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Comparative Study of Foliar Venation Pattern and foliar Anatomy of Two Species of Anaphalis Dc. (Asteraceae)

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ABSTRACT

Leaves are the indissoluble and constant part of a plant body. The gross form of angiosperm leaves include features like size, shape, nature of margin, form of the apex, base and petiole, positioning of glands and nature of venation pattern. They have the potential of diagnostic characters within their venation. The objective of this paper was to describe and compare the foliar morphology, venation pattern and foliar anatomy of *Anaphalis margaritacea* (L.) Benth. & Hook. f. and *Anaphalis triplinervis* Sims. ex C. B. Clarke. to distinctly identify the plants. The major venation pattern from fresh as well as dried leaves along with areoles, minor veins, marginal venation, veinlet endings, the 'very tips' and other associated characters were analyzed for the two species. Three primary veins, sparsely placed areoles arranged in the longitudinal direction of the lamina, free vein endings consisting of one spirally thickened tracheid and some free vein endings associated with sclerotracheoids atre the distinctive characters of *A. margaritacea***.** Whereas, in *A. triplinervis***,** the number of primary veins are five with dense areolation, arranged randomly. The free vein endings consist of four to five spirally thickened tracheids and covered with distinct sclerenchmatous sheath cells throughout. Other characters of the leaf of two species in addition to the above stated features can be elaborated asan identification key.

Introduction

Generally the floral characters of the plants are used for the identification of angiosperms. In 1974 Radford et. al. have pointed out that due to lack of detailed classification features of leaf, they are neglected organs in taxonomic and comparative morphological studies. The arrangement of veins in the lamina is an important component of the study of leaf architecture. Though the leaves are present in plants for much greater part of its life span than the flowers and fruits, still its use in identification of plants is very limited.After the publication of venation pattern terminologies by Ettingshaesun (1861) and systemization of these terminologies by Hickey (1973) attempts have been made to study the leaf architecture of some dicotyledonous families (Sehgal & Paliwal,1974; Jain, 1978). In 1976, Melville gave the venation pattern terminologies for both monocots and dicots. In the recent past attempts have been made to study the leaf architecture of different species belonging to different genera

under family Asteraceae by Banerjee & Deshpande (1973), Banerjee (1978) Ravindranath &Inamdar (1982), Akinubi et al. (2014) and others. A similar kind of work was done by Maitra and Mukherjeein 2017 on 4 species of *Spilanthes.*

So farno work has been done on leaf architectural pattern of the members of*Anaphalis* sp. In this paper the foliar anatomical characters of. *Anaphalis margaritacea*(L.) Benth. & Hook. *f*. and *Anaphalis triplinervis* Sims ex C. B. Clarke. are studied and considered as an additional tool for identification ofthis genus

Materials and Methods

Materials were collected from Darjeeling districts of West Bengal and East Sikkim in sets of 4-5 in numbers and identified with the local floras. These specimens were matched with the collections of the Department of Botany, University of Kalyani, Nadia. Author citations were matched following literature of Brummitt and Powell (1992) and website of the International Plant Names Index (www.ipni.org) and The Plant List (www.theplantlist.org).The voucher specimens were kept at the departmental herbarium of Government General Degree College, Keshiary. The specimens are listed in Table 1.

For future use matured leaves are preserved in FAA solution where the ratio of commercial formaldehyde: acetic acid: 70% alcohol is 5:5:90. Ten leaves of each species collected from five different plants were cleared following the techniques of Dilcher, 1974.

For studying theleaf vascularization, the entire lamina along with petiole was soaked overnight in 4% NaOHand transferred to chloral hydrate solution (Arnott,1959) and finally stained with 1% aqueous safranin. After gradual dehydration, permanent slides were prepared and mounted in Canada balsam. For representation of the vein orders upto 3rd order, magnifications with 5x eye piece and 4x objective of the microscope has been used and for higher order venations a suitable median portion of the lamina, 1/3 from apex and 2/3 from base of the laminawere selected and the vascular pattern drawn from the 3rd order onwards along with epidermis and trichomes, under mirror type camera Lucida (Fig.1). For microscopic photographs Olympus CX21i microscope and camera UCMOS 10000 KPA of Topcam were used (Plate 1).

In the same way, marginal venation pattern and free vein endings have been sketched under higher magnification (10x eyepiece and 40x objective) of the microscope. For anatomical descriptions, work ofMetcafe and Chalk (1950) has been followed and for foliar venation patternpublications ofthe followingauthors were consulted[Hickey (1973, 1979) Melville (1976), Prabhakar and Ramayya (1982), Annamani and Prabhakar (1991a;b, 1993, 1994a;b), Ferzana*et al* . (1991)and Dilcher (1974)].

Table 1. List of Specimens Collected

S. No.	Name of Species	Localities	Field No.
1.	*Anaphalis margaritacea* (L.) Benth.& Hook. *f.*	Happy Valley Tea Estate, Darjeeling Dist. Rishav, Darjeeling District Juluk, East Sikkim	76 112 145
2.	*Anaphalis triplinervis* Sims ex C. B. Clarke.	Tipindara, Rishav, Darjeeling District Juluk, East Sikkim	116 143

OBSERVATIONS

The identity of the two species of *Anaphalis* viz. *A. margaritacea* and *A. triplinervis* has been established on the basis of leaf morphology (Table 2) and anatomy (Table 3).An artificial key to the species was alsodeveloped:

Table 2. Leaf Morphology of *Anaphalis* spp.

Name of the species / Characters	*A. margaritacea*	*A. triplinervis*
Leaves	Leaves alternate, simple, narrowly linear or oblong,1 - 3 cm long x 0.1-0.5 cm wide, acute at apex, margin entire,auriculate at base, herbaceous, densely woolly on lower surface, less on upper side, sessile.	Leaves alternate, simple, lanceolate to elliptic-lanceolate,3-5 cm long x 1-1.5 cm wide; acute to acuminate at apex, margin entire, cuneate at base, leathery, usually 3-veined, woolly-white above and rusty-grey beneath, sessile.

DISCUSSION

Leaf anatomy according to Carlquist (1961) provides a variety of features that could be used for taxonomic purposes. This is evident from the leaf characters present in the two species of *Anaphalis*. Leaf morphology to leaf anatomy of both the species have lots of contrasting feature expressed in the observation table 2 and 3. The most eminent among them are the leaf shape, primary vein number, quaternary veins, areolar type, areolar density, veinlets and their association, epidermis and trichomes as mentioned inTable 2 and 3.Figure 1 and Pate 1 are also there to substantiate the observation. The most noticeable character among all, is the veinlets association with sclerotracheoids in *A. margaritacea*whereas in *A. triplinervis*,sclerenchymatous sheath cells covering each and every order of veins (Fig.-1C, C' and Plate 1 B, B').

CONCLUSION

It is proved that leaf venation pattern and leaf anatomical characters are stable in the studied species of *Anaphalis* Dc. From the present findings it may be concluded that leaf architecture characters are good taxonomic markers in plant identification and classification. However, it is also clear from the studies that these characters of leaves are potentially significant to delimit the taxa and

Table 3. Leaf anatomy of *Anaphalis* spp.

Name of the species / Characters	*A. margaritacea*	*A. triplinervis*
Venation type	Perfect-acrodromous	Palmate-acrodromous
Primary vein (1°)	3, basal, moderate, middle onestraight, lateral 2 slightly curved converging at the apex, unbranched.	5, basal, perfect, moderate, middle onestraight, lateral four curved, unbranched.
Secondary veins (2°)	10-14 pairs as connecting to primary veins, curved, branched or unbranched .	14-17 pairs as connecting to primary veins, curved, branched or unbranched
Intramarginal veins	Present as extreme lateral, continued from base to apex, merging with the primary laterals near the apex	Present as extreme lateral, primary, continued from base to apex.
Tertiary veins (3°)	Connecting the secondaries, mostly composite	Connecting the secondaries, mostly unbranched.
Quaternary veins (4°)	Rarely present	Thick, straight and relatively orthogonally oriented
Highest vein order (as free vein endings)	3°.	5°
Areoles	Areoles imperfect.	Areoles perfect
Areole shape	Mostly tetrangular rarely pentangular	Mostly tetrangular, rarely pentangular
Areoles/ sq. cm	1100	2678
Nature of free vein endings within the areole	Short in size, consisting of one spirally thickened tracheid of normal shape; the very tip normal and pointed or blunt due to coupling of very tip with sclerotracheoid	Short to medium in size, consisting of four to five spirally thickened tracheids of normal shape; the very tip normal and pointed, completely covered with sclerenchymatous sheath cells.
Associated features	The areoles in the margin linear-oblong, tetragonal, arranged according to long axis of leaves without free vein endings.	The areoles in the margin alike to the normal venation of laminar part
Upper epidermis	Consisting of compactly arranged thin walled corrugated to sinuate, elongated rhomboidal cells. Cell count ˜ 710 per sq. mm	Consisting of compactly arranged thin walled ovoid cells. Cell count ˜ 2253per sq. mm
Lower epidermis	Consisting of thin walled corrugated to undulated rectangular cells, associated with stomata. Cell count ˜ 800 per sq. mm	Consisting of compactly arranged thin walled ovoid cells, associated with stomata. Cell count ˜ 1972 per sq. mm
Stomata	Hypostomatic leaves with anomocytic stomata, stomatal frequency 160 per sq. mm	Hypostomatic leaves with anomocytic stomata, stomatal frequency 207 per sq. mm
Trichomes	Nonglandular flagellate trichome with one celled oval foot, 2-3 celled stalk whose basal cell is cylindrical and broader than median cell, head unicellular flagellate.	Nonglandular, flagellate trichome with one celled oval foot, 2-3 celled oblong cylindrical stack and unicellular long flagellate head.

on the basis of which identification keys are provided.

Key to the studied species of *Anaphalis* based on leaf morphology

1a. Leaves narrowly linear or oblong, 1–3 cm long x 0.1–0.5 cm wide, auriculate at base, herbaceous. ……***A. margaritacea***

1b.Leaves lanceolate to elliptic lanceolate, 3–5 cm long x 1–1.5 cm wide, cuneate at base, leathery, rusty-grey beneath. …***A. triplinervis.***

Key to the studied species of *Anaphalis* based on leaf anatomy

1a. Primary veins 3, areoles quadrangular and pentagonal, arranged usually in the longitudinal direction of the lamina, free vein endings consists of one spirally thickened tracheids, devoid of sheath cells, may associated with sclerotracheoids…..…***A. margaritacea***

1b. Primary veins 5, areoles quadrangular, arranged randomly, free vein endings consists of four to five spirally thickened tracheids, covered with sclerenchymatous sheath cells throughout…… ***A. triplinervis.***

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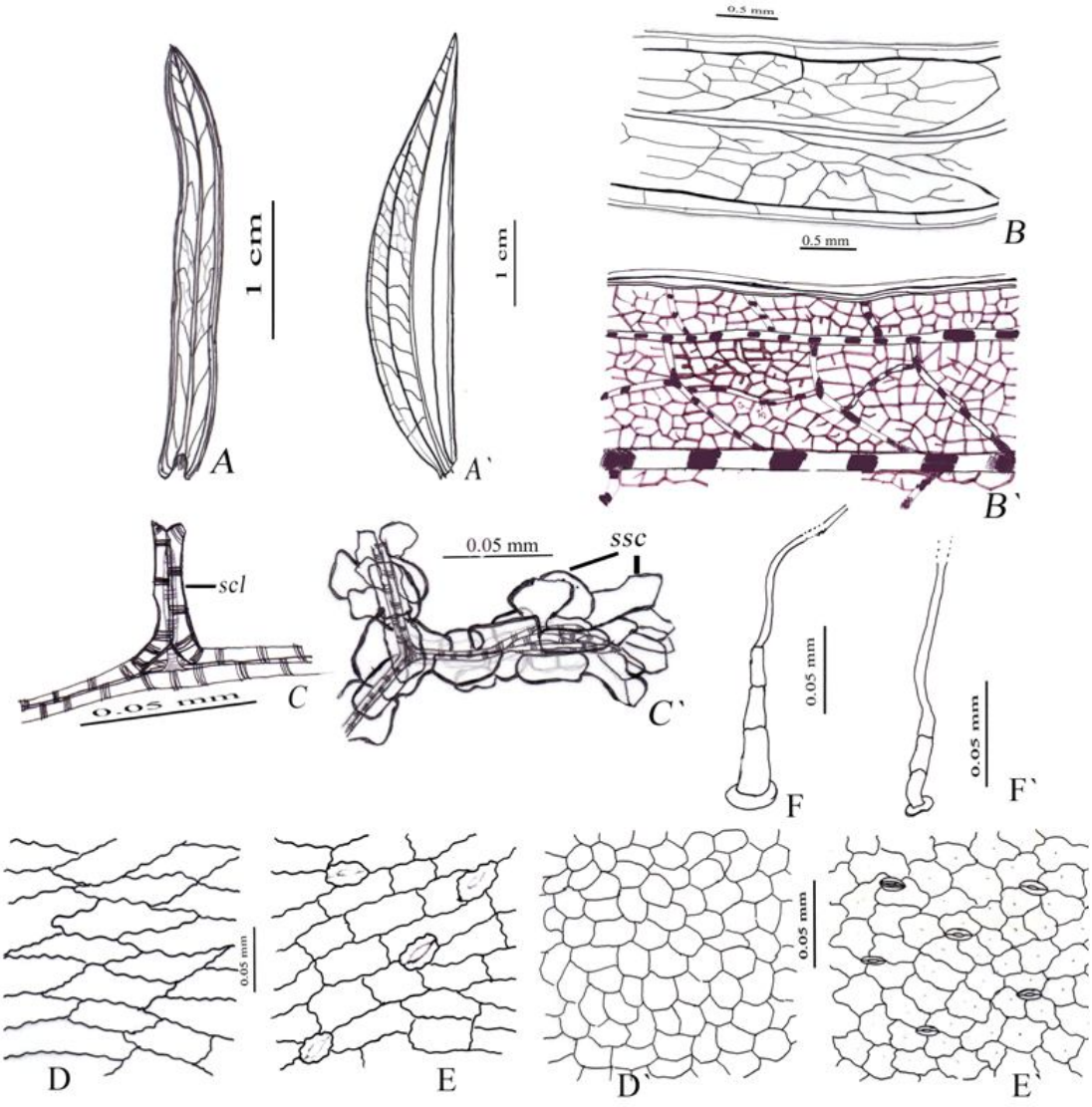

Fig 1: Foliar venation and foliar anatomy of *Anaphalis margaritacea* and *Anaphalis triplinervis*: A-F: *A. margaritacea*: A`-F`: *A. triplinervis*; A& A`. Leaf with major venations*; B-Fand B`-F` are the camera lucida diagrams.* *B & B`. Minor* venations; C & C` Free vein endings; D & D`. Upper epidermis; E & E` . Lower epidermis; F & F`. Foliar trichome;

(scl - Sclerotracheoids, ssc - Sclerenchymatous sheath cells.)

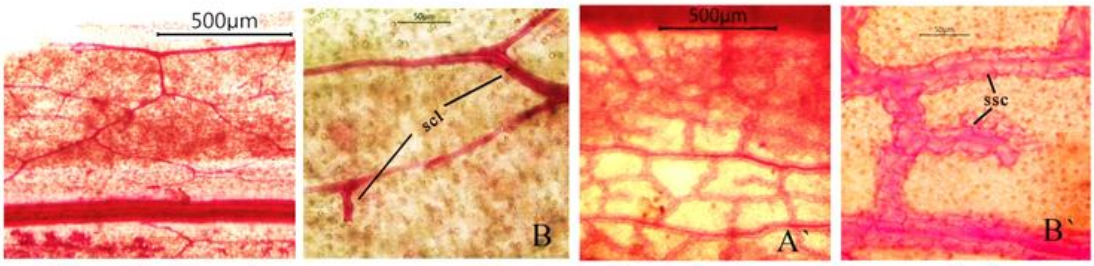

Plate 1: Venation, patterns and vein endings in the species studied: A-B. *Anaphalis margaritacea*: A. Venation pattern; B. Vein endings A`-B`. *Anaphalis triplinervis :* A`. Venation pattern; B`. Vein endings (scl - Sclerotracheoids, ssc - Sclerenchymatous sheath cells.)

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