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Leaf anatomy and essential oil characters of *Cinnamomum pauciflorum* Nees. - a potential spice crop from North-East India

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Abstract

The anatomy and essential oil characters of leaf of *Cinnamomum pauciflorum*, a new promising source of cinnamon was studied. Eighteen components accounting for 99.8% of the total oil were identified by GC analysis of the oil. Cinnamaldehyde was the major component in the oil.

Keywords: anatomy, *Cinnamomum pauciflorum*, essential oil, leaf.

Cinnamomum pauciflorum Nees. (Lauraceae) has been identified as a promising crop of spice value from north east India (Nath 1998; Baruah 2000). It is a bushy aromatic shrub attaining a height of 1.5–5.0 m and occurs wild in eastern Himalayas, particularly in north-eastern region up to an altitude of 1800 m above MSL. In North-East India, it grows sporadically in some restricted pockets especially in Arunachal Pradesh, Assam Hills, Manipur, Meghalaya and Nagaland. A chemical race of *C. pauciflorum* having safrole as the major component in its essential oils extracted from the leaf, stem bark and lateral roots has been reported from China (Chen *et al.* 1992). However, in another chemical race, cinnamaldehyde has been reported as the only major component in the essential oils of leaf, stem and root bark of the species growing in North-East India (Nath *et al.* 1996). The significance of this chemical race is that besides having the properties of cinnamon in its bark, the mature leaves and root bark also possess the same properties, ie, warm, pungent and sweet taste which has not been found in leaves and root bark of true cinnamon (*C. verum* Presl. Syn. *C. zeylanicum* Blume.). The stem bark is used by the local tribal people of Meghalaya as a substitute of true cinnamon and is sold in the local markets as 'dalchini' or 'deinglorthia'. The present communication reports the results of studies on anatomical and essential oil characters of leaf of *C. pauciflorum.*

Live plant germplasm and voucher specimens (RRLJ 1844) of *C. pauciflorum* were maintained and preserved in the Experimental Botanic Garden and Herbarium, respectively, of Regional Research Laboratory (CSIR), Jorhat, Assam (India). Studies on epidermis and venation of the material were carried out following the methods described by Baruah & Nath (1999) and Barua *et al.* (1994), respectively.

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Table 1. Quantitative data on foliar epidermal structures of *Cinnamomum pauciflorum*

Character	Upper surface	Lower surface
No. of epidermal cell mm^{-2}	2271.00	2539.00
Length of epidermal cell (μm)	28.27	22.81
Breadth of epidermal cell (μm)	17.23	10.43
No. of stomata mm^{-2}	-	802.00
Stomatal Index	-	24.00

The quantitative data for each character were examined and recorded taking into account the average of 12 readings. For studying the characteristics of leaf powder and TS of lamina and petiole, routine methodologies were followed (Anozia 1986).

The leaves were collected from a single tree from Cherrapunjee (1250 m) of Meghalaya, during September 1999. Shade dried leaves were hydro-distilled using a Clevenger-type apparatus for 3 h. The oil thus obtained was dried over anhydrous sodium sulphate and stored in sealed vial under refrigeration. Refractive index and optical rotation of the oil were determined using a Carl Zeiss 3300 g, Abbe's refractometer and A100 automatic digital polarimeter, respectively. Density of the oil was determined by classical weighing method with the help of pycnometer. GC analysis was performed using a Varian 3700 GC equipped with FID, fitted with a 25 m x 2.2 mm stainless steel column packed with 15% SE-52 coated on 80/100 mesh Chromosorb W-HP. Nitrogen was used as the carrier gas at a flow rate of 30 ml min^{-1}. The column temperature was programmed as follows: 90^0C (12 min), 90–220^0C (@ 20^0C/min) and 220^0C (20 min). Component identification was done by comparison with reference compounds, peak enrichment and matching their 70 eV EI mass spectra with those of library search data.

Anatomy

The epidermis is composed of a single layer of cells covered by thick cuticle. Epidermal cells are tetragonal to polygonal in outline, slightly to moderately sinuous and variable in size. Stomata are confined only in the lower surface of leaf, ie, 'hypostomatic'. The stomata are of sunken type, randomly distributed and irregularly oriented (Fig. 1A, 1B). Sunken type of stomata are however, reported in six Philippine (Santose 1930) and eight North-East Indian (Baruah & Nath 1997) *Cinnamomum* species. The quantitative data on epidermal characters are presented in Table 1.

Major venation pattern (Fig. 1C) acrodromous; primary (mid) vein moderate and straight, lateral primary veins suprabasal and perfect, running in convergent arches and gradually disintegrated at the tip, secondary veins faint, 1–2 mm apart. Areoles constituting the minor venation pattern are tetragonal to polygonal in outline and variable in size at apical, middle and basal regions of the lamina. Average absolute areoles number (in 000) is 75.3. Veinlet entering in areole absent (Fig. 1D–F). Veinlet entering in areole are also reported absent in linalool and citral chemotypes of *C. sulphuratum* Nees (Baruah *et al.* 1999), although most of the *Cinnamomum* species possessed veinlet entering with simple or branched veinlet endings (Baruah 2000; Barua *et al.* 1994). The quantitative data on venation characters are presented in Table 2.

The dried leaves with petioles when powdered are greyish-green to greenish-yellow

Table 2. Quantitative data on venation structures of *Cinnamomum pauciflorum*

Character	Apical region	Middle region	Basal region
Size of areoles (μm)	207.49 x 139.99	247.50 x 180.00	259.99 x 169.99
Frequency of areole mm^{-2}	38.20	34.20	32.20
Absolute areole number (in '000)	82.51	73.87	69.55
No. of veinlet entering in areole mm^{-2}	0.00	0.00	0.00

with strong pungent fragrance like 'cinnamon' spice. Under microscope (280 x), it exhibited small groups of epidermal cells with stomata, portions of vascular elements, small groups of palisade cells, simple to compound starch grains containing cells, stone cells and other cell contents.

The leaf lamina is dorsiventral in structure. TS of the leaf through midrib shows that both upper and lower epidermis are uniseriate, while at the mid-vein region the epidermis are biseriate and covered with thick cuticle. The epidermal cells are thick walled, ellipsoid to oval in shape and comparatively smaller in size than its neighbouring tissues. Stomata are confined to the lower surface, lying in depression of epidermal cells or sunken in nature. No stoma was noticed at the mid-vein or its adjacent lamina portion. Below the biseriate epidermal layer is a continuous zone of oval to rectangular parenchymatous tissue. It is followed by 3–5 layers of polygonal to thick walled sclerenchymatous tissue surrounding the vascular bundles. The xylem is present towards the upper side and the phloem towards the lower side. The xylem bundles are arranged in 10–12 radial rows. The lamina portion next to uniseriate upper epidermal layer is followed by a layer of palisade cells which are filled with chloroplast. The palisade cells are radially-elongated and are arranged in a row. The palisade ratio is 2. The palisade cells measure 54.60–62.52 µm x 14.56–21.84 µm. In between the palisade cells and lower epidermal cells there are several layers of loosely arranged mesophyll cells with intercellular spaces known as spongy parenchyma. The spongy parenchyma cells also contain chloroplast pigments and measure 43.68–54.60 µm x 21.84–29.12 µm (Fig. 1G).

In outline, TS of petiole exhibits concave adaxial side with slightly out curving edges, while the abaxial side is sharply convex. Epidermis is uniseriate and cutinized. The hypodermis is parenchymatous. Air cavities are present in the hypodermis at regular intervals. Balasubramanian *et al.* (1993) reported the presence of air cavities in the petiole of *C. tamala* (Ham.) Th. Nees & Eberm., while these are absent in *C. camphora* (L.) Bercht. & Presl., *C. macrocarpum* Hooker f. and *C. zeylanicum*. Stone cells are present isolated or in groups of 2–5, and distributed in the ground tissue at different orientations like that of *C. macrocarpum* and *C. zeylanicum* (Balasubramanian *et al.* 1993). Oil and mucilage cells are also present in the ground tissue. The vascular bundle is arc shaped with incurving edges surrounded by a thick continuous zone of sclerenchyma at the abaxial size (Fig. 1H). The xylem is present towards the adaxial side whereas phloem towards the abaxial side ie, adjacent to the sclerenchymatous zone.

Essential oil

The oil obtained from the leaves was (4%, DWB) a yellow mobile liquid possessing a sweet spicy odour. The physico-chemical constants of the oil are as follows: Refractive index (25^0C) = 1.6045, optical rotation (25^0C) = -1.15 and specific density (30^0C) = 1.0466. Eighteen components representing 99.8% of the total oil were identified (Table 3). Cinnamaldehyde was the predominant component (94%) in the oil. Nath *et al.* (1996) reported only cinnamaldehyde as the major

Table 3. Composition of leaf essential oil of *Cinnamomum pauciflorum*

Component	% composition
Benzaldehyde	trace
α-Pinene	0.30
Camphene	trace
β-Pinene	0.25
p-Cymene	trace
1,8-Cineole	0.10
Cis–Trans linalool oxide	0.07
Linalool	2.30
Borneol	0.80
Terpinen-4-ol	0.16
α-Terpineol	0.15
(Z)-Cinnamaldehyde	0.70
(E)-Cinnamaldehyde	94.00
Eugenol	0.40
Cinnamyl acetate	0.34
(Z)-Nerolidol	0.11
Caryophyllene oxide	0.14

Trace=< 0.05%

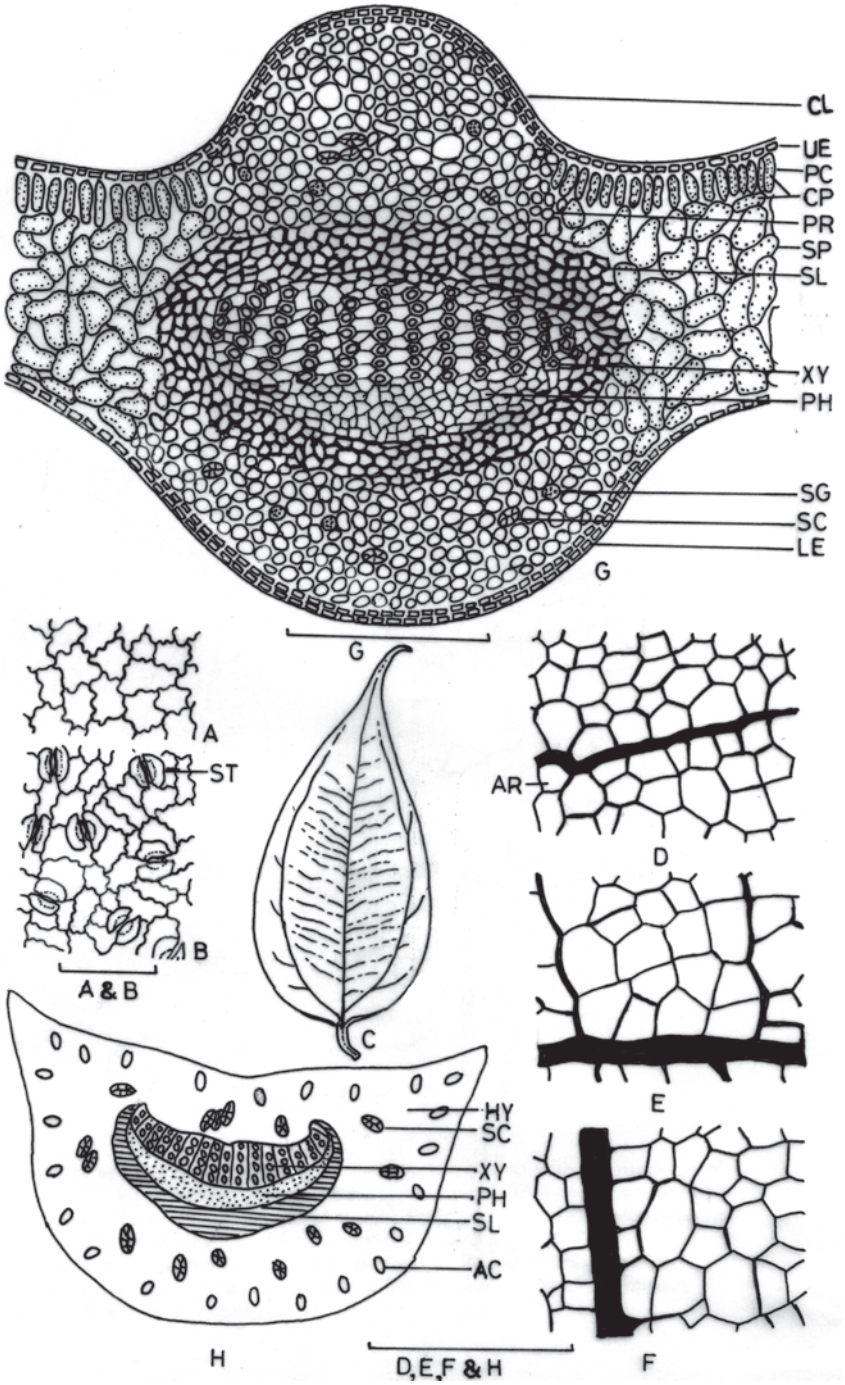

Fig. 1. Anatomical structures of the leaf of *Cinnamomum pauciflorum* (CL=cuticle, UE=upper epidermis, PC=palisade cells, CP=chloroplast pigments, PR=parenchyma, SP=spongy parenchyma, SL=sclerenchyma, XY=xylem, PH=phloem, SG=grains starch, SC=stone cells, LE=lower epidermis, ST=stomata, AR=areole, HY=hypodermis, AC=air cavity). A & B - Epidermal structures. A - Astomatiferous upper surface. B - Stomatiferous lower surface. C - Major venation pattern. D–F - Minor venation pattern at Apical (D), Middle (E) and Basal (F) regions. G - TS of Lamina. H - TS of petiole (Diagrammatic). Scale Bar: A & B=50mm; D–F, H=1mm; G=2mm.

component in the leaf oil of *C. pauciflorum* from North-East India, in contrast to safrole, which was the major component of the leaf oil of the species from China (Chen *et al.* 1992).

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