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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF THE UMBELLIFERAE FAMILY

Part III. *Pimpinella anisum, Pimpinella acuminata and Pimpinella stewartii.*

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The essential oils of *Pimpinella anisum, P. acuminata* and *P. stewartii* were tested against the standard cultures of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella dysentery,* and *Vibrio cholera.* The optical density measured spectrophotometrically at 530 nm in liquid media was taken as an index of the growth of bacteria. The oil of *P. anisum* showed good inhibitory activity against all the five pathogens. Whereas *P. stewartii* had a notable inhibitory effect against *Shigella dysentery* and *P. acuminata*, inhibited the growth of *S. aureus* only. The antimicrobial activity of different species of the Umbelliferae family does not seem to be genera related.

Key words: Antimicrobial Activity, Essential Oils, Minimum Inhibitory Concentration.

INTRODUCTION

On worldwide basis the Pimpinella genus of the Unbelliferae family consists of about 150 species, out of which six have been reported to grow wild in Pakistan. The physicochemical analysis of these has already been reported by Bhatty *et al* [1]. Among the several species of N.O. Umbelliferae being tested for their antimicrobial activity, three are from the genus Pimpinella, viz. *P. anisum, P. acuminata* and *P. stewartii. P. anisum* has been widely used as a carminative agent [2] in the local matria medica. It has been shown to retard oxidative decomposition of food [3] and increase the latters keeping quality. It has also been used in many drugs [5]. Anethole present in the essential oil of anise (about 85%) is known to help a rapid drug absorption from the intestine [6]. Anise oil has also been used in treating diarrhoea and flatulence in domestic animals [7]. Kurvez and Hornok [8] tested this oil against seven microorganisms and found it as an effective antibiotic agent. Literature survey shows that the oils of *P. acuminata* and *P. stewartii* have not been studied for their antimicrobial properties. In order to find out as to whether, being present in the same genera they also have antimicrobial activity like anise and that the antimicrobial activity of the members of the Umbelliferae family is genera related, the oils of *P. anisum, P. acuminata* and *P. stewartii* were investigated against five pathogenic bacteria viz. *S. aureus, E. coli, S. typhi, S. dysentery,* and *V. cholera* by the spectrophotometric method.

MATERIALS AND METHOD

Materials:

1. *Cultures.* The National Institute of Health, Islamabad and the Drug Testing Laboratory, Lahore kindly provided these Laboratories the standard culturs of *S. aureus 6538-P, E. coli* ATCC-M/200, *S. typhi, S. dysentery* and *V. cholera.*

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 *P. anisum, P. acuminata,* and *P. stewartii* were obtained by the steam distilation of their seeds.

Preparation of media and inoculum. The procedure for the preparation of media and inoculum has already been reported in Part I of these series [9]. After incubation of the tubes at 35° for 20 hr., the absorbence was measured at 530 nm using Hitachi Model 100-20, UV-Vis. spectrophotometer. The turbidity or the optical density was taken as an index of the bacterial growth.

RESULTS AND DISCUSSION

The means of the optical density of four sets are shown in Tables 1-5 and Fig. 1-5. The optical density is plotted against the quantity of an oil in ppm. The Minimum inhibitory Concentration (MIC) is the concentration of the oil

which caused an eightfold decrease in the growth of the organism. The bacteriumwise results are as follows:

1. *S. aureus.* The linear graph of inhibition shown by *P. anisum* resembles that of *T. ammi* [10] but the former is slower in activity. It shows a two-fold inhibition at 800 ppm. (Table 1, Fig. 1). Its MIC is about 2400 ppm. At

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

Amount of essential oil (in ppm)	Optical density (mean)		
	P. anisum	*P. acuminata*	*P. stewartii*
0	429	421	436
400	296	410	419
800	196	377	418
1200	138	349	370
1600	97	310	340
2000	79	158	253
2400	52	86	226

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

Amount of essential oil (in ppm)	Optical density (mean)		
	P. anisum	*P. acuminata*	*P. stewartii*
0	422	438	426
400	287	406	399
800	238	381	370
1200	193	353	320
1600	176	302	288
2000	151	268	269
2400	126	227	241

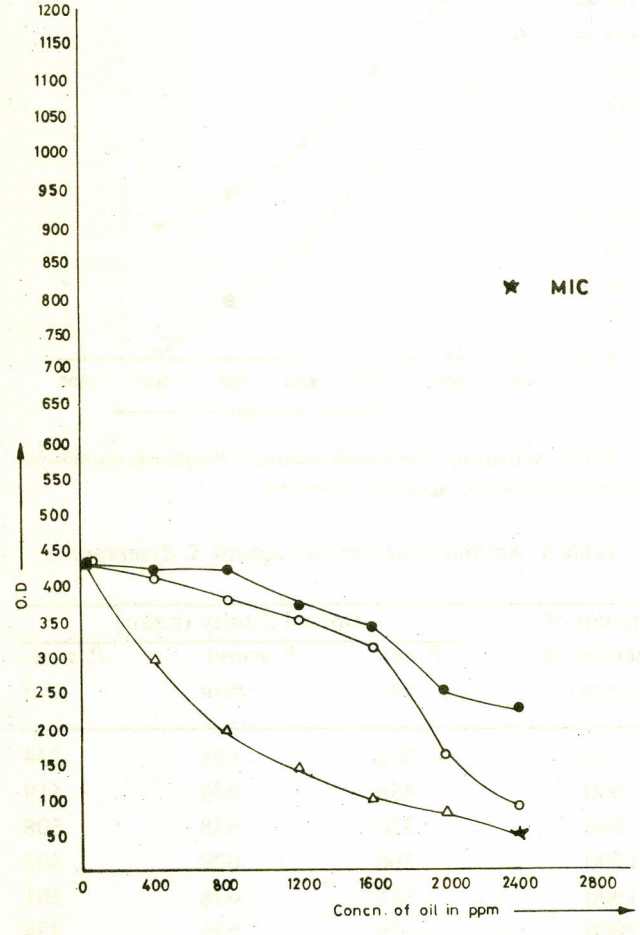

Fig. 1. Activity of △*Pimpinella anisum*, ○*Pimpinella acuminata*, ●*Pimpinella stewartii*, against *S. aureus.*

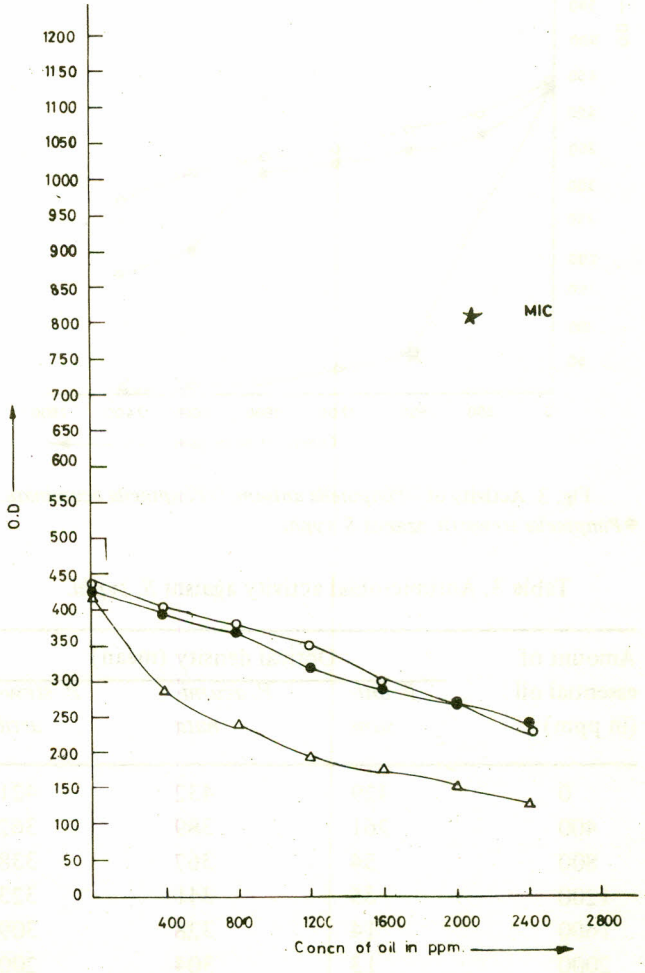

Fig. 2. Activity of △*Pimpinella anisum*, ○*Pimpinella acuminata*, ●*Pimpinella stewartii*, against *E. coli.*

early stages both the oils of *P. acuminata* and *P. stewartii* show similar effect, very slow inhibition, but later on after 1600 ppm. the rate of inhibition increases suddenly; especially that with *P. acuminata* which at 2400 ppm nearly reaches the MIC level.

2. *E. coli. P. anisum* shows gradual inhibition against *E. coli*, but it is not very much effective as that shown by *T. ammi* [10] or *C. cyminum* [9] (Table 2, Fig. 2). It causes a twofold inhibition of the organism at 1000 ppm. Both *P. accuminata* and *P. stewartii* again show a simillar

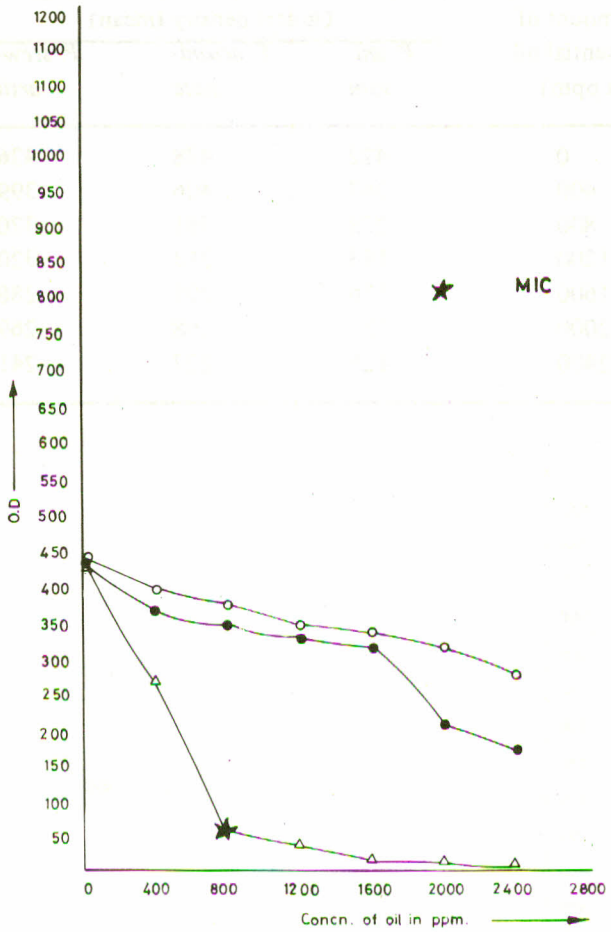

Fig. 3. Activity of △*Pimpinella anisum*, ○*Pimpinella acuminata*, ●*Pimpinella stewartii*, against *S. typhi*.

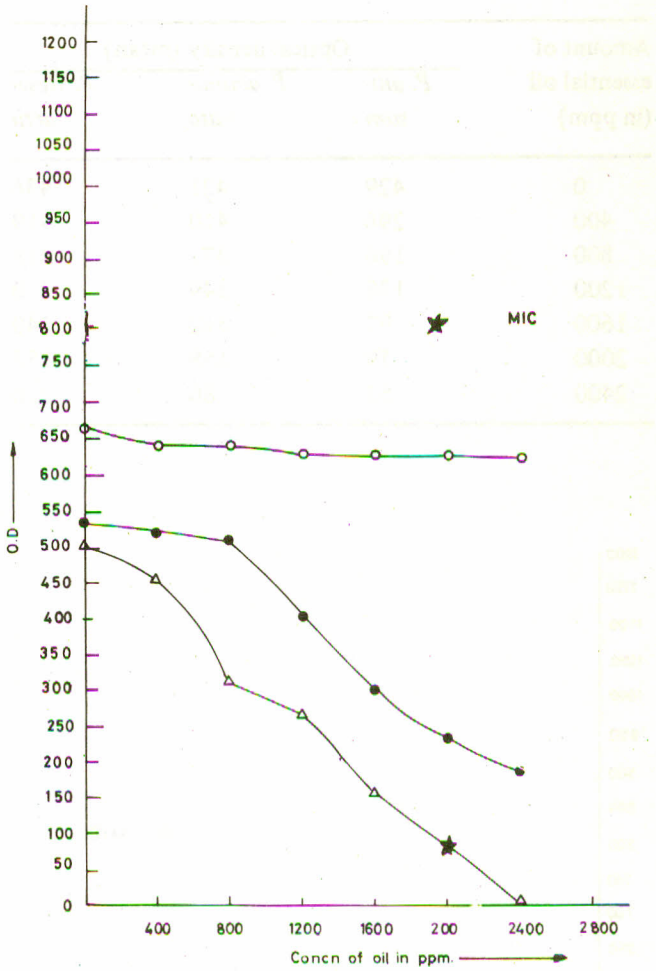

Fig. 4. Activity of △*Pimpinella anisum*, ○*Pimpinella acuminata*, ●*Pimpinella stewartii*, against *S. dysentery*.

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

Amount of essential oil (in ppm)	Optical density (mean)		
	P. anisum	*P. acuminata*	*P. stewartii*
0	429	432	421
400	261	389	362
800	54	367	338
1200	35	341	323
1600	14	328	309
2000	13	304	200
2400	0	272	168

Table 4. Antimicrobial activity against *S. dysentery*.

Amount of essential oil (in ppm)	Optical density (mean)		
	P. anisum	*P. acuminata*	*P. stewartii*
0	502	665	534
400	456	639	519
800	310	638	508
1200	266	629	402
1600	155	628	301
2000	79	626	234
2400	0	623	188

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

Amount of essential oil (in ppm)	Optical density (mean)		
	P. anisum	*P. acuminata*	*P. stewartii*
0	939	922	914
400	530	922	780
800	379	921	688
1200	320	920	661
1600	239	919	637
2000	199	876	625
2400	136	861	605

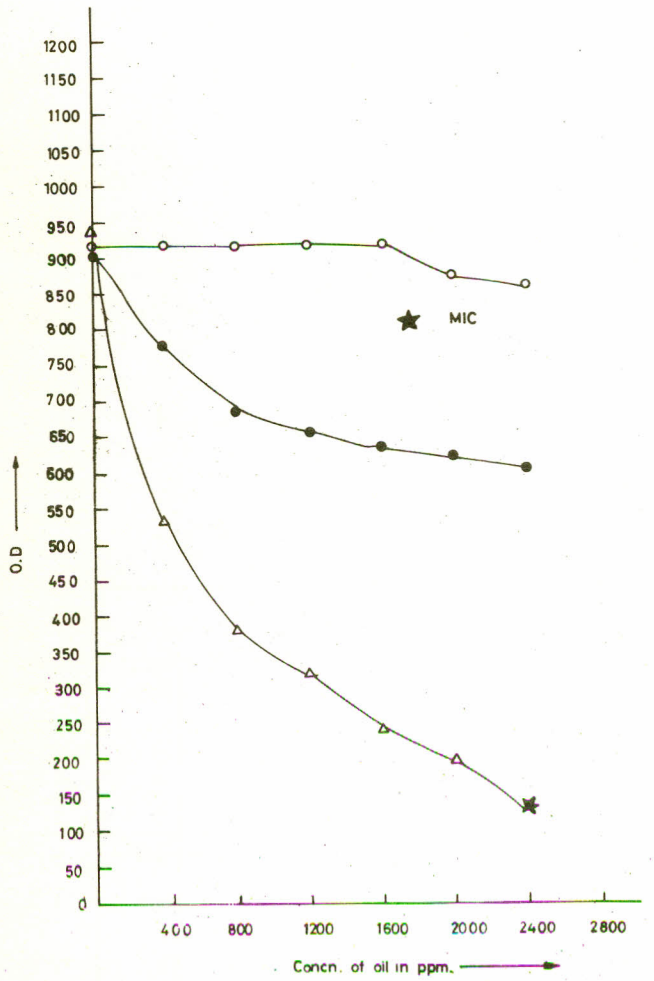

Fig. 5. Activity of △*Pimpinella anisum*, ○*Pimpinella acuminata*, ●*Pimpinella stewartii*, against *V. cholera.*

slow type of inhibition of the organism, both causing a twofold inhibition at 2400 ppm.

3. *S. typhi. P. anisum* shows a notable inhibitory activity against *S. typhi.* After showing a twofold decrease in growth at about 500 ppm it rapidly reaches the MIC level at 800 ppm (Table 3, Fig. 3). Its activity is like that of *A. graveolens* [10] (both have high concentrations of anethole). Upto 1600 ppm both *P. acuminata,* and *P. stewartii* show an identical type of rather slow inhibition, but afterwards the inhibition by *P. stewartii* increases and at 2000 ppm it shows a twofold inhibition of the organism.

4. *S. dysentery.* A gradual effective inhibition of the organism is seen by *P. anisum* but it is less than that of *D. carota* [10]. Its MIC is 2000 ppm. A complete inhibition of the organism is seen at 2400 ppm. *P. acuminata* shows no effect on this bacterium (Table 4, Fig. 4), whereas *P. stewartii* after 800 ppm makes an effective linear graph. At 2100 ppm it causes a twofold inhibition of the organism.

5. *V. cholera.* The activity of *P. anisum* against *V. cholera* is more than that of *A. graveolens* [10] and less than that of *C. sativum* [9]. At about 600 ppm the inhibition becomes two-fold (Table 5, Fig. 5). Its MIC is nearly 2400 ppm. *P. acuminata* has no marked effect on the growth of the bacterium, whereas *P. stewartii* inhibits the organism but its inhibition is much less than that by *P. anisum.*

These results show that only *P. anisum* is an effective essential oil against all the five pathogens but its activity is less than those by *C. cyminum* [9] and *T. ammi* [10]. *P. accuminata* has no marked effect against any of the organisms, except *S. aureus,* whereas *P. stewartii* is effective against *S. dysentery* and *V. cholera.* Against other three organisms its activity resembles that of *P. acuminata,* especially in the early stages. From the results we conclude (i) that despite some resemblance between *P. acuminata,* and *P. stewartii,* the activity does not seem to be genera related; and (ii) *P. anisum* being an effective antimicrobial agent can successfully be used in medicine against these pathogenic bacteria.

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