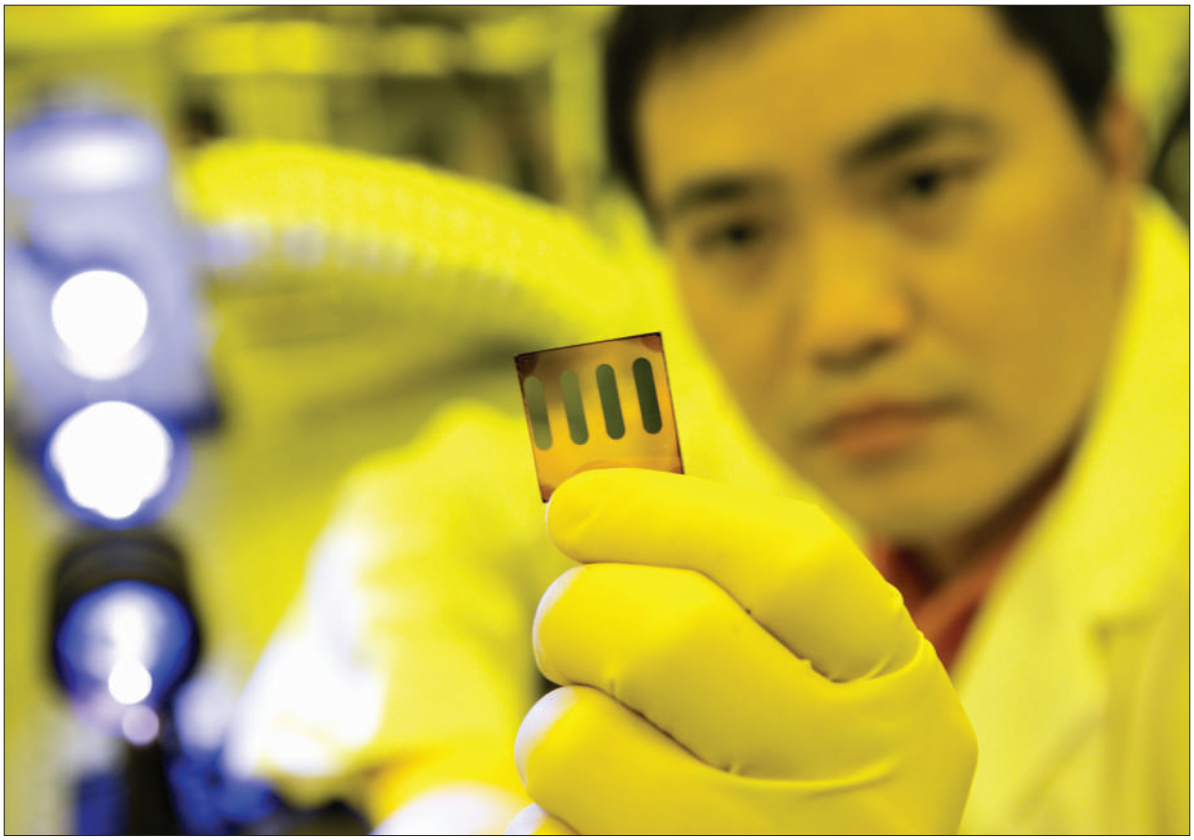

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Research Article
Systematic Significance of Petiole Anatomical Characteristics in Some Members of Asteraceae from Some Parts of Nigeria

C. Ekeke and C.A. Ogazie

Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria

Abstract

Background and Objective: Petiole anatomy of 30 species of Asteraceae belonging to 24 genera was undertaken to investigate variations in vascular bundle arrangement at the basal and distal of the petiole including the petiole outline to determine their taxonomic significance. **Materials and Methods:** Methods used in this study were petiole sectioning using a sliding microtome, staining, mounting and observation under a light microscope. **Results:** Our findings showed that all species studied have mainly three major vascular bundles and minor ones which formed either an open separate arc or open continuous/united arc with rib traces. Five different types of vascular bundle arrangements were described based on the number, size, arrangement, presence or absence of accessory and/or interspersed vascular bundles and 8 petiole outlines. Among the vascular bundle types, type-1 occurred in 19 species and 12 genera, type-2 in 8 species and 7 genera, type-3 in 3 species and 3 genera, type-4 in 12 species and 11 genera type-5 in 1 specie and 1 genus. The type-5 vascular bundle is restricted to *V. conferta*. The petiole outline type-1 occurred in 9 species, type-2 in 5 species, type-3 in 16 species, type-4 in 5 species, type-5 in 11 species, type-6 in 3 species, type-7 and 8 in 1 specie each. Twenty three of the species had a leaf-like wing at either distal or basal end of the petiole. **Conclusion:** These characters are fairly different and diagnostic especially the presence and number of accessory and interspersed vascular bundles among the taxa especially at the genus and species level.

Key words: Asteraceae, petiole anatomy, petiole outline, vascular bundle, taxonomy

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Corresponding Author: C. Ekeke, Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Petiolar anatomical features are one of the key parameters used in the identification and classification of many plant families and have been used in differentiating plants of different species, genera and families[1-5], but are imperfectly studied[2]. In recent times, there is increased interest and research on the use of petiole anatomy as a complementary tool in plant taxonomy and significant progress has been made in using this line of evidence in plant classification[6-8]. Different authors have also considered the arrangement of vascular bundles in different portions of the petiole to actually have taxonomic relevance[2,3,9]. Metcalfe and Chalk[2-3] emphasized that the distal end (the portion immediately below the lamina) has considerable taxonomic features compared to other parts of the petiole.

Asteraceae is considered one of the largest plant family with approximately 950-1500 genera and 25,000 species in different habitats[10,11]. Eighty-four genera and 273 species occur in West Africa[12]. They exhibit mainly herbaceous habits, although arboreous and voluble herbaceous habits also occur[13,14]. Because of the variety of habits, the family presents various anatomical structures and in some cases, ecological specialization may occur[3,15] but the anatomy of the petiole being sparsely or not affected[2]. Among the members of this family, considerable works have been done on the secretory structures being restricted in distribution and as an important diagnosis character[3,16] by occupying distinct positions in different organs of the plant[17,18]. The anatomical features such as epidermal and lamina characteristics of some members of this family have been described to have various types of glandular or covering trichomes, papillae on the abaxial epidermis, having anomocytic, anisocytic and rarely heliocytic stomata, presence of hydathodes, presence of hypoderm, vascular bundles with parenchymatic sheath composed by large cells[1,19]. Also, this family presents species of great importance in the fields of nutrition, cosmetics and pharmacy due to the production of essential oils[20,21].

The present study aims to complement the above treatments by examining a relatively large number of species from Nigeria. In total, the petiole of 30 Asteraceae species was studied in order to provide additional data for the identification of the species.

MATERIALS AND METHODS

Source of the sample used: Representative specimens deposited in the University of Port Harcourt Herbarium (UPH) were collected with the permission of the Curator and investigated in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. This study was conducted from June, 2017 to July 2019. The information about the specimens studied is presented in Table 1.

Area of study: This research study was conducted in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria from June, 2017 to July, 2019.

Petiole anatomy: The petiole anatomy of the studied taxa was carried out by cutting a transverse section of the petioles of each of the taxa at the proximal, median and distal regions. The transverse sections were cut with the aid of a Reichert sliding microtome at a thickness of 8-15 µm. The sections were stained with safranin O and counter-stained in alcian blue, after which they were made to pass through different concentrations of ethanol for differentiation and dehydration. The sections were then mounted in a 25% dilute glycerin solution for examination under the microscope. Anatomical descriptions were made[22,23]. Photomicrographs of the different sections were made using a trinocular microscope (Amscope-T340B) fitted with a digital camera.

RESULTS

The results of current study's findings on the anatomical characteristics of the cross-sections of the petiole at distal and basal portions of the taxa studied are presented in Table 2 and 3 and Fig. 1-5.

Vascular bundle arrangement and sizes in the petiole: Based on the number of vascular bundles (vb), size, arrangement, presence or absence of accessory vascular bundles (rb) and presence of interspersed vascular bundles (Int), they are categorized in the vascular system at the distal and basal portions of the Asteraceae species studied into 5 types namely:

- **Type 1:** Three large (major) vascular bundles in an open separate arc with two accessory vascular bundles in distal and base of the petiole in *V. cinerea* (Fig. 1n, 2n) and *S. trilobata* (Fig. 2j, 4j), *E. alba* (Fig. 1f, 3f) and *E. aureus* (Fig. 2e, 4f) and the petiolar base of *A. africana*, *A. uliginosa*, *A. conyzoides*, *C. sumatrensis*, *E. pretamissa*, *S. nodiflora*, *T. procumbense*, *C. cinereum*, *A. hispidum*, *L. taraxacifolia*, *L.aurita*, *S. uliginosa*, *C. odorata*, *T. procumbense*, *S. nodiflora*, *S. filicaulis*, *A. houstonianum*, *L. taraxacifolia* and *S. costata* (Fig. 1(a-c, e, g, j, l), Fig. 2(d, f, i, m, o), Fig. 3(d, j, l) and Fig. 4(c, g, i, l)), respectively

Table 1: List of Voucher specimens studied

Species name	Ascension number	Tribe	Super tribe
Conyza sumatrensis (Retz.) E. H. Walker.	0912	Astereae	Asterodae
Erigeron aureus Greene	0815	Astereae	Asterodae
Launaea taraxacifolia (Willd.) Amin. MS. ex C. Jeffrey	1271	Cichorieae	-
Ageratum conyzoides Linn.	0466	Eupatorieae	Helianthodae
Ageratum houstonianum Mill.	0923	Eupatorieae	Helianthodae
Chromolaena odorata (Linn.) R. M. King and Robinson	0987	Eupatorieae	Helianthodae
Mikania cordata (Burm. f.) B. L. Rod.	1344	Eupatorieae	Helianthodae
Acanthospermum hispidum DC.	0419	Heliantheae	Helianthodae
Acmella uliginosa (Sw.) Cass.	0981	Heliantheae	Helianthodae
Aspilia africana (Pers.) C. D. Adams	0453	Heliantheae	Ecliptinae
Eclipta alba (L.) Hassk	0785	Heliantheae	Helianthodae
Eleutheranthera ruderalis (Sw.) Sch. Bip.	0771	Heliantheae	Helianthodae
Melanthera scandens (Schumach. And Thonn) Roberty	0106	Heliantheae	Helianthodae
Sphagneticola trilobata (L.) Pruski	1016	Heliantheae	Helianthodae
Spilanthes costata Benth.	1641	Heliantheae	Helianthodae
Spilanthes filicaulis (Schum and Thonn) C. D. Adams	0853	Heliantheae	Helianthodae
Spilanthes uliginosa Sw.	1411	Heliantheae	Helianthodae
Synedrella nodiflora Gaertn.	0761	Heliantheae	Helianthodae
Tithonia diversifolia (Hemsl.) A. Gray	0673	Heliantheae	Helianthodae
Tridax procumbens Linn.	1001	Heliantheae	Helianthodae
Wedelia trilobata (Linn.) Hitchc.	0974	Heliantheae	Helianthodae
Laggera aurita (Linn. f.) Benth. Ex C. B. Cl.	1704	Inuleae	Helianthodae
Crassocephallum crepidioides (Benth.) S. Moore	1187	Senecioneae	Senecionodae
Emilia praetermissa Milne-Redhead	0902	Senecioneae	Senecionodae
Cyanthillium cinereum (Lin.) H. Rob.	0316	Vernoieae	-
Ethulia conyzoides Linn. f.	0679	Vernonieae	-
Vernonia amygdalina Delile	1051	Vernonieae	-
Vernonia biafrae Oliv. and Hiern. (G and H)	0389	Vernonieae	-
Vernonia cinerea (Linn.) Less.	0890	Vernonieae	-
Vernonia conferta Benth.	0566	Vernonieae	-

- **Type 2:** Three large (major) vascular bundles in an open separate arc without accessory vascular bundles or the accessory vascular bundles occurring in the leaf-like wings in distal and base of the petiole in *S. filicaulis* (Fig. 2c, 4c) and at the petiolar base of *C. crepidioides*, *A. conyzoides*, *C. sumatrensis*, *S. filicaulis*, *A. hispidum*, C. *crepidioides*, *L.aurita* and *S. uliginosa* (Fig. 2k, Fig. 3c, e, Fig. 4c, k, m, o)
- **Type 3:** Many vascular bundles including 3 large (major) ones in an open separate double arc with 1-3 accessory vascular bundles and interspersed vascular bundles in distal and base of the petiole in *M. scandense* (Fig. 1i, 3i) and petiolar base (basal portion) of *C. odorata* and *T. diversifolia* (Fig. 1d, 3k)
- **Type 4:** The open separate arc having 5-7 vascular bundles of different sizes. Arc without accessory vascular bundles at petiole base of *E. pretamissa* (Fig. 3g) and at the distal and base of the petiole in *V. amygdalina* (Fig. 1m, 3g), arc with 1-2 accessory vascular bundles in *E. ruderalis*, *V. biafrae* and *E. conyzoides* (Fig. 1h, o, h), with 3-accessory vascular bundles in distal and basal portions of the petiole in *W. trilobata* (Fig. 2n, 4n) and petiolar base (basal portion) in *V. biafrae* (Fig. 3o) or with smaller interspersed vascular bundles petiolar base (basal portion) *T. diversifolia*, *M. cordata*, *E. aureus*, *E. conyzoides* and *S. costata* (Fig. 1k, 2b, f, g, l) and in distal portion in *A. africana*, *E. ruderalis*, *V. conferta* and *C. cinereum* (Fig. 3a, n, 4b, d)
- **Type 5:** Vascular bundle forming a continuous open arc with accessory vascular bundles at both ends of the arc in distal and base of the petiole in *V. conferta* (Fig. 2a, 4a)

The mean sizes of the vascular bundles at the distal portion of the petioles varied from 22.5 µm in *Ethulia conyzoides* to 97.5 µm in *Erigeron aureus* while at the basal end it varied from 22.5 µm in *Sphagneticola trilobata* to 80.8 µm in *Melanthera scandense* (Table 2, 3). Also, the thickness of the abaxial and adaxial parenchyma, adaxial and abaxial epidermises and the thickness of the petioles varied among the species studied and are diagnostic especially when combined with other anatomical features.

Table 2: Description of the distal portions of the petiole in the Asteraceae species studied

							Size of Vb (µm)		Thickness of PC (µm)			Thickness (µm)		
Tribe	Subtribe	Species names	Winged	Vb type	Ad outline	No. of Vb	Range	Mean	Adaxial	Abaxial	A/B	ABEP	ADEP	Petiole
Astereae	Asterodae	*Acanthospermum hispidum* DC.	+	1	3	5 (2 minor)	22-85	42.3	33-35	90-95	0.37	5-7	4-8	233-235
Astereae	Asterodae	*Acmella uliginosa* (Sw.) Cass.	+	1	4	6 (3 minor)	25-127	81.0	263-270	115-120	2.27	10-13	6-8	502-507
Cichorieae	-	*Ageratum conyzoides* Linn.	+	1	5	5 (2 minor)	19-49	33.3	88-94	54-60	1.60	2-3	2-3	195-201
Eupatorieae	Helianthodae	*Ageratum houstonianum* Mill.	+	1	3	3	75-94	80.8	148-150	146-148	1.01	9-11	8-10	413-415
Eupatorieae	Helianthodae	*Aspilia africana* (Pers.) C. D.	+	4	3	5 (2 minor)	25-88	54.8	88-98	88-114	0.92	5-6	7-9	332-335
Eupatorieae	Helianthodae	*Chromolaena odorata* (Linn.) R.	-	3	3	11 (8 minor)	28-130	81.5	189-185	90-95	2.02	9-10	14-15	450-455
Eupatorieae	Helianthodae	*Conyza sumatrensis* (Retz.) E. H.	-	4	5	11 (6 minor)	23-113	68.8	217-224	127-132	1.70	4-6	4-6	482-500
Heliantheae	Helianthodae	*Crassocephallum crepidioides*	+	1	4	3	14-38	26.3	13-23	11-28	0.92	3-4	2-4	89-92
Heliantheae	Helianthodae	*Cyanthillium cinereum* (Lin.) H.	-	1	2	5 (2 minor)	42-109	71.3	127-130	146-150	0.87	6-9	8-10	415-420
Heliantheae	Ecliptinae	*Eclipta alba* (L.) Hassk	+	1	1	12 (9 minor)	31-142	79.8	130-135	149-152	0.88	6-10	4-7	399-402
Heliantheae	Helianthodae	*Eleutherantheraruderalis* (Sw.)	+	1	3	7 (4 minor)	17-101	60.8	97-100	154-160	0.63	4-6	6-8	394-402
Heliantheae	Helianthodae	*Emilia praetermissa* Milne-	+	4	3	7 (4 minor)	28-105	64.3	99-103	140-142	0.72	4-6	6-10	378-392
Heliantheae	Helianthodae	*Erigeron aureus* Greene	+	3	3	14 (11 minor)	22-178	97.5	153-160	140-148	1.19	2-3	2-3	524-536
Heliantheae	Helianthodae	*Ethulia conyzoides* Linn. f.	+	1	4	5 (2 minor)	9-40	22.5	8-12	39-42	0.25	2-3	2-3	76-82
Heliantheae	Helianthodae	*Laggeraaurita* (Linn. f.) Benth.	+	2	1	5 (2 minor)	55-92	70.3	109-120	129-140	0.85	6-8	4-7	350-360
Heliantheae	Helianthodae	*Launaea taraxacifolia* (Willd.)	+	4	2	5 (2 minor)	18-72	44.3	61-65	82-90	0.73	3-4	4-5	242-248
Heliantheae	Helianthodae	*Melanthera scandens* (Schumach)	+	1	1	5 (2 minor)	52-78	64.3	78-80	91-95	0.85	8-10	10-13	292-300
Heliantheae	Helianthodae	*Mikaniacordata* (Burm. f.) B. L.	+	1	2	5 (2 minor)	34-100	63.5	88-92	91-100	0.94	3-5	2-4	320-342
Heliantheae	Helianthodae	*Sphagneticola trilobata* (L.)	+	4	3	12 (9 minor)	38-136	69.8	153-164	173-180	0.90	7-8	6-8	600-610
Heliantheae	Helianthodae	*Spilanthes costata* Benth.	-	1	4	5 (2 minor)	14-100	53.3	161-170	182-190	0.89	18-20	25-27	494-500
Heliantheae	Helianthodae	*Spilanthesf ilicaulis* (Schum and	+	4	1	8 (5 minor)	10-74	45.0	75-80	62-64	1.23	2-4	2-4	230-240
Inuleae	Helianthodae	*Spilanthesuliginosa* Sw.	+	1	5	3	47-84	62.3	119-123	83-85	1.44	2-4	3-5	305-310
Senecioneae	Senecionodae	*Synedrella nodiflora* Gaertn.	+	2	2	3	51-71	56.8	144-150	98-100	1.48	6-8	9-10	334-340
Senecioneae	Senecionodae	*Tithonia diversifolia* (Hemsl.) A.	+	1	4	5	54-81	61.5	182-190	104-110	1.74	8-10	7-10	400-410
Vernonieae	-	*Tridax procumbens* Linn.	-	1	5	10 (6 minor)	30-141	69.0	165-168	173-175	0.96	4-6	4-6	497-500
Vernonieae	-	*Vernonia amygdalina* Delile	+	4	5	7 (2 minor)	25-147	70.8	211-226	181-190	1.18	2-4	2-3	561-578
Vernonieae	-	*Vernonia biafrae* Oliv. And Hiern.	-	4	5	8 (2 minor)	46-174	86.5	228-234	181-190	1.25	9-12	6-8	607-612
Vernonieae	-	*Vernoniacinerea* (Linn.) Less.	+	4	3	11 (6 minor)	20-86	47.5	234-240	192-200	1.21	3-4	2-3	525-531
Vernonieae	-	*Vernonia conferta* Benth.	-	1	3	5 (2 minor)	29-98	57.8	129-132	152-160	0.84	10-13	6-9	419-424
Vernonieae	-	*Wedelia trilobata* (Linn.) Hitchc.	-	5	5	3 (2 minor)	24-108	58.8	394-400	196-200	2.01	3-5	4-5	242-250

vb: Vascular bundle, A: Adaxial, B: Abaxial, PC: Parenchyma

Table 3: Description of the basal portions of the petiole in the *Asteraceae* species studied

							Size of Vb (µm)		Thickness of PC (µm)			Thickness (µm)		
Tribe	Subtribe	Species Name	Winged	Vb type	Ad outline	No. of Vb	Range	Mean	Adaxial	Abaxial	A/B	ABEP	ADEP	Petiole
Astereae	Asterodae	*Acanthospermum hispidum* DC.	-	2	6	6 (3 minor)	24-75	45.0	22-31	22-44	0.80	4-6	4-6	160-165
Astereae	Asterodae	*Acmella uliginosa* (Sw.) Cass.	-	1	2	5 (2 minor)	36-107	65.3	64-107	100-154	0.67	3-6	4-5	356-360
Cichorieae	-	*Ageratum conyzoides* Linn.	-	2	1	5 (2 minor)	36-122	71.0	170-176	180-184	0.95	4-6	3-4	535-540
Eupatorieae	Helianthodae	*Ageratum houstonianum* Mill.	-	1	1	11 (8 minor)	26-104	59.8	58-80	64-81	0.95	3-4	2-4	258-260
Eupatorieae	Helianthodae	*Aspilia africana* (Pers.) C. D.	-	4	5	6 (3 minor)	21-88	53.3	64-68	94-98	0.69	3-7	3-4	269-272
Eupatorieae	Helianthodae	*Chromolaena odorata* (Linn.) R.	-	1	5	9 (6 minor)	27-116	74.3	158-160	94-98	1.66	4-6	3-5	442-448
Eupatorieae	Helianthodae	*Conyza sumatrensis* (Retz.) E. H.	-	2	5	7 (4 minor)	39-103	62.5	150-154	130-135	1.15	4-5	4-6	425-430
Heliantheae	Helianthodae	*Crassocephallum crepidioides*	+	2	5	9 (6 minor)	51-71	62.0	130-134	89-92	1.46	4-5	5-8	322-330
Heliantheae	Helianthodae	*Cyanthillium cinereum* (Lin.) H.	-	4	8	5 (2 minor)	59-91	69.5	131-134	98-100	1.34	4-7	6-8	316-320
Heliantheae	Ecliptinae	*Eclipta alba* (L.) Hassk	-	1	3	7 (4 minor)	24-74	44.0	43-45	145-150	0.30	4-7	4-6	307-310
Heliantheae	Helianthodae	*Eleutheranthera ruderalis* (Sw.)	-	4	3	8 (5 minor)	28-119	69.8	70-75	87-90	0.82	5-7	6-8	303-307
Heliantheae	Helianthodae	*Emilia praetermissa* Milne-	-	4	3	7 (4 minor)	14-85	51.0	116-120	113-116	1.03	8-10	6-8	327-334
Heliantheae	Helianthodae	*Erigeron aureus* Greene	-	1	2	6 (3 minor)	25-70	42.3	51-55	59-62	0.88	4-5	2-3	187-194
Heliantheae	Helianthodae	*Ethulia conyzoides* Linn. f.	-	4	5	8 (3 minor)	25-133	77.0	166-170	149-153	1.11	4-6	4-6	445-450
Heliantheae	Helianthodae	*Laggera aurita* (Linn. f.) Benth.	+	2	3	5 (2 minor)	16-39	55.8	72-75	81-85	0.89	3-6	7-9	234-240
Heliantheae	Helianthodae	*Launaea taraxacifolia* (Willd.)	+	1	1	5 (2 minor)	19-66	39.8	103-115	68-75	1.52	3-5	2-4	235-243
Heliantheae	Helianthodae	*Melanthera scandens* (Schumach.	+	3	3	17 (14 minor)	15-144	80.8	143-156	119-124	1.23	4-5	3-4	456-475
Heliantheae	Helianthodae	*Mikania cordata* (Burm. f.) B. L.	-	4	1	11 (4 minor)	23-113	68.8	224-240	94-100	2.39	3-4	2-4	414-435
Heliantheae	Helianthodae	*Sphagneticola trilobata* (L.)	+	1	4	7 (4 minor)	9-40	22.5	31-35	53-60	0.58	1-2	1-2	119-126
Heliantheae	Helianthodae	*Spilanthes costata* Benth.	-	2	1	3	55-92	65.0	96-100	98-100	0.99	7-9	4-5	282-294
Heliantheae	Helianthodae	Spilanthesf ilicaulis (Schum)	-	1	3	7 (4 minor)	18-72	44.3	55-60	80-85	0.70	3-4	5-7	228-231
Inuleae	Helianthodae	*Spilanthes uliginosa* Sw.	-	2	1	5 (2 minor)	52-78	59.8	114-120	178-182	0.65	8-11	10-14	434-440
Senecioneae	Senecionodae	*Synedrella nodiflora* Gaertn.	-	1	3	6 (3 minor)	34-100	63.5	98-100	102-110	0.93	4-6	5-7	328-334
Senecioneae	Senecionodae	*Tithonia diversifolia* (Hemsl.) A.	-	3	6	12 (9 minor)	38-136	69.8	215-210	191-204	1.08	3-4	3-4	571-580
Vernonieae	-	*Tridax procumbens* Linn.	-	1	3	5 (2 minor)	14-100	53.3	60-65	133-140	0.46	20-25	28-32	378-384
Vernonieae	-	*Vernonia amygdalina* Delile	-	4	5	7	46-174	78.5	255-261	151-155	1.69	4-6	5-6	598-600
Vernonieae	-	*Vernonia biafrae* Oliv. and Hiern.	-	4	1	8 (3 minor)	20-86	47.5	173-180	183-190	0.95	6-8	4-6	477-480
Vernonieae	-	*Vernonia cinerea*	+	1	6	5 (2 minor)	29-98	57.8	71-75	97-100	0.74	12-15	6-10	300-305
Vernonieae	-	*Vernonia conferta* Benth.	-	5	7	3 (2 minor)	24-108	58.8	389-400	250-254	1.57	2-4	2-4	772-780
Vernonieae	-	*Wedelia trilobata* (Linn.) Hitchc.	+	4	3	8 (5 minor)	10-74	45.0	27-30	45-50	0.60	2-3	1-2	156-160

vb: vascular bundle, A: Adaxial, B: Abaxial, PC: Parenchyma

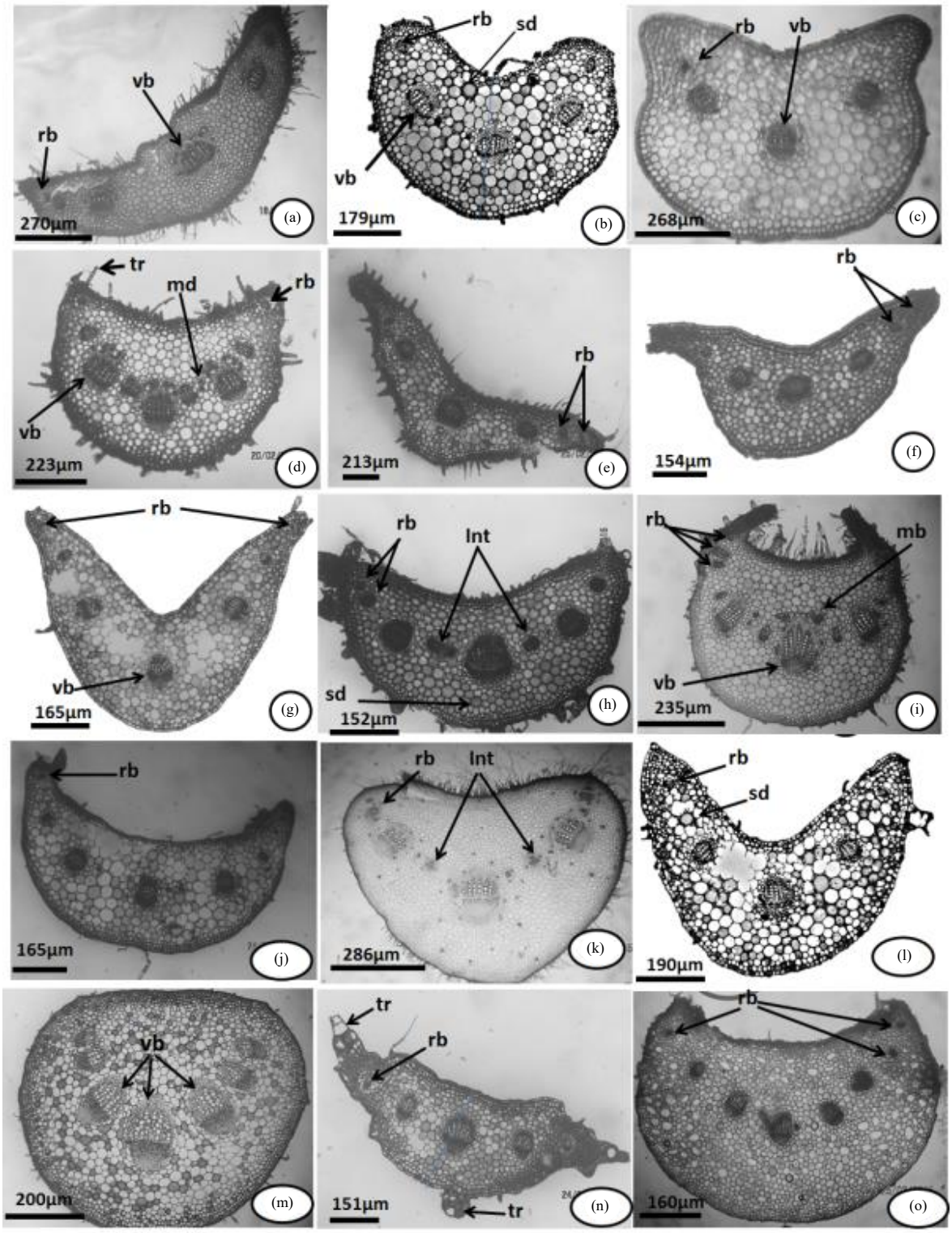

Fig. 1(a-o): Transverse section of basal portion of the petiole in Asteraceae studied, (a) *A. africana*, (b) *A. uliginosa*, (c) *A. conyzoides*, (d) *C. odorata*, (e) *C. sumatrensis*, (f) *E. alba*, (g) *E. pretamissa*, (h) *E. ruderalis*, (i) *M. scandense* (leaf-like wings), (j) *S. nodiflora*, (k) *T. diversifolia*, (l) *T. procumbense*, (m) *V. amygdalina*, (n) *V. cinerea* and (o) *V. biafrae*

rb: Rib trace, mb: Medullary vascular bundle, vb: Vascular bundle, tr: Trichome, sd: Secretory duct, Int: Interposed vascular bundles

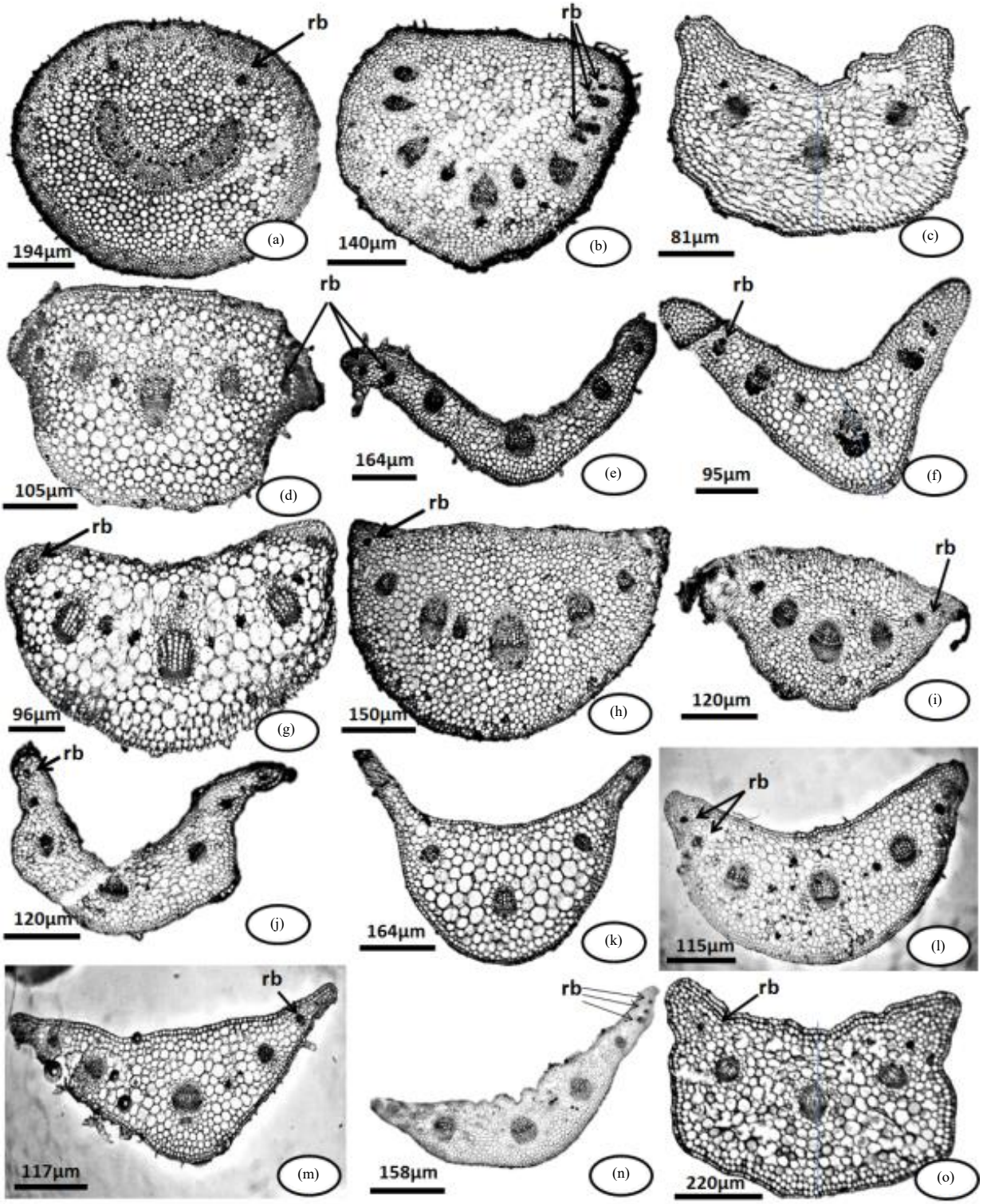

Fig. 2(a-o): Transverse section of basal portion of the petiole in Asteraceae studied continued, (a) *V. conferta*, (b) *M. cordata*, (c) *S. filicaulis*, (d) *C. cinereum,* (e) *A. hispidum,* (f) *E. aureus,* (g) *A. houstonianum,* (h) *E. conyzoides*, (i) *L. taraxacifolia* (leaf-like wings), (j) *S. trilobata* (leaf-like wings), (k) *C. crepidioides,* (l) *S. costata* (m) *L. aurita,* (n) *W. trilobata* and (o) *S. uliginosa*

rb: Rib trace

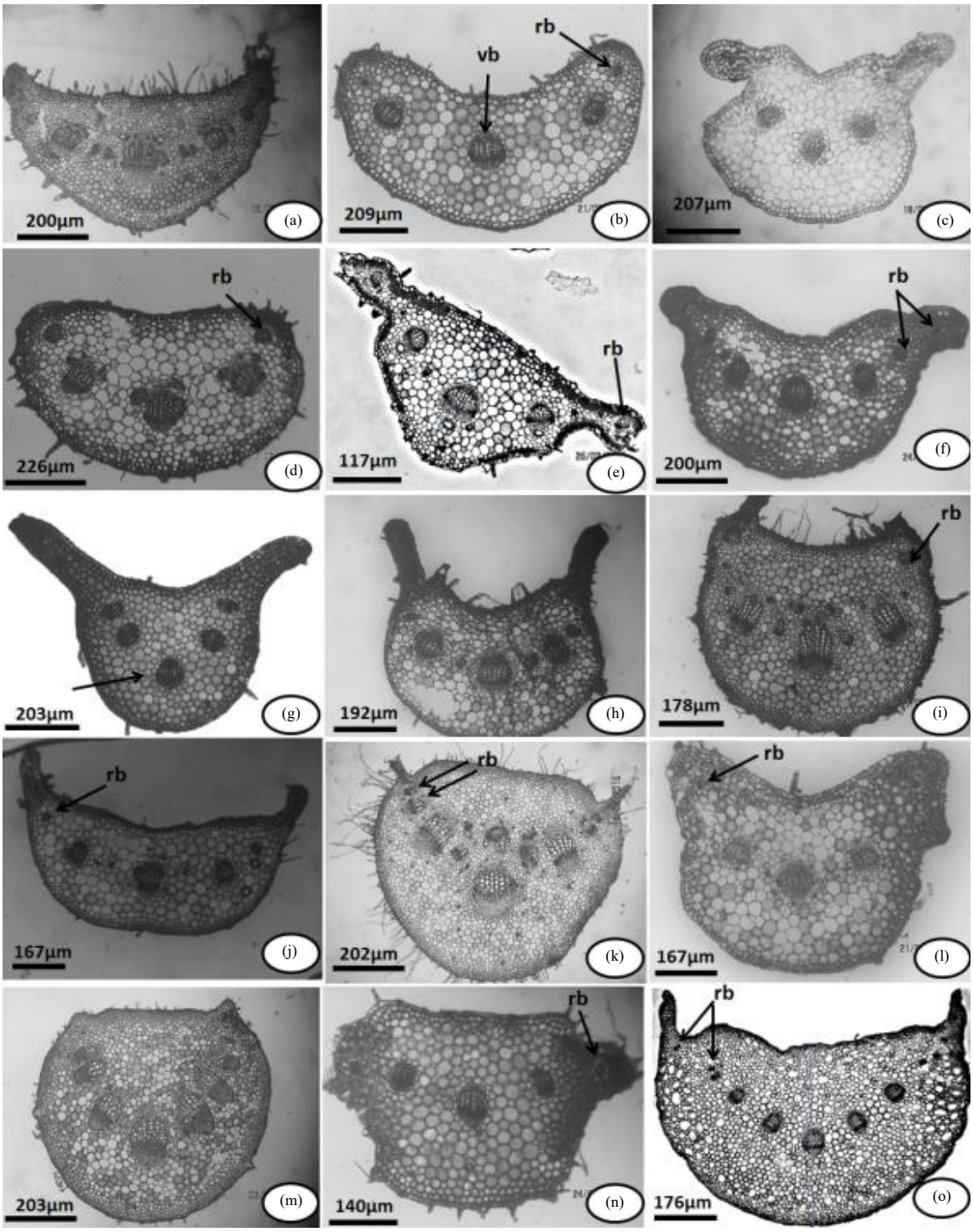

Fig. 3(a-o): Transverse section of distal portion of the petiole in *Asteraceae* studied, (a) *A. africana* (leaf-like wings), (b) *A. uliginosa*, (c) *A. conyzoides* (leaf-like wings), (d) *C. odorata*, (e) *C. sumatrensis* (leaf-like wings), (f) *E. alba* (leaf-like wings), (g) *E. pretamissa* (leaf-like wings), (h) *E. ruderalis* (leaf-like wings), (i) *M. scandense* (Note: leaf-like wings), (j) *S. nodiflora* (leaf-like wings), (k) *T. diversifolia* (leaf-like wings), (l) *T. procumbense* (m) *V. amygdalina*, (n) *V. cinerea* and (o) *V. biafrae* (leaf-like wings)
rb: Rib trace, vb: Vascular bundle

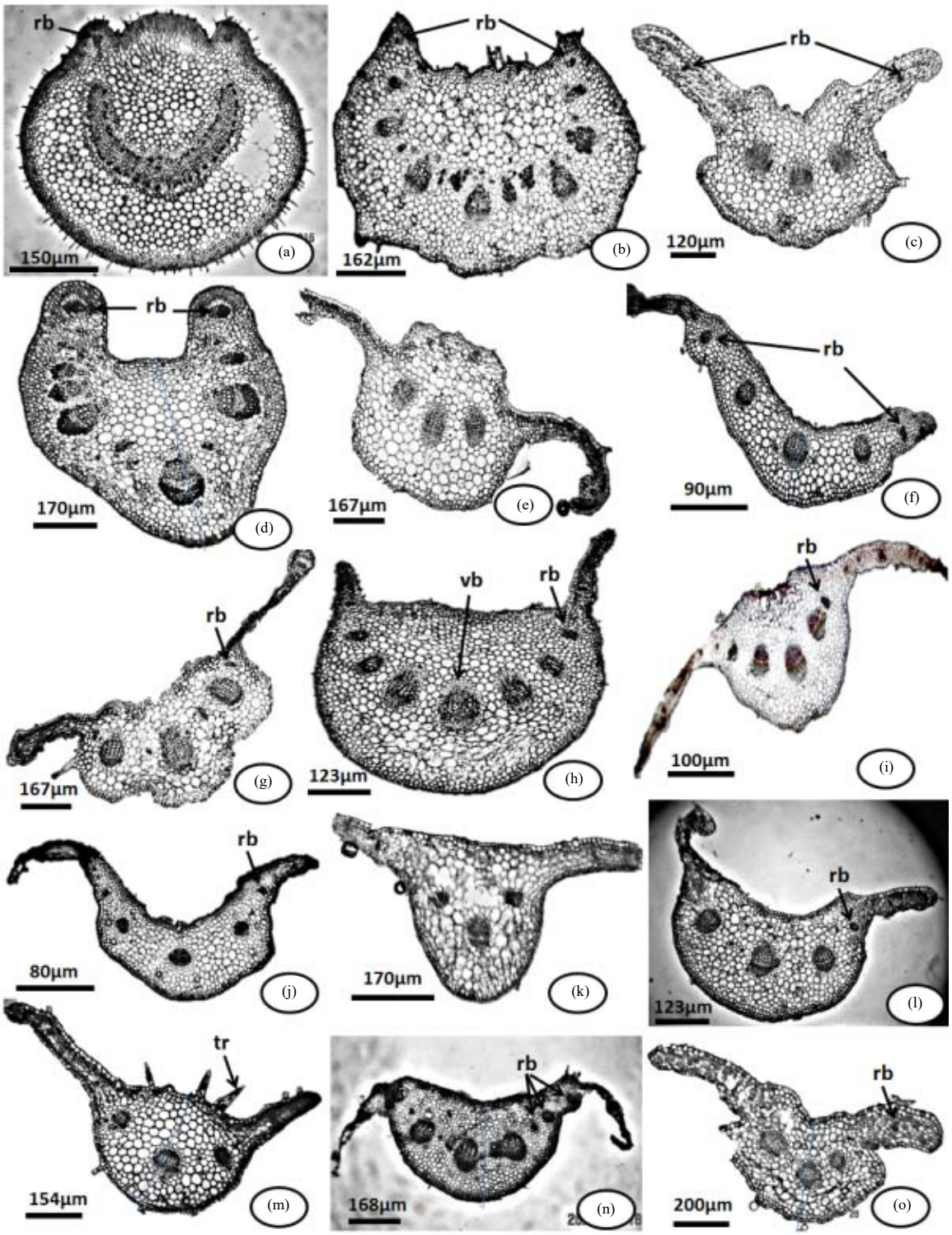

Fig. 4(a-o): Transverse section of distal portion of the petiole in Asteraceae studied continued, (a) *V. conferta*, (b) *M. cordata*, (c) *S. filicaulis* (leaf-like wings), (d) *E. aureus* (leaf-like wings), (e) *C. cinereum* (leaf-like wings), (f) *A. hispidum,* (g) *A. houstonianum* (leaf-like wings), (h) *E. conyzoides* (leaf-like wings), (i) *L. taraxacifolia* (leaf-like wings) and (j) *S. trilobata* (leaf-like wings), (k) *C. crepidioides*, (l) *S. costata* (m) *L. aurita*, (n) *W. trilobata* and (o) *S. uliginosa*, rb: Rib trace, vb: Vascular bundle, tr: Trichome

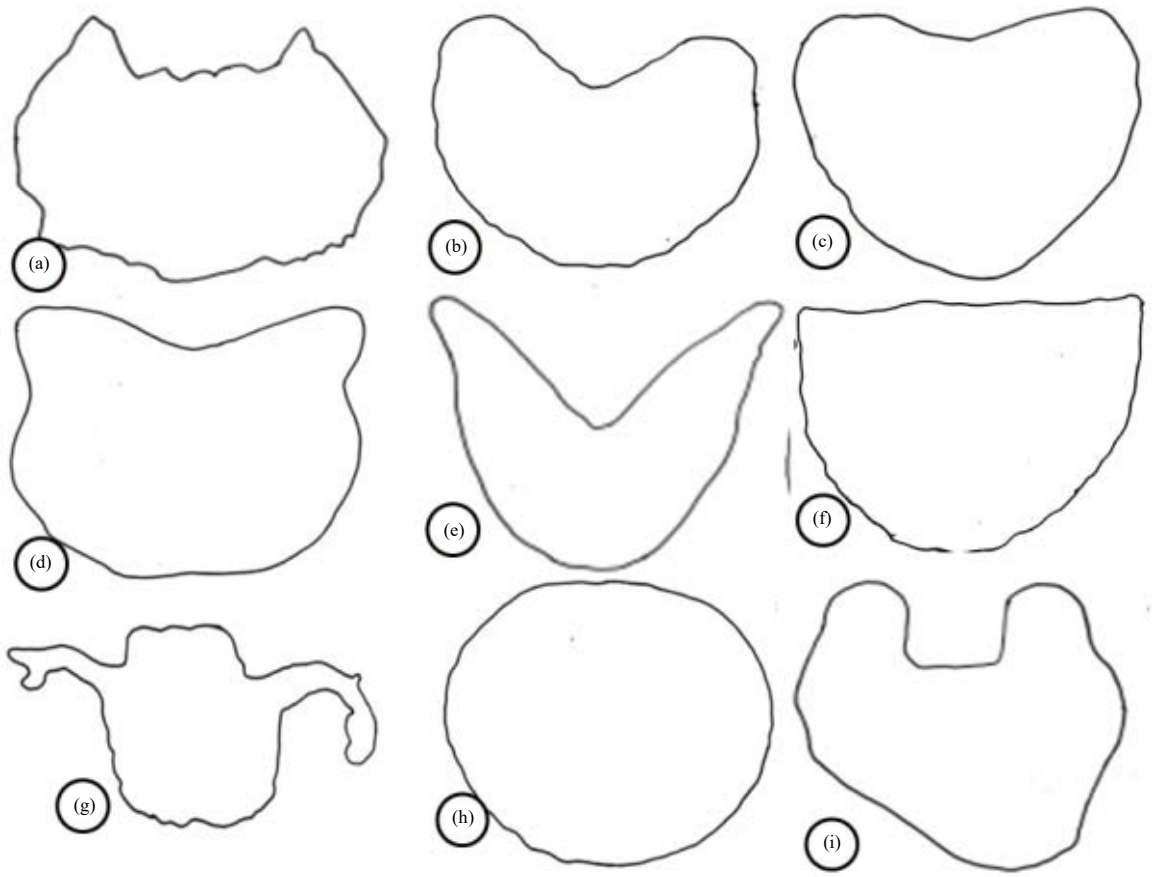

Fig. 5(a-if): Adaxial cuticle shapes of petiole in the taxa studied, (a) Undulating or wavy outline, (b-d) Deeply concave or slightly concave outline, (e) V-shaped outline, (f) Flat or relatively flat surface, (g) Slightly elevated with a relatively flat surface, (h) Convex surface and (i)-U-shaped surface

Adaxial petiole outline: The surface of petiole of the species studied are densely hairy in *A. africana*, *C. odorata*, *C. sumatrensis*, *E. ruderlis*, *M. scandense*, *T. diversifolia* and *V. conferta* (Fig. 1a, d, e, h, i, k, Fig. 2a, Fig. 3a, h, i, k, 4a) and fairly/sparsely hairy or glabrous in the other species. The adaxial outline of the petiole of the different species investigated showed 8 different forms namely:

- **Type 1 (Fig. 5a):** Adaxial cuticle outline showed undulating or wavy outline in basal portion of *A. africana*, (Fig. 1), distal portions of *W. trilobata*, *A. conyzoides*, *V. biafrae*, *M. cordata*, *A. houstonianum* and *L. taraxacifolia* (Fig. 2n, Fig. 3c, o, Fig. 4b, g, i) and at both basal and distal portions of *S. filicaulis* and *S. uliginosa* (Fig. 2c, o, Fig. 4c, o)
- **Type 2 (Fig. 5b):** Adaxial cuticle outline deeply concave in basal portions of *A. uliginosa*, *S. nodiflora*, *C. crepidioides*, *S. costata* (Fig. 1b, j, 2k, l) and distal portions of *A. uliginosa* and *E. aureus* (Fig. 3b, 4f)
- **Type 3 (Fig. 5c, d):** Adaxial cuticle outline slightly concave in the basal and distal portions of *E. alba* (Fig. 1f, i), *E. ruderalis* (Fig. 1h, m) and *M. scandense* (Fig. 1i, 3i). This type of outline also occurred in *A. conyzoides*, *C. odorata*, *C. sumatrensis*, *T. diversifolia*, *V. cinerea*, *A. houstonianum*, *E. pretamissa*, *S. nodiflora*, *T. procumbense*, *S. costata* and *W. trilobata* (Fig. 1c-f, h, i, k, n, Fig. 2g, Fig. 3f-j, l, Fig. 4l, n)
- **Type 4 (Fig. 5e):** Adaxial cuticle outline V-shaped in the basal portion of *E. pretamissa*, *T. procumbense*, *A. hispidum* and *E. Aureus* (Fig. 1g, l, Fig. 2e, f) and in basal and distal portions of *S. trilobata* (Fig. 2i, 4j)
- **Type 5 (Fig. 5f):** Adaxial cuticle outline flat or relatively flat surface in basal and distal of *C. cinereum* (Fig. 2d, 4d) and basal petiolar portions of *V. amygdalina*, *V. conferta*, *M. cordata*, *E. conyzoides*, *L. taraxacifolia* and *L. aurita* (Fig. 1m, Fig. 2a, b, d, h, i, m) and in the distal petiolar portions of *A. africana*, *C. sumatrensis*, *E. conyzoides* and *C. crepidioides* (Fig. 3a, e, Fig. 4d, h, k)

- **Type 6 (Fig. 5g):** Adaxial cuticle outline slightly elevated with a relatively flat surface in distal petiolar portions of *T. diversifolia*, *V. cinerea* and *A. hispidum* (Fig. 3k, n, 4e)
- **Type 7 (Fig. 5h):** Adaxial cuticle outline convex in *V. conferta* (Fig. 4a)
- **Type 8 (Fig. 5i):** Adaxial cuticle outline U-shaped in *C. cinereum* (Fig. 4n)

Among the species studied, it was observed that some of them have leaf-like wings. The leaf-like wings were observed either at the distal or basal portions of the petiole or at both ends. This feature makes the species distinguishable and was found mainly at the distal portion of the petiole in most of the species studied, however, in some species like *M. scandense*, *C. crepidioides*, *A. conyzoides* and *C. sumatrensis* in Fig. 1i, 2k, 3c and e.

DISCUSSION

Studies on the vascular bundle arrangement and anatomical characteristics in Asteraceae are well documented[2,3] however, comprehensive study on the petiolar vascular bundle arrangement is still lacking. Nevertheless, the number of vascular bundles, shape, arrangement and petiole outline are consistent in angiosperms and could be used in plant identification and classification[24-26]. Also, petiole anatomy including vascular bundle arrangement has been used in delimiting families such as Malvaceae[25], Dipterocarpaceae[24,26] and Annonaceae[27]. Furthermore, Noraini *et al.*[28] used the petiole outline in the identification of some species of *Coelotegia* (Bombacaceae) and Olowokudejo[29] in Cruciferae.

The vascular bundles in the species studied consist mainly of three large (major) ones and smaller (minor) ones. The vascular bundles in all the species studies formed an open separate arc except in *V. conferta* were the vascular bundle formed an open continuous/united arc (Fig. 2a, 4a). Remarkable among the family is the presence of 3 large (major) vascular bundles which is similar to those observed in Asteraceae[2]. Though the majority of the species have 3 main (lager) vascular bundles, few of them have more than 3 vascular bundles at either the distal or basal areas of the petiole. The 3 larger vascular bundles were observed more on the basal end of the petiole than the distal ends. *M. cordata* has 11 vascular bundles at both distal and basal ends but with 5 and 7 larger ones at the distal and basal ends, respectively. Also, *V. brafrae* and *V. conferta* have 5 and 1 larger vascular bundles each at the distal and basal ends, respectively. Furthermore, *E. conyzoides* and *V. amygdalina* have 4 and 6 larger vascular bundles at the distal ends, respectively (Table 2, 3). In other taxa, the vascular bundles are distinct numbering 3-7 without the smaller or minor ones. These observations were recorded in *A. hispidum*, *A. conyzoides*, *C. crepidiodes* and *L. aurita* which have 3 vascular bundles each. *E. praetermissa* has 5, *S. filicaulis* 3 and *V. amygdalina* (7) at the basal ends of their petioles. These differences made these species distinct from the remaining species studied. Among the taxa with three larger vascular bundles, there are variations in the number of smaller or minor ones. These variations in the number, size and arrangement of vascular bundles are genetically controlled[30] and could be used in delimiting the taxa studied especially when combined with other characters such as the shape of the adaxial cuticle and vascular bundle type. At the genus level especially the taxa with more than one species like *Ageratum*, *Spilanthes* and *Vernonia*, we observed that the for among the species of *Ageratum* studied, the number of bundles and types at both distal and basal ends of the petiole are different but the outline of the adaxial cuticles are the same. Also, they have wing-like petioles. Similar observations were made for *Spilanthes* and *Vernonia*. Subsequently, at the family level, these features are specific to any genus, however, at the genus level, these features such as vascular bundle type and number are distinct and could serve as a diagnostic character among the taxa studied.

Notable among these is the presence of interspersed vascular bundles in *A. africana*, *M. scandense*, *C. odorata* and *T. diversifolia.* This feature was not observed among other members of this family studied and therefore made these species different from other members of this family studied. A similar observation has been made on members of Asteraceae[2]. Metcalfe and Chalk[3] pointed out that petiolar anatomy in Asteraceae at the distal end has simple separate vascular bundles, varying in number and may be complex or simple. They found complex vascular bundles in some genera such as *Arctium, Cirsium* and *Eupatorium* (*Chromolenea*) and further noted that some genera have partly double arc of vascular bundles on the adaxial portion while some of the species are having separate vascular bundle arc accompanied by medullary strands, arc phloem around the inner edge of the xylem or petiole covered with thick cuticle or with leaf-like wings. Mainly the distal portions of the majority (73.33%) of the species studied have leaf-like wings while 76.67% of them have this structure at the basal portion. Some of the species studied like *M. cordata*, *A. uliginosa*, *T. procumbens*, *C. odorata*, *V. amygdalina*, *V. conferta* and *C. cinereum* have leaf-like wings at both basal and distal portions of the petiole while others have trichome at the edges of the petiole. The presence of these leaf-like wings has been reported major characteristics of Asteraceae[3].

In this study, the petioles of the taxa studied have some close similarities however; some of the species could be differentiated from others based on this feature especially at the genus level. Generally, vascular bundle type 1 was found in two species viz. the distal part of *A. africana* petiole and the base of *C. odorata* petiole. Type 2 was found in a majority of the species such as distal and portions of *E. alba*, at the distal portion of *A. conyzoides*, *E. pretamissa* and *V. cinerea* and the base of *C. conyza* and *S. nodiflora.* The type 3 vascular bundle arrangement was only found in the distal portion and base of *V. amygdalina*. This feature however made this species distinct from all the other species studied. In the same way, vascular bundle type 4 was observed in the distal part of only two species (*T. procumbense* and *V. cinerea*).

According to Noraini *et al.*[28] petiole outline can be used for the identification of some species in genus *Coelostegia* (Bombacaceae). Another study on the petiole anatomy of selected species and genera in the family Cruciferae also has identified the differences of petiole outline on 46 taxa that can be used in taxonomic classifications[29]. Chia[31] stated that the petiole outline in *M. erythrocarpa* and *M. laurifolia* are rounded in shape.

In this study, we observed eight (8) types of petiole outlines. Among the members of this family studied, we noted that the morphological and anatomical features of the petiole could be used to delimit them. Though some of the species studied had similar vascular bundles, the outline or cross-section and presence/absence of leaf-like wing are further used to distinguish them. Among the 24 genera and 30 species of Asteraceae studied, we observed five vascular bundle types and eight petiole outlines (cross-sections).

Among the vascular bundle types, Type-1 occurred in 19 species and 12 genera, type-2 in eight species and seven genera, type-3 in 3 species and three genera, type-4 in 12 species and 11 genera and type-5 in one species and one genus. The type-5 vascular bundle is restricted to *V. conferta* and is found at both distal and basal ends of the petiole in this species. Other ones occurred singly or in association with other vascular bundle type and are not restricted to a species. We noted that among the *Spillanthes* species studied, the vascular arrangement at the distal end of the petiole could be used to distinguish them. *S. filicaulis* had an only type-2 vascular bundle, *S. costata* had type-4 and type-1 vascular bundles and *S. uliginosa* had type-2 and type-1 vascular bundles. Also, in the genus *Vernonia*, they have different vascular bundles types but *V. amygdalina* and *V. brafrae* had a similar vascular type and could be further differentiated base on the cross-sectional outline of the petiole.

Also, the petiole outline type-1 occurred in 9 species, type-2 in 5 species, type-3 in 16 species, type-4 in 5 species, type-5 in 11 species, type-6 in 3 species, type-7 and 8 in 1 species each. Twenty three of the species had a leaf-like wing at either distal or basal end of the petiole while seven of the species do not have a leaf-like wing.

CONCLUSION

In this study, it is evident that the arrangement, number and sizes of vascular bundles in the petiole were fairly different and diagnostic especially the presence and number of accessory vascular bundles that varied among the species. Also, the presence and number of interspersed vascular bundles varied sparingly among the taxa especially at the genus and species level.

SIGNIFICANCE STATEMENT

This study discovers the importance of petiole anatomical information in the classification of Asteraceae that can be beneficial to diagnose the presence and number of accessory vascular bundles which shows variation. This study will help the researcher to uncover the critical areas of arrangement, number and sizes of vascular bundles in the petiole. Thus a new theory to find the number of interspersed vascular bundles may be arrived at.

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