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Research Paper :

Fractionation of lemon grass oil and anti-microbial activity of various fractions

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ABSTRACT

The oil of lemon grass (*Cymbopogon flexuosus*) was fractionated under reduced pressure into ten (10) fractions and the sensitivity potential of the bacteria *Salmonella typhi* and *Staphylococcus aureus* to these fractions were investigated. The oil sample was first evaluated for anti-microbial properties using the cup plate method. The oil sample showed high antimicrobial activity on the *Salmonella typhi* and *Staphylococcus aureus*. The oil was then fractionated and each fraction was collected at its boiling point range under vacuum 5-10 mm Hg. The sensitivity potential of the test organisms was then carried out in these fractions. The results obtained indicated that *Salmonella typhi* was highly sensitive to the fractions 5, 6 and 7 (boiling point) 90-92^0 C and moderately sensitive to all other fractions while *Staphylococcus aureus* showed no sensitivity to the entire fractions.

Key words : Fractionation, Boiling points, Inhibitory effects, and Sensitivity potential

Lemon grass (*Cymbopogon flexuosus* L.) is a aromatic species of *Cymbopogon* belonging to the family Graminae. It has been known for its essential oil for a long time and widely distributed to different agro-climatic zones of the country. This aromatic grass is perennial in nature and once planted properly can give economic yield for a number of years (4-5) depending upon the management practices, climate, soil fertility, etc. This crop is sensitive to environmental conditions *i.e.* rainfall, humidity, temperature and soil fertility. Therefore, there is wide variation in both yield and quality of the oil produced at different locations. In India, this plant is found growing in wasteland, saline soils, alkaline soils, hill slopes and marginal lands of semiarid regions with low to moderate rainfall. The essential oil is obtained from the distillation of whole plant. Lemon grass is of four high yielding varieties like CKP- 25, Kalam Krishna, Pragati and Praman. There are three distinct varieties of palmarosa that grow side by side in wild condition. The three varieties are Trishna,Tripta and PRC-1.

Essential oil is the volatile oil produced by steam, or water distillation of whole plant material. The vapours are condensed to yield a water condensate and an essential oil that can be separated off, usually by gravity. Essential oil is a complex mixture of hundred constituents. These constituents can be separated into single isolate by using fractional distillation unit. Fractionation is a process in which the oil is redistilled in vacuum so individual components, or fractions, are separated out as they evaporate one after the other. This is possible because fraction or constituent has its own rate of volatility based on time and temperature.

A sample of lemon grass oil produced in New Zealand was found by Lis Bal Chin *et al*. (1996) to possess the following major constituents, Limonene 4.6%, Neral 26.1%, Geranial 42.5%. Chagonda and Chalchat (1997) found that the main constituents of an oil of citratus produced from plants grown in Zimbabwe have neral 30% and geranial 41% with two other less major constituents *i.e.* Myrcene and geraniol. In 1997, Bhattacharya *et al.* reported four *C. flexuosus* cultivars OD-19, Pragati, Cauvery, SKK –7 rich in neral/ geranial, one rich in geraniol GR-1 and one hybrid (CKP 25 *C. flexuosus* x *C.khasianus* hackstapf ex. Bor.) rich in neral/ geranial that has been released for commercial cultivation. In addition to the above named lemon grass cultivars, Kulkarni (2000) listed SD 68 and Krishna as additional cultivars grown in India. Baratta *et al*. (1998) screened a commercial oil of lemongrass for its antimicrobial and antioxidant properties. Chisowa *et al.* (1998) analyzed an oil of *C. citratus* produced from plants grown in Zambia for the chemical investigation of the oil. Chalchat *et al.* (1998) also reported that oils produced from *C. citratus* from plants collected in the Ivory Coast were found to posses the volatile component. In the same year, Liu *et al.* (1998) determined the composition of an oil of *C. citratus* produced from plants grown in China. A commercial sample of lemongrass oil purchased in Austria was subjected to GC MS analysis by Oberhofer *et al.* (1999) for its volatile composition. Lemongrass oil was produced from *C. citratus* in Zimbabwe from four consecutive years. Chagonda *et al.* (2000) analysed the

oil and the oil compositions were found to varying range. Pino and Rosado (2000) analyzed the oil of *C. citratus* by GC MS produced from plant grown in Cuba. An oil of *C. citratus* produced from plant grown in Nigeria was found by Kasali *et al*. (2001) and that oil contained many miner compounds. Man had used various parts and extracts of plants and herbs as antimicrobial agents from earlier time. A great number of antimicrobial agents from plant sources already exist for various purposes and application. However, the continuous search for new ones should be sustained process, since the target microorganisms sometimes often evolve into new genetic variants, which subsequently become resistant to existing agent.

The anti microbial and the antibacterial activities have been reported on the methanolic extract of lemon grass species. There has been little or no documented scientific evidence on their fractionated samples on the test organisms. Thus, the aim of this study is to fractionate the oil into their various components and test the same on the *Staphylococcus aureus* and *Salmonella typhi* for anti-microbial activity.

MATERIALS AND METHODS

Lemon grass oil was collected locally and subjected to GC analysis by using Hewlett Packard 5890 series II gas chromatograph equipped with flame ionization detector (FID) and Carbowax 20mm polar fused silica capillary column (30m x 0.32mm.). The injector and detector temperatures were maintained 210^0C and 220^0C, respectively. Nitrogen was used as carrier gas; flow rate 1.5 ml/min. The amount of sample injected was 0.1ml (split ratio 60:1). The oven temperature as 60-210^0C was programmed at 3c/min.

After the analysis of lemon grass oil, it was then separated into various fractions using a 5-litre capacity glass fractionating column equipped with 3-nech flask capacity 5litres, column 2"x4"with stainless steal wire sulzer packing, reflux divider 2", condenser 2"x24", receivers of 250ml, 500ml, 1000ml along with 300liters per min. capacity vacuum pump. Glass reaction unit of 5 liters capacity fitted with Teflon stirrer, thermometer pocket and condenser and 250 ml capacity hydrogenation apparatus, shaker type Perfit Model were also used to carry out chemical conversion of fractions obtained from fractionation of oil into highly valuable components (Table 2). The experiment was carried out at Fragrance and Flavour Development Centre, Kannauj, U.P.

Table1 : International Standards for Lemon grass Oil (ISO 4718-1981)

Properties	Specifications
Appearance	Clear, mobile liquid
Colour	Pale yellow to yellowish brown
Relatively density at $20/20^0$ C	Minimum: 0885 Maximum:0. 905
Refractive index at 20^0C	Minimum: 1.4830 Maximum: 1.4890
Optical Rotation at 20^0 C	Range: -3^0 to $+1^0$
Miscibility in 70% (V/V) ethanol at 20^0C	One (1) Volume in three (3) volumes of 70%(V/V) to give a clear solution, which sometimes becomes opalescent on further dilution.
Carbonyl value	Minimum: 268- corresponding to 73% of carbonyl compounds, expressed as Citral.
Residue from vacuum distillation	Maximum: 10%(m/m)

Table 2 : Quality parameters of Lemon grass oil used in the processing

Sr. No	Quality parameters	Values
1.	Refractive index at 27^0C	1.4852
2.	Specific gravity at 27^0C	0.8895
3.	Limonene	4.1%
4.	Linalool acetate	1.13%
5.	Linalool	3.03%
6.	Citral-a	32.59%
7.	Citral-b	41.26%
8.	Geranyl acetate	3.67%
9.	Nerol	0.47%
10.	Geraniol	2.44%

Lemon grass oil contains a number of fragrant fractions of which citral-a, citral-b, limonene, linalool, geraniol, nerol, alpha pinene, beta pinene, nerol and ggeranyl acetate are the major components. Fractional distillation of lemon grass oil yielded 10 fractions that were tested on *Salmonella typhi* and *Staphylococcus aureus*. Pure isolates of test microorganisms *Salmonella typhi* and *Staphylococcus ureus* were obtained from Chandigarh. These organisms were preserved on sterile nutrient agar and kept at 4^0C in a refrigerator for further analysis.

Prior to commencement of the sensitivity potential tests, *Staphylococcus aureus* and *Salmonella typhi* were subcultured from the sterile nutrient agar slants into sterile nutrient broth and incubated at 37^0C for 24 Hour. The antimicrobial sensitivity test was carried out using

the cup plate method described. Nutrient agar was used as the medium. Twenty eight gram per liter of the agar was autoclaved along side with the Petri dishes at 121^0 C for 15 min. Sterile plates containing the nutrient agar were aseptically swabbed with a loopful of the test organisms using a sterile drug cotton swab. Wells were then punched into the agar with the aid of sterile cork borers. The wells measuring 3 mm (internal diameter) were 2.5 mm deep. The wells were carefully made without disturbing the medium. Three drops of the essential oil fractions were then put inside the wells using a sterile Pasteur pipette. The plates were incubated at 37^{0}C for 24 h and the zones of inhibition were measured with a transpent meter rule. The same procedure was followed with the different essential oil fraction. The experiments were carried out in triplicates. Control experiment with the original oil was carried out.

RESULTS AND DISCUSSION

There are at least not less than 10 fractions obtained from lemongrass oil (Table 3). The results of this investigation reveled that both bacteria were highly susceptible to the original lemongrass oil. This is in support with what was reported on the methanolic extract of this plant on *Staphylococcus aureus* and *Salmonella typhi* showed indication of high susceptibility to the original lemon grass (*Cymbopongon flexuous*) oil. It was observed that some of the fractions were highly inhibitory such that they inhibit growth absolutely of the microorganisms after 24 of incubation. The result in Table 4 shows that, *Salmonella typhi* was remarkably susceptible to fraction 5, 6 and 7 (boiling point 90 –92^{0}C). This suggests that fraction 5, 6 and 7contain the main active component of the oil responsible for its antimicrobial property of *Salmonella typhi*.

On the other hand, *Staphylococcus aureus* was not susceptible to all the fraction of lemongrass oil. The entire fractions and lemongrass oil are having no inhibitory effect upon the growth of *Staphylococcus aureus* (Table 5). However, the oil and fractions showed very high inhibitory effect on *Salmonella typhi*. It was also recorded that the residue of the fraction showed little or no inhibitory effect. These observations tend to suggest that the fractions (except the residue) posses antibacterial properties (high inhibitory effect) on *Salmonella typhi*. The present study has demonstrated that the oils either in original form or at their different fractions have significant inhibitory effect on the test organisms.

Table 3 : Fractionation of lemon grass oil

Fractions No.	Vacuum in mm Hg	Pot temperature (^{0}C)	Vapour temperature (^{0}C)	Yield of fractions
1	750	90-120	68-70	4.50%
2	752	120-128	70-74	2.00%
3	752	128-130	74-84	3.50%
4	754	130-135	84-90	12.50%
5	755	135-140	90	25.00%
6	755	140	90	38.50%
7	755	140-148	90-92	3.50%
			Residue	5.50%

Table 4 : The inhibitory effect of various fractions on *Salmonella typhi*

Fractions	Fractions temperature (^{0}C)	Inhibition zone (mm) at 30^{0}C
1	68-70	19.54
2	70-74	27.23
3	74-84	38.97
4	84-90	51.46
5	90	58.43
6	90	65.31
7	90-92	69.03
8	92-95	41.20
9	95-99	37.34
10	Residue	16.40
	Pure oil	53.90

Table 5 : The inhibitory effect of various fractions on *Staphylococcus aureus*

Fractions	Fractions temperature (^{0}C)	Inhibition zone (mm) at 30^{0}C
1	68-70	No growth
2	70-74	No growth
3	74-84	No growth
4	84-90	No growth
5	90	No growth
6	90	No growth
7	90-92	No growth
8	92-95	No growth
9	95-99	No growth
10	Residue	2.81
	Pure oil	No growth

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