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# ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF UMBELLIFERAE FAMILY Part VII. Heracleum candicans, Prangos pabularia and Peucedanum ferulaefolium Fruit Oil

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In continuation of our studies on antimicrobial activity of esential oils of umbelliferae family, oils from the seeds of *Heracleum candicans*, *Prangos pabularia* and *Peucedanum farulaefolium*, were tested against *Staphylococcus-aureus*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibrio cholerae*. The oil of *Peu. ferulaefolium* shows excellent activity against all the five pathogens at quite low doses. The oil of *H. candicans* showed moderately good activity whereas the oil of *Pr. pabularia* is not very effective against these bacteria.

Key words: Heracleum candicans, Prangos pabularia, Peucedanum ferulaefolium.

### INTRODUCTION

Many species of Umbelliferae family, mostly aromatic, and therapeutically active, grow wild in Pakistan. Heracleum is a large genus consisting of about 70 species and occurs in temperate regions. some species of this genus are used in indigenous systems of medicines and contain psoralens and other coumarins as constituents [1, 2] *Heracleum candicans* (morchar) grows wild in Muree, Hazara, Chitral, Gilgit, Swat and Kashmir [3]. The plant, used mostly as a veterinary medicine, exhibits stimulant properties. It increases the rate of respiration and blood pressure. The gas chromatographic analysis of its fruit oil, by Bhatty *et. al.* [3], shows that esters are its main constituents while lactones have been reported by Sarin and Kapoor [4], coumarins constitutes 2 to 6.5% of the oil [3].

The genus Prangos consists of some 30 species growing in the mediterranean, Central, and West Asian regions. Only two species Pr. bucharica and Pr. pabularia have been reported in Pakistan [5]. The latter species, Pr. pabularia (Mushain), grows wild in Pakistan, Afghanistan, India and Tibet. In Pakistan it grows in Chitral, Swat, Gilgit and Singash [5]. The fruit of the plant used in local remedies, as carminative, stimulant and diuretic and for the treatment of *Fasiola hepatica* infection in sheep. The flowers and leaves are said to be insect repellent. The fruit oil contains more than 20% coumarins and resinous material [5]. Koul and Dhar [6] have also identified some coumarin glucosides in the fruit and these have been found to be effective against some bacteria [7].

Peucedanum is another genus of the Umbelliferae family. It comprises some 120 species distributed in Eu-

rope, Asia and Africa. The species *Peu. ferulaefolium* (wild dill) grows wild in Afghanistan and Pakistan [8]. It is used as a substitute of dill in local remedies for indigestion. The fruits give nearly 3% yield of pleasant smelling oil. Main components of the oil are methyl-eugenol (50%), santene (30%) and coumarins (10%) [8, 9].

In continuation of our earlier work [10], the activity of their fruit oils against five pathogens has been studied.

#### MATERIAL AND METHOD

(The material and method are described in detail in Part-I of this series [10]).

Material. Cultures: Standard cultures of staphylococcus aureus, ATCC 6538-P, Escherichia coli M 200, (causes urinary tract infection) Salmonella typhi, Shigella dysenteriae and Vibrio cholerae were obtained from the National Institute of Health, Islamabad and the Drug Testing Laboratory, Lahore.

*Media.* 2% agar was added in merck's glucose broth medium (Art. 8338) to make slants for stock culture and oxoid antibiotic medium No. 3 was used for culture broth.

*Essential oils*. The essential oils were obtained by the steam distillation of the fruits (distillation time 8-12 hr.) [3, 5, 8].

Preparation of media and inoculum, measurement of inhibition. The media and inoculum were prepared according to the method described previously [10]. After incubating the tubes for 20 hr. at 35°, optical density, taken as index of bacterial growth, was measured spectrophotometrically at 530 nm. using a Hitachi Model 100-20 UV-Vis spectrophotometer.

## RESULTS AND DISCUSSION

The quantity of essential oil used, in parts per million (ppm) vs the optical density of the culture is persented in Tables 1-5 and Fig. 1-5. The oil concentrations showing no growth was taken as minimum inhibitory concentration (MIC) and the results are presented below:

Staphylococcus aureus. 400 ppm concentration of the oil of *H. candicans*, causes 67% inhibition of *S. aureus*. This inhibitory activity increases to 80% at 800 ppm and to 91% at 1200 ppm., whereas 1600 ppm of this oil causes

Table 1. Activity of essential oil against, S. aureus.

Amt. of oil in ppm.	Optical density (mean)*		
	H. candi- cans	Pr. pabu- laria	Peu. ferula- efolium
400	133	308	33
800	82	274	6
1200	36	240	0
1600	0	211	0
2000	0	203	0
2400	0	187	0

Table 2. Activity of essential oil against, E. coli.

Amt. of oil	Optical density (mean)*		
in ppm.	H. candi- cans	Pr. pabu- laria	Peu. ferula- efolium
400	188	306	118
800	87	188	87
1200	32	169	61
1600	0	160	49
2000	0	154	31
2400	0	142	2

Table 3. Activity of essential oil against, S. typhi.

Amt. of oil	Optical density (mean)*		
in ppm.	H. candi-	Pr. pabu-	Peu. ferula-
	cans	laria	efolium
0	405	408	441
400	336	233	67
800	281	198	2
1200	192	195	0
1600	23	174	0
2000	7	167	0
2400	0	155	0

100% inhibition. The MIC of *H. candicans* against this bacterium is nearly 1000 ppm.

*Pr. pabularia* oil in contrast is low in its activity against this organism. At 400 ppm., there is 25% inhibition which slowly increases to 54% at the maximum dose of 2400 ppm. (Table 1, Fig. 1).

Table 4. Activity of essential oil against, S. dysenteriae.

Amt. of oil in ppm.	Optical density (mean)*		
	H. candi- cans	Pr. pabu- laria	Peu. ferula- efolium
400	422	415	493
800	169	413	15
1200	24	404	3
1600	8	399	0
2000	0	397	0
2400	0	395	0

Table 5. Activity of essential oil against, V. cholerae.

Amt. of oil in ppm.	Optical density (mean)*		
	H. candi- cans	Pr. pabu- laria	Peu. ferula- efolium
400	579	834	510
800	527	820	285
1200	492	818	7
1600	441	803	0
2000	382	754	0
2400	257	528	0

\*Mean of 4 sets of test tube.



Fig. 1. Activity against Staphylococcus aureus of -- Heracleum candicans, -0- Prangos pabularia  $-\Delta$ - Peucedanum ferula-efolium \* MIC.



Fig. 2. Activity against Escherichia coli of -o- Heracleum candicans, -o- Prangos pabularia - $\Delta$ - Peucedanum ferula-efolium \* MIC.



Fig. 3. Activity against Salmonella typhi of -- Heracleum candicans, -0- Prangos pabularia -Δ- Peucedanum ferula-efolium \* MIC.



Fig. 4. Activity against Shigella dysentry of -o- Heracleum candicans, -o- Prangos pabularia - \Delta- Peucedanum ferula-efolium \* MIC.



Fig. 5. Activity against Vibrio cholera of -•- Heracleum candicans, o- Prangos pabularia -Δ- Peucedanum ferula-efolium \* MIC.

The oil of *Peu. ferulaefolium* is highly active against this pathogen. At 400 ppm. the oil inhibits the growth by 92%. The MIC is nearly 300 ppm.

*Escherichia coli.* There is 55% growth inhibition of *E. coli* by 400 ppm oil of *H. candicans*. The inhibition rises to 80% at 800 ppm, to 92% at 1200 ppm. Total inhibition was observed at 1600 ppm concentration of the oil. The MIC of the oil against this pathogen is 1000 ppm.

The oil of *Pr. pabularia* is less in its activity in this case too. At 400 ppm., it causes 25% inhibition of *E. coli*. At 800 ppm, however, the activity is 54% which reaches up 65% at a dose of 2400 ppm.

The essential oil of *Pue. ferulaefolium* is highly active against this pathogen as well. At 400 ppm it causes 73% inhibition, and reaches 93% at 2000 ppm. Almost 100% inhibition is attained at 2400 ppm. (Fig. 2, Table 2).

Salmonella typhi. At 400 ppm., the essential oil of H. candicans causes less than 20% growth inhibition of typhoid organism. The inhibition increases to more than 50% at 1200 ppm and is 94% at 1600 ppm. There is almost complete inhibition at 2000 ppm. The MIC of H. candicans against this pathogen is about 1500 ppm.

The activity of the essential oil of *Pr. pabularia* against *S. typhi* seems independent of the amount of oil. Nearly two fold inhibition is caused at initial doses and remains so even when the quantity of oil is increased.

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The oil of *Peu. ferulaefolium* is highly active against this pathogen also. At 400 ppm the inhibition is 85%. Almost total inhibition occurs at the next higher dose. The MIC of *Peu. ferulaefolium* is about 500 ppm (Fig. 3, Table 3).

Shigella dysenteriae. At initial dose of 400 ppm the oil of *H. candicans* is not very active against *Sh. dysenteriae*. There is only 28% inhibition at this concentration. It slowly reaches 71% at 1200 ppm. However at 1600 ppm of the oil, the inhibition suddenly becomes 96% and almost complete inhibition is attained at the next higher dose. The MIC of this oil agaisnt this pathogen is about 1000 ppm.

The oil of *Pr. pabularia* shows no significant activity against this pathogen. The inhibition is only 10% to 14% even upto the highest dose.

The oil of *Peu. ferulaefolium* causes 19% inhibition at 400 ppm. However it suddenly rises to 98% at 800 ppm concentration and becomes complete at next higher dose. The MIC is about 700 ppm. (Fig. 4, Table 4).

Vibrio cholerae. The oil of H. candicans is moderately active against V. cholerae. At 400 ppm dose, there is nearly 50% inhibition which however increases to 73% at 2400 ppm. Keeping in view the high rate of growth of this pathogen this activity is quite significant (as the growth of this pathogen during the same incubation time and nutritional conditons is almost double than the other bacteria).

The oil of *Pr. pabularia* shows only 9% to 18% inhibition from the concentration of 400 ppm to 2000 ppm. However at the maximum dose of 2400 ppm the inhibition become 42%.

*Peu. ferulaefolium* is very effective agaisnt this pathogen as well. There is nearly two fold inhibition at 400 ppm. At 800 ppm, the inhibition is 70%. Ther is almost complete inhibition at 1200 ppm. of this oil (Fig. 5, Table 5). The activity of this oil against *V. cholerae* is even more than that of *C. cyminum* [10].

The results show that the oil of *Peu. ferulaefolium* is highly active against all the five pathogens at quite low concentrations.

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