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DIFFERENTIATION OF THE VARIETIES OF CYMBOPOGON ON THE BASIS OF ITS CHEMICAL CONSTITUENTS

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Abstract. A number of species of the genus *Cymbopogon* were grown at the nursery of the department. Their free amino acids, lipids and phenol contents were studied by chromatographic methods. Although some relationship was shown in different species no definite differentiation was possible on chemical basis alone.

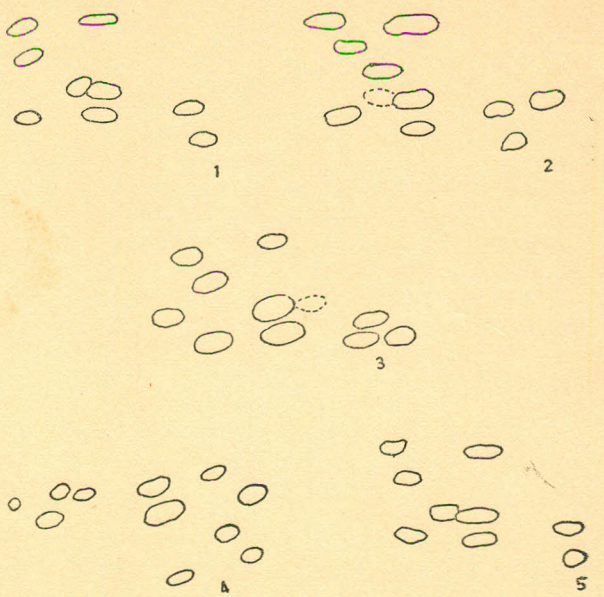
The importance of the genus *Cymbopogon* is mainly on account of its essential oil contents, e.g. geraniol, lemon grass oil and oil of citronella.[1–6] Of the 26 species of *Cymbopogon*, only six grow in Pakistan and five of these were studied in the present investigations. In the evolution of this genus, hybridization seems to have played an important role.[7,8] In demonstrating the hybridization and interspecific relationship the composition of lipids, free amino acids and phenolic compounds have been employed.[9–13] Gupta[14] studied the essential oils of 15 diploid and polyploid species of this genus from Kashmir and India, and has provided a satisfactory evidence of hybrid origin of different species. The differences in composition of essential oil, however, are not characteristic enough of the various species. In the present investigation the variations that occur in the lipids, free amino acids and phenolic constituents of different species of *Cymbopogon* namely, *C. caesius*, *C. olivieri*, *C. jwarancusa* and *C. distans* were studied. These results were compared with the variability in the species.

Experimental

All the species of the *Cymbopogon* studied were grown at the nursery of the Department.

Free Amino Acids. About 100 g leaves from each species were ground to paste and exhaustively extracted with 80% ethanol at room temperature (3×24 hr extract). The extract was evaporated to a small volume. The insoluble material was separated by centrifugation. From the clear supernatant, the qualitative estimation of amino acids was carried out by paper chromatography using (a) butanol–pyridine—water (1:1:1, v/v) and (b) phenol–water—ammonia (37.5:5.0: 0.03, v/v) in the first and second dimension respectively. After drying the paper was sprayed with Cd–ninhydrin (0.5 g ninhydrin in 50 ml acetone, to this was added 0.5 g cadmium acetate solution, dissolved in 1 ml glacial acetic acid and 5 ml distilled water), dried in an oven for 10 min at 80°C and developed in a tank containing conc H_2SO_4 for 3 hr. The spots observed are shown in Figs. 1–5.

Lipids. For the isolation of lipids, stripped leaves from each species were dried, ground to powder and extracted with ether in Soxhlet apparatus at 40°C. The ether extract was dried and dissolved in 1 ml hexane-benzene mixture (9:1, v/v). The lipids were separated from this material on silica column (25×1 cm), and eluted with hexane–benzene mixture, till the fractions gave negative test for lipids. The lipid fractions from each species were evaporated to small volume and further separation was achieved on silica gel thin layer plates using solvent system petroleum ether–ether–acetic acid (80:20: 1.5, v/v). The plates were developed in iodine tank, and the iodine-sensitive spots are shown in Fig. 6.



Spots showing free amino acids present in: Fig. 1. *Cymbopogon caesius*. Fig. 2. *C. olivieri*. Fig. 3. *C. jwarancusa*. Fig. 4. *C. distans*. Fig. 5. *C. parkeri*.

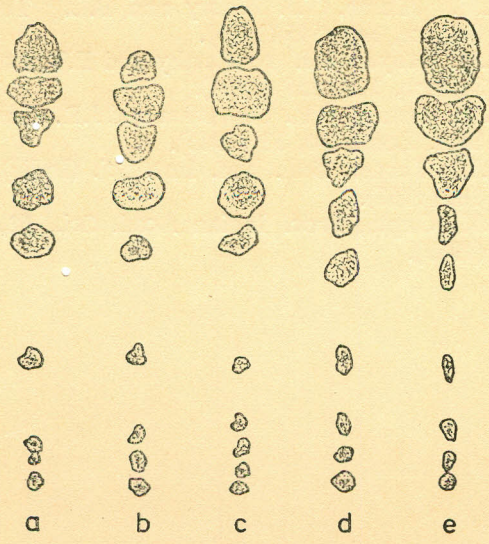


Fig. 6. Spots showing the lipid contents in: (a) *Cymbopogon olivieri*; (b) *C. parkeri*; (c) *C. caesius*; (d) *C. jwarancusa*; and (e) *C. distans*.

Phenolic Compounds. For the isolation of phenolic compounds, the solvent from the alcoholic extract was evaporated and the yellowish green pigment removed by several washings with toulene. The clear extract was partitioned with ether. The ether portion containing phenolic compounds, acids and steriods was shaken with NaOH solution to extract all the phenols, and the NaOH portion was further partitioned with ether to obtain phenolic compounds after neutralising with conc HCl. The ether extract was evaporated to a small volume and an aliquot was used for acending paper chromatography (Whatman No.1) using butanol–acetic acid–water (12:3:5, v/v) system. The paper was examined under UV-lamp and the flourescent spots were marked numerically. The spots found in each species are given in Table 1.

Results

As a whole all the species are more or less similar in their chemical make up, although some interspecific variation is present.

Free Amino Acid. The spots in the paper chromatograms for free amino acies were found to be same in all the species. The number of spots observed ranged from 9 to 11 spots. Interspecific variation was found to be similar to interspecific variation (Figs. 1–5).

Lipids. Thin layer chromatography of total lipid contents also demonstrated limited interspecific variation. The types of lipids found in all the species are similar with the exception of one spot that was observed in *C. jwarancusa* and *C. distans* which was absent in the rest of the species (Fig. 6).

Phenolic Compounds. The results of phenolic compounds indicates a considerable variation from species to species, however, total interspecific variation was not observed.

TABLE 1. PHENOLIC COMPOUNDS IN FIVE SPECIES OF *Cymbopogon.*

	1	2	3	4	5	6	7	8	9	10	11	12
C. parkeri		+			+	+		+				+
C. Olivieri			+			+			+			+
C. jwarancusa			+			+		+				+
C. caesius		+				+	+				+	+
C. distans	+					+	+	+		+	+	+

In all 12 different kinds of spots of phenolic compounds observed under UV-lamp two of these spots were common to each of the five species.

Cymbopogon olivieri had four spots, three of which were common to *C. jwarancusa.* The later had five spots, as does *C. caesius. C. distans* had a total of seven spots of which four were similar to *C. caesius* of the remaining three spots, one was similar to *C. caesius* and *C. jwarancusa,* whereas two were not observed in any other species studied. In *C. parkeri* five spots were observed, two spots common to *C. caesius, C. jwarancusa,* and *C. distans.* The fourth spot was similar to *C. jwarancusa* and *C. distans.* The fifth spot was the only one different from all the other species. A summary of the results is shown in Table 1.

Discussion

The present investigations have given an indication that *C. jwarancusa* and *C. olivieri* shared a large number of spots of the amino acids, the phenolic compounds and the lipids among each other and showed a close relationship. Similarly *C. caesius* and *C. distans* were found to be more closely related than any other species studied, and *C. parkeri* was closely related to *C. olivieri–C. jwarancusa* group rather than the *C. caesius–distans* group.

Although these studies have provided evidence of relationships among the various species of *Cymbopogon,* however, it would appear that neither free amino acid nor phenolic compounds or lipids provide clear cut differences between different species of this genus, and cannot be a criteria for the classification of different species.

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