Petiole anatomy of Indian species of the genera *Smilax* L. and *Heterosmilax* Kunth. (Smilacaceae).

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Abstract: Petiole anatomy of 12 species, 11 species of *Smilax* L. and one species of *Heterosmilax* Kunth., of the family Smilacaceae have been studied. Anatomical features of the petiole provide supporting evidences for the infra-specific circumscription of the occurring in India.

Keywords: Petiole anatomy, taxonomy, *Smilax*, *Heterosmilax*, India

Introduction

*Smilax* L. (1753: 1028) is the type genus of family Smilacaceae, with ca. 350 species [1] mainly distributed in tropical and temperate areas throughout the world, but mostly confined to Asia and America [2]. The genus *Heterosmilax* is a small south eastern Asiatic genus that is closely allied to the genus *Smilax* [3]. *Heterosmilax* is comprised of about 11 species recorded from southern China, Myanmar, Thailand, Indo-China, and western Malaysia, with one species extending to the Ryukyu Archipelago, and Assam and Khasia in India [4]. In India the family represented by 33 species of *Smilax* L. and another four species *v.r.* *elicrisium, S. decipiens, S. bolganga* and *S. singaporensi* as doubtful species and two species *v.r.* *S. multibhi, S. villandia* as imperfectly known species [5]. Out of 33 species reported by Hooker only 24 are from the present political boundary of India which includes five species from South India and 17 from northeast India [6]. However, like other monocots of NE India the Smilacaceae is yet to be properly documented and studied. Recently, from north eastern part of India several important studies have been performed on genus *Smilax* and *Heterosmilax* [7-9].

The identity of the genus *Smilax* is often difficult due to close morphological similarity of the species and dioecious nature of the plants. Most often it is unlikely that both male and female plants occur in the same locality and this very often leads to difficulty in ascertaining the proper identity of the members of the genus. Reproductive characters often are also not reliable for species delimitation [10]. Therefore, alternative attributes are required to resolve identity of the species with certainty.

In recent years, anatomical characters including the petiole characters have been widely used in solving taxonomic problems in vascular plants [11-15]. The structure of petiole shows differences between genera and species. In the present study, anatomical characters of the petiole of 12 species belonging to the family Smilacaceae were examined and compared.

Materials and Methods

Plant materials were collected from different localities of Northeastern India (Table 1). The collected plant materials were fixed in F.A.A. and then preserved in 70% alcohol. Permanent transverse hand sections of middle part of petiole were made and stained with Safrin and Fast Green [16] dehydrated with an ethanol gradient (70% in three steps to 100%) and finally incubated in xylol (99.5%). The stained dehydrated sections were then mounted on slides using DPX and studied under binocular research and compound Nikon microscope.

Results and Discussion

Cross sections of petioles are shown in Plate No. I, Figs. a-i. There are three kinds of tissue in each cross section (Figs. a-i). The exterior parenchymatous cortical tissue at the periphery of petiole is bounded by a single layer of epidermis. The centre of the petiole is occupied primarily by parenchymatous ground tissue. Vascular bundles are the third tissue.

*Smilax perfoliata* [Fig. a]

The petiole in t.s. is shallowly grooved with two short acuminate lateral outgrowths and acuminate in abaxial surface. Epidermis comprises of single layer of isodiametric cells. The 20-25 randomly distributed vascular bundles of variable size are confined to the central part of the petiole and are of different in size.

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Smilax ovalifolia [Fig. b]  
In t. s. the adaxial side of the petiole is deeply grooved with two wide obtuse lateral projections, whereas the abaxial face is acute. Epidermis consists of single layer isodiametric cells. The hypodermis is irregular in thickness and comprises of isodiametric parenchymatous cells with dark cell content. The 45-50 conjoint, collateral vascular bundles of variable size are randomly distributed and surrounded by 3-4 layers of sclerenchyma.

Smilax orthoptera [Fig. c]  
In t. s. petiole is flat on adaxial side without lateral projections and almost round on abaxial side. Epidermis is of single layer with thin cuticle. The hypodermis is of two-layers of chlorenchymatous cells. The 12-13 vascular bundles of variable size are arranged in two circles around a central zone.

Smilax ocreata [Fig. f]  
In cross section the adaxial side of the petiole is horizontally flat and nearly round on adaxial side. The single epidermal layer has thin exterior cuticle layer. Hypodermis varies in thickness with dark cell content. The 20-22 conjoint, collateral vascular bundles of variable size are randomly distributed and are surrounded by 3-4 layers of schlerenchyma.

Smilax lanceolata [Fig. g]  
The outline of the petiole in t. s is horse-shoe shaped with a shallow groove on adaxial side and abruptly acute on abaxial side. Epidermal cells are circular and cutic layer is thick. Hypodermal layer is absent. The numbers of vascular bundles are 8-10 and are of different size.

Smilax aspericaulis [Fig. h]  
The outline of the petiole in t. s is slightly wavy forming angles at points with adaxial side forming a v-shaped groove and acuminate on abaxial side. The epidermal cells are cutinized, irregular in sized and several layered on the adaxial side but numbers of layers on lateral and abaxial sides are less. The 6-7 vascular bundles of variable size are encircled by 3-4 layers of sclerenchyma. Tanniferous cells are distributed in ground tissue and in vascular bundles.

Smilax arisanensis [Fig. i]  
The outline of the petiole in t. s. is circular with shallowly U-shaped groove on the adaxial side and acuminate on the abaxial side. The epidermal layer is single cell thick and cuticle is thin. Hypodermis is single layered and number of vascular bundle of variable size is 18-20.

Smilax zeylanica [Fig. j]  
The adaxial epidermis of the petiole in t. s. is slightly inclined with two unequal lateral projections and acuminate on abaxial side. Epidermis consists of single layer of thick-walled oval cells with thick cuticle. Hypodermis is distinct and consists of either single or double layers. Numbers of vascular bundles are 14-16 and their size is variable.

Smilax sp. [Fig. k]  
As exhibited in t. s. the adaxial side of the petiole is deeply grooved with two lateral projections overlapping each other and the abaxial face is round. Epidermis consists of single layer of thick-walled round cells with cuticle. The 5-7 variable size vascular bundles are arranged in a linear pattern and each of them are surrounded by 3-4 layers of sclerenchyma.
**Heterosmilax japonica** [Fig. 1]  
Adaxial face of the petiole as exhibited in *S. arisanensis*, *S. ovalifolia*, *S. arisanensis*, *Smilax sp.* and *S. orthoptera* abaxial face have been recorded as round. The pattern of arrangement of vascular bundles also varies from linear to circular or even scattered among the species studied. The scattered vascular bundle has been recorded in *Smilax ovalifolia* and *S. perfoliatia*. The linear arrangement of the vascular bundle has been recorded in *S. china*, *S. glabra* and *Smilax sp.*. The numbers of vascular bundles vary from species to species (Table 2). The vascular tissue is capped by sclerenchyma layer in all the species. Sclerenchymatous layer are continuous in *S. aspericaulis*, *Smilax sp.* and *Heterosmilax japonica*. The ground tissue is parenchymatous in all the species studied but it is massive covering the central part except in *Smilax china*, *S. aspericaulis* and *Smilax sp.*. The tanniniferous cells are recorded in the cortex and in the ground tissue in *S. aspericaulis* and *Smilax sp.* respectively.

Scrutiny of the literature revealed that no work has so far been done on anatomy of petiole of species of *Smilacaceae*. Data obtained from the present study shows that anatomy of the petiole can be used for proper identification of these species. The significant attributes of the petioles for the genera *Smilax* and *Heterosmilax* are scattered vascular bundles and acumenation on abaxial side. The epidermis is consisting of single layer followed by 10-12 layers of parenchymatous cortex. The numbers of vascular bundles are 10-12 and their sizes are different.

### Table 1. Localities of studied *Smilacaceae* taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Locality</th>
<th>GPS Coordinates</th>
<th>Specimen acc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smilacaceae</td>
<td>Khetri, Kamrup, Assam</td>
<td>553 (GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Smilacaceae</td>
<td>Khetri, Kamrup, Assam</td>
<td>480 (GUBH, Assam, India)</td>
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<tr>
<td>Smilacaceae</td>
<td>Halakandi, Assam</td>
<td>531 (GUBH, Assam, India)</td>
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<tr>
<td>Smilacaceae</td>
<td>Kothd-iati, Ri-Bhoi, Meghalaya</td>
<td>533 (GUBH, Assam, India)</td>
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</tr>
<tr>
<td>Smilacaceae</td>
<td>Tinsukia, Assam</td>
<td>535 (GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Smilacaceae</td>
<td>Wall. ex A. DC.</td>
<td>587 (GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Smilacaceae</td>
<td>Karbi Anglong, Assam</td>
<td>651 (GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Smilacaceae</td>
<td>North Cacher Hills, Assam</td>
<td>487(GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Smilacaceae</td>
<td>Hayata East Stang, Arunchal Pradesh</td>
<td>226(GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Smilacaceae</td>
<td>East Stang, Arunchal Pradesh</td>
<td>622 (GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Smilacaceae</td>
<td>Tinsukia, Assam</td>
<td>532 (GUBH, Assam, India)</td>
<td></td>
</tr>
<tr>
<td>Heterosmilax</td>
<td>Kunth Karbi Anglong, Assam</td>
<td>538 (GUBH, Assam, India)</td>
<td></td>
</tr>
</tbody>
</table>

*Gauhati University Botanical Herbarium (GUBH)*

### Table 2. Comparative petiole anatomical features of the studied species

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Shape of the petiole</th>
<th>Nature of cuticle</th>
<th>Hypoderms</th>
<th>Sclerenchyma layers in vascular bundle</th>
<th>No. of vascular bundle layer</th>
<th>Larger size</th>
<th>Small size</th>
<th>Arrangement of vascular bundles</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. perfoliatia</td>
<td>shallowly grooved</td>
<td>acuminate</td>
<td>Thick</td>
<td>single layer</td>
<td>3-4 layers</td>
<td>15</td>
<td>10</td>
<td>scatteredly arranged</td>
</tr>
<tr>
<td>S. china</td>
<td>deeply grooved</td>
<td>acuminate</td>
<td>Thick</td>
<td>not distinguish</td>
<td>3-4 layers</td>
<td>3</td>
<td>5</td>
<td>linear</td>
</tr>
<tr>
<td>S. glabra</td>
<td>deeply grooved</td>
<td>acuminate</td>
<td>Thin</td>
<td>double layer</td>
<td>2-3 layers</td>
<td>3</td>
<td>8</td>
<td>linear pattern</td>
</tr>
<tr>
<td>S. ovalifolia</td>
<td>deeply grooved</td>
<td>acuminate</td>
<td>Thin</td>
<td>single layer</td>
<td>3-4 layers</td>
<td>22</td>
<td>20</td>
<td>scatteredly arranged</td>
</tr>
<tr>
<td>S. orthoptera</td>
<td>flat</td>
<td>round</td>
<td>Thick</td>
<td>double layer</td>
<td>2-3 layers</td>
<td>8</td>
<td>4</td>
<td>circular pattern</td>
</tr>
<tr>
<td>S. ovata</td>
<td>flat</td>
<td>round</td>
<td>Thick</td>
<td>single layer</td>
<td>3-4 layers</td>
<td>11</td>
<td>10</td>
<td>scatteredly arranged</td>
</tr>
<tr>
<td>S. lanceifolia</td>
<td>horse-shoe shaped</td>
<td>slightly acute</td>
<td>Thick</td>
<td>single layer</td>
<td>1-2 layers</td>
<td>8</td>
<td>2</td>
<td>circular pattern</td>
</tr>
<tr>
<td>S. aspericaulis</td>
<td>slightly waved</td>
<td>acuminate</td>
<td>Thick</td>
<td>single layer</td>
<td>5-6 layered</td>
<td>6-7</td>
<td>-</td>
<td>circular arranged</td>
</tr>
<tr>
<td>S. arisanensis</td>
<td>shallowly u-shaped</td>
<td>acuminate</td>
<td>thin</td>
<td>double layer</td>
<td>3-4 layers</td>
<td>10</td>
<td>8</td>
<td>scatteredly arranged</td>
</tr>
<tr>
<td>S. zeylanica</td>
<td>slightly inclined</td>
<td>acuminate</td>
<td>Thin</td>
<td>double layer</td>
<td>2-3 layers</td>
<td>12</td>
<td>2</td>
<td>scatteredly arranged</td>
</tr>
<tr>
<td>Smilax sp.</td>
<td>deeply grooved</td>
<td>O-shaped</td>
<td>round</td>
<td>Absent</td>
<td>3-4 layers</td>
<td>3</td>
<td>2</td>
<td>linear line</td>
</tr>
<tr>
<td>H. japonica</td>
<td>Flattish</td>
<td>acuminate</td>
<td>Thick</td>
<td>Absent</td>
<td>1 layer</td>
<td>4</td>
<td>8</td>
<td>circular line</td>
</tr>
</tbody>
</table>

**References**


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