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Antibacterial Activity of Hydrodistilled Essential Oil of *Psammogeton canescens* N.O. Umbelliferae

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Abstract: The essential oil of *Psammogeton canescens* obtained by hydro distillation was evaluated for its antibacterial activity against gram positive (*Bacillus megaterium*, *B. subtilis*, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Staphylococcus albus*, *S. aureus*, *Vibrio cholera*) and gram negative bacteria (*E. coli*, *Salmonella typhae*, *Shigella ferrarie*). The oil was found active against all the bacterial strains. However the activity of oil varies with its concentration and kind of bacteria. The oil showed activity even at 50µg concentration. The antibacterial activity was comparable with amoxil, streptomycin and kanamycin. The oil had bactericidal activity against all the bacteria under investigation except *Shigella ferrarie*, *Micrococcus leuteus* and *Vibrio cholera*. The MIC value of oil varies from 75.5-160 and 75.5-135mg ml^{-1} against gram positive and gram negative bacteria, respectively.

Key words: Essential oil, *Psammogeton canescens*, antibacterial activity, bactericidal, MIC

Introduction

The umbelliferae is a cosmopolitan family. The name umbelliferae derived from the latin word *Umbula* means a little shade and *alludes* to the flower being produced in parasol shaped clusters. Its inflorescence is umbel shaped. In most genera the umbel is compound whereas few have simple umbel. This family has about 300 genera and 3000 species out of which 56 genera and about 167 species are reported from Pakistan (Nasir and Ali, 1972). The umbelliferae plants are important chiefly for vegetable, volatile oil and drugs. The chemical composition of the essential oil of about half of the species met in Pakistan, is carried out at PCSIR (Waheed *et al.*, 1989; Seemal *et al.*, 1988; Ahmad *et al.*, 1987; Bhatty, 1982). The antimicrobial activities of essential oil of different plants against various microorganisms have been reported by many scientists (Qasim and Khan, 2001; Rahman and Gul, 2000; Yazdana *et al.*, 1997; Razia *et al.*, 1996; Syed *et al.*, 1986 & 1991). Naqvi *et al.* (1985, 1987) studied 163 plant species and found 98 of them positive for antibacterial activity. The use of plant for the treatment of human ailments date back to the prehistoric times.

Volatile oils are much effective constituent of plant against gram positive and gram negative pathogenic microorganism like *Staphylococcus aureus*, *E. coli*, etc. The sensitivity of different organisms varies with different oils.

In present work, the antibacterial activity, minimum inhibition concentration (bactericidal/bacteriostatic) of *Psammogeton canescens* oil was studied against three gram negative (*E. coli*, *Salmonella typhae*, *Shigella ferrarie*) and seven gram positive bacteria (*Bacillus megaterium*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Staphylococcus albus*, *S. aureus*, *Vibrio cholera*).

Materials and Methods

Essential oil: *Psammogeton canescens* plants were collected from Hazar gangi, National Park, Quetta, Balochistan, Pakistan, during the month of April, 1999. The aerial parts of the plants were brought to the laboratories and air dried. The oil was obtained from the plants by hydro distillation. Five kilogram dried and crushed plants were steam distilled in Dean Starke head (Guenther, 1952).

Test organisms: Ten standard cultures of bacteria were procured from drug testing laboratories, Government of Punjab, Pakistan. These cultures include seven gram positive bacteria (*Bacillus megaterium*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Staphylococcus albus*, *Staphylococcus aureus*, *Vibrio cholera*) and three gram negative bacteria (*E. coli*, *Salmonella typhae*, *Shigella ferrarie*).

Maintenance of cultures: The above mentioned cultures were maintained on nutrient agar slants, stored in refrigerator and subcultured after every 15 days.

Antibacterial activity: The antibacterial activity of the essential oil was achieved by agar diffusion method (Murtaza *et al.*, 1994). Mueller and Hinton agar (Ericson and Sherris, 1971) was used for testing the sensitivity of different strains towards the essential oil of *Psammogeton canescens*. Twenty four hours old cultures were spread on the surface of Mueller and Hinton agar plates. Four wells were dug in each plate with the help of sterile metallic borer. Stock solution of the essential oil 1mg/mL and dilutions of the stock solution containing 50, 100, 150 and 200µg were prepared in dimethyl sulfoxide (DMSO). Hundred µL of each dilution was added in their respective well. Control received 100µL DMSO only. Amoxil, streptomycin and kanamycin were used as standard drugs (100µg). The plates were incubated at 37°C for twenty four hours. After twenty four hours zones of inhibition were recorded and compared with standard drugs.

Determination of MIC and MBC: Minimum inhibitory concentration (MIC) was determined by the tube

dilution method using Mueller and Hinton broth (Ericsson and Sherris, 1971). Bactericidal and bacteriostatic characteristic of the essential oil at MIC was determined by diluting 1 mL of the culture from the MIC tube as well as 1 mL from above and below the MIC tubes. Diluted broth culture (0.1ml) was transferred to nutrient agar plates and incubated at 37°C for 18-24 hours for the determination of growth.

Results and Discussion

The essential oil of *Psammogeton canescens* showed antibacterial activity against all gram positive and negative strains even at 50µg concentration. The activity of essential oil varied with the concentration of oil and type of microorganism. Syed *et al*. (1987) have also reported dose dependence of inhibitory activity of umbelliferae member oils. The activity of oil at the same concentration of standard antibiotic was comparable for some bacteria (Table 1).

Among gram negative bacteria, the oil was much active against *E. coli*. The activity response to *E. coli* was more or less the same at 50µg as that of amoxil and kanamycin (100µg) whereas at 100 µg concentration the activity was equal to streptomycin (100µg). The activity of essential oil was comparable against *S. typhae* to kanamycin at 50µg and to amoxil at 150µg. Against *Shigella ferrarie*, the activity of 150µg oil concentration activity was more or less equal to amoxil and kanamycin (100µg). At 200 µg oil concentration antibacterial activity against *S. typhae* and *S. ferrarie* was little better than streptomycin (100µg). Qasim and Khan (2001) have reported the activity of *Carum copticum* oil against *E. coli*. The essential oil of Psammogeton canescens showed remarkable activity against gram positive bacteria, B. subtilis and S. albus with respect to standard antibiotics at 100µg concentration. The activity of oil at 50µg concentration against B. megaterium was better as compared with amoxil (100µg) and at 150µg with respect to streptomycin and kanamycin (100µg) against B. megaterium. The response of streptomycin was far better than oil even at 200µg against L. acidophilus. However, the activity of Psammogeton canescens oil at 100µg against L. acidophilus was only 8.9 % better than amoxil (100µg) and oil at 200µg showed 19.10 % greater activity than kanamycin (100µg). M. leuteus responded in same manner to oil, amoxil and kanamycin at 100 µg. The oil at 200 µg concentration has 16% better activity with respect to kanamycin against M. leuteus. The zones produced by 150 and 200 µg oil concentration was better than the standard antibiotics. The oil showed poorest activity against V. cholera as compared to standard antibiotics, amoxil, streptomycin and kanamycin. Antibacterial activity of essential oil of umbelliferae members, Carum carvi, Petroselinum crispum and Dorema ammoniacum has been reported by Syed et al. (1987) against gram positive bacteria. The activity of Petroselinum crispum may be due to its Coumarin contents (Florya and Kretsa, 1980).

The minimum inhibitory concentration (bactericidal/bacteriostatic) of oil and standard antibiotics against gram positive and negative bacteria is given in Table 2. The oil of Psammogeton canescens

Table 1: Antibacterial activity of *Psammogeton canescens* oil compared with amoxil, streptomycin and kanamycin

	Different concentrations of *Psammogeton canescens* oil (µg) used for zone of inhibition (Diameter in mm)				Standard antibiotic used (100 µg) for zone of zone of inhibition (Diameter in mm)		
Names of organisms	50	100	150	200	Amoxil	Streptomycin	Kanamycin
Gram negative bacteria							
E. coli	20.50	30.25	35.00	40.00	19.50	28.50	21.25
Salmonella typhae	18.60	25.60	30.75	34.50	27.50	33.50	19.75
Shigella ferrarie	15.50	20.75	25.50	30.25	25.00	28.50	25.50
Gram positive bacteria							
Bacillus megaterium	20.25	30.75	36.00	42.50	12.65	34.00	32.75
Bacillus subtilis	14.00	20.50	26.75	31.25	15.25	15.75	19.00
Lactobacillus acidophilus	11.75	15.25	19.00	26.50	14.00	31.50	22.25
Micrococcus leuteus	14.50	20.50	25.75	32.00	21.00	20.75	27.50
Staphylococcus albus	18.75	27.50	33.00	38.00	21.00	25.75	26.50
Staphylococcus aureus	12.50	15.25	21.25	27.50	17.25	19.50	23.75
Vibrio cholera	08.00	12.00	17.00	23.75	25.00	18.75	26.25

Table 2: Minimum inhibitory concentration (MIC) (Bactericidal/Bacteriostatic) of *Psammogeton canescens* oil compared with Amoxil, Streptomycin and Kanamycin

Names of organisms	*Psamogeton canescens*		Amoxil		Streptomycin		Kanamycin	
	MIC mg/mL	Charact-eristic	MIC mg/mL	Charact-eristic	MIC mg/mL	Charact-eristic	MIC mg/mL	Charact-eristic
Gram negative bacteria								
E. coli	120.00	Bactericidal	125.00	Bactericidal	72.00	Bactericidal	85.80	Bactericidal
Salmonella typhae	*75.50*	Bactericidal	75.00	Bactericidal	105.00	Bactericidal	90.00	Bactericidal
Shigella ferrarie	135.00	Bacteriostatic	80.00	Bactericidal	110.00	Bactericidal	95.00	Bactericidal
Gram positive bacteria								
Bacillus megaterium	78.0	Bactericidal	205.00	Bactericidal	85.50	Bactericidal	90.00	Bactericidal
Bacillus subtilis	95.00	Bactericidal	220.00	Bactericidal	82.20	Bactericidal	72.50	Bactericidal
Lactobacillus acidophilus	75.50	Bactericidal	145.00	Bactericidal	72.50	Bactericidal	75.30	Bactericidal
Micrococcus leuteus	150.00	Bacteriostatic	70.00	Bactericidal	80.50	Bactericidal	125.00	Bactericidal
Staphylococcus albus	98.00	Bactericidal	200.00	Bactericidal	95.00	Bactericidal	105.00	Bactericidal
Staphylococcus aureus	160.00	Bactericidal	180.00	Bactericidal	80.00	Bactericidal	92.50	Bactericidal
Vibrio cholera	120.00	Bacteriostatic	100.00	Bactericidal	75.00	Bactericidal	85.30	Bactericidal

showed bacteriostatic activity only against S. ferrarie, M. leuteus and V. cholera at 135, 150 and 120µg concentration, respectively. The increase of oil had the same effect above MIC against S. ferrarie, S. aureus and V. cholera. The MIC of oil for other bacteria where it had bactericidal effect, ranged from 75.5 to 160mg/ml for gram positive and 75.5 to 120mg/mL for gram negative bacteria.

The MIC values for amoxil was 70-220, streptomycin 72-110 and kanamycin 72.5-125mg/mL.

It can be safely concluded that Psammogeton canescens oil can be utilized as bacteriostatic/bactericidal drug against gram positive and negative bacteria and in soaps for dermatological use as Dorema ammonicum oil recommended for medicines (Heywood, 1971) and as therapeutic agent in or beside the existing antibiotics (Syed *et al*., 1987).

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