Detection of new trace constituents in the essential oils of Cymbopogon flexuosus

By Faiz Mohammad and M. C. Nigam, Central Institute of Medicinal and Aromatic Plants, Lucknow, India
and
Wasiur Rahman, Department of Chemistry, Aligarh Muslim University, Aligarh, India

The essential oil of lemongrass is derived chiefly from two important species of *Cymbopogon*, *C. flexuosus*, which has been the subject of numerous investigations,[1-10] and *C. citratus*. *C. flexuosus* (Stend.) Wats. (syn. *Andropogon nardus* var. *flexuosus* Hack), is native to India. Its two strains, SD-68 and OD-19, which have been cultivated at our Kukrail Research Farm, Lucknow, were examined by gas chromatography and the presence of new trace constituents was established for the first time.

The essential oil of lemongrass has been subjected to numerous investigations by earlier workers. Tiemann and Semmler isolated citral a and b.[2,3] Barbier and Bouveault[4] as well as Schimmel & Co.[5] observed the presence of citronellal, while Schimmel & Co.[7] reported n-decyl aldehyde in the oil of Indian lemongrass. Eleze found nerol and farnesol in these oils.[8] The essential oil of Malabar lemongrass was investigated in greater detail in 1961 by Ping-Hsien Yeh, who reported the presence of myrcene, p-cymene, methyl heptenone, geranic acid, and geranyl salicylate.[9] Ayyar, Kamath, and Rao identified geranyl acetate in noncitral parts of the lemongrass oils.[10] In 1962, Airth, Stringer, and Levi developed a method for determination of citral in lemongrass oils by barbituric acid condensation,[11] while Kasim Cemal Guven used paper chromatography for citral estimation.[12] Shukla, Nigam, and Handa developed a spectrophotometric method for estimation of citral in lemongrass oils by converting it into 2-4-dinitrophenyl-hydrazone.[13]

Recently, work has been undertaken in different laboratories all over the world to develop new strains of *Cymbopogon flexuosus* for higher citral content besides other useful constituents. Srikulvadhana, Jennings, and Deragules distilled essential oils from three selected mutants of *Cymbopogon flexuosus* from Guatemala in 1976 and used combined gas chromatography and mass spectrometry to examine them[14] Development of various strains of *Cymbopogon flexuosus* has since been reported in India also. One of the reports mentions two high-yielding chemotypes of *C. flexuosus*, designated RRL-54 and RRL-59.[15] The former was collected from Chandi hill and the latter from Kolar (South India). The oils from these two strains were combined and examined. They contained about 30.5% of geraniol and 19.45% of methyl eugenol with traces of citral.

A variety of *Cymbopogon flexuosus*, designated SD-68 and having citral content, was developed by Datta and Nigam at the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, by plant selection.[16] Another variety, designated OD-19, was developed at the Lemongrass Research Station, Odakali (South India).[17,18] None of these varieties have been examined by gas chromatography for their trace constituents.

Materials and methods

Two samples of *C. flexuosus* selections, SD-68 and OD-19, were distilled by hydrodistillation and the essential oils were obtained in yields of 0.49% and 0.43% respectively on the fresh weight basis. These were examined by gas chromatography.

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Gas chromatographic examination

Both the oils were gas chromatographed on Perkin Elmer gas chromatograph using a column of carbowax (10%) on chromosorb W and hydrogen as the carrier gas at a flow rate of 30 ml per minute. Column temperature was maintained at 150°C. Samples of 5 μl were injected in both cases and well-resolved peaks were identified by serial dilution technique and comparison with the standard data on relative retention times. The assessment of percentage was done by determination of area of peaks by sigma method.[19,20]

Results and discussion

The two oils, SD-68 and OD-19, were examined gas chromatographically (see figs. 1 and 2). The results of these studies can be seen in Table I.

A study of the trace constituents of these essential oils by gas chromatography revealed the presence of terpinen-4-ol, β-terpineol, α-terpinyl acetate, borneol, and nerolidol. The presence of a trace amount of nerolidol in one of the varieties, SD-68, has its own significance in establishing the chemotaxonomic differences between the two varieties. Coexistence of such trace constituents as borneol, β-terpineol, and α-terpinyl acetate also has its own significance, as these are also known to occur together in other essential oils and hence exhibit their biogenetic relationship. The complete absence of limonene, which is one of the important constituents of many other essential oils of *Cymbopogon* species, in this oil established one of the important criteria of identity of this essential oil.

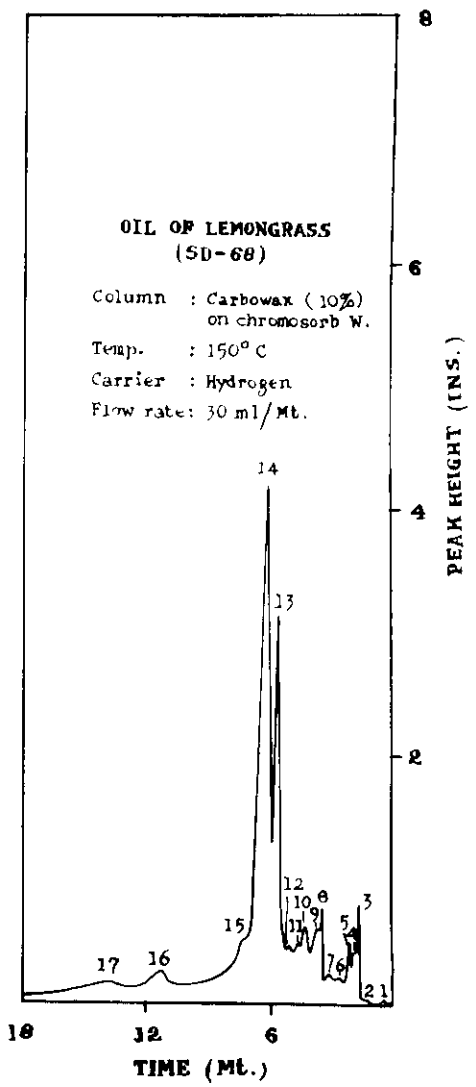

Figure 1

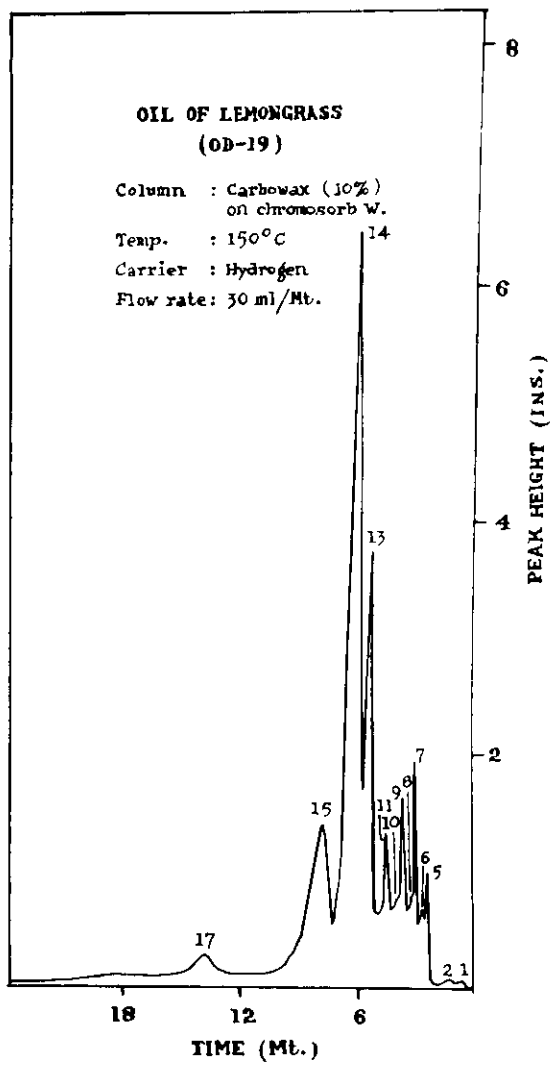

Figure 2

Table 1. Comparative chemical composition of two types of Cymbopogon flexuosus oil

Peak No.	Compound	RRT	Percentage SD-68	Composition OD-19
1.	myrcene	0.08	0.02	0.02
2.	p-cymene	0.18	0.04	0.04
3.	methyl heptenone	0.26	0.07	n.d.
4.	decanal	0.36	0.35	n.d.
5.	terpinen-4-ol	0.39	0.41	0.60
6.	beta-terpineol	0.43	0.17	0.40
7.	alpha-terpineol	0.49	0.38	2.25
8.	alpha-terpinyl acetate	0.54	1.20	0.90
9.	borneol	0.59	0.95	1.90
10.	geraniol + nerol	0.74	0.78	1.50
11.	citronellal	0.82	0.70	n.d.
12.	neral	0.88	34.90	27.70
13.	geranial	1.00	45.70	46.60
14.	farnesol	1.28	6.00	12.80
15.	nerolidol	1.88	trace	n.d.
16.	farnesal	2.32	2.40	3.00

n.d. = not detected

Acknowledgement

The authors are grateful to Dr. Akhtar Husain, Director, Central Institute of Medicinal & Aromatic Plants, Lucknow, for his keen interest in this work. The authors are equally grateful to Dr. R. K. Gupta for providing genuine oils for this work.

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