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LEAF MACRO- AND MICRO-MORPHOLOGICAL CHARACTERS AS TAXONOMIC TOOLS FOR SOME ASTERACEAE SPECIES

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Abstract

The morphological and epidermal characters of leaves of 10 Asteraceae species, collected from Dammam city in the eastern region of Saudi Arabia were examined to highlight their significance in identification and classification. Several morphological leaf characters played a crucial role in delimitation of the studied species, including leaf arrangement, type, shape, and the presence or absence of a petiole. Regarding anatomical features, two types of mesophylls were observed, and subsidiary bundles were present in both the petiole and blade. Six types of cuticular ornamentation and 12 different types of trichomes were found. The numerical analysis of the obtained characters demonstrated that the leaf characteristics were taxonomically important for classification and differentiation at the subfamily and generic levels.

Key words: Leaf morphology, Anatomy, Stomata, Trichomes, Scanning Electron Microscope, Asteraceae, Eastern region of Saudi Arabia.

Introduction

The Asteraceae (Compositae) is the second largest Angiosperm family with approximately 23,600 species and 1,620 genera (Stevens, 2001). It is widely distributed, especially in tropical and subtropical regions, mostly in open areas (Anderberg, 2007 and Funk *et al*., 2005). Many plants in Asteraceae family have economic importance as weeds, decorative, medicinal and edible plants (Olorode, 1984). Members of the family are easily recognized by their inflorescence types. The leaves are simple, pinnately lobed in some species, alternate or opposite, and prickly in some genera. In Saudi Arabia, the initial survey conducted by Migahid in 1978, recorded 61 genera and 120 species of Asteraceae. Subsequent studies have contributed to the family's knowledge. Additional species have been recorded resulting in an increase in the total number of species. According to Collenette (1999), the number rose to nearly 222 species distributed among 88 genera. Chaudhary (2000) further expanded the count to 242 species belonging to 87 genera. These figures include both wild and cultivated species, such as *Chrysanthemum coronarium* L., which is commonly cultivated. In a more recent study Al-Rehaily *et al*., (2015) reported 90 genera and 244 species of Asteraceae from Saudi Arabia. Mandaville (1990) reported 43 genera and 66 species of Asteraceae from the eastern region of Saudi Arabia.

The sub-familial classification of Asteraceae has been changed from time to time, as recognized by various scientists. Cronquist, (1955) initially identified two subfamilies and 10 tribes, whereas Thorne, (1983) classified the family into two subfamilies and 14 tribes. Bremer (1994) further divided it into four subfamilies and 17 tribes. Baldwin *et al*., (2002) and Panero & Funk (2002) proposed 10 subfamilies and 35 tribes. More recently, Funk *et al*., (2009) and Panero *et al*., (2014) revised the classification to 13 subfamilies and 44 tribes. These changes in subfamilial classification reflect the evolving understanding of the relationships and diversity within the Asteraceae family as more research and data become available. Different scientists have proposed varying classifications based on their analysis and interpretation of the available evidence.

The morphological characteristics of vegetative organs play a crucial role in taxonomic studies, providing a foundation for classification (Polhill, 1968; Pilbeam & Bell, 1979; Adedeji, 2004). Epidermal studies focusing on features such as stomata, trichomes, and cuticle ornamentation are particularly reliable for taxonomic purposes, as they remain unaffected by varying environmental conditions (Barthlott, 1990). Consequently, comparative studies of plant epidermal traits have proven to be trustworthy tools in taxonomy and systematics (Stace, 1969; Ogunkunle & Oladele, 2000; Metcalfe & Chalk, 1950, 1979; Naik & Nigrude, 1981; Palmer & Tucker, 1981; Adedeji, 2004; Adedeji & Illoh, 2004).

The characteristics of leaves, encompassing their morphology, anatomy, and epidermal features, play a vital role in plant classification. Numerous authors have conducted studies in this area (Baranova, 1972; Metcalfe & Chalk, 1979; Stace, 1984; Baranova, 1992; Yang & Lin, 2005; Lu *et al*., 2008; Mavi *et al*., 2011; Szymura & Wolski, 2011). The first comprehensive study on the general leaf anatomy of the Asteraceae family was conducted by Metcalfe & Chalk (1950), who observed significant anatomical variations in Asteraceae species, particularly in leaf structures, attributable to their diverse habitats. Subsequent studies have focused on different leaf characters of Asteraceae species, conducted by Napp-Zinn & Eble (1978), Castro *et al*., (1997), Neszmelyi *et al*., (1992), Ciccarelli *et al*., (2007), Maes *et al*., (2011), Majdi *et al*., (2011, 2013), Prasanth (2019) and Inceer & Ozcan (2011, 2021).

In Saudi Arabia, most studies conducted on the Asteraceae family have focused on the flora and the discovery of new species within the kingdom (Ghafoor & Al-Turki, 1999; Al-Rehaily *et al*., 2015 and Ibtisam & Doka, 2018). Additionally, several studies on the Asteraceae family in Saudi Arabia had been done other than leaf and epidermis characters as, Shahat *et al*., (2017), Ali *et al*., (2015), Qari *et al*., (2016), Al-Ahmadi, (2017) and Al-Fredan, (2019).

The anatomical and epidermal studies on the Asteraceae species of Saudi Arabia have not been done. Therefore, the aim of this study is to establish the taxonomic importance of foliar features for ten Asteraceae species growing in eastern region of Saudi Arabia.

Material and Methods

The leaf and epidermal characters of 10 Asteraceae species, belonging to two sub-families and six tribes as classified by Panero & Funk (2002), were collected fresh from various localities in Dammam city, located in the eastern region of Saudi Arabia (Table 1). The materials studied were identified using the taxonomic keys provided by Mandaville (1990) and Chaudhary (2000).

For anatomical investigations, each specimen was killed and fixed using the F.A.A. (formalin-glacial acetic acid - 70% alcohol) method, following the protocol established by Nassar & El-Sahhar (1998), with a ratio of 5:5:90 by volume. Hand sections of the leaves, petiole and blade were prepared at a thickness of 20-30 µm and stained using the technique developed by Dilcher (1974). The resulting sections were examined and photographed for further analysis.

For the study of leaf surface, including cuticular ornamentation, stomata, and trichome types, a scanning electron microscope (SEM) was used. Two leaves were mounted on metal stubs, coated with gold, and examined and photographed using a JEOL-SEM at accelerating voltages of 7 and 10 kV. The terminology regarding trichome types and epidermal characters was derived from relevant literature sources (Metcalfe & Chalk, 1979; Saha & Mukherjee, 2012; Perveen *et al*., 2016).

A total of 45 morphological and anatomical characters were analyzed to compare the studied leaf species. Numerical analysis was employed to determine the relationships between the species, and dendrograms were generated as shown in Figure (1). The statistical programs PRIMER 6, version 6.1.6, and SPSS version 16 were used for the analysis.

Results

Morphological features: Ten studied species of Asteraceae from Dammam in the eastern region of Saudi Arabia show varying morphological features between the lower and upper leaves (Table 2 and Plate 1).

Lower leaf: The lower leaf arrangements of the studied species exhibited different patterns: alternate in *Conyza linifolia*, *Pluchea dioscoridis*, *Senecio glaucus*, and *Ifloga spicata*; opposite in *Eclipta alba*; and rosette in the remaining species. Most of the leaves were petiolate, except for *Pluchea dioscoridis*, having sessile leaves, and in *Eclipta alba*, leaves were sub-sessile. The length of the petioles ranged from 0.6 mm in *C. linifolia* to 4.1 mm in *S. oleraceus*.

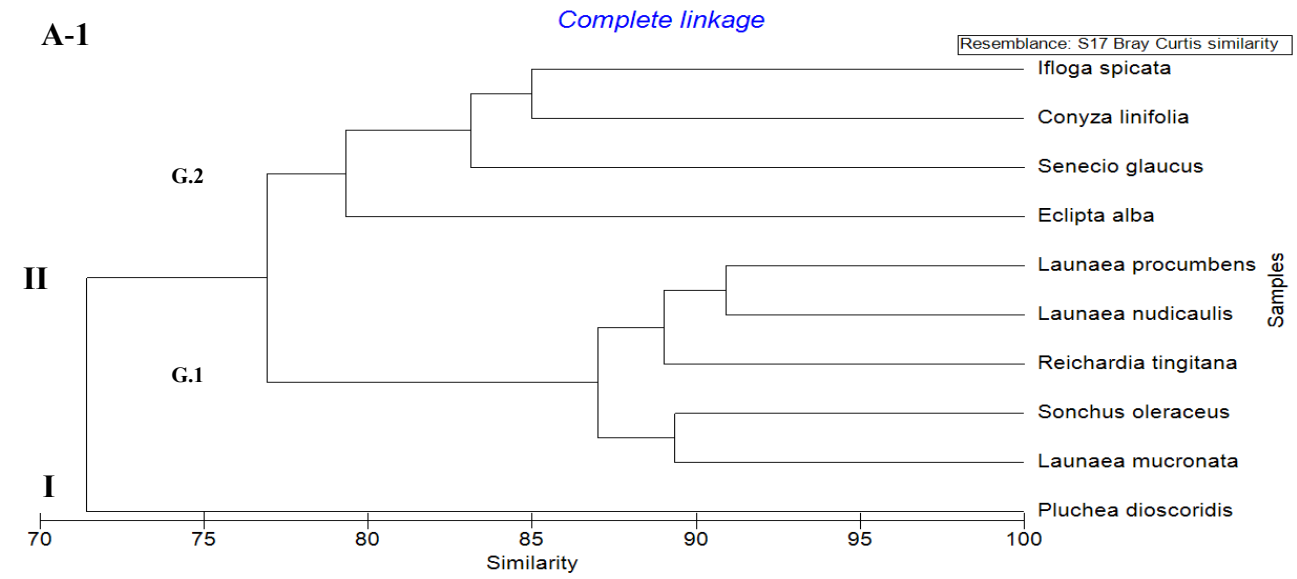
The leaves of most studied species were pinnate, except for 4 species (*Conyza linifolia*, *Pluchea dioscoridis*, *Ifloga spicata* and *Eclipta alba*) which had simple leaves. The shape of the leaf could be classified into four types: oblong, oblong-lanceolate, oblong-obovate, and elliptic-lanceolate. The leaf base is auriculate in three species (*Conyza linifolia*, *Pluchea dioscoridis* and *Senecio glaucus*). The length of the blade ranged from 2.25 cm in *Conyza linifolia* to 14.6 cm in *Launaea mucronata*, and the width ranged from 0.5 cm in *Conyza linifolia* to 4.45 cm in *Sonchus oleraceus*. The blade base was symmetric in all studied species, except in *Pluchea dioscoridis*, which was asymmetric.

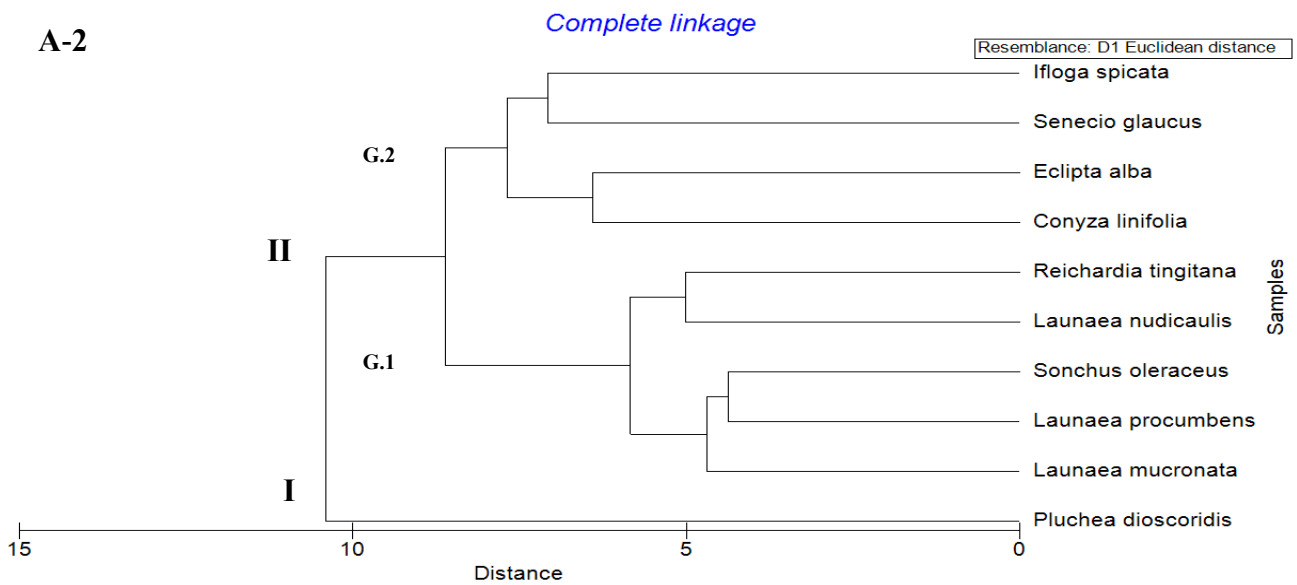
Upper leaf: The leaves of the majority of the studied species were alternate, except in *Eclipta alba*, which had opposite leaves. They were sessile or sub-sessile in most species, while *Ifloga spicata* and *Launaea nudicaulis* had petiolated leaves. The types of leaves were the same as the lower leaves, except in *Reichardia tingitana*, where the lower leaves were pinnatipartite and the upper ones were simple. The shapes of the leaves were of five types: oblong in two species, oblong-lanceolate in one species, oblong-obovate in five species, elliptic-lanceolate in one species, and linear in one species. The leaf base was typically auriculate in the studied species, except for *Conyza linifolia*, *Ifloga spicata*, and *Eclipta alba*. The length of the leaf blade ranged from 1.9 cm in *Ifloga spicata* to 8.38 cm in *Sonchus oleraceus*, and the width ranges from 0.1 cm in *Conyza linifolia* to 3.75 cm in *Sonchus oleraceus*.

Anatomical features: Anatomical features of petiole and blade cross section of studied species given in Table 3 and Plate 2.

Table 1. Collection data of the studied species of family Asteraceae from Eastern Region of Saudi Arabia, Sub-family & tribes according to Panero & Funk, 2002.

Sub-family	Tribe	Species	Locality and date
Asteroideae	Astereae	*Conyza linifolia* (Wild) Tackh	Rayan – Dammam, 1/2017
	Plucheae	*Pluchea dioscoridis* (L.) DC.	King Khaled street – Dammam, 5/2017
	Senecioneae	*Senecio glaucus* L.	Rayan – Dammam, 4/2017
	Gnaphalieae	*Ifloga spicata* (Forssk.) Sch.- Bip.	Rayan – Dammam, 4/2017
	Heliantheae	*Eclipta alba* (L.) Hassk.	Rayan – Dammam, 4/2017
Cichorioideae	Cichorieae	*Launaea mucronata* (Forssk.) Muschl	Rayan – Dammam, 3/2017
		Launaea nudicaulis (L.) Hook.f.	Rayan – Dammam, 3/2017
		Launaea procumbens (Roxb.) Romayya & Rajg.	Rayan – Dammam, 3/2017
		Sonchus oleraceus L.	Rayan – Dammam, 3/2017
		Reichardia tingitana (L.) Roth	Rayan – Dammam, 3/2017





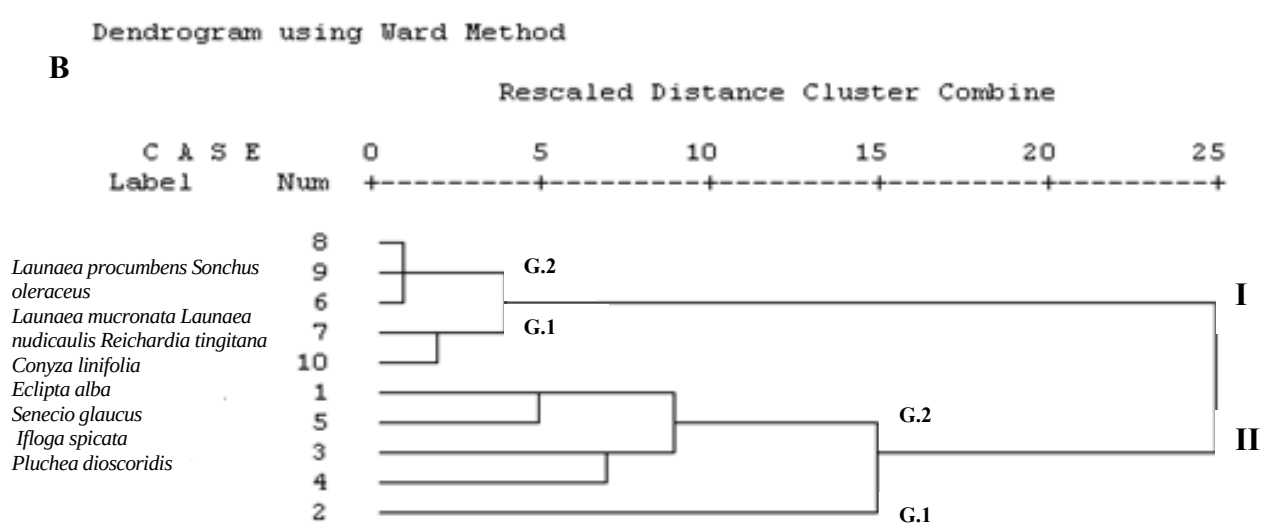


Fig. 1. Dendrograms showing the interrelationships between ten species of Asteraceae based on 45 characters of foliar characters. A: PRIMER Program; B: SPSS Program

Table 2. Macro-morphological characters of leaves for the studied Asteraceae species.

	Species / Characters		*Conyza linifolia*	*Pluchea dioscoridis*	*Senecio glaucus*	*Ifloga spicata*	*Eclipta alba*	*Launaea mucronata*	*Launaea nudicaulis*	*Launaea procumbens*	*Sonchus oleraceus*	*Reichardia tingitana*
Lower leaf	**Arrangement:** 1- Alternate 2- Opposite 3- Rosette		1	1	1	1	2	3	3	3	3	3
	Petiole:1- Sessile 2- Subsessile 3- Petiolate		3	1	3	3	2	3	3	3	3	3
	Petiole length (Cm.)	Mean	0.5-0.8 0.65	0	0.5-1.5 1	1-2 1.43	0.2-0.4 0.28	0.5-2.5 1.45	0.6-2.5 1.53	0.5-3 1.75	2.5-5 4.13	1-2.5 1.75
		Std.	±0.11	0	±0.38	±0.38	±0.08	±0.83	±0.76	±1.03	±1.19	±0.56
	Type: 1-Simple 2- Pinnatified 3- Pinnatipartite 4- Pinnatisect		1	1	4	1	1	3	2	2	3	2
	Outline: 1-Oblong 2- Oblong-lanceolate 3- Oblong-obovate 4- Elliptic lanceolate		2	3	3	3	4	2	1	1	2	1
	Base: 1-Normal 2- Aurticulate		2	2	2	1	1	1	1	1	1	1
	Blade length (Cm.)	Mean	2.25	6.38	3.93	2.83	2.78	14.63	6.25	5.75	11	3.13
		Std.	±0.36	±1.18	±0.38	±0.54	±0.23	±0.96	±1.48	±1.82	±0.94	±0.74
	Blade width (Cm.)	Mean	0.5	2.78	2.2	0.98	0.63	4.18	1.48	1.03	4.45	1.68
		Std.	±0.07	±0.64	±0.41	±0.15	±0.11	±0.77	±0.36	±0.37	±0.36	±0.61
	Blade base: 1-Symmetric 2- Asymmetric		1	2	1	1	1	1	1	1	1	1
Upper leaf	**Arrangement:** 1-Alternate 2- Opposite		1	1	1	1	2	1	1	1	1	1
	Petiole: 1-Sessile 2- Subsessile 3- Petiolate		2	1	1	3	2	1	3	1	1	1
	Petiole length (Cm.)	Mean	0.3	0	0	0.6	0.55	0	0.75	0	0	0
		Std.	±0.16	0	0	±0.07	±0.11	0	±0.18	0	0	0
	Type: 1-Simple 2- Pinnatified 3- Pinnatipartite 4- Pinnatisect		1	1	4	1	1	3	2	2	3	1
	Outline: 1-Oblong 2- Oblong-lanceolate 3- Oblong-obovate 4- Elliptic lanceolate 5- Linear		5	3	3	3	4	2	1	1	3	3
	Base: 1-Normal 2- Aurticulate		1	2	2	1	1	2	2	2	2	2
	Blade length (Cm.)	Mean	2.45	3.8	3.5	1.9	2.2	6.13	2.28	7.63	8.38	4.5
		Std.	±0.11	±1.16	±0.79	±0.66	±0.19	±1.52	±0.19	±2.16	±1.43	±0.94
	Blade width (Cm.)	Mean	0.15	1.8	2.2	0.63	0.65	2.38	0.9	2.3	3.75	1.55
		Std.	±0.05	±0.49	±0.59	±0.13	±0.11	±0.74	±0.07	±0.56	±0.56	±0.58

Table 3. Micro-morphological characters of leaves for the studied Asteraceae species.

	Species / Characters	*Conyza linifolia*	*Pluchea dioscoridis*	*Senecio glaucus*	*Ifloga spicata*	*Eclipta alba*	*Launaea mucronata*	*Launaea nudicaulis*	*Launaea procumbens*	*Sonchus oleraceus*	*Reichardia tingitana*
Petiole	**Outline:** 1- Arc shape 2- Half circle 3- Half circle with 2 ridges 4- Ob-triangle with 2 ridges	1	0	1	1	2	3	3	4	4	4
	Cuticle Thickening: 1- Thin 2- Thick 3- Very thick	2	0	3	2	1	2	2	2	1	2
	Cuticle Surface: 1- Smooth 2- Warty	2	0	2	1	1	2	2	2	1	2
	Epidermis:1- Radial 2- Mixed	2	0	1	2	2	1	2	2	2	2
	Ground tissue Type: 1- Parenchyma 2- Parenchyma and palisade 3- Parenchyma, palisade and collenchyma	2	0	2	2	3	2	2	2	3	1
	No. of vascular bundles	5	0	3	3	5	5	5	5	5	3
	Druses: 1- Present 2- Absent	2	0	2	2	2	2	1	2	2	2
Blade	Cuticl Thickening: 1- Thin 2- Thick 3- Very thick	2	2	3	1	1	2	2	2	1	2
	Cuticl Surface: 1- Smooth 2- Warty	2	2	2	1	1	2	2	2	1	2
	Epidermis:1- Radial 2- Mixed	2	2	2	2	2	1	2	2	2	2
	Mesophyll Type:1- Isolateral 2- Dorsiventral	1	2	2	2	2	1	2	2	2	2
	Mesophyll Midrib:1- Continuous 2- Dis-continuous	2	2	2	2	2	2	1	1	1	2
	No. of vascular bundles: 1- One 2- Three (1main & 2 lateral) 3- Five (1main & 4 lateral)	1	2	1	1	3	2	2	3	2	1
	Druses: 1- Present 2- Absent	2	2	2	2	2	2	1	2	2	2

Petiole anatomy: All the studied species of Asteraceae could be classified into four types based on the outline of their petioles: an arc shape in *Conyza linifolia*, *Senecio glaucus* and *Ifloga spicata*; a half-circle shape in *Eclipta alba*; a half-circle shape with 2 ridges in *Launaea mucronata* and *Launaea nudicaulis*; and an ob-triangle shape with 2 ridges in *Launaea procumbens, Sonchus oleraceus*, and *Reichardia tingitana*. The ground tissue exhibited three types: parenchyma and palisade tissue in six species, parenchyma only in one species (*Reichardia tingitana*), and collenchyma in combination with parenchyma and palisade tissue in two species (*Eclipta alba* and *Sonchus oleraceus*). The majority of the studied species had five vascular bundles (1 main bundle and 2 subsidiary bundles), except for *Senecio glaucus* and *Ifloga spicata*, which had three vascular bundles (1 main bundle and 2 subsidiary bundle). Schizogenous canals were present in all studied species, while druses are found only in *Launaea nudicaulis*.

Blade anatomy: The mesophyll of the blade in the studied species could be classified into two types: dorsiventral and isolateral. The dorsiventral type was predominant in 8 species, while the isolateral type was present in only two species. The palisade tissue was continuous in *Launaea nudicaulis*, *Launaea procumbens*, and *Sonchus oleraceus*, but discontinuous in the other studied species. The number of vascular bundles in the midrib region varied: one vascular bundle in *Conyza linifolia, Senecio glaucus, Ifloga spicata*, and *Reichardia tingitana*; three vascular bundles (1 main and 2 lateral) in *Pluchea dioscoridis, Launaea mucronata, Launaea nudicaulis*, and *Sonchus oleraceus*; and five vascular bundles (1 main and 2 lateral on each side) in *Eclipta alba* and *Launaea procumbens.*

Plate 1. Leave morphology of 10 species of Asteraceae as revealed by light microscopy: 1. *Conyza linifolia*; 2. *Pluchea dioscoridis*; 3. *Senecio glaucus*; 4. *Ifloga spicata*; 5. *Eclipta alba*; 6. *Launaea mucronata*; 7. *Launaea nudicaulis*; 8. *Launaea procumbens*; 9. *Sonchus oleraceus*; 10. *Reichardia tingitana*. A= Lower leaf; B= Upper leaf

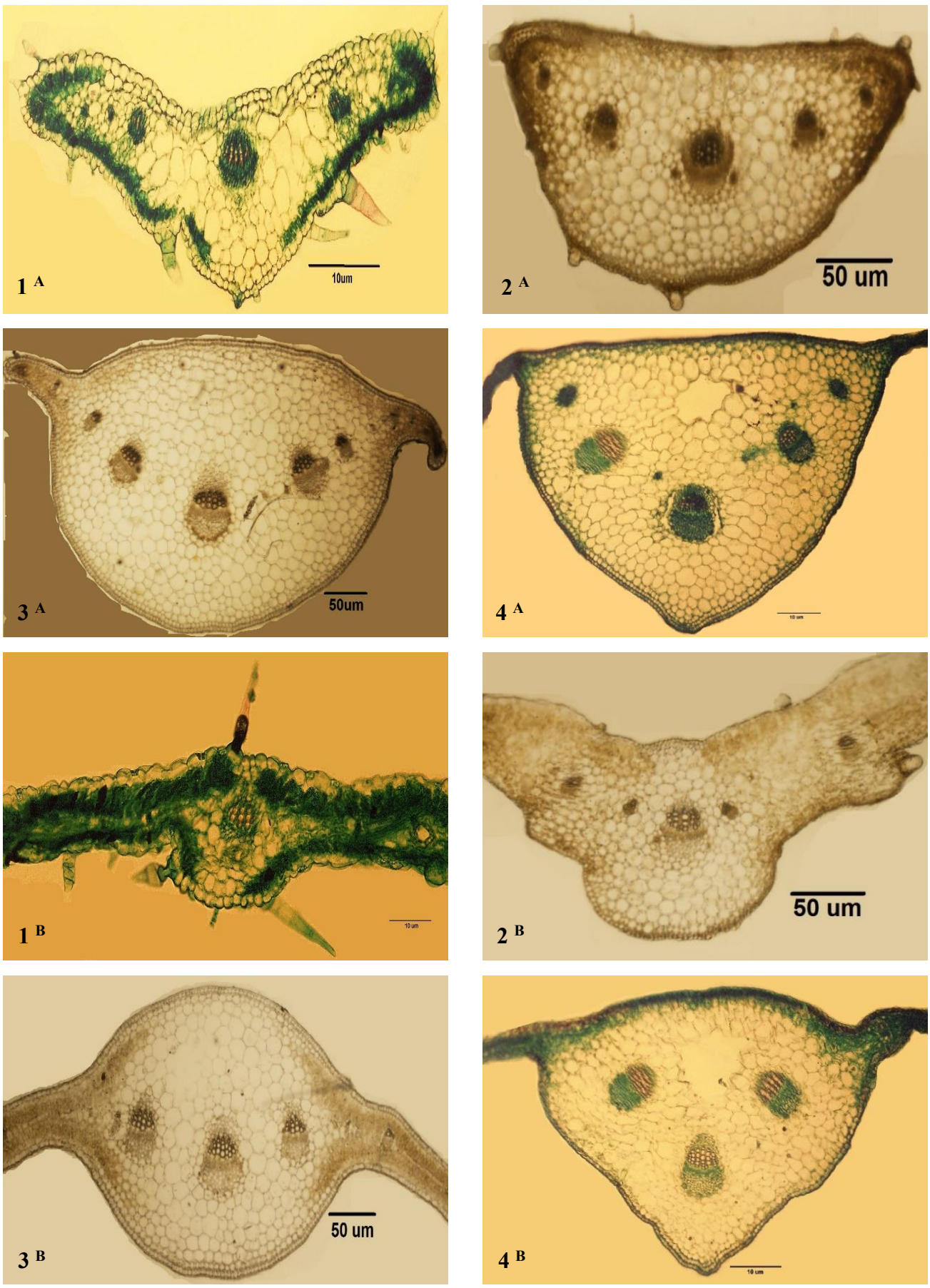


Plate 2. Main anatomical structure of 10 studied species of Asteraceae as revealed by light microscopy: 1. *Conyza linifolia*; 2. *Eclipta alba*; 3. *Launaea mucronata*; 4. *Sonchus oleraceus*.
A= Petiole cross section; B= Blade cross section

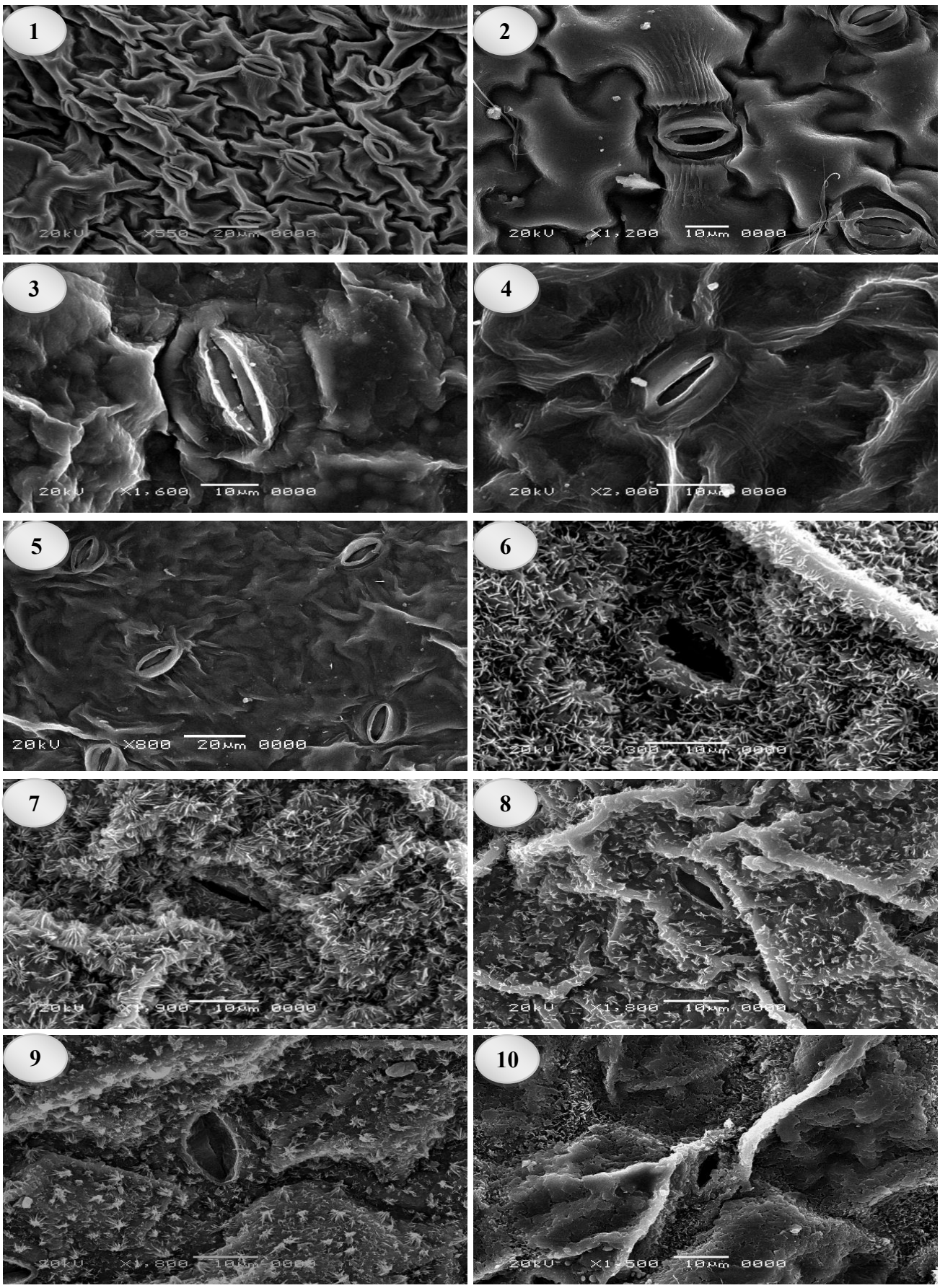


Plate 3. Stomatal characters and Cuticular ornamentation of 10 species of Asteraceae as revealed by SAM: 1. *Conyza linifolia*; 2. *Pluchea dioscoridis*; 3. *Senecio glaucus*; 4. *Ifloga spicata*; 5. *Eclipta alba*; 6. *Launaea mucronata*; 7. *Launaea nudicaulis*; 8. *Launaea procumbens*; 9. *Sonchus oleraceus*; 10. *Reichardia tingitana*.

Table 4. Stomatal characters and Cuticular ornamentation of studied species.

	Species / Characters	*Conyza linifolia*	*Pluchea dioscoridis*	*Senecio glaucus*	*Ifloga spicata*	*Eclipta alba*	*Launaea mucronata*	*Launaea nudicaulis*	*Launaea procumbens*	*Sonchus oleraceus*	*Reichardia tingitana*
Stomatal apparatus	**Level:** 1- Semi-depressed. 2- Depressed 3- At level.	1	3	1	1	3	2	2	2	3	1
	Outline: 1- Elongated. 2- Sub-orbicular	1	2	1	1	1	2	2	1	1	2
	Aperture shape: 1- Elliptic. 2- Oblong 3- Ovate	1	1	1	2	1	3	1	1	1	1
	Aperture width: 1- Narrow 2- Wide	1	2	1	1	2	2	1	2	2	2
	Stomatal rim: 1- Slightly raised. 2- Raised. 3- Flat	2	2	2	3	2	2	1	2	1	1
	Inner stomatal rim: 1- Smooth. 2- Sinuolate erose. 3- Sinuolate	1	2	3	3	2	3	3	2	3	3
	Peri-stomatal rim: 1- Overlapping-stout. 2- Overlapping. 3- Stout. 4- Absent	1	2	3	1	4	4	2	4	4	2
Cuticular ornamentation		1	1	3	6	2	4	4	5	4	4

Cuticular ornamentation: 1- Favulariate plane with slightly striated, 2- Favulariate Plane, 3- Favulariate- granulate, 4- Favulariate plane with upright sacles of wax, 5- Favulariate plane with short branched scales, 6- Slightly striated

Stomatal characters and cuticular ornamentation: In all the studied species, two compound types of stomata were observed: anomocytic and anisocytic. Stomatal leveling exhibited three types: at level in *Eclipta alba* and *Sonchus oleraceus*, semi-depressed in *Conyza linifolia, Senecio glaucus, Ifloga spicata*, and *Reichardia tingitana*, and depressed in the remaining species. The stomatal outline ranged from sub-orbicular to elongate, with elongated being the predominant type in six of the studied species. The aperture shape showed 3 types: elliptic, oblong, and ovate, with elliptic being the primary shape in eight of the studied species, oblong in *Ifloga spicata*, and ovate in *Launaea mucronata*. Aperture was wide in most studied species and narrow in *Conyza linifolia, Senecio glaucus, Ifloga spicata*, and *Launaea nudicaulis*. The stomatal rim exhibited three types: flat in *Ifloga spicata*, slightly raised in *Launaea nudicaulis, Sonchus oleraceus,* and *Reichardia tingitana*, and raised in the remaining species. The inner stomatal rim displayed three types: smooth, sinuolate erose, and sinuolate. The peri-stomatal rim was absent in four of the studied species, while the remaining species exhibited three types of peri-stomatal rim: overlapping-stout, overlapping, or stout. Overlapping-stout was observed in *Conyza linifolia* and *Ifloga spicata*, overlapping in *Pluchea dioscoridis, Launaea nudicaulis*, and *Reichardia tingitana*, and stout in *Senecio glaucus* only.

Six types of cuticular ornamentation was observed. *Conyza linifolia* and *Pluchea dioscoridis* exhibited favulariate plane with slightly striated ornamentation. *Eclipta alba* showed favulariate Plane ornamentation. *Senecio glaucus* displayed favulariate-granulate ornamentation. *Launaea mucronata*, *Launaea nudicaulis, Sonchus oleraceus*, and *Reichardia tingitana* exhibited favulariate plane with upright sacles of wax ornamentation. *Launaea procumbens* displayed favulariate plane with short branched scales ornamentation. Lastly, *Ifloga spicata* showed slightly striated ornamentation (Table 4 and Plate 3).

Table 5. Trichomes types of the studied species.

Species / Trichomes types		*Conyza linifolia*	*Pluchea dioscoridis*	*Senecio glaucus*	*Ifloga spicata*	*Eclipta alba*	*Launaea mucronata*	*Launaea nudicaulis*	*Launaea procumbens*	*Sonchus oleraceus*	*Reichardia tingitana*
Non-glandular	1	2	2	1	2	2	2	2	2	1	2
	2	2	2	2	2	2	2	2	1	2	2
	3	2	2	2	2	2	1	1	2	2	2
	4	1	2	2	2	2	2	2	2	2	2
	5	1	2	2	2	2	2	2	2	2	2
	6	1	2	2	2	2	2	2	2	2	2
	7	2	2	2	2	1	2	2	2	2	2
	8	2	2	2	1	2	2	2	2	2	2
Glandular	9	1	1	1	1	2	1	1	1	1	2
	10	2	2	1	2	2	2	1	2	2	2
	11	2	2	2	2	2	2	1	1	1	2
	12	2	1	2	2	2	2	2	2	2	2

1= Present; 2= Absent

Types of trichomes: Trichomes play a crucial role in taxonomic identification of different species (Adedeji *et al*., 2007). The studied species of Asteraceae exhibited approximately 12 types of trichomes, comprising 8 non-glandular and 4 glandular types (Table 5). There was significant variation in the distribution of trichome types among the species, with most species possessing both glandular and non-glandular trichomes. *Pluchea dioscoridis* exclusively displayed glandular trichomes, while *Eclipta alba* only had non-glandular trichomes. Additionally, *Reichardia tingitana* was found to be glabrescent (without trichomes). Certain trichome types were unique to specific species. For example, multicellular papillose trichomes were specific to *Launaea procumbens*, while *Conyza linifolia* exhibited bicellular blunt apical cell with short basal cell, multicellular acute apical cell with branched basal cell, and multicellular tuberculated acute apical cell with flat basal cell. *Eclipta alba* was characterized by multicellular tuberculated acute apical cell with round curly basal cell, and *Ifloga spicata* possessed flagellate filiform trichomes. Moreover, *Pluchea dioscoridis* had glandular capitate trichomes with multicellular stalks and unicellular heads, which were distinct to that species.

The following types of trichomes have been recorded on the leaves of the studied species:

A. Non-glandular

1. Unicellular papillose (Plate 4, Fig. 1A); *Senecio glaucus* and *Sonchus oleraceus*
2. Multicellular papillose *(*Plate 4, Fig. 2A); *Launaea procumbens*
3. Unicellular with blunt apical cell and short broad basal cell (Plate 4, Fig. 3A); *Launaea mucronata* and *Launaea nudicaulis*
4. Bicellular blunt apical cell with short basal cell (Plate 4 Fig. 4A); *Conyza linifolia*
5. Multicellular acute apical cell with branched basal cell. (Plate 4, Fig. 5A); *Conyza linifolia*
6. Multicellular tuberculated acute apical cell with flat basal cell. (Plate 4, Fig. 6A); *Conyza linifolia*
7. Multicellular tuberculated acute apical cell with round curly basal cell. (Plate 4, Fig. 7A); *Eclipta alba*
8. Flagellate filiform trichome (Plate 4, Fig. 8A); *Ifloga spicata*

B. Glandular

1. Unicellular stalk and unicellular head. (Plate 4, Fig. 10 B); *Conyza linifolia*, *Pluchea dioscoridis*, *Senecio glaucus*, *Ifloga spicata*, *Launaea mucronata*, *Launaea nudicaulis*, *Launaea procumbens* and *Sonchus oleraceus*
2. Unicellular stalk and multicellular head. (Plate 4, Fig. 9 B); *Senecio glaucus* and *Launaea mucronata*
3. Multicellular stalk and multicellular head. (Plate 4, Fig. 10 B); *Launaea mucronata*, *Launaea nudicaulis* and *Launaea procumbens*
4. Capitate with multicellular stalk and unicellular head (Plate 4, Fig. 11 B); *Pluchea dioscoridis*

Trichome ornamentation: Trichome ornamentation is rarely used for leaf anatomical diagnoses but is well worth consideration for diagnostic value between families or genera. The following types of trichome ornamentations were recorded on the leaves of studied species:

1. Smooth in *Sonchus oleraceus, Conyza linifolia, Ifloga spicata* and *Senecio glaucus.* (Plate 4, Fig. 1 A,4A, 8A, 9A).
2. Warty (roundish warts) in *Launaea mucronate, Launaea procumbens* and *Pluchea dioscoridis*. (Plate 4, Fig. 3 A, 10A, 11A).
3. Tuberculated in *Launaea procumbens, Conyza linifolia* and *Eclipta alba* (Plate 4, Fig. 2A, 5 -7A).

The key: The recorded data in Tables 2-5 were used to construct the following bracketed key to the 10 species of Asteraceae.

1 → Leaf sessile with asymmetric blade base and glandular Capitate trichome with multicellular stalk and unicellular head ……. ***Pluchea dioscoridis***

● → Leaf petiolate or subsessile with symmetric blade base and glandular Capitate trichome is absent ..…….. **2**

2 → Leaf subsessile, opposite with elliptic lanceolate shape, outline of petiole in cross section half circle and favulariate plane, cuticular ornamentation with Multicellular tuberculated acute apical cell with round curly basal cell ……. ***Eclipta alba***

● → Leaf petiolate, alternate or rosette ……..…… **3**

3 → Lower leaf alternate with arc shape petiole outline ……...……..…….. **4**

● → Lower leaf rosette and petiole, outline not arc shape ……..……...……. **6**

4 → Leaf pinnatisect with stout peri-stomatal rim and favulariate-granulate cuticular ornamentation .... ***Senecio glaucus***

● → Leaf simple with overlapping-stout peri-stomatal rim and different cuticular ornamentation …..……..…….. **5**

5 → Leaf simple with oblong-obovate shape and base not auriculate vascular bundles in petiole 3, mesophyll dorsiventral, slightly striated cuticular ornamentation and flagellate filiform trichome ...……...…..... ***Ifloga spicata***

● → Leaf simple with oblong-lanceolate shape and aurticulate base in lower and with linear and normal base in upper leaf, vascular bundles in petiole 5, mesophyll isolateral, favulariate plane with slightly striated cuticular ornamentation and multicellular acute apical cell with branched basal cell trichome .....…..… ***Conyza linifolia***

6 → Leaf pinnatipartite with oblong-lanceolate shape ……..……...……. **7**

● → Leaf pinnatified with oblong shape …..……..……..….. **8**

7 → Outline of petiole in cross section ob-triangle with two ridges, Mesophyll continuous dorsiventral, stomata atlevel, elongated with elliptic aperture and flat stomatal rim …….. ***Sonchus oleraceus***

● → Outline of petiole in cross section half circle, Mesophyll discontinuous isolateral, stomata depressed, sub-orbicular with ovate aperture and raised stomatal rim ..……..... ***Launaea mucronata***

8 → Upper leaf simple with oblong-ovate shape, vascular bundles in petiole cross section 3, mesophyll discontinuous with semi-depressed stomata ...……...… ***Reichardia tingitana***

● → Upper leaf pinnatified with oblong shape, vascular bundles in petiole cross section 5, mesophyll continuous with depressed stomata ……..……. **9**

9 → Upper leaf petiolate, petiole outline in cross section half circle with two ridges, vascular bundles in blade 3 and favulariate plane with upright scales of wax cuticular ornamentation ……..…….…… ***Launaea nudicaulis***

● → Upper leaf sessile, petiole outline in cross section obtriangle with two ridges, vascular bundles in blade 5 and Favulariate plane with short branched scales ….……..… ***Launaea procumbens***

Numerical analysis

The foliar features of the studied species were examined using both light and scanning electron microscopy. A total of 45 characters were selected for numerical analysis, utilizing the clustering method as a tool to determine the relationships between the species and the taxonomic significance of foliar characters in the Asteraceae family.

The statistical program Primer was employed to analyze the data, employing agglomeration of Schedule measure Euclidean distance and similarity with complete linkage between groups (Fig. 1A). The results classified the studied species into two main clusters and two groups: *Pluchea dioscoridis* in cluster (I), while the remaining species were grouped in cluster (II). Group (I) comprised *Launaea nudicaulis, Launaea procumbens, Launaea mucronata, Sonchus oleraceus*, and *Reichardia tingitana*, while group (II) consisted of *Eclipta alba, Senecio glaucus, Conyza linifolia*, and *Ifloga spicata*.

Additionally, the results obtained from the SPSS program using clustering methods, particularly analyzed by the ward method and measure distance cluster combine (Fig. 1B), revealed that the species were grouped into two major clusters, each was divided into two groups. Cluster (I) comprised 5 species: *Launaea procumbens, Sonchus oleraceus*, and *Launaea mucronata* in group (I), and *Launaea nudicaulis* and *Reichardia tingitana* in group (II). Cluster (II) encompassed 5 species: *Pluchea dioscoridis* in group (I), while *Conyza linifolia, Eclipta alba, Senecio glaucus,* and *Ifloga spicata* were recorded in group (II).

Discussion

The leaves' characters, including morphology, anatomy, and epidermal features, play an important role in plant taxonomy Ozcan *et al*., (2015). The morphology of the studied leaves exhibits various distinguishing characteristics among the species, such as leaf arrangement, type, shape, and the presence or absence of a petiole, as supported by Pilbeam & Bell (1979) and Adedejj (2004).

For the anatomical features in this study, the main type of mesophyll observed was dorsiventral, which was found in 8 species, while isolateral mesophyll was observed in only 2 species. This finding aligns with previous studies conducted by Metcalf & Chalk (1979) and Kadereit & Jeffry (2007). The presence of subsidiary bundles was recorded in the petioles of upper and lower leaves of all studied species and in the blades of some species. This observation was consistent with findings reported by Inceer & Ozcan (2011), Ozcan *et al*., (2014), and Ozcan *et al*., (2015).

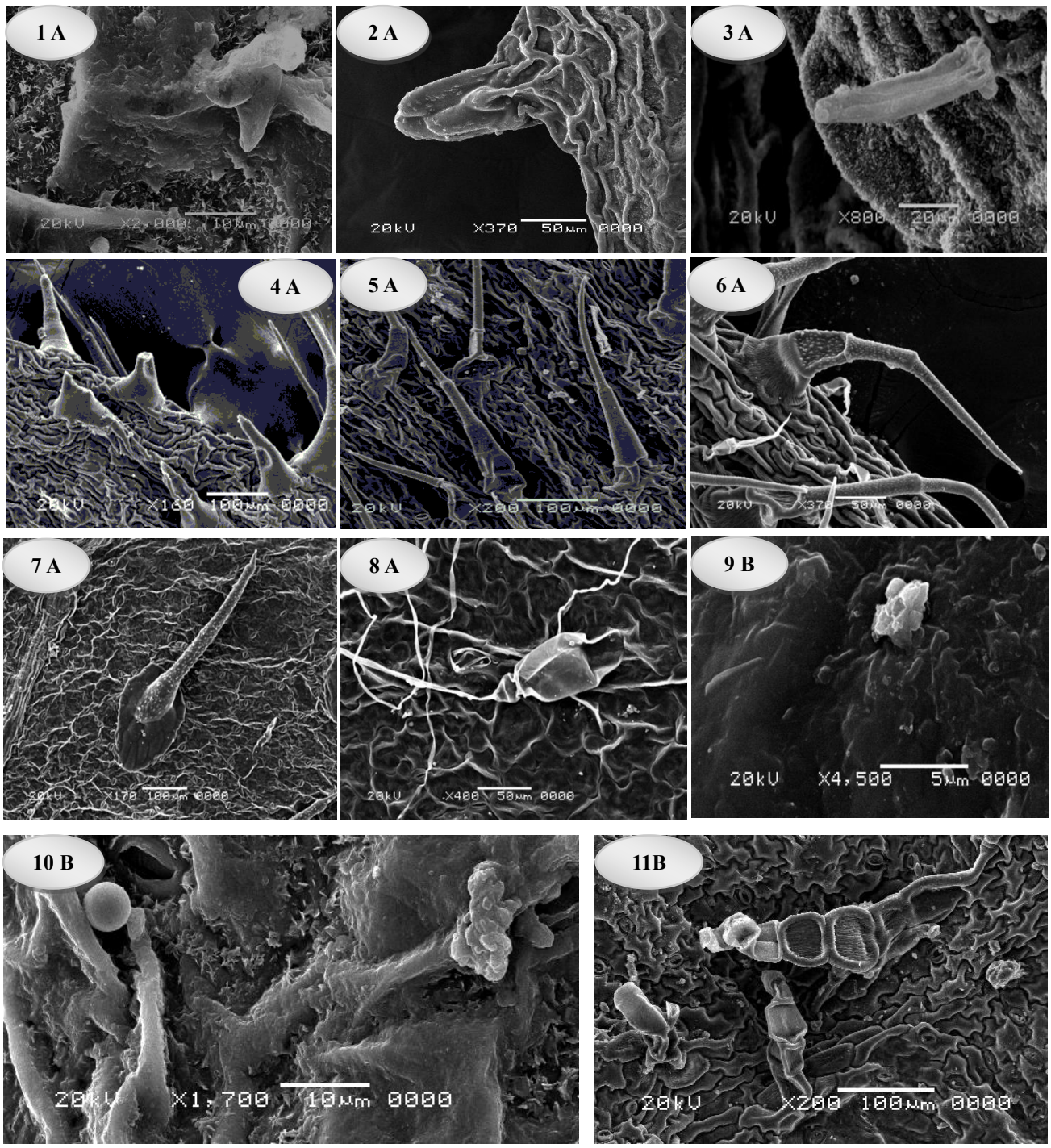


Plate 4. Main types of non-glandular and glandular trichomes for 10 species of Asteraceae as revealed by SAM: 1. *Sonchus oleraceus*; 2. *Launaea procumbens*; 3. *Launaea mucronata*; 4, 5, 6. *Conyza linifolia*; 7. *Eclipta alba*; 8. *Ifloga spicata*; 9. *Senecio glaucus*; 10. *Launaea procumbens*; 11. *Pluchea dioscoridis*.
A- Non-glandular; B- Glandular

Stomata in the studied species were typically anisocytic-anomocytic, which was in agreement with Metcalf & Chalk (1979), Kadereit & Jeffry (2007), and Ozcan *et al.*, (2015). The cuticular ornamentation observed in the studied species could be categorized into 6 types: 1) Favulariate plane with slightly striated, 2) Favulariate Plane, 3) Favulariate-granulate, 4) Favulariate plane with upright sacles of wax, 5) Favulariate plane with short branched scales, and 6) Slightly striated. These characteristics are useful for distinguishing between different species within the same genus. A total of 12 types of trichomes were recorded in the studied species, including 8 non-glandular and 4 glandular trichomes. While most species possessed both types of trichomes, *Pluchea dioscoridis* exclusively exhibited glandular trichomes, and *Eclipta alba* exhibited non-glandular trichomes, as reported by Perveen *et al.*, (2016). In the case of *Conyza* species, previous studies by Shabeena *et al.*, (2014) and Perveen *et al.*, (2016) described the trichomes as multicellular, long, hair-like, unbranched,

and non-glandular, with either blunt tips and swollen bases or pointed tips and flat bases. In this current study, bicellular blunt apical cells with short basal cells, multicellular acute apical cells with branched basal cells, and multicellular tuberculated acute apical cells with flat basal cells were observed in *Conyza linifolia*. The numerical analysis results classified the studied species into two major clusters. Cluster (I) *comprised Launaea procumbens, Sonchus oleraceus, Launaea mucronata, Launaea nudicaulis,* and *Reichardia tingitana*. Cluster (II) included *Pluchea dioscoridis, Conyza linifolia, Eclipta alba, Senecio glaucus,* and *Ifloga spicata*. This classification aligns with the classification proposed by Panero & Funk (2002).

Conclusion

The studied species exhibited variation in their leaf morphology and anatomy, which are crucial for plant classification and identification. This variation includes leaf arrangement, types, shape, and the presence or absence of a petiole. Some species showed differences between their upper and lower leaves in terms of leaf types and petiole length. Anatomical features, such as petiole outline, mesophyll type and the number of vascular bundles in the petiole and blade played a significant role in the identification of the studied species. Cuticular ornamentation and different types of trichomes also contributed to species differentiation. The numerical analysis results highlighted the importance of leaf characters for taxonomical classification and differentiation at the subfamily and genus levels. Overall, the morphological and anatomical features of leaves, as reported in this study, demonstrate significant importance in identifying different species within the same family.

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