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ESSENTIAL OILS OF GRAMMINEAE FAMILY HAVING ANTIBACTERIAL ACTIVITY

Part -I. (*Cymbopogon citratus, C. martinii* and *C. Jawarancusa* Oils)

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The essential oils of three indigenous species of Grammineae family, and Cymbopogon genus, i.e. *Cymbopogon citratus, C. martinii,* and *C. jawarancusa,* had been tested for their antibacterial activity against *Escherichia coli, Staphylococcus aureus, Shigella flexneri,* and *Salmonella typhi,* Para-A by spectrophotometeric method. The essential oil of *C. citratus* was found to be highly active even at lowest concentration and caused complete inhibition of *S. aureus* at less than 400 ppm. The other bacteria also exhibited high response to this essential oil. *C. martinii* oil was more active against *S. flexneri* and *S. typhi* while the essential oil of *C. jawarancusa* also had appreciably high activity against both of these bacteria, but less than the former two oils. The activity of above mentioned oils might have been attributed to their major constituents, like citral, geraniol and piperitone. Conditions should be searched to produce varieties of these grasses with higher concentrations of these active components.

Key words: Essential oils, Antibacterial, Cymbopogon.

Introduction

Grasses are the most widespread and cheapest natural source of preventive and therapeutic medication, not only for the herbivorous animals, grazing it as fodder but, their essential oils spread in vast area and clean the microbial air pollution for benefit of other beings also.

The nature's, most abundant feed keeps, the animals grazing it in fields, healthy and fit. It prevents them from flatulance, diarrhoea, [1], and from disturbances of circulatory system [2].

Grammineae family which consists of different types of grasses, is the most wide spread family. It's genus Cymbopogon has many species distributed in Asia with wild and cultivated varieties. Lemon grass, Rosha grass and Khavi grass are indigenous to South Asian countries.

Lemon grass (*C. citratus*) is commonly cultivated in Pakistan. The local variety contains 70% citral [3]. The essential oil of lemon grass not only possesses fresh lemon like smell but has detergent and antiseptic properties. It is used in soaps, cleansers, moist tissues, and skin lotion. Dube *et al.* [4] found the oil active against some pathogens by zone inhibition method. Onawunmi *et al.* [5] described the phenomenon, as the spheroplast rupturing effect of this oil.

Another important species of this genus is Rosha grass or *Palma rosa* (*C. martinii,* var: motia) the grass contains geraniol as its major constituent, with a pleasant rose like odour [6]. The varieties present in Punjab possess 80 to 87% of geraniol [3]. The oil has been reported to be moderately active against some bacteria by zone inhibition method [4].

Khavi grass (*C. jawarancusa*) is another commonly known wide spread species of this genus. This species has its importance due to its main component piperitone [7], which can be converted *in vitro* to some commercially important alcohols. The variety present in Pakistan contains about 80% piperitone [3]. This grass is richly distributed in different areas of Punjab, like Talagang, Bhakkar and Multan.

We have tested the essential oils of the above mentioned three species of grasses i.e. Lemon, Rosha, and Khavi, for their antibacterial activity in emulsified broth, using spectrophotometric method. The test bacteria were *Escherichia coli, Staphylococcus aureus, Shigella flexneri* and *Salmonella typhi* Para-A.

Materials and Methods

Standared bacterial cultures: (1). *Escherichia coli,* (2). *Staphylococcus aureus,* (3). *Shigella flexneri,* (4). *Salmonella typhi Para-A.* (Obtained from the Nuclear Institute of Agricultural Biology, Faisalabad (NIAB), and National Institute of Health, Islamabad (NIH). The Fermentation Section of PCSIR, Lahore, also provided us the culture of *E. coli* isolated and standardized in its laboratories).

Media. (1). Oxoid's antibiotic medium no. 3 (Broth medium for test). (2). Merc's glucose broth, plus Merck's agar (Solid medium for stock culture slants). (3). Tween-20 or polysorbate-20 (Emulsifier).

Grasses for essential oils. (1). *C. citratus* (Forest Div. Lahore), (2). *C. martinii,* (Var motia) (Ayub Agric. Res. Inst., Faisalabad), (3). *C. jawarancusa.* (Talagang).

(The leave-cuttings were steam distilled to obtain their respective essential oils).

Spectrophotometer. (Single unit Ultraspec-II, LKB-automatic Spectrophotometer (530 nm).

Preparation of media and inocculum. (1). Dose of essential oil. (2). Incubation — 35° for 20 hrs. (3). Spectrophotometric readings.

The complete method in detail has been reported earlier in our work with Umbelliferae family [8]. Emulsified broth medium was used for test cultures. After incubation (35°, 20 hrs.) the optical density was taken by spectrophotometer (Ultraspec-II, LKB). The percentage inhibition for each dose of oil was calculated by comparing the optical density. (While tube with zero dose was considered to have zero inhibition or 100% growth). Inhibition thus was plotted against dose in parts per million (ppm) of essential oils (Table 1- Graphs 1-A).

Discussion

Activity of C. citratus oil. Lemon grass (*C. citratus*) oil has been used in Pakistan and China for stomach ailments, and digestions. It seems that the lemon flavour of oil enhances the apetite, and digestion [1], and it also subsides the over production of intestinal flora [5]. The strain of *E. coli*, we tested might have been a resistant strain. Though 90% inhibition was caused at 2400 parts per million (ppm) of oil (Table 1, Graph 1-A), but we had expected a more rapid and sharp inhibition, at lower doses. The essential oil was remarkably active against *Staph-aureus*, while it had been more famous for gastro-intestinal tract treatment.

The lemon grass oil at less than 400 ppm had caused 100% inhibition of *Stph-aureus* (Table 1, Graph 1-A). No growth was observed even at the lowest concentration of oil. The oil was also highly active against *Shigella flexneri*, and 100% inhibition was achieved at 1600 ppm, while 73% inhibition of *S. typhi* para-A, a very fatal strain of enterobacteriaceae, was achieved at a low dose of 800 ppm. Subsequent higher doses completely inhibited the bacterial growth, and as reported by Onawunmi *et al.* [5], if this effect is repture of bacterial cell membrane, then the inhibition is bacteriocidal, rather than bacteriostatic.

Activity of C. martinii oil. Rosha grass (*C. martinii*) oil had little effect on *Staph aureus*, but it was highly active

Table 1.

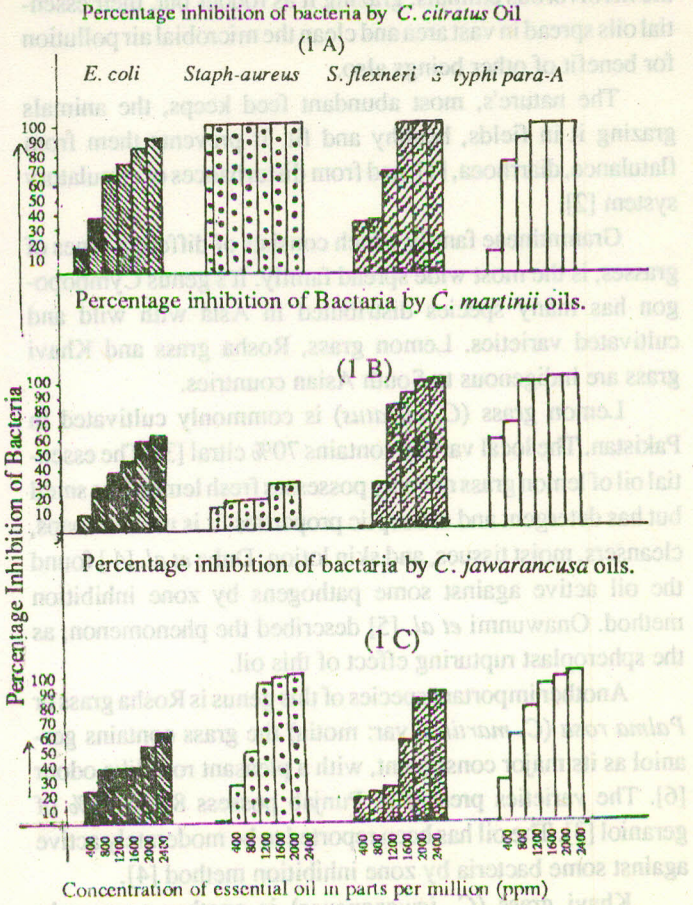
Amt. of oil in ppm.	Percentage inhibition of diff. bacteria by *C. citratus* oil			
	E. coli	*S. aureus*	*S. flexneri*	*S. typhi* Para-A
0	0	0	0	0
400	15	100	32	12
800	36	–	34	73
1200	65	–	66	91
1600	73	–	100	100
2000	84	–	–	–
2400	90	–	–	–

Table 2.

Amt. of oil in ppm.	Percentage inhibition of diff. bacteria by *C. martinii* oil			
	E. coli	*S. aureus*	*S. flexneri*	*S. typhi* Para-A
0	0	0	0	0
400	10	14	0	58
800	28	16	30	69
1200	38	17	82	97
1600	45	19	90	98
2000	58	28	99	100
2400	61	30	100	–

Table 3.

Amt. of oil in ppm.	Percentage inhibition of diff. bacteria by *C. jawarancusa* oil			
	E. coli	*S. aureus*	*S. flexneri*	*S. typhi* Para-A
0	0	0	0	0
400	21	2	15	27
800	36	25	18	58
1200	37	48	22	78
1600	38	95	54	92
2000	51	98	82	97
2400	62	100	86	100



Graph 1. Essential oils of grammineae family having anti-bacterial activity. (Meena *et al. PCSIR Lhr.).*

against enterobacters, especially *Shigella flexneri*, and *S. typhi* Para-A, i.e. more than 80% inhibition of Shigella and 97% inhibition of Salmonella was attained at 1200 ppm. The effect might have been attributed to geraniol [9], which is the major component of this oil, as such, and as its esters (Table 2, Graph 1-B).

Activity of C. jawarancusa oil. Khavi grass (*C. jawarancusa*) oil had simillar activity against *E. coli*, as had Rosha grass oil. This bacterium responded alike to the oils of both species (Table 3, Graph 1-C). Khavi oil was highly active against *Staph aureus*, contrary to the oil of Rosha, and 2000 ppm of oil caused 82% inhibition of *Shigella flexneri*, while similar inhibition of *S. typhi* was attained at about 1200 ppm of oil. Piperitone is the major constituent of this oil [3], it might be converted in the medium to some more active alcohols like thymol or menthol, as it does *in vitro*, when it is reduced or oxidised.

As the major component of the essential oil of each of the grass species discussed above is more than 70%, the activity seems to be related with that major component i.e. citral, geraniol, and piperitone, [9] which are also the main precursors in the synthesis of other more important products like ionones, vitamin-A, menthol, and thymol[3]. To obtain the natural rich sources of these commercially important components, the botanists, the agriculturists, and the genetic technologists should come forward to produce the varieties richer in active components, so that the chemists and industrialists find these more feasible to utilize.

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