Structure and distribution of extrafloral nectaries (EFN) in Clerodendrum L. (Verbenaceae)

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Abstract
Extrafloral nectaries in 12 species of Clerodendrum L. have been studied and classified structurally into 'Philippinum' and 'Viscosum' types. A key for identification of the species studied based on their structure and distribution is provided.

Key words: Extrafloral nectaries, taxonomy, Clerodendrum.

1. Introduction
Extrafloral nectaries (EFNs) have a wide occurrence in angiosperms1-3. Morphologically and taxonomically EFNs are of considerable interest besides their role in biology of the plants concerned. Within the genus Clerodendrum, they have so far been reported in C.philippinum, C.splendens, C.aculeatum, C.squamatum, C.trichotomum and C.foetidum4-13.

The present study deals with the structure and distribution of the EFNs in 12 species of the genus Clerodendrum.

EFNs literally are those nectaries that are present on the vegetative region. However, some which are present on the floral parts, as in Clerodendrum and others4, are often included in the category. Therefore, their classification on the basis of their topographic distribution as proposed by earlier workers is not considered to be appropriate1,14. In the present study, they are classified on morphological basis. Accordingly, the EFNs in Clerodendrum are recognised as 'Viscosum' and 'Philippinum' types.

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Table I
Distribution of extrafloral nectaries in Clerodendrum L.

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Species</th>
<th>Leaf</th>
<th>Petiole</th>
<th>Stem</th>
<th>Bract</th>
<th>Sepal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adaxial</td>
<td>Abaxial</td>
<td>Adaxial</td>
<td>Abaxial</td>
<td>Adaxial</td>
</tr>
<tr>
<td>1</td>
<td>C. aculeatum Griseb.</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>C. culeiforme Linn.</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>C. indicum (Linn.) O.Kuntze.</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>C. inermes Gaertn.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>C. minorulose Teijm &amp; Binn.</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>6</td>
<td>C. nepifolium Wall.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>C. phillipinum Schauer.</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>C. phlomidis Linn.</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>C. serratum G.Don.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>C. splendens G.Don.</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>C. thomsonae Balf.f.</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>C. viscosum Vent.</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>


2. Material and methods
Mature vegetative and floral parts of 12 species of Clerodendrum (Table I) were fixed in Carnoy's fixative. Subsequently, epidermal peels of leaf, petiole, stem, bracts and sepals were taken following Leelavathi and Ramayya. Microtome sections (10-15 µm thick) of the organs were prepared following the traditional technique. Sections were stained with crystal violet and haematoxylin or basic fuchsina. The terminology used to describe the EFNs is after Ramayya and Padma Rao.

3. Observations
As stated earlier, the extrafloral nectaries of Clerodendrum have been distinguished based on structure, into two types: i) Philippinum, and ii) Viscosum. In the Philippinum type, the stalk cells are always more than three, being usually 13 to 33, forming into a single tier (Figs 1, 8, 9). On the other hand, in the Viscosum type, the stalk cells are 1 to 3, juxtaposed and subtend the head at its centre (Figs 2-7). In the Philippinum type, the anticalinal walls in transection of the head are conspicuously thickened, whereas such thickenings are absent in the Viscosum type. The EFNs of Philippinum type are either in straight flush with the epidermis or sunken in it, while those of Viscosum type emerge above the surface of the epidermis. Further details of the structure of the two types of nectaries are presented here.

Philippinum type: Foot: Compound, 10- to 40-celled, distinct, 1-tiered, cells squarish to rectangular, 8 to 16 µm high; contents slightly dense; walls thin. Stalk: 13- to 33-celled, 1-tiered, cells rectangular to squarish, 8-16 µm high, contents opaque; periclinial walls thin, anticlinal walls thick, seeming biconvex in shape. Head: Patelliform or circular, up to 60-cell wide, 1-tiered, covered with a cuticular vesicle which usually collapses at maturity. Cells
Figs 1–9 Photomicrographs of transection of extrafloral nectaries in Clerodendrum L. 1. A portion of EFN (Philippinum type) on leaf abaxial in C. calamusum (x 320). 2 & 3, EFNs with 1- and 2-tiered head, respectively, on abaxial side of the leaf in C. aculeatum (x 113 & x 103, respectively). 4 A portion of EFN enlarged on abaxial side of the leaf in C. viscosum (x 316). 5 & 6 EFNs on leaf abaxial in C. splendens with single stalk cell (x 163 & x 152, respectively). 7 Viscosum-type EFN with two stalk cells on abaxial side of the leaf in C. viscosum (x 123). 8. EFN on abaxial side of the sepal in C. philippinum (x 74). 9. Philippinum-type EFN abaxial side of the leaf in C. philippinum (x 127).

f = foot, h = head; s = stalk; v = vesicle.
linear, 20 to 48 \(\mu m\) high; 176 \(\mu m\) to 2 mm broad; contents dense; walls thin; surface smooth (Figs 1, 8, 9).

**Distribution:** This occurs in *C.philippinum* and *C.calamitosum*. In these taxa, they are restricted to the abaxial side of the leaf. In the former, they are present in the bract and sepal while in the latter on the abaxial side of the leaf and on the petiole. In *C.philippinum*, the EFNs on the bract and on the abaxial side of sepal are relatively few but conspicuous.

**Viscosum type: Foot:** Compound, 5- to 18-celled, distinct, 1-tiered, cells squarish to polygonal, 8 to 20 \(\mu m\) high; contents dense; walls thin. **Stalk:** 1 to 3 cells broad, 1-tiered, narrower than the head cells, conspicuously large, much broader than long, occupying the central position of the head, 24 to 52 \(\mu m\) high, contents opaque; anticlinal walls thick. **Head:** Patelliform or circular, multicellular, 18-33-celled wide, 1-tiered, enclosed by a thick cuticular vesicle which usually collapses at maturity, cells linear. 24 to 36 \(\mu m\) broad; but gradually becoming squarish to even rounded towards the periphery; contents dense; wall thin (Figs 2-7).

**Distribution:** Occurs on both the leaf surfaces in *C.viscosum*, but is confined only to the adaxial in *C.aculeatum*, while in the rest of the species restricted to abaxial side of the leaf. On the other hand, EFNs are totally absent on the leaf in *C.inerme*, *C.minahassae* and *C.neriifolium* (Table I). They occur on the petiole in *C.aculeatum*, *C.minahassae* and *C.phlomidis*, but in the last two, only on the stem.

In *C.phlomidis*, EFNs are also seen on abaxial side of the bract. Sepals invariably possess them in the species studied but restricted to adaxial side of the sepal in *C.aculeatum*, *C.neriifolium*, *C.splendens* and *C.thomsonae* and towards the abaxial side in the rest (Table I).

However, the EFNs on the floral parts in *C.indicum* and *C.minahassae* could not be studied due to non-availability of material.

**4. Discussion**

**General features:** The EFNs occurring in the species of *Clerodendrum* presently studied are trichomic in character as held by Dop and Duffas. From a morphological viewpoint, the EFNs are variously treated by different authors. They are described to consist of either stalk and body or stalk and head. But the present study shows that the EFNs can be marked into foot, stalk and head, like any other trichome type.

In the EFNs, the basal embedded part represents the foot, which has been otherwise reported as 'cells in the basal region' or 'supply tissue'. The foot is many celled and 1-tiered. It could also be compound in structure, but this requires confirmation through a study of its development.

The stalk has also been variously described. For instance, it is treated as a layer of polygonal cells, with the vertical walls suberised, 'filter tissue', 'merely 2-celled
structure\textsuperscript{19}, '2-layered'\textsuperscript{12} or '1–4-celled structure'\textsuperscript{8}. Presently, the stalk has been found to be 1-layered, consisting of 1–3 cells in the Viscosum type, while many celled in the Philippinum type, but in both, the vertical walls are fairly thickened. Chavan and Deshmukh\textsuperscript{19} reported the stalk to be uniseriate and 2-celled in \textit{C.aculeatum}, which is presently seen to be only 1-celled (Figs 2, 3). In \textit{C.splendens}, the stalk has been reported as 2-layered containing many cells\textsuperscript{12} but it has been now found to be 1–3 celled, the cells forming a single tier.

The head of the EFNs in \textit{Clerodendrum} has been previously described as consisting of 'Palisade-like epidermis'\textsuperscript{6,9,19} 'Palisade-like epithelial cells'\textsuperscript{11,12} or 'Secretory mass made up of 2–3 rows of numerous narrow cells'\textsuperscript{7}. In all the species currently investigated (Table I), the head is, however, found to be uniformly 1-tiered and palisade-like confirming the earlier observations excepting in \textit{C.aculeatum} (Fig. 3) wherein it is also 2-tiered.

The broadest EFN (up to 2 mm) is found in \textit{C.philippinum} (Fig. 8) while the narrowest (64 \textmu m) in \textit{C.aculeatum} (Fig. 3). Among the cells comprising the EFNs, the broadest (203 \textmu m) are found in the stalk (Figs 4, 7) and the narrowest (about 7 \textmu m) in the head in \textit{C.viscosum}. In width, the head is up to 60 cells as found in the EFNs of the Philippinum type in \textit{C.philippinum}, while it is 18–33 celled in the Viscosum type as found in \textit{C.aculeatum} and \textit{C.splendens}. The lateral walls are the thinnest (1.5–3 \textmu m) in the secretory tissue of the head, while those in the stalk are the thickest (up to 10 \textmu m) in \textit{C.viscosum}.

Distribution: The organographic distribution of EFNs in \textit{Clerodendrum} is presented in Table I. They occur on the leaf, petiole, stem, bract and sepal, while they are absent on other parts. Further in a given species, all the plant parts listed (Table I) may not possess EFNs. They are common on the leaf and sepal and less frequently on the petiole, bract and stem (Table I) in the species studied.

Though the EFNs are diffuse in distribution, they are denser at proximal end abutting the main veins, in the leaf abaxial, whereas sparser elsewhere. Of the 12 species presently studied, the EFNs are conspicuous on the bract and on abaxial side of the sepal only in \textit{C. philippinum} (Fig. 8).

The distribution of the EFNs in the taxa studied from organ to organ is found to be variable and hence taxonomically significant. Based on the above, a key for the identification of all the taxa studied is provided except in \textit{C.indicum}, in which as stated elsewhere, information on their distribution in the floral parts could not be made for want of material.

5. Key for the identification of the \textit{Clerodendrum} species based on structure and distribution of EFNs.

I. A. Extrafloral nectaries (EFNs) absent . . . \textit{C.serratum}.

I. B. EFNs present.

II. A. EFNs Philippinum type.

III. A. EFNs present on petiole . . . \textit{C. calamitosum}
III. B. EFNs absent on petiole . . . *C. philippinum*

II. B. EFNs of Viscosum type.
   IV. A. EFNs present on leaf.
      V. A. EFNs occurring on both surfaces of leaf . . . *C. viscosum*
      V. B. EFNs occurring on either abaxial or adaxial leaf surfaces.
      VI. A. EFNs occurring on adaxial leaf surface only . . . *C. aculeatum.*
      VI. B. EFNs occurring on abaxial leaf surface only.
      VII. A. EFNs present on petiole . . . *C. phlomidis.*
      VII. B. EFNs absent on petiole.
      VIII. A. EFNs up to 210 μm wide . . . *C. thomsoniae.*
      VIII. B. EFNs up to 1 mm wide . . . *C. splendens.*

IV. B. EFNs absent on leaf.
   IX. A. EFNs present on petiole . . . *C. minahassae.*
   IX. B. EFNs absent on petiole.
   X. A. EFNs present on sepal abaxial only . . . *C. inerme.*
   X. B. EFNs present on sepal adaxial only . . . *C. neriifolium.*

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