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## ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF UMBELLIFERAE FAMILY

### Part VI. Stewartiella baluchistanica, Psammogeton canescens and Ducrosia anethifolia

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The essential oils of Stewartiella baluchistanica, Psammogeton canescens, and Ducrosia anethifolia were studied for their inhibitory activity against Staphgloccus aureus, E.coli, Salmonella typhi, Shigella dysentery, and Vibrio cholera in emulsified broth by spectrophotometric method. All the oils showed inhibitory effect but the oil of P. canescens was found to be the most effective.

Key words: Stewartiella baluchistanica, Psammogeton canescens, Ducrosia anethifolia.

#### INTRODUCTION

In continuation of our studies of the antimicrobial activity of the essential oils of N.O. Umbelliferae [1], we report here the results of three more species, namely *Stewartiella baluchistanica, Psammogeton canescens,* and *Ducrosia anethifolia.* The chemical analysis of these species has been reported previously [2-4].

The genus Stewartiella comprises only one species, i.e. Stewartiella baluchistanica, which is endemic to the Baluchistan region. Bhatty et. al. [2] were the first to determine the chemical and physical properties of its essential oil and the first ever antimicrobial properties of this oil are being reported in this paper. P. canescens (Khushbue) is one of the seven species of the genus Psammogeton found in the deserts of Iran, Central Asia, Afghanistan, Pakistan and India. The oil of this high yielding plant has a very pleasant fruity smell and its composition was also reported by Bhatty and co-workers [3] for the first time.

The third genus, *Ducrosia* comprises only 5-6 species. *Ducrosia anethifolia* (gwartkh) grows wild in the NWFP and Baluchistan and is now being successfully cultivated in Quetta, Murree and Lahore. The major constituent of this oil is chrysanthenyl acetate [4], quite uncommon in the Umbelliferae family. The oil has been reported to be active only against gram-positive bacteria [5,6]. The activity of the essential oils of these species against grampositive, as well as gram-negative bacteria, viz. *S. aureus*, *E. coli*, *S. typhi*, *S. dysentery*, and *Vibrio cholera* is being reported in the paper.

#### MATERIAL AND METHOD

#### Materials.

1. Cultures. Pure standard cultures of S. aureus ATCC 6538-P, E. coli-M/200, S. typhi, S. dysentery, and V. cholera were provided by the National Institute of Health, Islamabad, and the Drug Testing Laboratories, Lahore.

2. *Media*. Merck's agar medium was used for stock culture slants, and Oxoid's Antibiotic Medium No. 3 was used for liquid broth.

3. Essential oils. The essential oils from the stalks and leaves of S. baluchistanica and from the seeds of P. canescens and D. anethifolia were obtained by steam distillation.

#### Preparation of media and inoculum.

The method for the preparation of media and inoculum has been reported earlier [1]. The tubes were incubated for 20 hr. at  $35^{\circ}$  and the optical density taken as index of bacterial growth, was measured at 530 nm on Hitachi Model 100-20 UV-Vis., spectrophotometer, and was plotted against quantity of essential oil in ppm.

#### RESULTS AND DISCUSSIONS

The optical density of growth medium against the quantity of essential oil used in ppm. has been shown in Tables 1-5 and Fig. 1-5. The concentration of oil that caused an eightfold inhibition of growth of the bacteria was taken as its Minimum Inhibitory Concentration (MIC). The bacterium-wise results are presented below.

1. S. aureus . S. baluchistanica actively inhibited S. aureus at 400 ppm. (Table 1, Fig. 1). This activity was

Table 1. Activity of essential oils against S. aureus.

Amount of	Optical density (mean)*			
oil (ppm.)	S. baluchistanica	P. canescens	D. anethifolia	
	anofin are as	ALIGHTS DER 20		
0	440	446	487	
400	66	26	179	
800	46	0	15	
1200	32	0	0	
1600	18	0	0	
2000	12	0	0	
2400	7	0	0	



Fig. 1. Antimicrobial activity of the essential oils of  $-\Phi$ -Stewertiella baluchistanica  $-\Phi$ -Psammogeton canescens;  $-\Phi$ -Ducrosia anethifolia, against Staph. aureaus.  $\star$  MIC.

greater than that of *Coriandrum sativum* [1] and less than that of *Cuminum cyminum* [1]. Its MIC was 600 ppm where as *Psammogeton canescens* was equal to *Cuminum cyminum* [1], both in activity and MIC, i.e. 300 ppm. *Ducrosia anethifolia* had a dose-dependent inhibition against *S. aureus.* At about 300 ppm it caused twofold inhibition of the organism, whereas at 800 ppm there was complete inhibition. Its MIC was about 650 ppm and it resemblea *F. vulgare* [1] in activity.

2. E. coli. S. baluchistanica at 400 ppm showed a 25% inhibition of E. coli. However at 800 ppm nearly 75 %

inhibition was achieved, with a 90 % inhibition at 1200 ppm. The activity increased further with increase in the concentration. The MIC level was nearly 1000 ppm (Table 2, Fig. 2). This activity was greater than that of *Coriandrum sativum* [1]. The oil of *P. canescens* also caused a rapid inhibition against this organism i.e. more than 90 % inhibition at 400 ppm. Its MIC was about 350 ppm. The activity of this oil against this urinary tract infectious bacterium was 'far more than the activity of other oils reported previously [1,7-9], with the exception of *C. cyminum* [1] and *Trachyspermum* ammi [7], which had

Table 2. Activity of essential oils against E. coli.



Fig. 2. Antimicicrobial activity of the essential oil of -- Sterwartiella baluchistanica; -- Psammogeton canescens, -- Ducrosia anethifolia; against E. coli.  $\neq$  MIC.

greater activities. D. anethifolia showed a linear rate of inhibition. Its activity resembled that of B. persicum [1]. The MIC of this oil against E. coli was 2200 ppm. which was close to that of Anethum graveolens [7].

3. Salmonella typhi. Like Apium graveolens [7]. the oil of S. baluchistanica showed a dose -dependant linear rate of inhibition (Table 3, Fig. 3). About 50 % inhibition occurred at 1200 ppm. The activity then increased rapidly and about 94 % inhibition was achieved at 1600 ppm with an MIC of 1550 ppm. Both P. canescens, and B. persicum [1] were equally active against this human pathogen. Both caused 50 % inhibition at 400 ppm, and

Table 3. Activity of essential oils against S. typhi

Amount of oil in ppm.	Optical Density (mean)*			
	S. balu chistanica	P. canescens	D. anethifolia	
To appliedo	ini pomadi any a	10000 1000 10000 1000	and a size the	
0 '	419	440	483	
400	325	227.	395	
800	278	36	306	
1200	201	12	227	
1600	36	0	168	
2000	29 .	0	93	
2400	13	0	32	

- 800 700
  - 600
- 500 Q 450

O

350

400



Fig. 3. Antimicrobial activity of the essential oils of -- Stewartiella baluchistanica, -- Psammogeton canescens -- Ducrosia anethifolia; against Salmonella typhi. \* MIC.

about 94 % inhibition at 800 ppm, with an MIC of 700 ppm. Ducrosia anethifolia had a linear rate of inhibition. 400 ppm of this oil caused a 25 % inhibition. A 50 % inhibition was observed at 1200 ppm and about 95 % inhibition at 2400 ppm. The MIC was 2200 ppm. The activity of this oil, against this organism, was more than that of A. graveolens [7].

4. Shigella dysentery. Against S. dysentery, the oil of S. baluchistanica resembled D. carota [7] in activity. At 1900 ppm., a 50 % inhibition was achieved and increased upto 85 % at 2400 ppm. (Table 4, Fig. 4). Although P. canescens was not much effective at 400 ppm, yet at 800

Table 4. Activity of essential oils against S. dysentery.

Amount of	Optical density (mean)*			
oil (ppm.)	S. baluchistanica	P. canescens	D. anethifolia	
0	592	678	626	
400	516	585	405	
800	465	224	37	
1200	408	17	0	
1600	365	5	0	
2000	279	0	0	
2400	118	0	0	

\* Average of four readings

800



Fig. 4. Antimicrobial activity of essential oils of -- Stewartiella baluchistanica, -- Psammogeton canescens; -- Ducrosia anethifolia; against Shigella dysentry. \* MIC.



Fig. 5. Antimicrobial activity of the essential oil of  $-\Phi$ -Stewartiella baluchistanica;  $-\Phi$ -Psammogeton canescens;  $-\Phi$ -Ducrosia anethifolia against Vibro cholera.  $\star$  MIC.

ppm, the oil showed about 70 % inhibition, which is quite noticeable. It showed almost complete inhibition at 1200 ppm, with an MIC of 1000 ppm. The activity was more than that of *F. vulgare* [1]. *D. anethifolia* was more active than *P. canescens* against this bacterium. At 800 ppm, it caused a 94 % inhibition, equal to that of *F. vulgare* [1]. The MIC was less than 700 ppm.

5. Vibrio cholera. S. baluchistanica exhibited about 40 % inhibition of V. cholera at 400 ppm. (Table 5, Fig. 5). The activity then slowed down and 50 % inhibition was observed at 1600 ppm. At 400 ppm, the activity of P. canescens was equal to that of P. anisum [8]. A 50 % inhibition similar to Carum carvi was observed at 800 ppm. The activity gradually increased with the amount of oil and at 2400 ppm, it caused about 90 % inhibition of the bacterium. This activity was more than that of S. baluchistanica. The oil of D. anethifolia also showed a gradual, and dose

Table 5. Activity of essential oils against V. cholera.

Amount of oil (ppm.)	Optical density (mean)*			
	S. baluchistanica	P. canescens	D.anethifolia	
.0	996	906	938	
400	759	576	* 755	
800	647	458	664	
1200	565	401	608	
1600	506	367	548	
2000	437	306	526	
2400	421	162	463	
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\* Average of four readings.

dependant activity against this organism. The activity was less than that of *S. baluchistanica*.

It has been observed that all three oils possessed effective inhibitory activity against the studied pathogenic bacteria. Whereas *P. canescens* was the most inhibitory of the three. Quite contrary to the previous observations [5,6] *D. anethifolia* very effectively inhibited gram-negative bacteria as well, especially *S. dysentery*. These results are much more encouraging than the previous reports.

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