into coarse powder. The coarsely powdered material (500g) was extracted exhaustively with methanol (4 L) using Soxhlet apparatus . The solvent was removed under reduced pressure to obtain a solid mass (180g)⁸. It was then preserved in a desiccator until further use.

Diuretic activity

Male rats (wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. The method of Lipschitz et

al^{6,7} was employed for the assessment of diuretic activity. Four groups of six rats in each were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received normal saline (25ml/Kg, p.o.); the second group received furosemide (20mg/Kg, i.p.) in saline; the third and fourth groups received the methanol extract at the doses of 500, 1000mg/Kg, respectively, in normal saline. Immediately after administration the animals were placed in metabolic cages (2 per cage), specially designed to separate urine and feces, kept at room temperature of $25 \pm 0.5^\circ\text{C}$ through out the experiment. The urine was collected in measuring cylinders up to 5hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na^+ , K^+ and Cl^- in the urine. Na^+ , K^+ concentrations were measured by Flame photometry⁸ and Cl^- concentration was estimated by titration^{9,10} with silver nitrate solution (N/50) using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20mg/Kg of furosemide per kg body weight in series of supportive experiments. Results are reported as mean \pm SD, the test of significance ($p < 0.01$ and $p < 0.05$) was statistically (Table 1)

Microorganisms and Media

The following bacterial strains used were *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* and fungal species *Candida albicans*, *Aspergillus niger*, for the present study. The bacterial and fungal cultures were maintained on Muller Hinton Agar Medium and Sabouraud dextrose agar slants respectively, which were stored at 4°C . Eight microorganisms maintained on nutrient agar base were used to assess the antimicrobial activity of the plant extracts. The fungi were maintained on Sabouraud dextrose agar, which is often used with antibiotics for the isolation of pathogenic fungi.

Antimicrobial screening

The anti bacterial activity of the extract was performed by disc diffusion method on nutrient agar plates^{11,12,13}. Three to five similar colonies were selected and transferred to 5ml broth with a loop and the broth cultures were incubated for 24 h at 37°C . The methanol extract was dissolved in DMSO with a magnetic stirrer. For screening, sterile 6-mm diameter filter paper discs were impregnated with 100-500 μg of the methanol extract and then placed in Muller Hinton Agar Medium. The inoculum for each organism was prepared from broth cultures and concentration of cultures was 1×10^5 colony forming units/ml. The antibacterial Amikacin (10 $\mu\text{g}/\text{ml}$) and antifungal Griseofulvin (20 $\mu\text{g}/\text{ml}$) was used as reference standards as recommended by the National Committee for Clinical laboratory standards¹⁴. The selected microorganisms included *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* at concentrations of 100, 250 and 500 $\mu\text{g}/\text{disc}$ respectively. The antifungal activity against *Aspergillus niger*, *Candida albicans* at the same concentrations.

Statistical analysis

The statistical analysis were carried out by student 't' test¹⁵, P considered significant. All the values are reported as Mean \pm SEM.

Results and Discussions

The methanol extract 500 and 1000mg/Kg p.o. showed significant increase in excretion of sodium, potassium and chloride ions in the urine in a dose dependent manner. The obtained effect was comparable to that of furosemide (20mg/Kg) (Table 1). It was observed that methanol extracts exhibited antifungal activity against *Aspergillus niger*, *Candida albicans* in a dose dependent manner. Tested extracts at higher concentration exhibits comparable antimicrobial activity with that of standard drugs. (Table 2). From the result we conclude that *Petroselinum crispum* possess diuretic and anti microbial activity. Further study required to isolate active constituents responsible for the activity.

Table 1: Diuretic activity of methanol extract of *Petroselinum crispum*

Experimental group	Dose	Urine volume (ml/kg)	Total sodium (mEq/I)	Total potassium (mEq/I)	Total chloride (mEq/I)
Control (normal saline)	25ml/kg , p.o	0.69±0.13	94.4±9	48.2±8.3	98.3±8.32
Standard (furosemide)	20mg/kg , p.o	3.2± 1.3*	130.1±0.21*	94.5±2.3	150.1±0.10*
ME	500mg/kg , p.o	1.0±0.15	98.2±7.3	110.1±9.4*	119.2±13.2*
ME	1000 mg/kg , p.o	1.3±0.25*	108.3±2.5**	127.1± 7.3*	155.2±11.8*

Values are mean ± SEM , n=6, *p<0.01, **p<0.05 when compared to normal saline (control).

ME: Methanol extract of *Petroselinum crispum*

Table 2: Antimicrobial activity of methanol extract of *Petroselinum crispum*

Organism	Diameter of zone of inhibition (mm)			
Bacteria	Methanolic extract			Standard (Amikacin) (10µg/ml)
	A	B	C	
<i>Bacillus subtilis</i>	9.5±0.5	11.3±1.3	15.6±1.2	20.5±0.5
<i>Staphylococcus aureus</i>	11.4±0.8	13.6±1.2	16.7±0.6 *	21.7±1.6
<i>Micrococcus luteus</i>	8.5±1.2	10.2±0.6	13.5±1.2	19.3±0.7
<i>Escherichia coli</i>	NI	9.7±1.5	12.5±1.3	18.6±1.3
<i>Pseudomonas aeruginosa</i>	8.5±1.2	10.3±0.8	13.4±0.5	20.2±0.2
<i>Salmonella typhi</i>	NI	7.2±1.4	8.5±0.5	14.5±0.6
Fungi				Standard Gresiofulvin (20µg/ml)
<i>Aspergillus niger</i>	7.3±0.7	10.1±1.2	12.5±0.3	19.2±0.7
<i>Candida albicans</i>	12.8±1.1	14.6±1.7	20.1±0.7	24.1±1.2

All the values indicate mean ±SD (n=3) the concentrations were A : 100µg/disc, B :250µg/disc, C :500µg/disc. NI- NO INHIBITION ZONE.

Statistical value *p<0.05 when compared to standard.

References

1. Yoganarashiman SN, Medicinal Plants of India, Vol.2, 2000, p. 406
2. Chopra RN, Nayar SL and Chopra I.C, Glossary of Indian Medicinal Plants. National Institute of Science Communication, 1999, p.189
3. The Wealth of India, Council of Science and Industrial Research, New Delhi, Vol 1991, p.328
4. Dictionary of Medicinal Plants, Central Institute of Medicinal and Aromatic Plants, Lucknow, 1992, p.336
5. Karaman I., Sahin F., Gulluce M., Ogutuc H., Sengul M and Adiguzel A.J. *Ethanopharmacol*, 2003, 85, 231-235.
6. W.L.Lipschitz, Z.Haddian and A.Kerpscar. *J.Pharmacol.Exp.Ther*, 1943, 79, 97-110.
7. T.Murugesan, L. Manikandan, K.B.Suresh, M.Pal and B.P.Saha. *Indian J.Pharm.Sci*, 2000, 62 (2), 150-151.
8. Vogel, In *Textbook of Quantitative Chemical Analysis*, Addison Wesley Longmann., England, 5th Edn, 1989, p. 801.
9. Beckett AH and Stenlake J B, In *Practical Pharmaceutical Chemistry*, CBS Publishers and Distributors, New Delhi Part I, 1st Edn. ,1997, p.197.
10. Jeffery GH, Bassett J, Mendham J, and Denny RC, *Vogels Textbook of Quantitative Chemical Analysis*, Addison Westley Longman Ltd, England 5th Edn, 1989, p. 801
11. Murray PR, Baron EJ, Pfaller MA, Tenover FC and Tenover H. *Manual of Clin .Microbiol*, 1995, 6, 45-56.
12. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of Yeast. NCCLS document M27-A2 National Committee for Clinical Laboratory Standards, Wayne Pa.2002, pp.45-65.
13. Pelczar M, Chan ECS and Krieg NR., In *Microbiology*, Int Edn. McGraw-Hill, New York, 1993, pp.578.
14. Mackeen M .M, Ali A .M, El-Sharkawy S .H, Salleh M .Y , Lajis N .H and Kawazu K. *Int J Pharmacogn*, 1997, 35, 237-243.
15. P.Amritage ., Eds., In; *Statistical Methods in Medical Research*, Blackwell Scientific Publications, London, 1971, pp. 217.
