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MORPHOLOGY, COMPOSITION AND SEASONAL VARIATION OF LEAF EPICUTICULAR WAXES OF SOME SELECTED TREE SPECIES

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The morphology of the leaf epicuticular waxes, composition and the seasonal variation was studied in seven selected tree species. Among the seven species studied, the maximum wax content associated with the leaves of *Dolichandrone atrovirens*, while the minimum in *Lannea coromandelica*. Epicuticular waxes showed marked variations from month to month and these differed from one plant species to the other. Relatively higher wax content wax observed in all the species during summer. Leaf surface waxes appeared like rods, needles, crystalline rods, plates and granular coating. The infrared spectra of the waxes revealed the presence of mixture of alcohols, phenols, alkanes, carboxylic acids, ketones and esters.

Key words : Morphology, composition, leaf epicuticular waxes, tree species.

The development of epicuticular waxes by the land plants is consistent with shifting of life from an aquatic environment to land habit. The leaf epicuticular waxes are deposited on the outer surface of the cuticle in a specific and characteristic pattern. The development of epicuticular waxes greatly depends on the environmental factors such as light, temperature and humidity (Hull *et al.*, 1975). The amount of wax on the leaf surface varies widely among the plant species and environment (Eglington & Hamilton, 1967). Interaction of various environmental factors particularly temperature, high light intensity, humidity and precipitation have been shown to result in the decreased levels of wax from summer to winter (Rao & Reddy, 1980). Surface morphology of the plant epicuticular waxes have been studied in wide range of plant species with scanning electron microscope (Davis, 1971; Krishnamurthy & Rajachidambaram, 1986). Epicuticular waxes of most higher plants appear as crystalline bodies or amorphous layer of great diversity of form and thickness (Martin & Juniper, 1970) . The crystalline waxes on specific organs of individual plants consist predominantly of a single structural form such as plates, tubes, ribbons, rods, filaments or dendrites (Baker, 1982). The identification of the components of leaf epicuticular waxes have been made with infrared analyser (Fernandes *et al.*, 1964).

However, the information on the composition and morphology of the surface waxes is scanty in some of the tropical tree species. As such the present study compares the composition, seasonal variation and morphology of seven tree species distributed in Deccan plateau of peninsular India.

MATERIALS AND METHODS

Seven forest tree species selected for the present study are *Chukrasia tabularis, Dolichandrone atrovirens, Eugenia jambolana, Gmelina arborea, Lannea coromandelica, Terminalia arjuna* and *Terminalia bellerica*. Seeds of the selected tree species used in the present study were obtained from the forest department of Tirupati division. Healthy seeds with uniform size were selected and sown in polythene bags containing a mixture of three parts of red sandy loam soil and one part of farmyard manure. Tree saplings were grown in an open air under natural photoperiodic conditions in the experimental botanical garden. Saplings at the age of nine months were transplanted into the experimental site. A total of 200 saplings of each plant species were transplanted in a plot of 120 m x 15 m and with a spacing of 3m x 3m between the plants and rows. The saplings were grown under natural conditions and the present study was initiated on saplings at the age of two years. The plant samples were collected for one year starting from June, 1990 to May, 1991.

The climatic data of the experimental site during the period of the present study are presented (Table 1). The climate prevailed during the study was mainly characterised by scanty and erratic rain fall with monthly minimum and maximum temperatures ranging 16.3°C - 26.5°C and 28°C - 38.3°C respectively. The relative humidity also showed monthly variations during the experimental period, the minimum and maximum

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Table 1: Monthly climatic variables at the field site during the growth period (from 1-6-1990 to 1-5-91). (Each value represents monthly average of one year)

Months	Temperature (°C)		Relative Humidity (%)		Rainfall (mm)	No. of rainy days	Sun shine hr/day
	Maxi-mum	Mini-mum	Maxi-mum	Mini-mum			
Jun	36.8	25.7	58.5	38.0	48.75	4.0	5.75
Jul	34.95	24.7	67.5	43.0	111.6	8.0	5.55
Aug	34.7	25.1	65.0	44.5	20.1	3.0	5.4
Sep	34.8	24.3	73.0	50.5	141.55	8.0	6.0
Oct	31.1	21.85	81.0	60.5	124.55	7.0	6.25
Nov	30.3	19.35	80.5	60.5	242.05	8.5	7.0
Dec	28.8	17.8	83.0	60.5	91.8	2.5	7.65
Jan	30.3	16.25	77.0	44.0	5.85	0.5	9.35
Feb	33.2	18.85	77.0	37.0	-	-	10.0
Mar	35.75	22.8	73.0	33.0	21.05	2.0	9.65
Apr	38.25	25.8	65.5	29.5	30.3	0.5	9.55
May	36.75	26.45	68.5	42.5	121.35	7.5	8.35

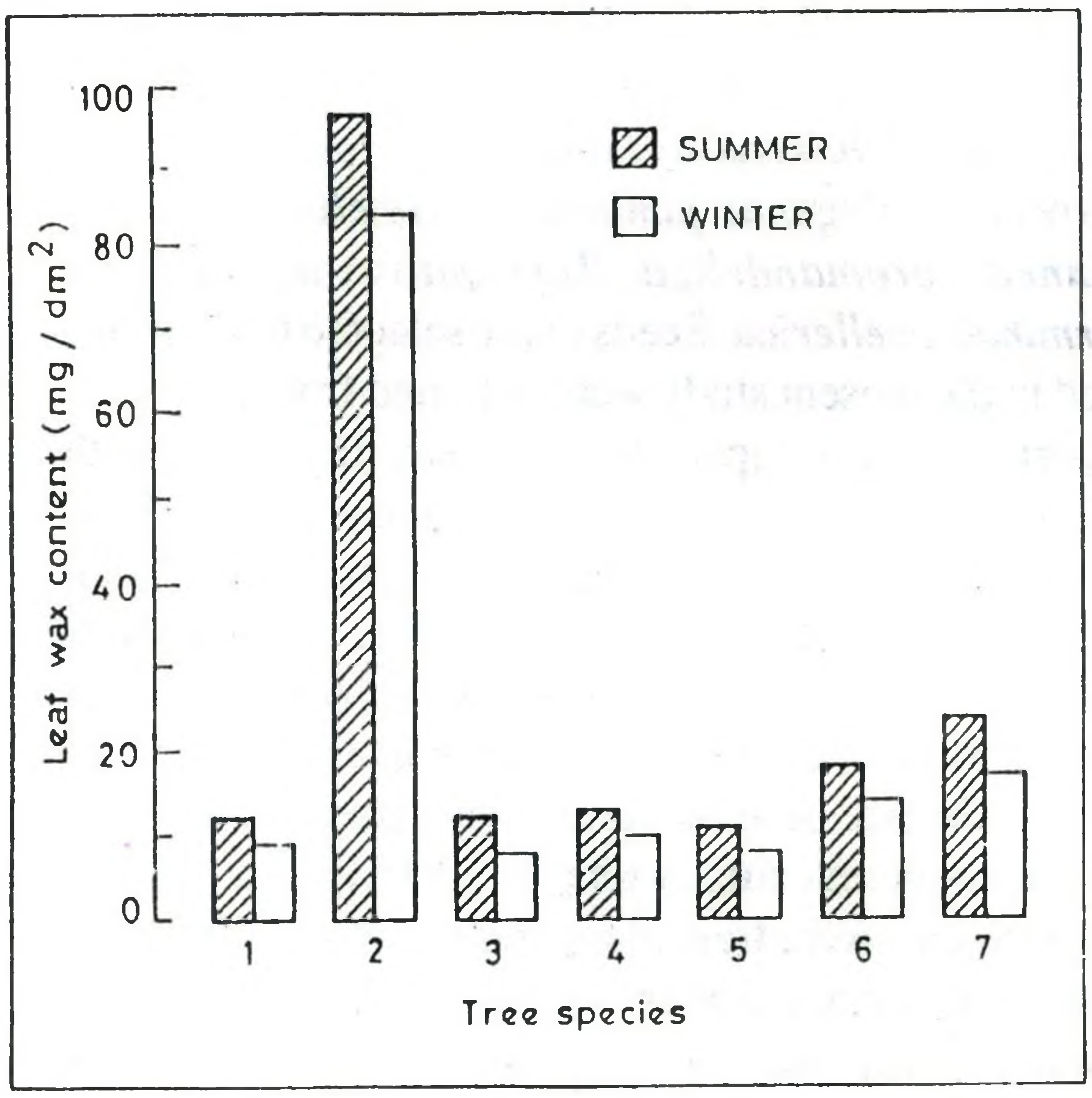


Figure 1. Wax content of seven tree species during summer and winter seasons.
1. *Chukrasia tabularis*, 2. *Dolichandrone atrovirens*, 3. *Eugenia jambolana*, 4. *Gmelina arborea*, 5. *Lannea coromandelica*, 6. *Terminalia arjuna*, 7. *Teminalia bellerica*

ranges namely 29.5% - 60.5% and 58.5% - 83.0% respectively. Data on the monthly average of rain fall showed maximum during September - November as well as in May and July. During the rest of the experimental period rain fall was very low. The seasons in the present study are based on climatological data of one year starting from June, 1990 to May, 1991. The year was divided into high light period/summer/dry period

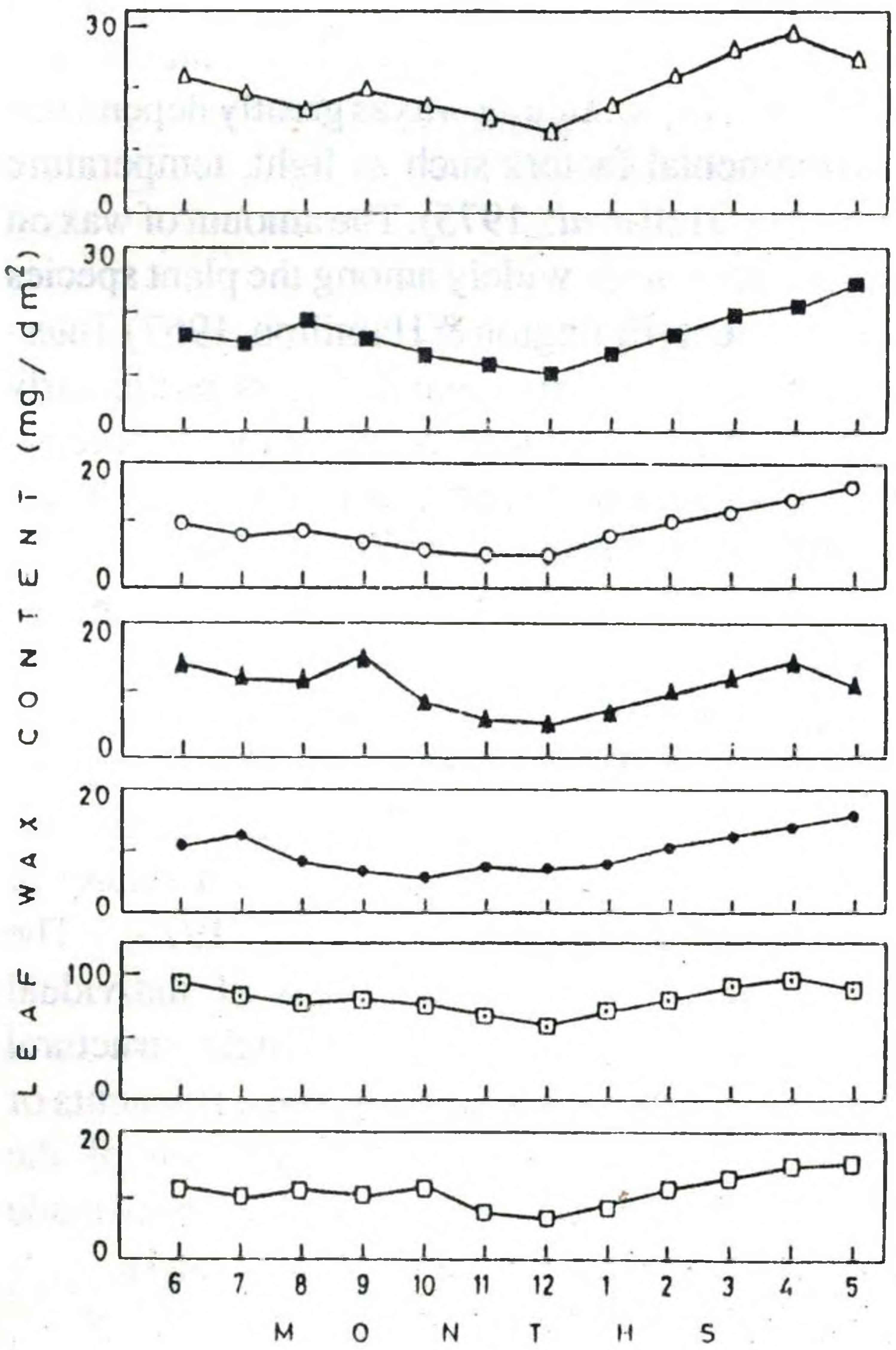


Figure 2. Monthly variations in leaf epicuticular waxes of seven tree species.

Chukrasia tabularis	(o)	*Dolichandrone atrovirens*	(o)
Eugenia jambolana	(●)	*Gmelina arborea*	(Δ)
Lannea coromandelica	(O)	*Terminalia arjuna*	(■)
Terminalia bellerica	(Δ)		

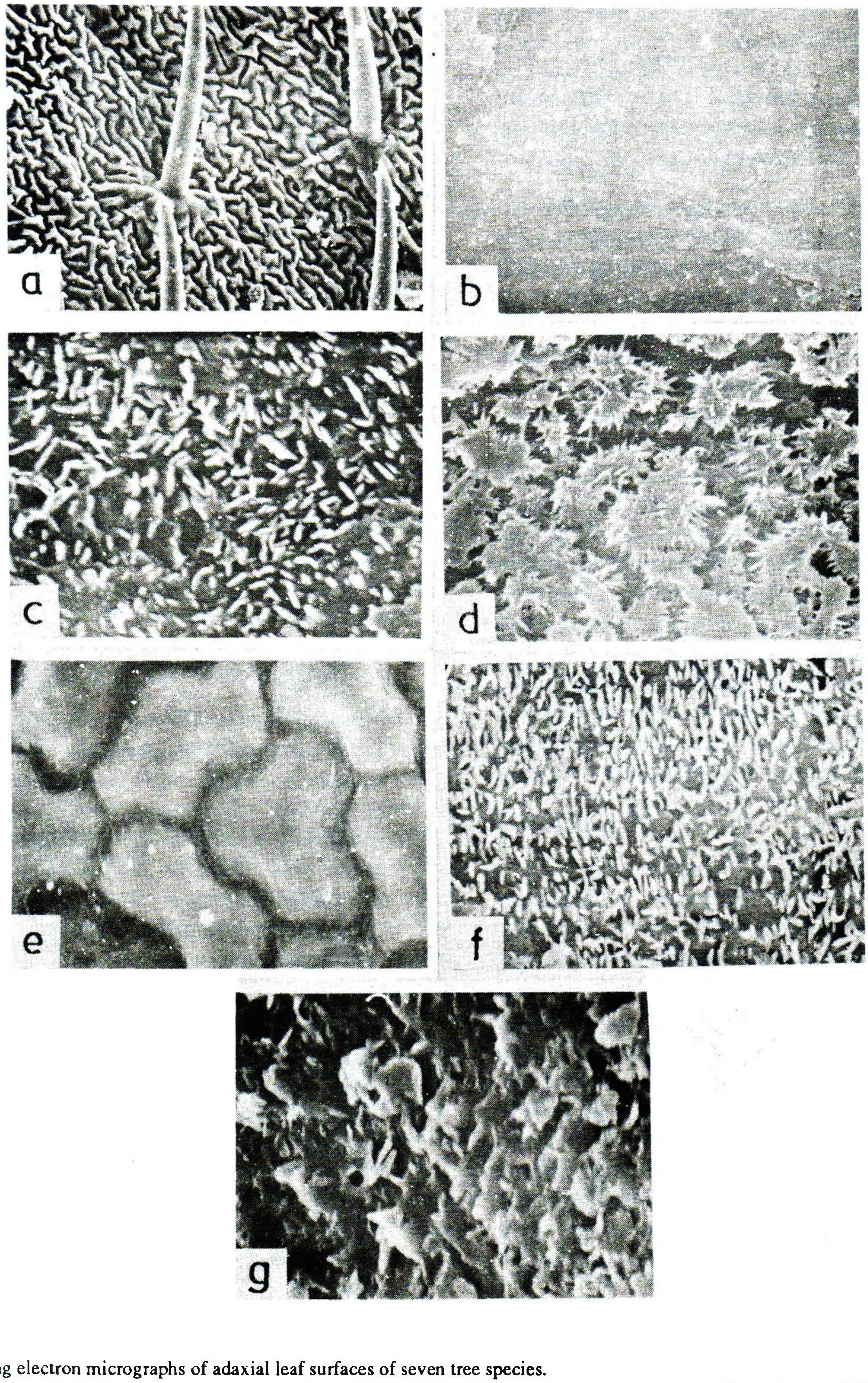
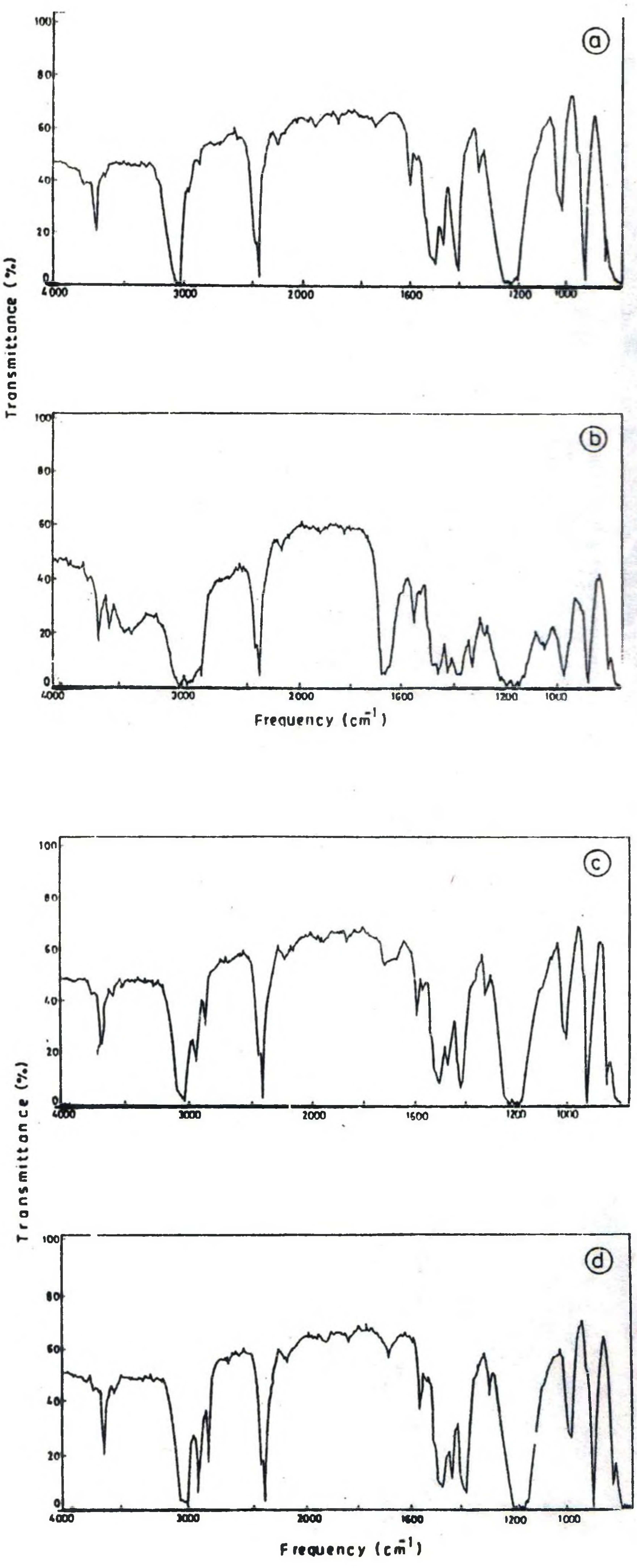
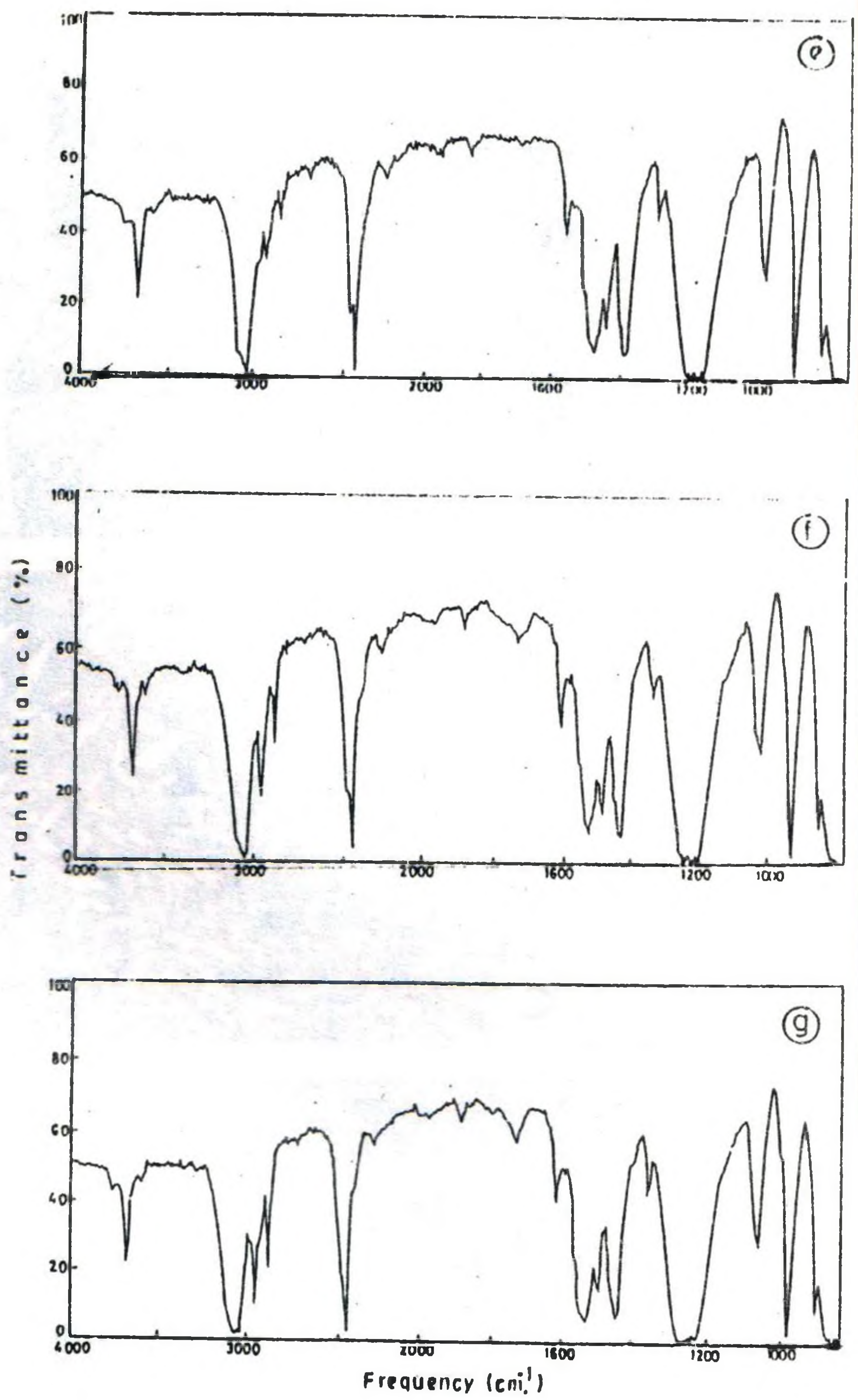


Figure 3. Scanning electron micrographs of adaxial leaf surfaces of seven tree species.
(a) *Chukrasia tabularis* x 200, (b) *Dolichandrone atrovirens* x 3000, (c) *Eugenia jambolana* x 7200, (d) *Gmelina arborea* x 7200, (e) *Lannea coromandelica* x 1000, (f) *Terminalia arjuna* x 7200, (g) *Teminalia bellerica* x 7200





(February-July) and low light period/winter/wet period (August-January).

Epicuticular Waxes : Leaf samples were collected at the end of each month. Fully mature leaves were taken for the estimation of leaf epicuticular waxes in a random manner from five plants of each species. The method followed is essentially that of Ebercon *et al.* (1977).

Scanning Electron Microscopy : The morphology of the epicuticular waxes on the adaxial surface of the mature leaves was studied with Scanning electron microscope (JEOL JSM -35 model). The leaves were dried in blotting paper were cut into about 5 mm pieces

Figure 4. Infrared spectra of leaf epicuticular waxes
(a) *Chukrasia tabularis*, (b) *Dolichandrone atrovirens*, (c) *Eugenia jambolana*, (d) *Gmelina arborea*, (e) *Lannea coromandelica*, (f) *Terminalia arjuna*, (g) *Terminalia bellerica*

and mounted on 10 mm brass specimen holder with nail polish coated with gold for 10 min. at 1.2 Kv.

Infrared absorption spectral analysis : Infrared absorption spectra of 0.5% (W/V) solutions of surface waxes in carbon tetrachloride were determined with Perkin-Elmer 783 Infrared Spectrophotometer. Peaks were identified with the help of a standard chart.

RESULTS

High temperature, low humidity and longer sunshine hours with scanty and erratic precipitation with minimum number of rainy days existed during the hot summer season. During the winter season the temperature declined with increasing humidity, precipitation and number of rainy days with shorter sunshine hours (Table 1). The highest amount of wax content (96.61 mg/dm^2) was observed in *Dolichandrone atrovirens* and the lowest one (10.48 mg/dm^2) in *Lannea coromandelica.* The surface wax content is always higher in all the seven tree species during summer when compared to winter (Fig. 1). The epicuticular wax content of the seven tree species showed monthly variations (Fig. 2). Further, the surface wax content did not showed their maximum values during the same period of a year in the tree species studied.

Scanning Electron Micrographs of adaxial leaf surfaces : The wax on the adaxial surface of *chukrasia tabularis* leaves was like fine rods of irregular shape and appears to arise vertically or obliquely from the epidermal surface. The trichomes seem to be interspersed within the wax rods (Fig. 3a). The wax was found as a simple layer of granular coating in which a continuous film is formed without any interspaces. The wax deposited as non-crystalline and forms without any interspaces. The wax deposited as non-crystalline and forms a uniform film over the surface of *Dolichandrone atrovirens* leaves (Fig. 3b). The adaxial surface was of *Eugenia jambolana* showed an aggregate wax coating, consisted of a less dense accumulation of crystalline rods or needles and these rods seem to arise horizontally form the surface of the leaves (Fig. 3c). The wax coating on the adaxial surface of *Gmelina arborea* leaf is made up of a large number of crystalline plates arranged irregularly. Each plate consists of a needle like outgrowths (Fig. 3d). The wax coating is made up of a large discrete irregular rod like structures and these irregular rod like are smooth without any fine structure. They are closely fitted in the furrows and ridges of each other and are found on the adaxial surface of *Lannea coromandelica* (Fig. 3e). The aggregate wax coating, consisted either of a dense aggregates of fine rods or needles and these rods seem to arise horizontally from the surface of the leaves of *Terminalia arjuna* (Fig. 3f). The adaxial surface of leaves in *Terminalia bellerica* showed a dense aggregates of irregular plate like projections. Some of the plates are involucral projections or folds (Fig. 3g).

Infrared absorption spectral analysis : The infrared absorption spectral analysis of leaf surface waxes (Figs. 4a-g) of the tree species examined in the present study showed a broad and intense band around 3650 cm^{-1} indicating the presence of free hydroxy group of alcohols and phenols. A strong band at 3000 cm^{-1} was considered as a characteristic absorption for the presence of alkanes of the waxes. The presence of a carboxylic acid moiety in the form of carboxylate ion displayed a strong band around 2450 cm^{-1}. A broad band around 1580 cm^{-1} was attributed to the presence of β-diketones in the enol form in all waxes. In addition to this, band at 1720 cm^{-1} represents keto group in *Dolichandrone atrovirens* (Fig. 4b) which is absent in leaf waxes of rest of the six tree species. In all waxes esters exhibited their presence around 1240 and 1110 cm^{-1}. Thus the presence of these functional groups in epicuticular waxes of all the seven tree species in the present study contained a mixture of alcohols, phenols, alkanes, carboxylic acids, β-diketones and esters.

DISCUSSION

The present study indicates the influence of environmental factors on the leaf epicuticular waxes. A high level of wax was observed in all the seven tree species during summer. Higher levels of wax during summer season have been attributed to increased light intensity and temperature which corroborates with others findings (Baker, 1974; Rao & Reddy, 1980). Similarly Hunt & Baker (1982) found heavier wax deposition in plant grown in dry habitats than those grown in wet habitats. Interaction of various environmental factors namely temperature, high light intensity, humidity and precipitation seems to be responsible for decreased levels of wax from summer to winter (Rao & Reddy, 1980).

Based on the results, it is evident that all seven tree species showed morphological characteristics of epicuticular waxes deposited on the adaxial surface of leaves. In the present study based on the quantification of epicuticular waxes in different seasons, it is evident that there exists interaction between epicuticular waxes and the environmental conditions. Therefore, specific morphological features of waxes may also vary with changes in the climatic conditions. A characteristic pattern of epicuticular waxes found in the present study may be useful to evaluate the plant climate interactions, if any. Epicuticular waxes of most higher plants appear as crystalline bodies or amorphous layer of great diversity of form and thickness (Martin & Juniper, 1970). This statement seems to be quite true in the seven tree species studied. The crystalline waxes on specific organs of individual plants consists predominently of a single structural form such as plates, tubes, ribbons, rods, filaments or dendrites (Baker, 1982).

The infrared absorption spectral analysis of waxes in the seven tree species contained a mixture of alcohols, phenols, alkanes, carboxylic acids, β-diketones and esters. In addition to this, a keto group is present in *Dolichandrone atrovirens* (Fig. 3b) which is absent in leaf waxes of remaining six tree species. Such analysis of epicuticular waxes may help in understanding plant - climate interactions.

The results of the present study clearly indicate that environmental factors can modify the levels of leaf epicuticular waxes. Further, results of the present study shows the scope to understand the plant - climate interactions using scanning electron microscope and infrared absorption spectral analysis of epicuticular waxes.

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