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Interspecific differences in the constituents of essential oils of *Cymbopogon*

G RAJENDRUDU and V S RAMA DAS
Department of Botany, Sri Venkateswara University, Tirupati 517 502, India.

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Abstract. Gas chromatography of essential oils of *Cymbopogon* showed striking interspecific differences in their major constituents. The two species of lemongrass, *C. flexuosus* and *C. pendulus* contained large amounts of citral while the citronella grass species, *C. nardus* and *C. winterianus* showed comparable amounts of geraniol, citronellal and citronellol. The essential oil of *C. martini* was composed only of geraniol and citronellol and was therefore quite different to the other species. The gas chromatography profiles enable the identification of various species yielding essential oils characterised by their specific constituents.

Keywords. *Cymbopogon;* essential oil; lemongrass; citronellagrass; gas chromatography.

1. Introduction

Among the aromatic plants, a few species of *Cymbopogon* are important grasses yielding essential oils such as lemongrass oil, citronella oil and palmarosa oil which are extensively used in perfumery, pharmaceutics and chemical industry. The lemongrass oil is mainly obtained from the shoots of *C. flexuosus* (East Indian lemongrass), *C. citratus* (West Indian lemongrass) and *C. pendulus* (Jammu lemongrass) (Atal and Bradu 1973). Two closely-related types of citronella oils, distilled from the shoots of *C. nardus* (Ceylon type) and *C. winterianus* (Java type) are known in trade for their essential oils. The palmarosa oil is mainly extracted from the species of *C. martini* var. motia. These aromatic grasses can be distinguished morphologically (Rajendrudu 1980) by the shape and length of their shoot system and chemically (Guenther 1950) by the composition of essential oils obtained from them.

The early efforts of identifying the chemical constituents of the essential oils were dependent on fractional distillation procedures and many of the known components could well have been artifacts formed on heating. A detailed comparative study of the essential oils of *Cymbopogon* using recent analytical methods is still limited although a few attempts were made on varietal differences of citronella oil using gas-liquid chromatography (GLC) methods (Weijesekera *et al* 1973). Therefore, the present investigation was directed to study the interspecific differences in the major constituents of essential oils of *Cymbopogon* using advanced analytical methods.

2. Material and methods

2.1 *Plant material*

The plants, *Cymbopogon flexuosus* (D.C) Stapf, *Cymbopogon martini* (Roxb.) Wats var. motia, *Cymbopogon* nardus (L.) Rendle, *Cymbopogon pendulus* Wats. and *Cymbopogon winterianus* Jowitt were obtained from the Regional Research Laboratory (CSIR), Bhubaneswar. Plants were raised on soil supplemented with farm yard

manure from their cuttings in the experimental botanical garden under natural photoperiodic conditions with a day/night temperature regime of 32/23°C. The light intensity at the soil surface was about 180 nE cm^2sec^{-1} (400-700 nm, PAR) at 12 noon on a clear day.

2.2 *Extraction of essential oil*

The essential oils were extracted by harvesting the plants at flowering and by direct steam distillation based on the principle of Guenther (1948) under laboratory conditions using a glass still. Distillation was carried out for 4-5 hr including the time required to heat the water in the retort to the boiling point. The essential oils collected in the receiver were taken out, cleared and weighed to calculate the percentage of oil yield. For further studies, the oil was redistilled under reduced pressure.

2.3 *Gas chromatography (GC)*

Analytical GC of the essential oils was made on a Hewlett Packard Gas chromatograph model 5830 A using a thermal conductivity detector with 18850 A Gas chromatograph HP terminal. Carbowax 20M, 10% in Chromosorb W (6′ × 1/8″) column was employed to detect the major constituents of essential oils. Injection temperature of 200° C, detector oven temperature of 230° C and the carrier gas (hydrogen, 30 ml/min) were used throughout the study. Samples of redistilled oil (0.5 μl) were injected with programming from 60 to 200° C at 4° C/min. Infrared spectra were run as smears with NaCl optics in infrared spectrophotometer (Perkin Elmer model 297). Tentative peak identification was assigned by comparing the corresponding retention data with those of authentic compounds using the same column. The quantity of the constituents was estimated by peak area integration and summation with data system (Hewlett Packard model 18850 A).

3. Results and discussion

Cymbopogon is a perennial grass exhibiting densely tufted habit with stems clasping and filiform leaves. The inflorescence bearing columns of *C. flexuosus*, *C. martini*, *C. nardus* and *C. winterianus* reached a height of 1.3m to 1.6m while *C. pendulus* with the inflorescence reached about 2m (table 1). The leaves of *C. flexuosus* and *C. pendulus* were narrow (16.3-18.4 mm width) and erect with a conspicuous midrib. The two types of citronella grasses were morphologically distinguished by long and narrow-leaves with a prominent midrib in *C. nardus* and shorter and broader leaves in *C. winterianus*. In *C. martini* the leaves were small but the number per plant was higher (table 1).

The oil obtained by steam distillation of the fresh grass was occasionally cloudy and, therefore, was stored in narrow test tubes for 10-15 hr to clarify the oil. The average oil

Table 1. Morphological variations between the species of *Cymbopogon* (± S.E.).

Plant species	Leaf length (cm)	Leaf width (cm)	No. of leaves/ plant	Total height of the plant (m)
C. flexuosus	88.2 ± 8.52	1.84 ± 0.23	8.7 ± 0.78	1.46 ± 0.09
C. martini	22.6 ± 2.09	2.06 ± 0.18	12.9 ± 0.70	1.63 ± 0.08
C. nardus	133.0 ± 7.80	2.20 ± 0.13	8.3 ± 0.41	1.54 ± 0.09
C. pendulus	75.3 ± 5.62	1.63 ± 0.17	9.4 ± 0.75	2.03 ± 0.12
C. winterianus	117.7 ± 4.58	2.89 ± 0.14	9.7 ± 0.58	1.32 ± 0.06

yield of each plant species is shown in table 2. A range of 0.24 to 0.93% (w/w) of oil from freshly harvested leaves of *Cymbopogon* was obtained at laboratory distillation conditions using a glass still. A maximum oil (0.93%, w/w) was obtained from leaves of Java citronella. The inflorescence of each of the plant species contained more essential oil on fresh weight basis except in Java citronella (table 2).

Table 2. Oil yield (% w/w) on fresh weight basis from the leaves and inflorescence of *Cymbopogon* (± S.E.).

Plant species	Leaves	Inflorescence
C. flexuosus	0.24 ± 0.03	0.43 ± 0.03
C. martini	0.28 ± 0.02	0.56 ± 0.05
C. nardus	0.35 ± 0.03	0.41 ± 0.04
C. pendulus	0.38 ± 0.04	0.40 ± 0.03
C. winterianus	0.98 ± 0.06	0.68 ± 0.05

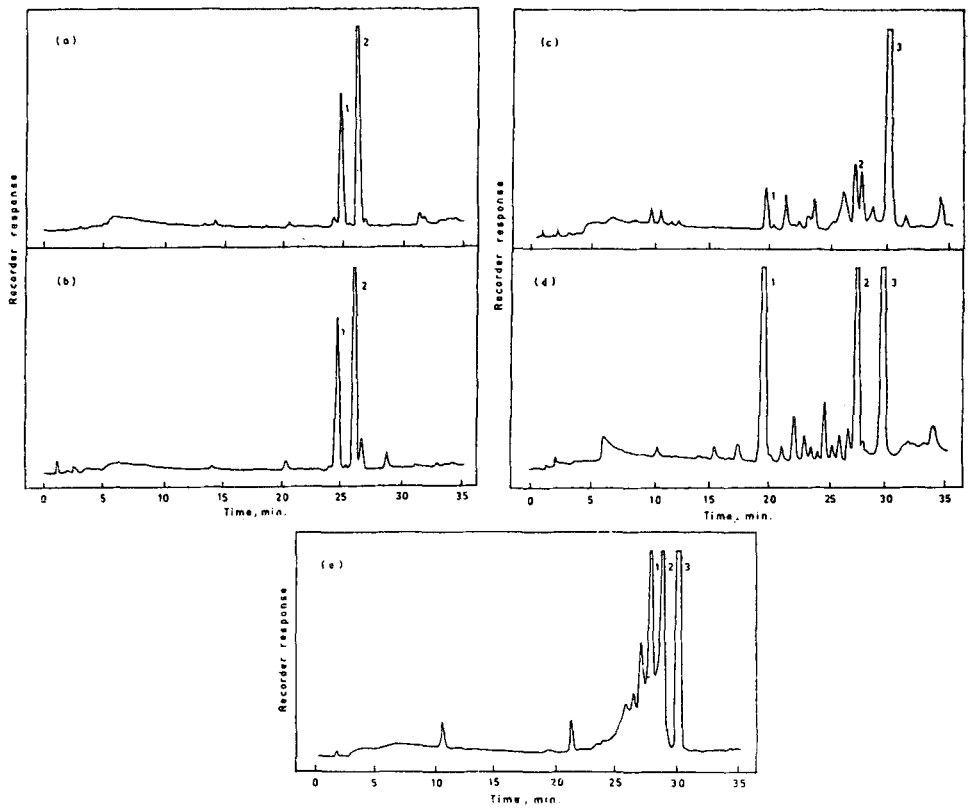

Figure 1a-b. Gas chromatograms of lemongrass oils extracted from (**a**) *C. flexuosus* and (**b**) *C. pendulus*. Two major peaks correspond to (1) alpha- and (2) beta- ionones of citral.

Figure 1c-d. Gas chromatograms of citronella oils extracted from (**c**) *C. nardus* and (**d**)*C. winterianus*. The major peaks correspond to (1) citronellal, (2) citronellol and (3) geraniol with other unidentified minor components.

Figure 1e. Gas chromatogram of palmarosa oil extracted from *C. martini* var. motia. The major peaks correspond to (1) citronellol, (2) unidentified component and (3) geraniol.

Table 3. Interspecific differences in the major constituents of essential oils (%).

Plant species	Citral	Citronellal	Citronellol	Geraniol	Others
C. flexuosus	84.4	—	—	—	15.6
C. martini	—	—	19.9	34.5	45.6
C. nardus	—	5.7	3.9	64.3	26.1
C. pendulus	85.1	—	—	—	14.9
C. winterianus	—	27.1	21.8	30.8	20.4

Typical gas chromatograms of essential oils from five species of *Cymbopogon* are shown in figure 1. The major constituents were identified based on retention data, infrared spectra and thin layer chromatography as compared to authentic compounds. The analytical data of the major constituents of oils are shown in table 3. The two lemongrass oils distilled from *C. flexuosus* and *C. pendulus* had very high citral content with alpha- and beta- ionones. These ionones from the oil are used in flavours, cosmetics, perfumes and the manufacture of synthetic vitamin A. The Ceylon citronella showed a high proportion of geraniol (64.3%) and a little citronellal (5.7%) as against 30.8% geraniol and 27.1% citronellal in Java type. In contrast the palmarosa oil obtained from *C. martini* var. motio contained comparable amounts of geraniol and citronellol with other unidentified compounds. Thus the GC analysis enables the identification of the species yielding essential oils with major constituents of economic value. The present results show striking interspecific differences in the chief constituents of essential oils of *Cymbopogon* consistent with the variation in their morphology.

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