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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF THE UMBELLIFERAE FAMILY

Part 1. *Cuminum cyminum, Coriandrum sativum, Foeniculum vulgare* and *Bunium persicum* Oils

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The essential oils of *Cuminum cyminum, Coriandrum sativum, Foeniculum vulgare,* and *Bunium persicum* have been tested against the standard strains of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella dysentery,* and *Vibrio cholera.* Optical density, measured spectrophotometrically at 530 nm, was taken as an index of the growth of bacteria in the liquid medium. While *Cuminum cyminum* has shown remarkable activity against all the pathogens at quite low concentrations, the other oils also exhibit such an activity but with larger amounts.

INTRODUCTION

Due to the rapid adaptation of bacteria to prevalent antibiotics, plants and plant products are being widely surveyed for their antimicrobial activity [1]. Nearly seven hundred plant species were studied by Dornberger and co-workers [2] and about 30% of these were found to have antimicrobial properties. Out of the active ones, 23% were active against gram positive, 16% against gram negative bacteria, 7.5% against mycobacteria and the rest against fungi and yeast. Plants exhibiting biological activity mostly belonged to N.O. Liliaceae, Ronunculaceae, Rosaceae [2], Umbelliferae [3], Nymphaceae [4], Zingiberaceae [5] and Myrsinaceae [6].

Although various types of compounds such as tannins [7], lactones [8], flavones [9], glycosides [10], ploye nes, saponins, alkaloids [3], quinones and anthraquinones [11] have been found to have antimicrobial activity, the maximum antibacterial activity of a plant is associated with its essential oil [12]. Though nearly each component has an antibiotic activity of its own [13], the activity of the whole oil is more than that of any individual component [14] The order of decreasing effectiveness of these components is aldehyde, alcohol, ether and acid. The presence of α, β double bonds enhances the activity [13] which phenomenon can perhaps be attributed to oxidation. Activity also depends upon the peroxidation number of an essential oil [15].

The inhibitory activity of an essential oil may be due to protein precipitation action [16], by the inactivation of enzymes [17], by the deformation of morphology [18], or by the lytic phenomenon [2]. The formation of a surface layer of an oil around the bacteria by adsorption may also kill them [19].

Among the medicinal plants Umbellifers have long been studied for their antimicrobial activity [11, 20, 21] and have been found to be quite effective against many bacteria. Effective anti-bacterial drugs from Umbellifers like *Heracleum* spp. [22] and *Peucedanum* spp. [23] have been prepared in China. Therapeutic mixtures containing Umbellifer oils have also been introduced as drugs in Germany [24]. Similarly antiseptic and anti-inflammatory compositions containing these oils have been found to be quite effective [25].

Plants of Umbelliferae family are widely distributed throughout Pakistan, especially in Baluchistan and the Frontier regions. While only 6 or 7 species out of 172 reported in Pakistan are cultivated, the rest of the species grow wild mostly in the mountainous regions. The chemistry of a large number of the essential oils of Umbelliferae has been studied and the results already reported [26]. In the present studies the essential oils of some 30 species of this family have been studied for their activity against standard control strains of *Staph. aureus, Escherichia coli, Salmonella typhi, Shigella dysentery* and *Vibrio cholera,* The results for *Cuminum cyminum* (cumin or *safed zera*), *Coriandrum sativum* (coriander or *dhania*), *Foeniculum vulgare* (fennel or *saunf*) and *Bunium persicum* (*zira siyah*) are being reported in this paper. Results of other species will be published as the work progresses.

EXPERIMENTAL

Materials

1. *Cultures.* Standard cultures of *Staph. aureus* 6538-P, *Escherichia coli* ATCC-M/200, *Salmonella typhi*, *Shigella dysentery* and *Vibrio cholera* were provided by the Drug Control Division of the National Institute of Health, Islamabad.

2. *Media.* Merck's agar medium for stock culture slants and Oxoid Antibiotic Medium No. 3 (Liquid broth) were used.

3. *Essential oils.* The essential oils of *Cuminum cyminum, Coriandrum sativum, Foeniculum vulgare* and *Bunium persicum* were obtained from seeds by steam distillation.

1. *Preparation of inoculum.* A suspension of bacterial culture was made on agar slant. A loopful of it was added to 250 ml of broth medium. After 24 hr. incubation at 35° one loopful from this culture was transferred to another flask of freshly prepared sterile 250 ml broth medium and incubated for 24 hr. at 35° when the culture was ready for inoculation.

2. *Preparation of media.* 500 ml of the media were prepared from Oxoid Antibiotic Medium No. 3 and 2 % Tween-20 were added as an emulsifier. The resultant medium was sterilized and cooled to room temperature. Five sets each consisting of seven plugged and sterilized tubes which were numbered from zero to six were taken. One of the sets was used as the *reference set* and the remaining four as *test sets.*

In each of the tubes of the reference set, 10 ml of sterilized medium were poured and the tube was carefully replugged. The rest of the medium in the flask was then inoculated by a loopful of one of the standard cultural suspension. 10 ml of the inoculated medium were poured in each tube of the four test sets. The essential oil was added to each tube of the five sets with the help of a micro-syringe as per Table 1.

Thus for each concentration of an essential oil there was a separate reference tube, so that the change in opacity, if any, in the medium due to the presence of different amounts of the oil was eliminated. The tubes were incubated at 35° for 20 hr. The absorbence of each set of the tubes after having been shaken was measured at 530 nm using Hitachi Model 100-20 UV-Vis Spectophotometer. Optical density was taken as an index of bacterial growth. Means of the optical density of four sets are shown in Tables 2-5 for each essential oil. Fig. 1-5 show the graphs wherein optical density has been plotted against the quantity of an oil in p.p.m.

Table 1. Amount of essential oil.

Tube No.	0	1	2	3	4	5	6
Amt. of oil in µl	0	4	8	12	16	20	24
Amt. of oil in ppm.	0	400	800	1200	1600	2000	2400

DISCUSSION

The antibacterial activity of essential oils has been studied in different ways, by using the oil as such, by vaporization in an alcoholic solution [27], oil in water dispersion medium [28], by the zone inhibition method using ethanol as the solvent [29], and by the usual ordinary filter paper disc diffusion method without any solvent [30]. The biological activity of an essential oil greatly depends upon the method of assay e.g. by the filter paper diffusion

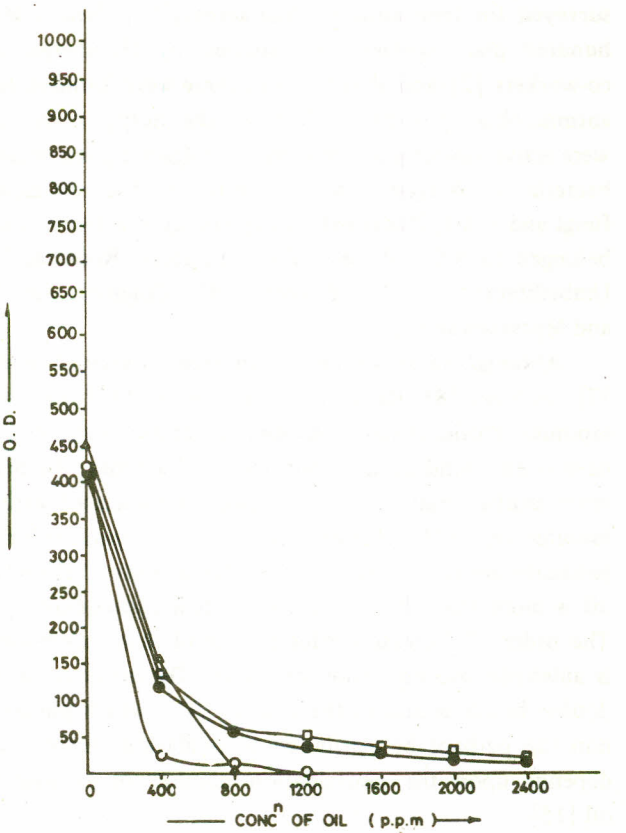

Fig. 1. Activity against *Staph aureus* of ○ *Cuminum cyminum;* ● *Coriandrum sativum;* △ *Foeniculum vulgare;* □ *Bunium persicum.*

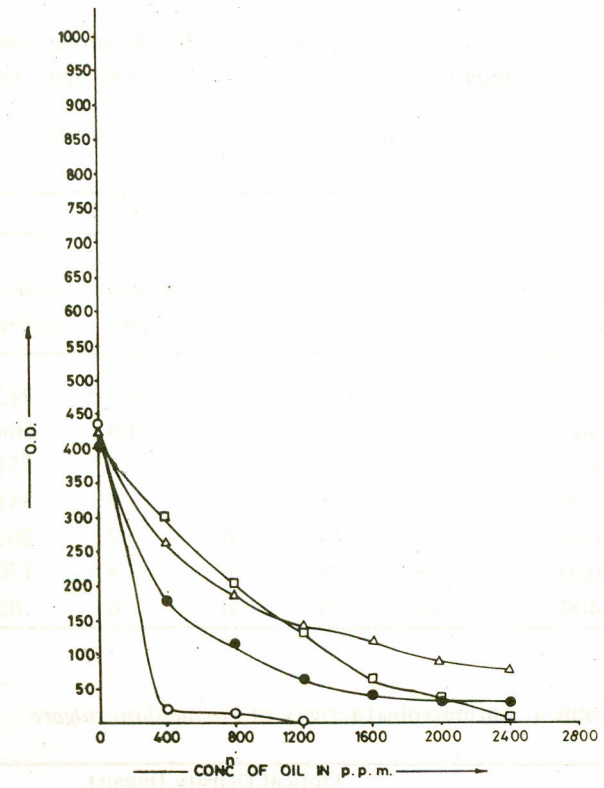

Fig. 2. Activity against *E. coli* of ○ *Cuminum cyminum;* ● *Coriandrum sativum;* △ *Foeniculum vulgare;* □ *Bunium persicum.*

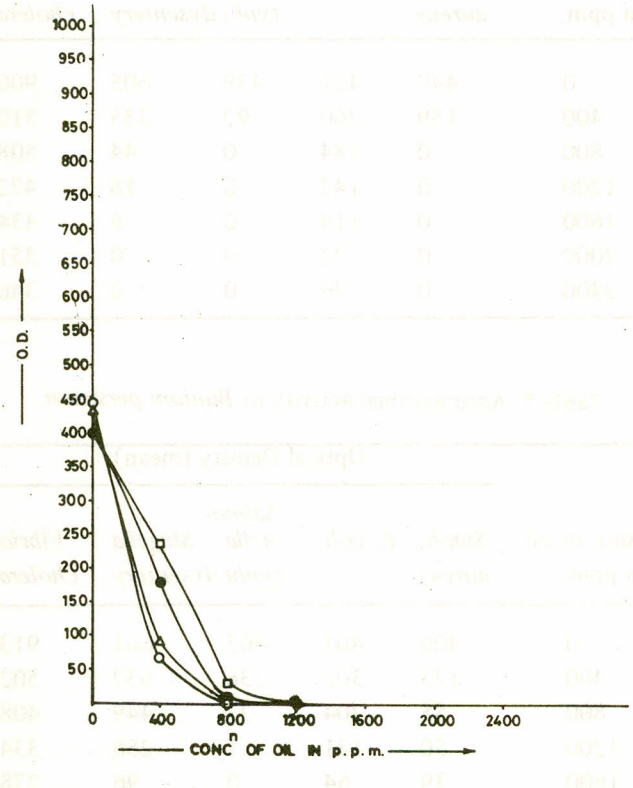

Fig. 3. Activity against *Salmonella typhi* of ○ *Cuminum cyminum;* ● *Coriandrum sativum;* △ *Foeniculum vulgare;* □ *Bunium persicum.*

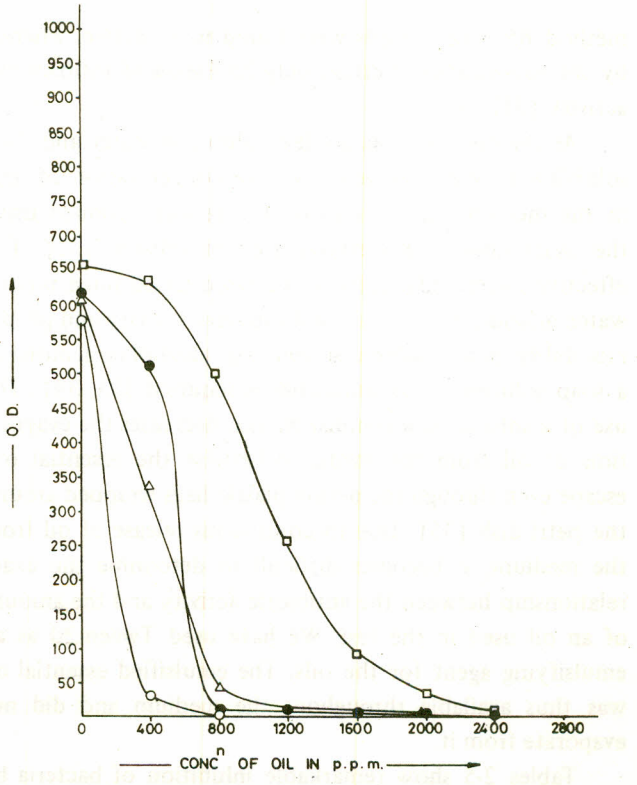

Fig. 4. Activity against *Shigella dysentery* of ○ *Cuminum cyminum;* ● *Coriandrum sativum;* △ *Foeniculum vulgare;* □ *Bunium persicum.*

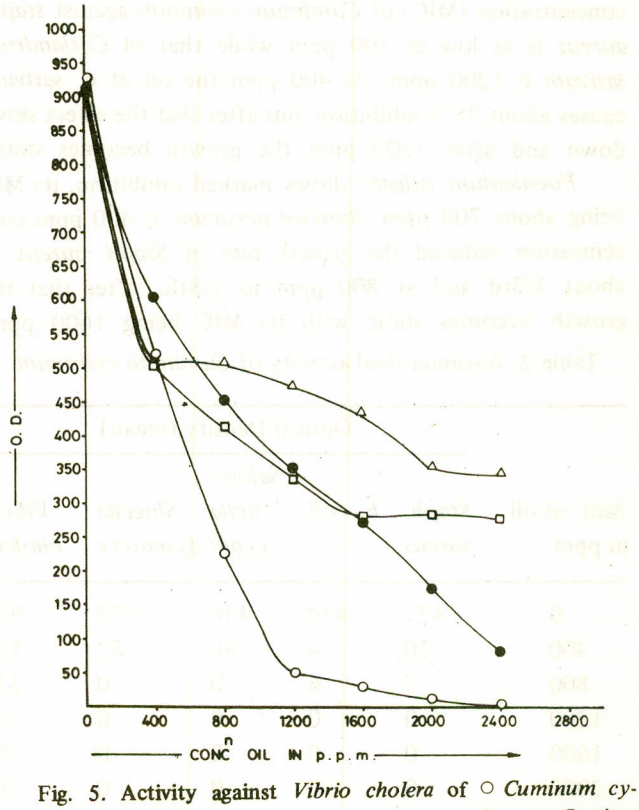

Fig. 5. Activity against *Vibrio cholera* of ○ *Cuminum cyminum;* ● *Coriandrum sativum;* △ *Foeniculum vulgare;* □ *Bunium persicum.*

method 85 % of the oils were found to be active, whereas by the vaporization method only 23% showed antibacterial activity [31].

As the essential oils are less soluble in water and their solubility in water decreases as the concentration of agar in the medium increases [14], the results obtained using the usual inhibition methods are not reliable [32]. For effective bactericidal activity the essential oil must be in a water miscible form. For good capillary activity and proper miscibility, some suitable solvent e.g. alcohol or acetone or a soap solution or an emulsifier is required [33, 34]. The use of a solvent or an emulsifier also decreases the evaporation of oil from the media, otherwise the essential oils escape even through the perlon plastic bags wrapped around the petri dish [35]. Due to continuous release of oil from the medium, it becomes difficult to determine the exact relationship between the antibiotic activity and the amount of an oil used in the test. We have used Tween-20 as an emulsifying agent for the oils. The emulsified essential oil was thus available throughout the medium and did not evaporate from it.

Tables 2-5 show remarkable inhibition of bacteria by some oils, while the others are moderately active. Bacteriumwise results are as follows:-

1. *Staph. aureus (6538-P).* The minium inhibitory concentration (MIC) of *Cuminium cyminum* against *staph. aureus* is as low as 300 ppm while that of *Coriandrum sativum* is 1200 ppm. At 400 ppm the oil of *C. sativum* causes about 75 % inhibition, but after that the effect slows down and after 1200 ppm the growth becomes static.

Foeniculum vulgare shows marked inhibition, its MIC being about 700 ppm. *Bunium persicum* at 400 ppm concentration reduced the growth rate of *Staph. aureus* to about 1/3rd and at 800 ppm to 1/8th. After that the growth becomes static with its MIC being 1600 ppm.

Table 2. Antimicrobial activity of *Cuminum cyminum.*

	Optical Density (mean)				
Amt. of oil in ppm.	*Staph. aureus*	*E. coli*	*Salmonella typhi*	*Shigella dysentery*	*Vibrio cholera*
0	421	436	438	578	928
400	20	14	66	52	523
800	7	6	0	0	228
1200	0	0	0	0	53
1600	0	0	0	0	32
2000	0	0	0	0	16
2400	0	0	0	0	0

2. *E. coli* (ATCC. M/200). Like *Staph. aureus*, the MIC of *Cuminum cyminum* for *E. coli* is 300 ppm. In-

Table 3. Antimicrobial activity of *Coriandrum sativum*

	Optical Density (mean)				
Amt. of oil in ppm.	*Staph. aureus*	*E. coli*	*Salmonella typhi*	*Shigella dysentery*	*Vibrio cholera*
0	407	406	403	629	912
400	118	184	179	509	604
800	57	114	14	12	551
1200	35	65	0	9	348
1600	27	44	0	7	262
2000	18	29	0	4	170
2400	14	29	0	0	82

Table 4. Antimicrobial activity of *Foeniculum vulgare.*

	Optical Density (mean)				
Amt. of oil in ppm.	*Staph. aureus*	*E. coli*	*Salmonella typhi*	*Shigella dysentery*	*Vibrio cholera*
0	449	421	439	605	900
400	159	260	92	335	510
800	0	184	0	44	508
1200	0	142	0	18	472
1600	0	119	0	6	434
2000	0	92	0	0	351
2400	0	76	0	0	346

Table 5. Antimicrobial activity of *Bunium persicum.*

	Optical Density (mean)				
Amt. of oil in ppm.	*Staph. aureus*	*E. coli*	*Salmonella typhi*	*Shigella dysentery*	*Vibrio cholera*
0	406	401	405	661	913
400	133	302	236	637	502
800	58	204	27	449	408
1200	50	131	1	256	334
1600	39	64	0	96	278
2000	29	40	0	31	278
2400	22	9	0	11	274

hibition of *E. coli* by *C. cyminum* is rapid and fast, whereas that by *C. sativum* is gradual. 400 ppm concentration of *C. sativum* reduces the growth rate of *E. coli* to about 1/2. After that the inhibition is gradual and becomes more or less static at 1200 ppm concentration of the oil. Its MIC is 1800 ppm. The inhibition of *E. coli* by *Foeniculum vulgare* is gradual and much less than that by *C. cyminum* and *Coriandrum sativum.* There is about 50 % inhibition by 600 ppm of the oil and about 75 % inhibition by 1000 ppm. Inhibition of *E. coli* by *Bunium persicum* is also gradual. It is even less than that by *F. vulgare* at lower concentration, but afterwards it increases. The MIC of *B. persicum* is 2000 ppm. Its activity is lower than that of *C. sativum* and much lower than that of *C. cyminum.*

3. *Salmonella typhi. Cuminum cyminum* has a very good inhibitory effect against *Salmonella typhi.* The MIC of this oil against *S. typhi* is 400 ppm and that of *Coriandrum sativum* is about 650 ppm. *F. vulgare* causes more than 80% inhibition at 400 ppm. The MIC of *F. vulgare* for *Salmonella* is about 450 ppm. *Bunium persicum* also shows good inhibitory activity against *Salmonella* but it is less than that of others. The MIC of *Bunium persicum* for *Salmonella typhi* is about 750 ppm.

4. *Shigella dysentery.* The growth rate of *Shigella dysentery* is faster than that of *Staph. aureus, E. coli* and *Salmonella. Cuminum cyminum* is remarkably active, its MIC against *Shigella dysentery* being 400 ppm. *Coriandrum sativum* shows quite low inhibition of the organism at 400 ppm but the inhibition increase rapidly with increased amount of the oil. The MIC of *C. sativum* against *S. dysentery* is about 750 ppm. *Foeniculum vulgare* shows about 50 % inhibition at the concentration of 400 ppm and 90 % inhibition of growth at 800 ppm. The MIC of *F. vulgare* is 850 ppm.

Bunium persicum is not very active against *Shigella* at 400 ppm but the inhibition increases gradually with the increase in the concentration of the oil. The MIC of this oil is 2000 ppm.

5. *Vibrio cholera.* Under the same conditions of incubation and media, *Vibrio cholera* if found to have a much more rapid growth rate. The maxium growth of *Vibrio cholera* is more than double as compared to the maximum growth of *Staph. aureus, E. coli* and *Salmonella.* Obviously it will need double the concentration of the oil for equal inhibition. At 500 ppm the oil of cumin lowers the growth rate to half, while its MIC is 1200 ppm.

Coriandrum sativum shows quite good inhibitory effect against *Vibrio cholera* at 400 ppm. The inhibition seems to be linear. It shows about 50 % inhibition at 800 ppm, 75 % inhibition at 1800 ppm and 90 % inhibition at 2400 ppm, which is also its MIC. *Foeniculum vulgare* is very active against *Vibrio cholera* at the concentration of 400 ppm. A 50 % inhibition is shown at 1300 ppm and about 66 % inhibition at 2000 ppm, after which the effect becomes static. *Bunium persicum* like *Foeniculum vulgare* is quite active at 400 ppm concentration. The activity afterwards slows down but is still better than that of *Foeniculum vulgare.* It shows 50 % inhibition at 600 ppm and 66 % inhibition at 1600 ppm after which the effect becomes static.

It is evident from the above that *Cuminum cyminum* has the widest range of activity. It is much more active against all the five pathogens as compared to other oils.

Coriandrum sativum comes second in activity. The activity of *Foeniculum vulgare* and *Bunium persicum* is nearly the same. For *Staph., Salmonella,* and *Shigella,* the oil of fennel is more active whereas for *E. coli* and *Vibrio* its activity is less than that of *Bunium persicum.*

Our results are in surprising contrast with those of Afzal and Akhtar [20]. They have shown *Cuminum cyminum* to be the least active oil. They have claimed that the activity of *Foeniculum vulgare* is equal to that of *Streptopenicillin* and more than that of tetracycline and penicillin.

Our results show that *Cuminum cyminum* is a very active oil. The difference in results seems to be due to the different methods of assay. The biological assay of an essential oil as has been mentioned earlier is not possible without its being properly dissolved in the medium by using an organic solvent or on being emulsified by using a good emulsifier, and thus preventing it from evaporation. Moreover in the absence of a solvent or emulsifier there is no capillary activity and consequently there can be no proper zone of inhibition. Another point that needs mentioning is that these authors have tried to compare essential oils with standard antibiotics by the inhibition zone method. While they used standard antibiotic discs which usually contain 1 mg/ml or even less antibiotics, they have used pure oils without any dilutions. The results thus obtained become misleading.

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