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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF UMBELLIFERAE

Part II. *Trachyspermum ammi, Daucus carota, Anethum graveolens,* and *Apium graveolens* Oils.

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The essential oils of *Trachyspermum ammi, Daucus carota, Anethum graveolens* and *Apium graveolens* 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. The oil of *Trachyspermum ammi* exhibits remarkable inhibitory activity against all five pathogens while the other three oils also how comparable antibacterial activity.

INTRODUCTION

The plants of N.O. Umbelliferae have since long been used in medicine. Their activity seems to be associated with the well known coumarins [1, 2]. However lactones [3], flavone – glycosides [4], phthalides [5], terpenes [6], alkaloids, polyenes and saponins [7] also seem to play an active role. Most of these components are steam volatile and are present in the essential oils.

The chemistry of the Umbellifers found in Pakistan has already been studied and reported [8]. Oils of some thirty different plants of this family are now being studied for their bactericidal and bacteriostatic activity. Results on the activity of four essential oils were reported [9] in the Part I of this series. Here we report the results of the activity of *Trachyspermum ammi* (Sprague, *Ajowan*), *Daucus carota,* (carrot), *Anethum graveolens* (dill, *sowa*), and *Apium graveolens* (celery, *ajmodh*), against five different pathogens, viz. *Staphylococcus aureus, Escherichia coil, Salmonella typhi, Shigella dysentery* and *Vibrio cholera.*

EXPERIMENTAL

Materials

1. *Cultures.* Standard cultures of *S. aureus 6538-p, E. coli ATCC-M/200. S. typhi, S. dysentery* and *V. cholera* were kindly 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 *T. ammi, D. carota* *An. *graveolens* and •*Ap. graveolens* were obtained from their seeds through steam distillation.

Preparation of Media and Inoculum. The media and inoculum were prepared according to the procedure previously reported [9]. After incubation of the tubes at 35° for 20 hr. absorbence was measured at 530 nm using Hitachi Model 100-20 UV-Vis spectrophotometer. Optical density was taken as an index of bacterial growth.

RESULTS AND DISCUSSION

The means of the optical density of four sets are shown in Tables 1-5. The graphs (Fig. 1-5) show the optical density plotted against the quantity of an oil in ppm. The concentration of the oil which causes an eight fold decrease in growth is taken as Minimum Inhibitory Concentration (MIC). The bacteriumwise results are described below.

1. *S. aureus.* The oil of *T. ammi* showed a gradual and effective activity against *S. aureus* (Table 1, Fig. 1). The MIC of the oil against this organism is about 1200 ppm and complete bactericidal effect is seen at 1600 ppm. *D. carota* is more effective against *S. aureus.* At 400 ppm. *D. carota* is far more effective than *T. ammi.* The MIC of former is 700 ppm but afterwords it shows a bacteriostatic effect. *An. graveolens* is the most effective oil against *S. aureus* at low concentrations. The MIC of this oil against *S. aureus* is the lowest, i.e. less than 600 ppm. It has bactericidal rather than baceteriostatic effect. At 800 ppm it inhibits the organisms completely. *Ap. graveolens* is also

**Anethum graveolens* •*Apium graveolens.*

very effective at low concentrations. After 400 ppm its inhibition becomes gradual. The MIC of *A. graveolens* against this bacterium is 1200 ppm.

2. *E. coli. E. coli* is markedly inhibited by *T. ammi* at low concentrations (Table 2, Fig. 2). The MIC of this oil against the organism is 300 ppm whereas a complete inhibition is seen at 400 ppm. The MIC of *D. carota* against *E. coli* is about 1800 ppm. The inhibition is gradual but quite effective.

Table 1. Antimicrobial activity against *S. aureus.*

Amount of essential oils (ppm)	Optical density (mean)			
	T. ammi	*D. carota*	*A. graveolens*	*A. graveolens*
0	400	408	465	413
400	238	146	116	119
800	124	40	10	71
1200	61	39	0	53
1600	12	29	0	21
2000	0	28	0	12
2400	0	19	0	7

Table 2. Antimicrobial activity against *E. coli.*

Amount of essential oils (in ppm)	Optical density (mean)			
	T. ammi	*D. carota*	*A. graveolens*	*A. graveolens*
0	411	428	443	424
400	12	283	179	255
800	11	184	156	194
1200	0	118	120	132
1600	0	70	81	114
2000	0	27	59	96
2400	0	18	56	82

Table 3. Antimicrobial activity against *S. typhi.*

Amount of essential oils (in ppm)	Optical density (mean)			
	T. ammi	*D. carota*	*A. graveolens*	*A. graveolens*
0	403	416	449	439
400	0	185	103	364
800	0	150	38	286
1200	0	135	31	197
1600	0	98	22	136
2000	0	85	14	99
2400	0	6	12	86

Table 4. Antimicrobial activity against *Shigella dysentry.*

Amount of essential oils (in ppm)	Optical density (mean)			
	T. ammi	*D. carota*	*A. graveolens*	*A. graveolens*
0	506	594	639	558
400	10	521	562	261
800	0	471	43	172
1200	0	394	24	102
1600	0	310	13	46
2000	0	253	0	40
2400	0	112	0	31

Table 5. Antimicrobial activity against *V. cholera.*

Amount of essential oils (in ppm)	Optical density (mean)			
	T. ammi	*D. carota*	*A. graveolens*	*A. graveolens*
0	908	961	998	1002
400	469	752	395	678
800	468	713	374	603
1200	413	667	293	561
1600	0	648	268	535
2000	0	604	242	512
2400	0	546	191	497

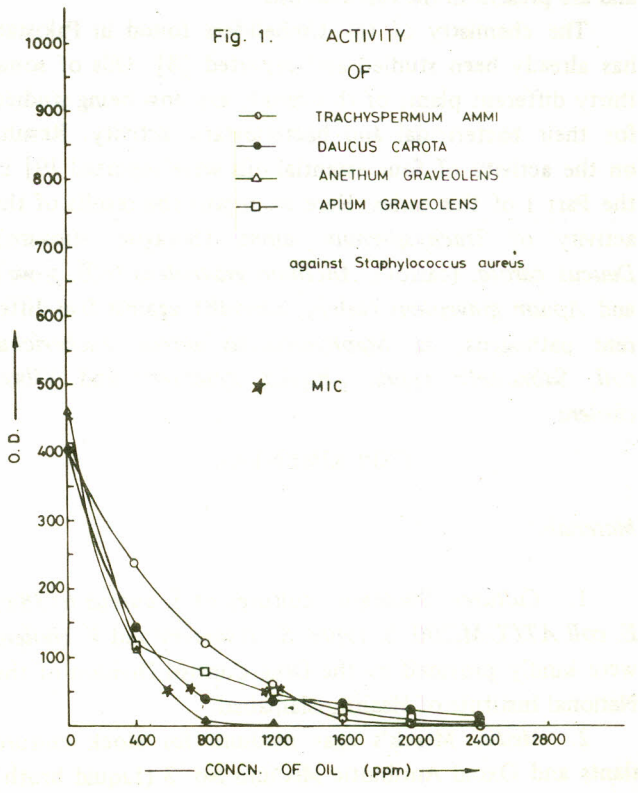
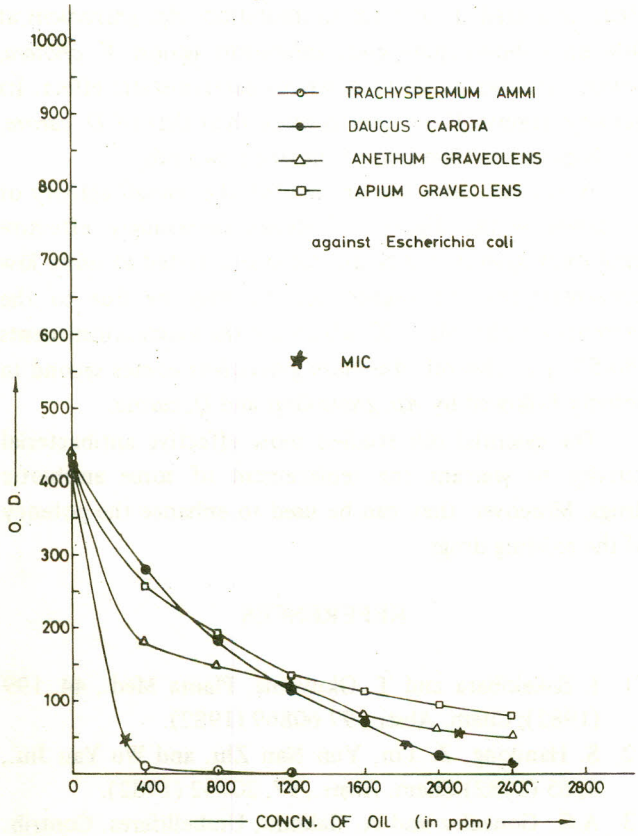


Fig. 2. ACTIVITY



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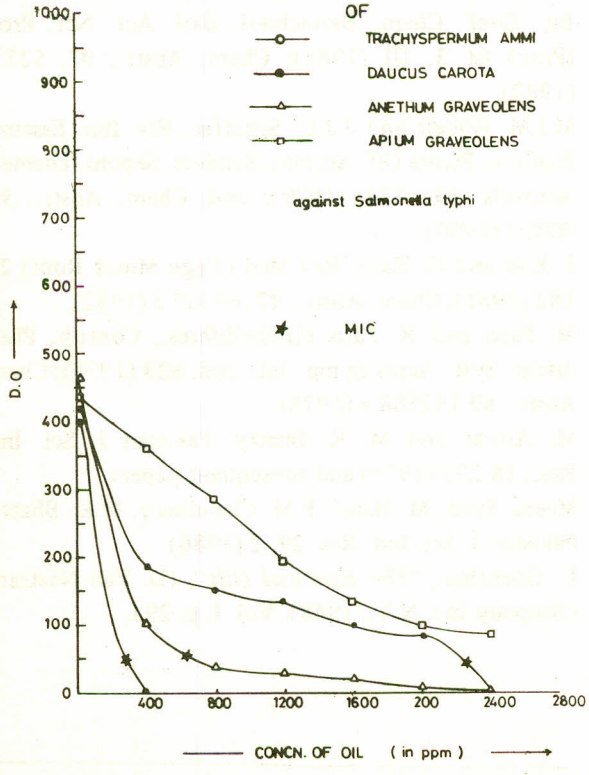


Fig. 4. ACTIVITY OF

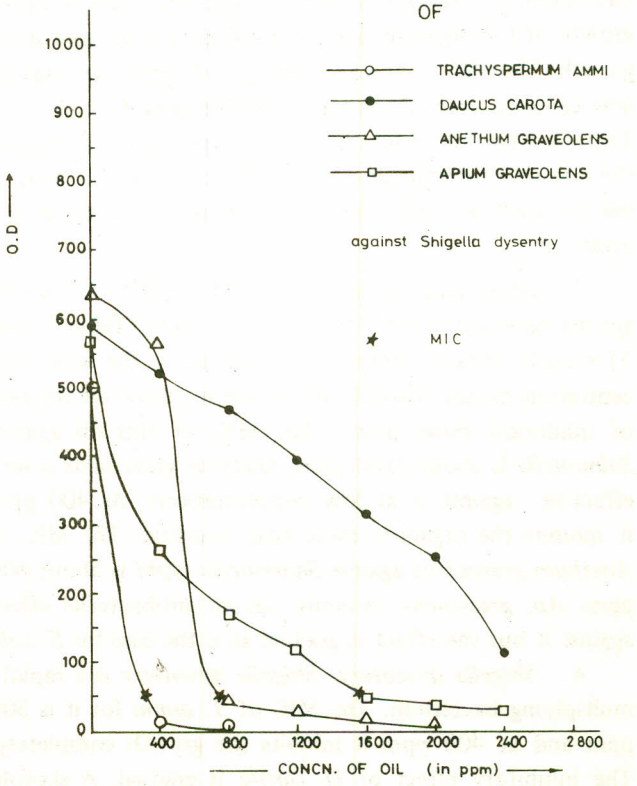
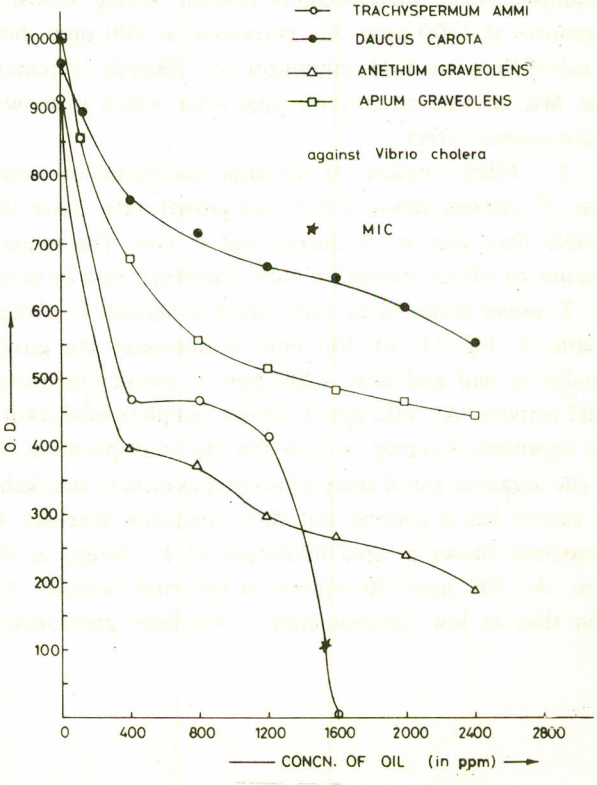


Fig. 5 ACTIVITY OF



Anethum graveolens is quite effective against the bacterium. At 300 ppm it causes a twofold decrease in the growth of the organism and after 400 ppm it shows a static growth. The MIC of the oil is about 2100 ppm. *Ap. graveolens* causes a gradual inhibition of the growth of *E. coli.* There is a twofold inhibition at 800 ppm. After 1600 ppm the rate of inhibition slows down. Of the four oils used, it has the least but still quite good inhibitory effect on this organism.

3. *Salmonella typhi.* Like *E. coli* the MIC of *T. ammi* against *Salmonella typhi* is about 300 ppm (Table 3, Fig. 3) a really notable inhibition. *D. carota* at the same concentration causes twofold inhibition but later on the rate of inhibition slows down. The MIC of this oil against *Salmonella* is about 2100 ppm. *Anethum graveolens* is very effective against it at low concentrations. At 400 ppm it inhibits the organism more than fourfold. The MIC of *Anethum graveolens* against *Salmonella typhi* is about 600 ppm. *Ap. graveolens* certainly has an antibacterial effect against it but the effect is gradual as is the case for *E. coli.*

4. *Shigella dysentery. Shigella dysentery* is a rapidly multiplying bacterium. The MIC of *T. ammi* for it is 300 ppm and at 400 ppm it inhibits the growth completely. The inhibitory effect of *D. carota* is gradual. A six-fold inhibition is seen at 2400 ppm. *An. graveolens* is not very effective at 400 ppm but at 800 ppm, which is also its MIC, it surprisingly causes a sixteen-fold inhibition. The inhibitory effect then becomes gradual, killing almost all organisms at 1600 ppm. *Ap. graveolens* at 400 ppm shows a more than twofold inhibition of *Shigella dysentery.* The MIC of this oil is 1500 ppm after which it shows a bacteriostatic effect.

5. *Vibrio cholera.* At the same conditions of incubation, *V. cholera* shows a very fast growth rate, more than double than that of *S. aureus* and *E. coli.* The required amount of oil for inhibition must, therefore, also be doubled. *T. ammi* seems to be very effective against *V. cholera,* (Table 5, Fig 5). At 400 ppm it decreases the growth rapidly to half and after 1200 ppm it shows fast bactericidal activity. At 1600 ppm it shows complete inhibition of the organism. Keeping in view the fast multiplication rate of the organism this display of activity is rather remarkable. *D. carota* has a gradual and slow inhibition whereas *An. graveolens* shows a rapid inhibition of *V. cholera* at 400 ppm. At 300 ppm the inhibition becomes twofold. It is seen that at low concentration, *Anethum graveolens* is more active than *T. ammi,* but at higher concentration there is a gradual increase in inhibition. *Ap. graveolens* at 400 ppm shows quite good inhibition against *V. cholera,* which afterwards slows down to bacteriostatic effect. Its activity against *V. cholera* is more than that of *D. carota,* but is quite less than that of the other two oils.

It is clear from the above that the overall activity of *T. ammi* is the highest. It shows surprisingly effective inhibition against nearly all pathogens tested at quite low concentrations. Its higher activity may be due to the presence of phenols [10] which are the major constituents (50-55%) of this oil. *Anethum graveolens* comes second in activity followed by *Ap. graveolens* and *D. carota.*

The essential oils studied show effective antibacterial activity to warrant the replacement of some antibiotic drugs. Moreover, they can be used to enhance the potency of the existing drugs.

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