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ANTIFUNGAL ACTIVITY BY LEMONGRASS ESSENTIAL OILS

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The inhibitory effect of lemongrass (*Cymbopogon flexuosus*, N.O. Graminae) essential oil, isolated from local and Thai cultivars, against pathogenic fungi has been studied. The antifungal activity has been compared with that of common antimycotic agents. The oils were first screened by disc diffusion method, and then the active concentration was calculated by flask culture method. No significant difference in the activity of local and Thai cultivars was found, although a two years old local oil showed the maximum inhibitory effect. It completely inhibited the growth of *Monilia sitophilia* at 500 ppm, *Penicillium digitatum* at 1000 ppm, *Aspergillus parasiticus* at 1500 ppm, *A. niger* and *A. fumigatus* at 2000 ppm.

Key words: *Cymbopogon flexuosus*, Essential oil, Antifungal, Lemongrass.

Introduction

Lemongrass has been used in medicines as a carminative, antispasmodic, antidiarrheal, antiflatulence, stomachic tonic, diuretic and expectorant. Locally it is applied in rheumatism, lumbago, and sprains [1-3]. It is reported to have a depressent effect on the C.N.S. [4] and also insect repellent activity against human ectoparasites, when used as a shampoo ingredient [5]. The oil has been found an important source of citral, used for the production of ionones andvitamin A [6].

Lemongrass oil has been reported by many scientists to possess appreciable antibacterial activity [7-12]. Onawunmi [13] studied the effect of lemongrass oil on antibacterial activity of phenoxyethanol, and reported that a mixture of lemongrass oil with phenoxyethanol (0.03:99.97) appreciably increased phenoxyethanol activity against *E. coli* and *S. aureus*. The oil was also reported to be effective against phytopathogenic fungi [14-15]. Misra *et al.* [16] found it effective against the species of Aspergillus (*A. flavus*, *A. fumigatus* and *A. parasiticus* at 3000 ppm, 2000 ppm and 900 ppm respectively).

Keeping in view the increasing dermatological problems in Pakistan, studies on the antimicrobial effectiveness of lemongrass essential oil, isolated from local and Thai cultivars, over three different seasons, were undertaken. These oil were tested against 8 pathogenic fungi and a yeast. The results are being reported in the communication.

Materials and Method

The essential oil of *Cymbopogon flexuosus* (commonly known as lemongrass) was obtained from local and Thai cultivars using Likens Nickerson apparatus. Four oils were kept for a certain period redistilled and then studied. The following oils were used for the present studies shown in Table1.

Microorganisms. The antimicrobial activity of these oils was determined against following fungi:-

Aspergillus niger, A. fumigatus, Candida albicans, Trichophyton tonsurance (isolated from patients), *A. parasiticus, Penicillium digitatum, Helminthosporium oryzae* (isolated from plants), *Monilia sitophilia* (isolated from seed) and *Saccharomyces cerevisiae* (isolated from food).

Antimicrobial activity determination. Oils were tested for their antifungal activity by two methods. Seven days old cultures of fungi and 48 hrs old cultures of yeast, were used to seed the media. Screening of the oils was first done by disc diffusion method [18], using Sabouraud's dextrose agar, having 1% Tween 20 as media. Sterilized discs of Whatmen No.1 filter paper (6 mm diameter) were soaked in respective oils (approx. 4.5 lit. oil/disc). A blank disc was used simultaneously. The zone of inhibition thus obtained after 48 and 72 hrs of incubation, at 30° were measured in millimeter (mm). Few fungicides (Table 2), already in use, were also tested under identical conditions for comparison. All the results were taken as the average of the triplicate.

In second set of experiments the oils were tested for their effective concentration by flask culture method [19]. Seven days old cultures of fungi was taken and spores were suspended aseptically in 0.05% sterilized Tween 80. One ml of

TABLE 1.

No.	Lemongrass cultivar	Distilled/ redistilled	Citral content (%)
A	Local	1985/87	80.01
B	Local	1987	71.01
C	Local	1989	80.09
D	Thai	1985/87	79.07
E	Thai	1987	77.85

this suspension was used to seed the 50 ml Sabouraud's dextrose broth, in a 250 ml Erlenmeyer flask, with 1% Tween 20 as an emulsifyer. Oils were added to bring concentrations of 2000, 1500, 1000, 500 and 100 ppm prepared in triplicate. Broth was then seeded with spore suspension, and the flasks were incubated at 30° for 14-31 days, depending on the mycelium formation. A control without essential oil and another with oil but without spore suspension were taken as 0 and 100% inhibition.

After incubation these flasks were heated at 121° for 30 sec. to kill spores and vegetative mycelia. The mycelium formed were then separated from the culture broth by filtering through pre-weighed (Whatmann No. 1), filter papers washed with distilled water, dried at 85° for 24 hrs and weighed. The percentage inhibition of the oils was then calculated and the concentrations of the oils giving approx. 80% inhibition of fungal growth were taken as the minimal inhibiting concentrations (MICs) samples were tested in triplicate and the average was taken.

Results and Discussion

The antifungal activity of 5 samples of lemongrass oil (from local and Thai cultivars) distilled in 1985, 87 and 89, were first screened against 8 pathogenic fungi and a yeast by the disc diffusion method [18]. The zones of inhibition were measured in millimeter and were compared with standard fungicides (Table 2).

Oil from local cultivar showed slightly better response than Thai cultivar by disc diffusion method (Table 2). The Thai oil showed a higher inhibitory effect only against *P. digitatum*. Among other fungicides screened, canesten was the most active drug against all the fungi tested, *C. albicans* was exceptional, in this case magenta paint and nystatin were more active. When compared lemongrass oil with these fungicides, they showed a better inhibition than benzoic acid, gentian violet, magenta paint, nystatin and tineafax. Only against *C. albicans*, magenta paint and nystatin were more effective. *M. sitophilia*, *T. tonsurance*, *H. oryzae* and *P. digitatum* were more sensitive towards the oil than canesten. There was no growth of *T. tonsurance* and *H. oryzae* except oil D, in the petri dishes (Table 2).

When tested by flask culture method a difference in pattern of activity from disc diffusion method was observed which may be ascribed to the oil vapours accumulated over the test media [20]. Oil 'A' (citral content 80.01%) had better inhibitory effect than oil 'B' (citral content 71.01%., Table 3 and 4). Only in the case of *T. tonsurance*, was the reverse true. The oil 'C' distilled 1989 showed almost same inhibitory effect as oil 'A'. (Table 5). It completely inhibited the growth of *M. sitophilia* at a conc. of 1000 ppm, MICs was found 500 ppm in both the cases. Both had caused 100% inhibition of *P. digitatum* at 100 ppm, *A. parasiticus* at 1500 ppm, *A. niger* and *A. fumigatus* at 2000 ppm, for these oils MICs were calculated to be 500, 1000 and 750 ppm respectively. At 2000 ppm. 77.38 and 78.49% inhibition of *T. tonsurance*, very common strain of scalp infection, was achieved by oil A and C. In case of Thai cultivar the same pattern was observed. The oil 'D' was more active than the oil 'E' (Table 6 and 7). Both caused 100% inhibition of *M. sitophilia* at 1000 ppm, MIC was found to be 500 ppm. Against *P. digitatum* 100% inhibition was achieved at 1000 and 1500 ppm, respectively, MICs were 500 and 1000 ppm. respectively for both. Only against *T. tonsurance* the oil 'E' was more active than oil 'D' (MIC 1000 ppm). From the above results, the activity of the oils seems to be related with the citral contents [11], which varies with the cultivar of the grass and time of harvesting. Leaf age and position also effect the amount and composition of the essential oil [21]. When kept for certain period the terpenes present in the oil got polymerized so with the increase of citral content on redistillation, the antifungal activity of oil also increases.

TABLE 2. ANTIFUNGAL ACTIVITY BY LEMONGRASS ESSENTIAL OIL (ZONE OF INHIBITION IN mm).

Organism	Local 1985 A	Local 1987 B	Local 1989 C	Thai 1985 D	Thai 1987 E	Benzoic acid	Canesten	Gentian violet	Magenta paint	Nystatin	Tineafax
A. niger	16.5	17.5	18.2	17.5	11.5	8.33	13.16	11.8	–	11.75	9.1
A. fumigatus	N.G	26.5	N.G	24.0	N.G	31.3	33.25	25.0	13.0	15.33	9.9
A. parasiticus	9.0	9.0	19.0	8.25	10.5	8.0	19.0	11.55	7.0	9.30	10.88
C. albicans	13.0	11.33	13.0	11.5	13.5	7.5	14.83	8.0	15.5	15.93	10.50
M. sitophilia	23.75	17.25	25.0	16.0	25.0	–	15.0	12.16	–	11.75	–
T. tonsurance	N.G	N.G	N.G	20.0	N.G	8.5	27.5	18.83	8.0	15.66	17.10
H. oryzae	N.G	N.G	N.T	N.G	N.G	–	25.0	22.0	8.0	15.83	–
P. digitatum	20.0	20.5	25.0	25.0	26.75	10.6	18.5	14.41	8.9	12.33	9.40
S. cerevisiae	18.1	19.5	N.T	11.5	11.5	8.0	27.0	17.00	–	12.00	8.00

Note:Benzoic acid 1%, Canesten 0.2g bisphenyl (2 chloro-phenyl)-1-imidazolylmethane/20 ml. Gentian violet 1%, Nystatin 100,000 units/ml. Tineafax, Zinc undecenoate 1%, -no-inhibition, N. G. no growth in plate, N.T. not tested.

TABLE 3. PERCENTAGE INHIBITION BY LOCAL LEMONGRASS OIL DISTILLED 1985 'A'.

Dose in ppm	*A. niger*	*A. fumigatus*	*A.parasiticus*	*M. sitophilia*	*T.tonsurance*	*H.oryzae*	*P.digitatum*
2000	100.00	100.00	100.00	100.00	77.38	98.43	100.00
1500	95.94	96.77	100.00	100.00	60.53	98.31	100.00
1000	94.14	89.51	92.11	100.00	35.56	96.90	100.00
500	20.22	73.38	17.43	100.00	5.29	97.19	98.22
100	14.73	38.70	2.32	0.00	2.20	42.65	12.81

TABLE 4. PERCENTAGE INHIBITION BY LOCAL LEMONGRASS OIL DISTILLED 1987 'B'.

Dose in ppm	*A. niger*	*A. fumigatus*	*A.parasiticus*	*M. sitophilia*	*T.tonsurance*	*H.oryzae*	*P.digitatum*
2000	95.69	87.01	94.38	100.00	98.05	96.02	100.00
1500	95.59	84.64	94.22	100.00	95.14	94.96	100.00
1000	17.06	75.52	23.44	100.00	95.36	89.47	100.00
500	7.16	17.18	15.91	98.9	68.93	89.20	92.74
100	0.32	0.00	8.40	0.70	24.27	33.77	7.84

TABLE 5. PERCENTAGE INHIBITION BY LOCAL LEMONGRASS OIL DISTILLED 1989 'C '.

Dose in ppm	*A. niger*	*A. fumigatus*	*A.parasiticus*	*M. sitophilia*	*T.tonsurance*	*H.oryzae*	*P.digitatum*
2000	100.00	100.00	100.00	100.00	78.49	99.49	100.00
1500	97.31	95.07	100.00	100.00	70.01	98.93	100.00
1000	79.34	90.52	97.24	100.00	50.49	97.95	100.00
500	35.13	79.03	20.73	97.21	20.02	34.64	95.00
100	10.27	35.70	15.19	2.50	5.40	23.72	15.20

TABLE 6. PERCENTAGE INHIBITION BY THAI LEMONGRASS OIL DISTILLED 1985 'D'.

Dose in ppm	*A. niger*	*A. fumigatus*	*A.parasiticus*	*M. sitophilia*	*T.tonsurance*	*H.oryzae*	*P.digitatum*
2000	100.00	97.58	100.00	100.00	63.72	98.43	100.00
1500	95.33	94.35	100.00	100.00	53.13	97.26	100.00
1000	94.61	76.61	91.00	100.00	22.81	95.82	100.00
500	25.71	13.70	14.31	98.15	7.19	95.34	91.22
100	6.23	8.46	17.98	0.00	1.02	30.64	10.20

TABLE 7. PERCENTAGE INHIBITION BY THAI LEMONGRASS OIL DISTILLED 1987 'E'.

Dose in ppm	*A. niger*	*A. fumigatus*	*A.parasiticus*	*M. sitophilia*	*T.tonsurance*	*H.oryzae*	*P.digitatum*
2000	97.09	87.09	97.78	100.00	100.00	98.14	100.00
1500	97.31	84.18	96.62	100.00	94.17	96.75	100.00
1000	96.96	80.28	39.65	100.00	94.17	91.98	96.84
500	6.79	67.69	16.16	98.00	76.69	82.38	13.92
100	0.49	21.04	12.51	20.04	1.26	34.50	0.51

It is thus evident that fresh as well as old lemongrass oil, exhibited a high antifungal activity. The oil can be used in ointments, inhalers, soaps and other pharmaceuticals after pharmacological evaluation. This can also be used as preservative in food against spoilage.

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