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Effect of solvent on recovery and quality of lemongrass (*Cymbopogon flexuosus* stapf.) oil

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ABSTRACT - An experiment was carried out in laboratory to study the effect of solvent (acetone) on quantity and quality of essential oil of lemongrass (*Cymbopogon flexuosus* Stapf.) Material of four varities/genotype *i.e.* CKP-25, OD-19, OD58 and HL-2were procured form the research farm of CCS HAU, Hisar. Solvent (acetone) in various amounts *viz*., 3,6,9 and 12 ml was added in required amount of water thereby maintaining 0.2 per cent, 0.4 per cent, 0.6 per cent and 0.8 per cent level of acetone in water. The experiment was performed in three replicates for each variety. All distilled out essential oil was analyzed by gas chromatography (GC) fitted with flame ionization detector (FID) and equipped with capillary column thermo TR-WAX. The oil content increased from 0.57 per cent to 0.60 per cent in comparision to control (0.53%). Qualitative analysis of oil recovered by adding solvent (acetone) at various concentrations showed that desirable constituents *i.e.* citral-a, citral-b and total citral content decreased approximately by 2 per cent in comparison to control.

Key words - Cymbopogon flexuosus, Essential oil, Gas chromatography, Solvent effect

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Among all types of plants in the world, about 1500 species are considered aromatic and therefore, they are all significant for the production of essential oils. India is one of the important essential oil producing and exporting country of the world. India ranks 28th in imports and 14th in exports in global trade of essential oils. The place of pride among the essential oils earning foreign exchange package mainly goes to oil of Lemongrass, because it is the chief source of citral which find innumerable use in perfumery, cosmetics, flavouring, medicines and in the synthesis of vitamin A, geraniol and ionone. Essential oils are distilled volatiles, odoriferous substances of plant materials that have strong aromatic components. Essential oils find varied uses as carminative, antiseptic, sedative, central nervous system stimulants, anti-stress and muscle relaxants.

Lemongrass belongs to the genus *Cymbopogon* Spreng (family Gramineae Syn. Poaceae) consists of about 140 species (Chase and Niles, 1962). Lemongrass oil is mobile, yellow to reddish brown in colour and is a rich source of monoterpenoids. Due to presence of important chemical constituents, Lemongrass oil has commercial value. In a good quality Lemongrass oil (Guenther, 1977), contents of aldehydes *i.e.* citral-a (geranial) 40 – 48 per cent and citral-b (neral) 30 – 37 per cent should be maximum and geranyl acetate (6.7%), geraniol (2.9%), methyl heptenone (1.9%), citronellal (1.1%), â caryophyllene (0.32%) and â-elemene (0.075%) should be minimum. Citral is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citral-a) and neral (*cis*-citral, citral b). The objective of this study was to determine the effect of solvent addition during distillation and its effect on the quantity and quality of essential oil.

EXPERIMENTAL METHODOLOGY

The materials of four varieties/genotype of

Cymbopogon flexuosus grasses were obtained from the Research Farm of Medicinal, Aromatic and Under-Utilized Plants Section, Department of Plant Breeding, CCS HAU, Hisar. The four varieties/genotype were as follows: Varieties CKP-25, OD-19 and OD-58 and genotype HL-2. The crops were harvested during June, 2007.

Isolation of essential oil by adding solvent during hydro-distillation:

The essential oil of all four varieties was extracted by adding the solvent, acetone in water. To the weighed samples (1 kg each) of the freshly harvested grasses add various amounts *viz*., 3, 6, 9 and 12 ml of solvent in required amount of water by maintaining 0.2 per cent, 0.4 per cent, 0.6 per cent and 0.8 per cent concentration of acetone in water. In control sample no amount of acetone was added in water. Experiment was performed in three replicates. Extracted essential oil was collected in glass vials and anhydrous sodium sulphate was added to remove the traces of water.

Oil content (%) on fresh weight basis (FWB):

The percent oil content of collected essential oil on fresh weight basis (FWB) was calculated as per the following formula:

$$\text{Oil content on FWB (\%)} = \frac{\text{Oil content (ml)}}{\text{Fresh wt of sample (g)}} \times 100$$

The essential oils were stored in the glass vials and kept in refrigerator at 4^0C. The essential oils thus collected were subjected to Gas Chromatography (GC) analysis for the estimation of citral-a (geranial), citral-b (neral), geraniol and citronellal content.

Oil percentage on dry weight basis (DWB):

The weighed samples (1 kg each) of the freshly harvested grass of each variety/ genotype was kept in oven at 100^0C for drying till the constant weight was achieved, thereafter, dry weight of each sample was taken. The percent oil content of collected essential oil on dry weight basis (DWB) was calculated as per the following formula:

$$\text{Oil content on DWB (\%)} = \frac{\text{Oil content (ml)}}{\text{Dry weight of 1 kg fresh leaves}} \times 100$$

Estimation of essential oil:

The essential oils were analyzed on Shimadzu Gas Chromatograph (Model GC 17A) fitted with Flame Ionization Detector (FID) and a capillary column (30 m × 0.32 mm I.D. × 0.25 µm film thickness) with TR-WAX (polyethylene glycol) as the stationary phase. The GLC conditions for estimation of citral-a (geranial), citral-b (neral), geraniol and citronellal in essential oil of Lemongrass of various varieties/ genotype were: Oven temperatures were 70°C (2min) → 4°C min^{-1} → 90 (0min) → 10°C min^{-1} → 200°C (5 min), injection port 240 °C and detector 260°C. Flow rate of carrier gas was 1.3ml / min, make up gas 54 ml / min and split ratio 1:10.Flow of hydrogen was 60 KPa, oxygen 50KPa and nitrogen (auxillary gas) as 60KPa.

EXPERIMENTAL FINDINGS AND ANALYSIS

Replicated data of oil content (FWB and DWB), citral-a (geranial), citral-b (neral), total citral, geraniol and citronellal of above experiments was statistically analyzed and the results are presented in Table (1-6). The oil content on fresh weight basis ranged from 0.53 to 0.60 per cent whereas on dry weight basis ranged from 1.45 to 1.52 per cent. Range of various continues of oil were citral-a (46.2 to 47.7%), citral-b (28.3 to 29.5%), total citral (74.5 to 77.2%), geraniol (2.82 to 2.97%), citronellal (0.95 to 1.02%). Time period of 5 h was found to be best suited for complete extraction of oil from lemongrass. In present study the oil content on fresh weight basis (FWB) and dry weight basis (DWB) in Table 1 increased significantly on addition of solvent acetone @0.2 per cent, 0.4 per cent, 0.6 per cent and 0.8 per cent concentration in water during hydro distillation. Addition of solvent in excess *i.e.* 1 to 3 per cent resulted in sharp decrease in the oil content and almost nil in some varieties. Mean citral-a (geranial) content Table 2 in the

Table 1 : Oil content (%) on FWB and DWB using solvent (acetone) during hydro-distillation

Variety →	Oil content (%)*									
Concentration of acetone	CKP-25		OD-19		OD-58		HL-2		Mean	
in water ↓	FWB	DWB	FWB	DWB	FWB	DWB	FWB	DWB	FWB	DWB
Acetone (0.2%)	0.84	2.21	0.53	1.25	0.53	1.40	0.38	0.93	0.57	1.45
Acetone (0.4%)	0.86	2.26	0.54	1.27	0.54	1.42	0.39	0.95	0.58	1.48
Acetone (0.6%)	0.87	2.28	0.56	1.32	0.54	1.42	0.39	0.95	0.59	1.49
Acetone (0.8%)	0.88	2.32	0.56	1.32	0.56	1.47	0.40	0.98	0.60	1.52
Control (acetone 0%)	0.80	2.11	0.49	1.15	0.47	1.24	0.34	0.83	0.53	1.33
Mean	0.85	2.24	0.54	1.26	0.53	1.39	0.38	0.93		

*Average of three replicates
FWB = Fresh Weight Basis; DWB = Dry Weight Basis, CD at 5%,
FWB= Factor A (Solvent concentration) = 0.008, Factor B (Variety) = 0.007, Factor A × B = NS,
DWB = Factor A (Solvent concentration) = 0.008, Factor B (Variety) =0.007, Factor A × B=0.017

essential oil of four varieties decrease significantly and values were 47.2 per cent (0.2% concentration) 46.8 per cent (0.4% concentration), 46.5 per cent (0.6% concentration), 46.2 per cent (0.8% concentration) in comparison to 47.7 per cent (in control). Mean citral-b (neral) and total citral content (Table 3,4) in essential oil of lemongrass of four varities shows significantly decrease on addition of acetone in water during hydro distillation and values were 29.9 per cent (0.2%), 28.7 per cent (0.4%), 28.5 per cent (0.6%), 28.3 per cent (0.8%), 76.3 per cent (0.2%), 75.6 per cent (0.4%), 75.0 per cent (0.6%), 74.5 per cent (0.8%), respectively. Mean geraniol content (Table 5) also shows negative response whereas mean citronellal content (Table 6) in essential oil of four varieties of lemongrass was not affected significantly on addition of solvent acetone in water during hydro distillation.

This result is in agreement with several studies reported by different authors. Pino *et al.* (1997) reported the extraction of volatile compounds from *Pimenta dioica* leaf by using hexane and supercritical CO_2. The yield obtained by solvent extraction was 5.67 percent and 0.80 percent with supercritical CO_2 extraction. The difference in aroma of the extracts was quite noticeable and was attributed to qualitative and quantitative differences of the components. Catchpole *et al.* (2003) studied the overall yield and extraction efficiency in ginger, black pepper and chilli powder using near-critical carbon dioxide, propane and dimethyl ether for selected pungent components. Extraction of all spice types was carried out with acetone to compare overall yields. Subcritical dimethyl ether was effective in extracting the pungent principles from spices. Subcritical propane was the least effective solvent. Huopalathi (1986) analyzed the aroma extracted by mixing solvents n-pentane and diethyl ether with dill herb (*Anethum graveolens*).

Table 2 : Citral-(geranial) content (%) in essential oils of various varieties/genotype using solvent as additive during hydro-distillation

Variety → / Concentration of acetone in water ↓	Citral-a (geranial) content (%)*				
	CKP-25	OD-19	OD-58	HL-2	Mean
Acetone (0.2%)	44.8	47.0	46.3	50.6	47.2
Acetone (0.4%)	44.5	46.7	45.9	50.2	46.8
Acetone (0.6%)	44.2	46.4	45.7	49.8	46.5
Acetone (0.8%)	43.8	46.0	45.4	49.5	46.2
Control (acetone, 0%)	45.3	47.6	46.8	51.0	47.7
Mean	44.5	46.7	46.0	50.2	

Average of three replicates * indicates significance of value at P=0.05)
Factor A (Solvent concentration) = 0.171, Factor B (Variety) = 0.153, Factor A x B = NS

Table 3 : Citral-b (neral) content (%) in essential oils of various varieties/genotype using solvent as additive during hydro-distillation

Variety → / Concentration of acetone in water ↓	Citral-b (neral) content (%)*				
	CKP-25	OD-19	OD-58	HL-2	Mean
Acetone (0.2%)	30.1	28.6	28.4	29.3	29.1
Acetone (0.4%)	29.7	28.1	28.1	29.0	28.7
Acetone (0.6%)	29.5	27.9	27.9	28.7	28.5
Acetone (0.8%)	29.3	27.7	27.6	28.5	28.3
Control (acetone, 0%)	30.5	29.0	28.8	29.8	29.5
Mean	29.8	28.3	28.2	29.1	

Average of three replicates * C.D. (P=0.05)
Factor A (Solvent concentration) = 0.166, Factor B (Variety) = 0.149, Factor A × B = NS

Table 4 : Total citral content (%) in essential oils of various varieties/genotype using solvent as additive during hydro-distillation

Variety → / Concentration of acetone in water ↓	Total citral content (%)*				
	CKP-25	OD-19	OD-58	HL-2	Mean
Acetone (0.2%)	74.9	75.6	74.7	79.9	76.3
Acetone (0.4%)	74.2	74.8	74.0	79.2	75.6
Acetone (0.6%)	73.7	74.3	73.6	78.5	75.0
Acetone (0.8%)	73.1	73.7	73.0	78.0	74.5
Control (acetone, 0%)	75.8	76.6	75.6	80.8	77.2
Mean	74.3	75.0	74.2	79.3	

Average of three replicates* (C.D. = P=0.05)
Factor A (Solvent concentration) = 0.194, Factor B (Variety) = 0.174, Factor A x B = NS

Table 5 : Geraniol content (%) in essential oils of various varieties/genotype using solvent as additive during hydro-distillation

Variety → / Concentration of acetone in water ↓	Geraniol content (%)*				
	CKP-25	OD-19	OD-58	HL-2	Mean
Acetone (0.2%)	3.2	2.7	2.9	2.8	2.90
Acetone (0.4%)	3.1	2.8	2.9	2.7	2.88
Acetone (0.6%)	3.1	2.7	2.9	2.7	2.85
Acetone (0.8%)	3.2	2.6	2.8	2.7	2.82
Control (acetone, 0%)	3.3	2.8	3.0	2.8	2.97
Mean	3.18	2.72	2.90	2.74	

Average of three replicates* C.D. (P=0.05)
Factor A (Solvent concentration) = 0.077, Factor B (Variety) = 0.069, Factor A × B = NS

Table 6 : Citronellal content (%) in essential oils of various varieties/genotype using solvent as additive during hydro-distillation

Variety → / Concentration of acetone in water ↓	Citronellal content (%)*				
	CKP-25	OD-19	OD-58	HL-2	Mean
Acetone (0.2%)	1.0	1.1	1.0	1.0	1.02
Acetone (0.4%)	1.1	0.9	1.1	0.9	1.00
Acetone (0.6%)	1.0	1.1	0.8	0.9	0.95
Acetone (0.8%)	0.9	1.0	0.9	1.0	0.95
Control (acetone, 0%)	1.1	1.1	0.9	0.9	1.00
Mean	1.0	1.0	0.9	0.9	

Average of three replicates* C.D. (P=0.05)
Factor A (Solvent concentration) = NS, Factor B (Variety) = 0.063, Factor A × B = 142,

The main components of aroma extract was 3, 6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, -phellandrene, myristium, b-phellandrene and p-cymene. Purushotaman *et al.,* 1982; Purushotaman and Vasanth, 1982 studied the applications of solvent extraction method in chemical analysis of *Argyreia speciosa* and *Agyneia baciformis* by using hexane as solvent. Kabilan *et al.* (2002) studied the extraction of Fenugreek seeds using the solvents in the order of n-hexane, chloroform and methanol based on their polarity. Spiro and Chem (1994) studied the rates and extents of extraction by hexane, ethanol and two mixtures of hexane + ethanol of six essential oil constituents of the leaves of rosemary (*Rosmarinus officinalis*). The extracted compounds were a-pinene, camphene, b-pinene, limonene, 1,8-cineole and camphor.The probable reason of higher oil recovery may be that in the presence of small quantity of solvent (acetone) some of non-volatile chemical constituents present in Lemongrass crop get energy from solvent and becomes volatile and thereby increasing the oil content. Since increase in oil content may be due to increase of some undesired chemical constituents (which may be other than citral-a, citral-b, total citral, geraniol and citronellal) hence, the amount of citral-a, citral-b, total citral and geraniol content decreased significantly in the recovered oil.

Conclusion:

In our findings the oil content shows positive response due to the addition of acetone (upto 0.8%) in water during hydro distillation. The qualitative parameters of essential oil *i.e.* citral-a, citral-b and total citral content decreased significantly due to addition of solvent acetone in water during hydro distillation.

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