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ESSENTIAL OILS OF THE FAMILY GRAMINEAE WITH ANTIBACTERIAL ACTIVITY
Part 2. The Antibacterial Activity Of A Local Variety Of Cymbopogan Citratus Oil And Its Dependence On The Duration Of Storage

MEENA SYED, SHADAB QAMAR, M. RIAZ AND F.M. CHAUDHARY
PCSIR Laboratories Complex, Shahrah-e-Jalaluddin Roomi, Lahore-54600, Pakistan

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Fresh oil, as well as two, seven and twelve years old oils of a local variety of lemon grass (*Cymbopogon citratus*) were distilled and redistilled and tested against *Escherichia coli, Staphylococcus aureus, shigella flexneri, Salmonella, typhi, para-A* and *Klebsiella pneumoniae*. The oil that has been kept for 2 years, exhibited after redistillation, maximum activity, due to its high citral content. *S. flexneri* and *S. typhi* were inhibited effectively at low doses of the oil. The inhibition seemed to be affected greatly by citral content of the oil.

Key words: *Cymbopogon citratus*, Essential oil, Antibacterial citral.

Introduction

We have earlier reported that lemon grass oil, the essential oil of *Cymbopogon citratus* is highly active against different bacteria [1]. Kirtikar and Basu [2] described the pungent hot grass as a laxative, appetizer, anthelmintic, useful in bronchitis, leprosy, flatulence, gastric troubles, cholera, rheumatism, and fever. Zheng *et al.* [3] isolated limonene and geraniol from the grass and found these compounds as potential anticarcinogenic natural products. Mosquera and munoz [4] reported that *C. citratus* significantly reduces the microbial contamination of herbs. Dube and Rao [5] tested the oil against some bacteria and found it antibacterial active. Liu *et al.* [6] reported the major component of lemon grass oil as 40-50% citral, whereas the local variety we tested has more than 70% citral [7,8].

In the investigation described in the present paper we tested the antibacterial activity of local variety of lemon grass oil after keeping it at room temperature (from 15 to 45°) for two, seven and twelve years and redistillation of the oil Table-1.

TABLE 1.

Oil No.	Distilled redistilled	Citral content
A	Fresh Oil	71%
B	Oil redistilled after two years	80%
C	Oil redistilled after seven years	78%
D	Oil redistilled after twelve years	76%

We tested oil against *Escherichia coli, Staphylococcus aureus, Shigella flexneri, Salmonella, typhi para-A*, and *Klebsiella pneumoniae* by a *spectrophotometric* method [9].

Material and Method

Oils. A local variety of lemon grass was cultivated in the PCSIR Fields and the volatile oil was obtained using a Likens and Nickerson [10] appratus. Oil samples were redistilled after two, seven, and twelve years and tested against some pathogenic bacteria.

Bacteria. Eschericha coli, Staphylococcus aureus, Shigella flexneri Salmonella typhi para-A and *Klebsiella pneumoniae* were obtained pure cultures from the National Institute of Health, Islamabad.

Media. Oxoid antibiotic medium No.3 was used for the test, and Merck's agar medium for the slants.

Experimental. Preparation of Inoculum. A suspension of a bacterial culture was made on an 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 250 ml freshly prepared sterile broth medium and incubated for 24 hr. at 35°. Then the culture was ready for inculation.

Preparation of Media. 500 ml medium were prepared from the oxoid Antibiotic medium No.3, and 2% tween-20 added as an emulsifier. The medium so obtained was sterilized and cooled at room temperature.

Five sets of test, each consisting of seven plugged and sterilized tubes, bumbered from zero to six, were taken. One of the sets was used as reference and the remaining 4 for the tests. In each of the tubes of the reference set, 10 ml of sterilized medium were poured and the tube carefully replugged. The rest of the medium prepared, inoculated by a loopful of one of the standard bacterial suspension cultures. 10 ml of the inoculated medium were then poured into each tube of the 4 test sets. The essential oil was added to each tube of the five sets by means of a microsyringe Table 2.

TABLE 2. AMOUNT OF ESSENTIAL OIL

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

For each amount of essential oil there was a seperate reference tube, so that any change in opaity of the medium due to the presence of different amounts of oil was eliminated. The tubes were stirred using a tube mixer and incubated at 35° for 20 hr. The the tubes were stirred again and the absorbance of each set of tubes measured at 530 nm. using a LKB - ultraspec-2 spectrophotometer. Maximum optical density in the zero oil tube was taken as 100% growth of the bacteria. The rest of the tubes were compared with this growth and the percentage growth was calculated (Tables 3- 6).

TABLE 3. LOCAL LEMON GRASS OIL FRESHLY DISTILLED

Amt. of oil	Percentage growth of the test bacteria				
in ppm.	E.coli	S.aur.	S.flex	S.typh.pA	K.pneu.
0	100	100	100	100	100
400	85	103	60	88	70
800	54	42	54	28	45
1200	34	26	16	9	22
1600	27	15	0	0	10
2000	16	11	-	-	0
2400	10	7	-	-	-
MIC*	1600	1200	1200	800	1200

TABLE 4. LOCAL LEMON GRASS OIL REDISTILLED AFTER TWO YEARS STORAGE

Amt. of oil	Percentage growth of the test bacteria				
in ppm.	E.coli	S.aur.	S.flex	S.typh.pA	K.pneu.
0	100	100	1100	100	100
400	83	93	55	10	62
800	30	17	0	0	49
1200	12	15	-	-	46
1600	-	-	-	-	30
2000	-	-	-	-	25
2400	-	-	-	-	12
MIC*	800	800	800	400	1600

TABLE 5. LOCAL LEMON GRASS OIL REDISTILLED AFTER SEVEN YEARS STORAGE

Amt. of oil	Percentage growth of the test bacteria				
in ppm.	E.coli	S.aur.	S.flex	S.typh.pA	K.pneu.
0	100	100	100	100	100
400	82	96	63	68	79
800	56	64	21	7	52
1200	23	23	0	0	23
1600	12	13	-	-	18
2000	0	10	-	-	7
2400	-	0	-	-	0
MIC*	1200	1200	800	800	1200

TABLE 6. LOCAL LEMON GRASS OIL AFTER TWELVE YEARS STORAGE

Amt. of oil	Percentage growth of the test bacteria				
in ppm.	E.coli	S.aur.	S.flex	S.typh.pA	K.pneu.
0	100	100	100	100	100
400	84	98	73	72	85
800	54	84	24	12	63
1200	14	44	0	0	28
1600	9	27	-	-	19
2000	0	24	-	-	9
2400	-	21	-	-	0
MIC*	1200	1500	800	800	1200

Results and Discussion

. *Fresh oil. E.coli* exhibited 85% growth at 400 ppm of oil and about 50% at 800 pp. The growth gradually decreases to 10% at 2400 ppm of oil.The minimum inhibitory concentration (MIC) of fresh oil against *E. coli* was 1600 ppm. *S. aureus* became 7% at 2400 ppm. The MIC of the oil was 1200 ppm. At 800 ppm the growth of *S. flexneri* was reduced to the half and completely inhibited at 1600 ppm. its MIC was 1200 ppm.

The growth of *S. typh. pA*, was reduced to 28% at 800 ppm, to 9% at 1200 ppm and was completely inhibited at 1600 ppm. The MIC was 800 ppm, *K. pneu* showed 70% growth at 400 ppm and 45% at 800 ppm. The growth became 22% at 1200 ppm which was also its MIC. 2000 ppm, of oil inhibited the growth of the bacteria completely.

. *Oil redistilled after two years storage. E.coli* at 800 ppm oil showed 30% growth. This dose was also its MIC. 1600 ppm oil completely inhibited the growth. The redistilled oil after two years storage was more active than fresh oil against other organisms as well. 800 ppm of oil brought the growth of *S. aureus* to 17% and completely inhibited its growth at 1600 ppm. The MIC was 800 ppm the growth of *S. flex.* was reduced to 55% at 400 ppm and 100% inhibition attained at 800 ppm. *S. typh pA* showed 10% inhibition at 800 ppm. The oil gave about 50% inhibition of *Kl.pneu.* at 800 ppm. The growth gradually decreased to 12% at 2400 ppm, the MIC was 1600 ppm of oil.

Oil redistilled after seven years storage. About 50% inhibition of growth was observed for *E.coli* at 800 ppm of oil and 100% at 2000 ppm. The MIC was 1200 ppm, *S. aureus* showed a growth of 64% at 800 ppm of oil and 10% at 2000 ppm. The growth was completely inhibited at 2400 ppm. The MIC was 1200 ppm of oil. The seven year old, redistilled oil was very active against *S. flex.* 1200 ppm of oil inhibited the growth of the test organism completely. The MIC was 800 ppm. The oil brought down the growth of *S. typh pA.* to 7% at 800 ppm which was also its MIC. 100% inhibition took place at 1200 ppm. The growth of *K. pneu.* was reduced to

about the half at 800 ppm and was gradually decreased to zero at 2400 ppm. The MIC was 1200 ppm.

Oil redistilled after twelve years storage. This oil brought about an inhibition of about 50% of *E. coli* at 800 ppm. 2000 ppm of oil completely inhibited the growth. The MIC was 1200 ppm. More than 50% inhibition of *S. aureus* was attained at 1200 ppm of oil and it gradually became 21% at 2400 ppm. The mIC was 1600 ppm. The growth of *S. flex* was inhibited 100% at 1200 ppm whereas its MIC was 800 ppm. *Sa. typh pA's* growth was only 12% at 800 ppm and zero at 1200 ppm. The MIC was 800 ppm. The growth of *Kl.pnue.* was gradually decreased to 28% at 1200 ppm. This was also its MIC. 2400 ppm of oil completely inhibited the growth.

Maximum inhibition of bacterial growth was obtained with oils after two years storage and redistillation. In such volatile oils the monoterpene hydrocarbons seemed to be polymerized and the citral content thus increased to 80%. After long storage it seemed that some citral also was oxidized and that the inhibition of bacteria is comparatively low, but still remarkable enough to be considered. Lemongrass oil exhibited high activity against entropathogenic bacteria, especially *Sh. flex* and *Sa. typh pA*. It might be recommended to be used in diseases caused by these bacteria.

References

1. Meena Syed, M. Rashid Khalid and F.M. Chaudhary, Pak. j. sci. ind. res., **33** , 529 (1990).
2. K.R. Kirtikar and B.D. Basu, *Indian Medicinal Plants*, (Eds), (Blatter & Caius, Delhi, 1994), Vol.IV, pp.2681.
3. G.Q. Zheng, P.M. Kenney, L.R.T. Lam, J. Agric. Fd. Chem., **41** (2), 153 (1993).
4. M. Mosquera and R. Munoz, Politecnica, **17** (1), 227 (1992).
5. K.G. Dube, T.S.Rao, Chem. Petro-chem. J., **15** (1), 13, (1984).
6. C. Liu, J. Zhang, R. Yiao, L. Gan, Huarve Xuebao (Zengkan) 241 (1981), C.A. **98**, 104358 (1983).
7. S. Qamar and F.M. Chudhary, Pak. j. sci. ind. res., **35** (6), 246 (1992).
8. F.M. Chaudhary, Proc. First National Symp. Essential Oils Perfumes and Flavours, PCSIR Labs. Lahore, **11**, (1989).
9. Meena Syed, M. Hanif, F.M. Chaudhary and M.K. Bhatti, Pak. j. sci. ind. res., **29** (3), 183 (1986).
10. S.J. Likens and G.B. Nickcrson, Amer Soc. Brew. Chem. Proc., 5 (1964).