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Anatomical Investigation of Leaves of *Sida* L. in Uyo, Nigeria and the Taxonomic Implications

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Abstract

Micromorphological features of the leaves of eight common species of *Sida* in Uyo, Nigeria were examined, in order to determine the taxonomic relationship between them. Plant samples were randomly collected from four locations in Uyo metropolis for investigation. The leaves were hypoamphistomatic and the stomata anisocytic. Abaxial epidermal cells had undulating anticlinal walls except in *S. stipulata*. Abnormal stomata were found only in *S. stipulata*.. Druses, hydropoten cells and Trichomes occurred in all the species. Trichomes of *S. stipulata* were the shortest (30µm). Glandular trichomes occurred on the abaxial epidermis in all the species except *Sida* sp. where they were found on both adaxial and abaxial surfaces. The Stomatal Index (S.I.) was highest in *S. scabrida* (25%) and least in *S. stipulata* (18%). Unicellular trichome density was higher than other trichomes in all the species except in *Sida* sp. with a higher density of glandular and stellate trichomes. Striae were observed in all the species. *S. stipulata* differs from the other species probably due to environmental factors while *Sida* sp. is a possible new species or a hybrid whose parents can be determined by further investigations.

Keywords: Micromorphology, Trichomes, Druses, Hydropoten, Hypoamphistomatic.

Contents

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1. Introduction

The genus *Sida (*Malvaceae) consists of herbs or under shrubs with stellate hairs. Leaves toothed, stipules linear to leafy, flowers are either solitary, paired or in clusters, style as many as carpels. Hutchinson and Dalziel [1] They are very common weeds in the Uyo metropolis.

Studies by Kunnur and Kotresha [2] on foliar epidermis of some *Sida* species, supported by cytology and morphology of the plant have revealed that *S. rhombifolia* is distinct from *S.acuta, S. cordata* and *S. cordifolia*. According to them, trichomes are absent in *S. acuta.* In their work, [3] reported that the ordinary epidermal cells of the leaves of *Sida L.* were commonly polygonal or irregular in form with merely curved to nearly straight walls, often thickened. Stomata were anomocytic, paracytic, diacytic and aniso-cytic. The differences in most of the anatomical features were of little taxonomic importance to delimit deferent taxa under study with certainty. The foliar trichomes of *Sida* however possessed a remarkable diversity and provided a great deal of systematic evidence. There were six main types; typically peltate, stellate and forked trichomes present in all the species investigated, accompanied by either conical hair as in *S. cordata and S. yunnanensis*, by stalked capitate trichomes as in *S. alii and S. spinosa* or by multicellular and uniseriate trichomes as in *S. mysorensis*. Features of hairs were broadly regarded as useful for establishing the systematic relations within the family Malvaceae [4].

Nigerian *Sida* L. have been identified on the basis of morphological features such as carpel, floral, folial and stem morphology, Hutchinson and Dalziel [1] and Aworinde, et al. [5] also identified Nigerian *Sida* on the basis of their leaf characters such as apex, base, margin, shape and texture.

Uyo is the fast growing state capital of Akwa Ibom State, Nigeria. It is home to the campuses of the University of Uyo. Massive infrastructural development has been taking place in the campuses as well as in the city itself. However, species of *Sida* thrive as weeds for most of the year. Current information on the status of *Sida* is lacking particularly concerning Akwa Ibom State. This work seeks to examine the foliar micro-morphological characters of common *Sida* species and use same to delimit the taxa.

2. Materials and Methods

Species of *Sida* were collected from the following four locations in Uyo metropolis; University of Uyo annex, University of Uyo main campus , University of Uyo town campus and Shelter Afrique Estate in Mbiabong, Uyo. The species collected were taken to the herbarium for identification, authentication and processing for storage. During identification, one of the collections could not fit into the description in the Flora. It was therefore authenticated without a specific epithet pending the outcome of the investigations. Voucher specimens were all stored at the University of Uyo Herbarium in the Department of Botany and Ecological Studies.

Materials for foliar epidermal anatomy were prepared following the technique of Cotton [6]. The stored leaves were collected, rinsed before it was scrapped. The adaxial surface was scrapped to view the abaxial surface structures and abaxial was scrapped to view the adaxial surface structures. The scrapped area was then rinsed, stained with saffranin and the stain was allowed to stay for about 2-5mins before it was rinsed and mounted in glycerol and examined using an Olympus light microscope. Photographs were taken with a Motic micrograph unit.

Measurements of epidermal and stomata length and width were taken at x100 objective while trichome measurements were made at x10 objective. The L/W ratio was calculated and the Stomata Index (S.I) was also calculated using the formula $S.I = \frac{S}{E+S} \times 100$. Where S = Number of stomata per view and E = Number of epidermal cells per view [7]. Trichome types were noted, measurement and density was determined. Presence or absence of druses, striae and hydropoten cells were also investigated. All measurements were subjected to statistical analyses.

3. Results

The results obtained in this investigation are summarised in Table 1 and 2 and Plates 1-8. A total of 8 species of *Sida* were examined. As listed in Table 1 they include *Sida acuta* Burm. f., *S. alba* Linn., *S. corymbosa* R.E.Fries, *S. ovata* Forsk fl., *S. scabrida* Wight & Arn., *S. stipulata* Car., *S. urens* Linn.and *Sida* sp. Stomata were more on the abaxial surface but similar in number for all the species. The ratio of stomata length to width was mostly 1:1 for both the adaxial and abaxial surfaces except in *S. ovata* which had a ratio of 1:2 on both adaxial and abaxial surfaces. The stomata index (S.I.) was higher on the abaxial surface in all species and the highest was 25% in *S. scabrida* and the least was 18% in *S. stipulata*.

Epidermal cells were more on the abaxial surface and, the cells were longer and wider on the adaxial surface than on the abaxial surface. The L/W ratio for epidermal cells was 1:2 in all the species except in *S. stipulata* and *S. scabrida* which had 1:1 on the adaxial surface.

In Table 2 druses were present on both adaxial and abaxial surfaces of *S. stipulata, S. scabrida, S. corymbosa* and *S. acuta* but present on only the abaxial surface of *S. alba, S. urens* and *Sida species* while it was absent in *S. ovata.* Stellate and glandular trichomes occurred more on the abaxial surface than on the adaxial epidermides of all the taxa and glandular trichomes were present on the adaxial surface of only *Sida*. sp. Stellate trichomes were present on both the adaxial and abaxial epidermides of *S.alba, S. corymbosa, S. urens, S. ovata* and *Sida* sp. but present on only the abaxial surface of *S. scabrida, S.stipulata* and *S. acuta*. In terms of density, there were more unicellular trichomes in all the species except in *Sida* sp. which had more of stellate and glandular trichomes. *Sida alba* had the highest density of unicellular trichomes followed by *S. corymbosa* while *S.urens* and *Sida* sp. had the highest density of stellate trichomes. *Sida corymbosa* and *Sida* sp. had the highest density for glandular trichomes. Two-armed trichomes were observed in *S. acuta, S. urens* and *Sida* sp. (Plates 1, 7 and 8). Four-armed trichomes were observed in *S. alba, S. ovata* and *Sida* sp. (Plate 2, 4 and 8).

Hydropoten cells (Plates 1d;2f;3,c,f;4i;5d,g; 6g;7b,h;8e,m) and anisocytic stomata (Plates 1c,h;2e;3d,k;4b;5a,i;6f,i;7g;8c) were observed on both adaxial and abaxial surface in all the species. Striae were

observed in all the species as seen in *S. acuta* and *S. corymbosa* (Plates 1c,d and 3c,d) but were faint in *S. scabrida* (Plate 5b) and *Sida* sp. (Plate 8e).

4. Discussion

The stomatal types based on the terminology of Metcalf and Chalk [7] were mainly anisocytic in all cases. They also described leaves with more stomata on the abaxial epidermis as being hypoamphistomatic which was the case in all the species of *Sida* examined in this work. The epidermal cell walls were more undulating in the abaxial than the adaxial epidermises. *Sida acuta* Burm *f, S alba* Linn., *S. corymbosa* R.E.Fries, *S. ovata* Forsk fl, *S scabrida* Wight & Arn., *S.urens* Linn. and *Sida* sp. had more undulating abaxial cell walls (Plates 1,2,3,4,5,7 and 8) than *S. stipulata* Car. (Plate 6). Bassey and Nyananyo [8] as well as Bassey and Okoli [9] also noted that anticlinal walls of epidermal cells were undulating in the ferns they worked on. Bogaard [10] observed that cell wall undulation is a reflection of adequate habitat moisture and that undulating walls provide leaves with greater tensile strength. Samples of *Sida stipulata* used in this work were collected from locations such as an abandoned cement embankment at a building site. Others were collected completely exposed in the middle of a dry dirt road and frequently crushed by vehicles. Jones and Rowe [11] stated that the degree of undulation of epidermal anticlinal cell walls with other gross features were very useful characters for distinguishing sun and shade morphotypes and that the undulation pattern is frequently more pronounced in shade than in sun leaves. Most *Sida* species cannot be considered as shade plants since they often occur along roadsides.

Many anomalous stomata (aborted guard cells, half stomata –with only one guard cell with or without a pore and twin stomata) were observed in the epidermis in *S. stipulata* (Plate 6c-f). This could be due to the impact of cement or other chemical pollution from the collection site on the tissues. A large number of druses were also observed in the epidermis of *S. stipulata*. Jianping, et al. [12] used druses and other types of calciphytoliths (calcium oxalate crystals) to delimit species of *Camellia sinensis*. Webb [13] on the other hand, stated that the role of druses is to provide long-term storage of calcium because the crystals apparently can be mobilized and degraded as needed. The crystals might also serve as a calcium sink, immobilizing excess calcium because plants regularly absorb more calcium than needed. This may explain the presence of druses mostly around the vascular bundles on the epidermis in all the species of *Sida* (Plates 4g, h, 5h, 6i, 7e). A structural role for crystals as tissue stiffeners has been supported by Gary [14]. It has been suggested that the crystals could function as deterrents to herbivores [15]. In this role, chewing druses would be like chewing sand.

S. stipulata unlike the other species, was more or less glabrous with very scanty stellate and unicellular trichomes (Plate 6i and 6j respectively) on the abaxial surface only. These were visible only under the microscope. *Sida* sp. on the other hand, had the highest number of glandular trichomes and also had more stellate to unicellular trichomes (Table 2) compared to the other species (Plate 8a,f,g,h,i). *S. acuta* had the longest unicellular trichomes (80-125µm) and this contradicts the report of who stated that trichomes were absent in *S. acuta*. Jones and Rowe [11] observed that the frequency of trichomes help to distinguish sun and shade morphophytes. As a result, the absence or presence of trichomes could be influenced by the habitat in which the plant is found. Aworinde, et al. [5] in their work noted that *S. scabrida* was glabrous. However, both stellate and unicellular trichomes were found in this investigation (Table 2 and (Plate 5c). Hydropoten cells were common in both adaxial and abaxial epidermal tissue of all the species investigated (Plates 1d, 2g, 3c f, 4i, 5d, g, 6g, 7b, h, 8e, m.). Hydropoten cells are water drinking cells and therefore are characteristic features of hydrophytes [7]. The fact that most of the samples were collected from muddy roadsides constantly fed by runoff from large ponds on the road, could account for the occurrence of the hydropoten cells. Striae are depositions on the cuticle which are diagnostic in their distribution and orientation [16]. Their presence in all the species investigated shows that the species are closely related to each other. Ugbabe and Ayodele [17] used the occurrence of striations to delimit 2 of the species they worked on from others.

In conclusion, the presence of striae, hydropoten cells, Presence of unicellular trichomes, glandular trichomes on the abaxial surface, anisocytic stomata and an hypoamphistomatic epidermis in all the species investigated, justifies their grouping into the same genus. However, the higher density of stellate and glandular trichomes than unicellular trichomes and the occurrence of glandular trichomes on both adaxial and abaxial epidermises delimit *Sida* sp. from the other species and justify the authentication process. It is possible that this is a hybrid whose parents could be determined by further cytogenetic investigations. The glabrescent nature of the epidermises (with the shortest unicellular trichomes), lack of undulation of the epidermal cell walls and having the lowest stomatal frequency (S.I. =18%) in *S stipulata* also delimits it from all the other species. Further investigations are also required to confirm that these differences are due to environmental factors

Table-1. Stomatal and Epidermal Investigations in Species of *Sida* in Uyo.

S/N	Taxa	Epid. Layer	Stomatal Characters					Epidermal Characters			
			No	L (µm) ±S.D	W (µm) ±S.D	Ratio L/W	S.I	No.	L (µm) ±S.D	W (µm) ±S.D	Ratio L/W
1	S. acuta	Ad	6	17±1.63	12±0.97	1:1	15	34	43±7.50	20±4.63	1:2
		Ab	13	17±1.63	13±0.99	1:1	21	50	41±7.50	21±4.63	1:2
2	S. alba	Ad	6	17± 0.68	13± 0.99	1:1	14	37	42± 7.91	22± 5.86	1:2
		Ab	13	18± 2.21	12± 1.09	1:2	21	49	46± 7.12	24± 6.79	1:2
3	S. corymbosa	Ad	3	19±2.83	13±1.32	1:1	9	32	46±10.01	24±4.39	1:2
		Ab	14	18± 1.81	13 2.95	1:1	24	44	45±14.85	23±5.98	1:2
4	S. ovata	Ad	4	20± 0.66	13± 0.92	1:2	11	32	44± 6.37	27± 6.44	1:2
		Ab	13	20± 1.82	13± 0.95	1:2	22	45	39± 7.30	20± 4.82	1:2
5	S. scabrida	Ad	10	16± 0.85	13±0.76	1:1	16	54	31±5.89	21±4.15	1:1
		Ab	18	17±2.18	14±0.94	1:1	25	53	36±14.27	18±4.14	1:2
6	S. stipulata	Ad	6	23± 1.38	16± 1.66	1:1	16	32	44± 8.26	31± 6.38	1:1
		Ab	9	21± 1.96	13± 3.30	1:2	18	42	39±10.20	20± 5.53	1:2
7	S .urens	Ad	6	21± 1.87	15± 0.94	1:1	15	34	44± 7.79	26± 5.76	1:2
		Ab	12	19± 1.81	13± 0.83	1:1	22	42	43±10.11	19± 4.62	1:2
8	Sida sp.	Ad	6	19±1.09	13±1.32	1:1	13	40	49±10.48	20± 4.31	1:2
		Ab	13	18±1.59	14±0.72	1:1	21	49	40±8.63	23±6.33	1:2

Ad = adaxial surface Ab=Abaxial

Table-2. Information on Druses and Tricho mes

S/N	Taxa	Druses	Stellate Trichome			Unicellular Trichome				Glandular Trichomes		
			+ / -	No.	Density / mm²	+ / -	No	Length(µm)	Density / mm²	+ / -	No.	Density / mm²
1	*S. acuta Ad*	+	-		0	+	8	80-125	0.95	-		0
	Ab	+	+	2, two –armed trichome also present	o.636	+	2	50-60	0.318	+	12	0.318
2	*S. alba* Ad	-	+	2	0.318	+	25	73-105	3.50	-		0
	Ab	+	+	6, four and five-armed trichome present	0.636	+	23	43-83	0.636	+	3	0
3	*S. Corymbosa* Ad	+	+	5	0.636	+	14	50-124	1.59	-		0
	Ab	+	+	1	0.318	+	5	56-112	0.318	+	10	0.95
4	*S. ovata* Ad	-	+	1, four-armed trichome present	0	+	19	53-112	0.636	_		0
	Ab	-	+	3	0.318	+	5	33-90	0	+	2	0.318
S/N	Taxa	Druses	+ /-	Stellate No	Trichome Density / mm²	+ / -	No	Unicellular Length (µm)	Trichome Density / mm²	+ /-	No	Glandular Density / mm²
5	*S. scabrida* Ad	+	-		0	+	8	95-111	0.636	-		0
	Ab	+	+	2	0	+	9	50-70	0.318	+	15	0.636
6	*S.stipulata* Ad	+	-		0	-			0	-		0
	Ab	+	-	1	0	+	2	28-30	0.318	+	3	0.318
7	*S.urens* Ad	-	+	3, two & four- armed trichome also present.	0.318	+	18	50-77	1.27	-		0
	Ab	+	+	9	0.318	+	16	30-70	0.636	+	9	0.636
8	*Sida* sp. Ad	-	+	1, two-armed trichome	0	+	2	85-93	0.318	+	2	0
	Ab	+	+	18, of two, three, four and five-armed trichome	1.91	+	12	32-54	0.636	+	21	0.95

Ad. = Adaxial, Ab=Abaxial

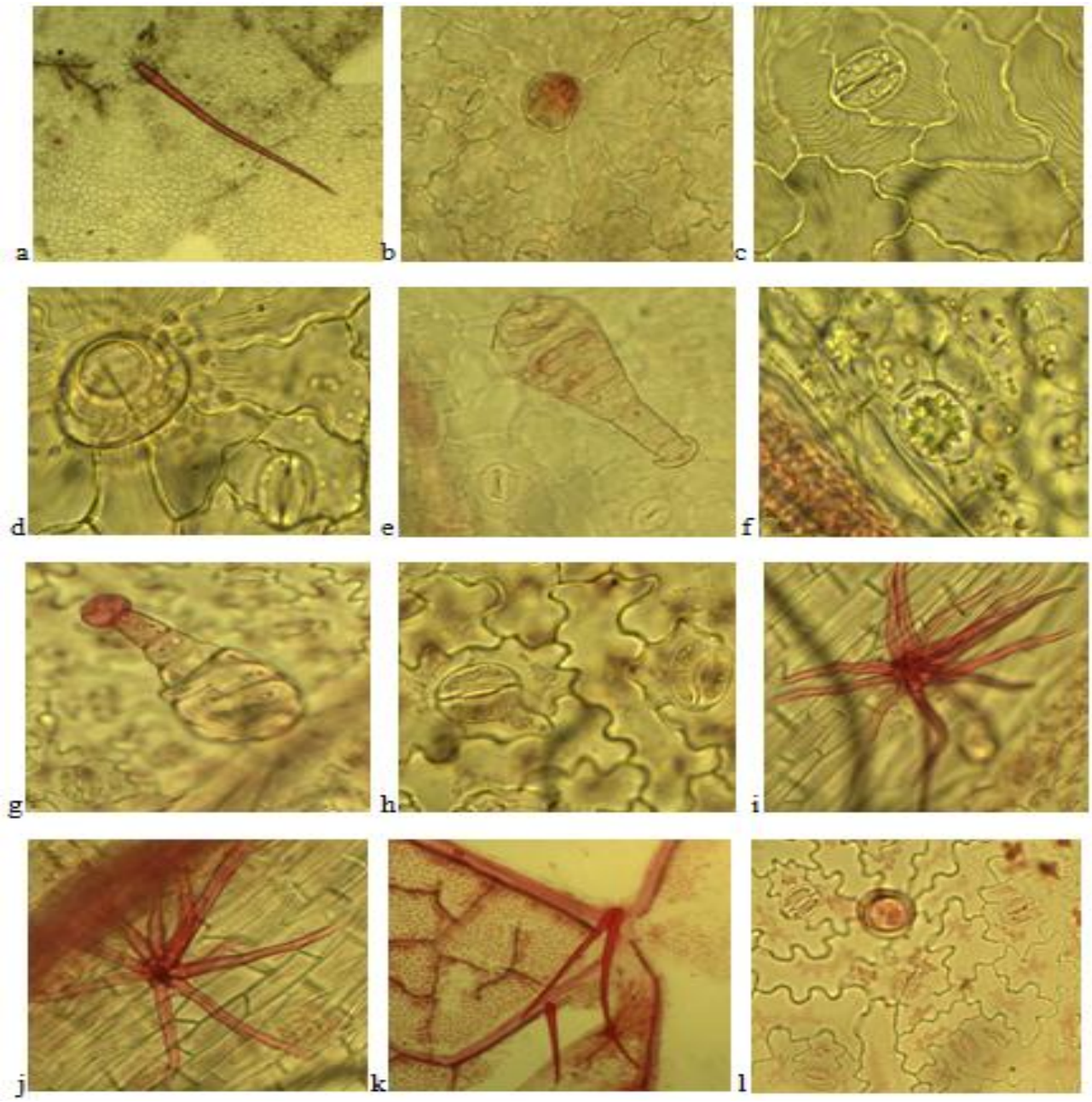

Plate-1. ***Sida acuta*** Burm. f.(Adaxial and Abaxial features). Adaxial features 1a: Unicellular trichome (x40) 1b: Basal cells of trichome (x400). 1c: Striae in epidermal and subsidiary cells (x1000). 1d: Hydropoten cell (x1000). 1e: Glandular trichome (x400). 1f: Druses (x1000). **Abaxial features** 1g: Glandular trichome (x40). 1h: Undulating cell walls(x1000) 1i&j: Stellate trichomes (x400). 1k: Two-armed trichomes (x100). 1l: Hydropoten cell and Epidermal cells (x400)

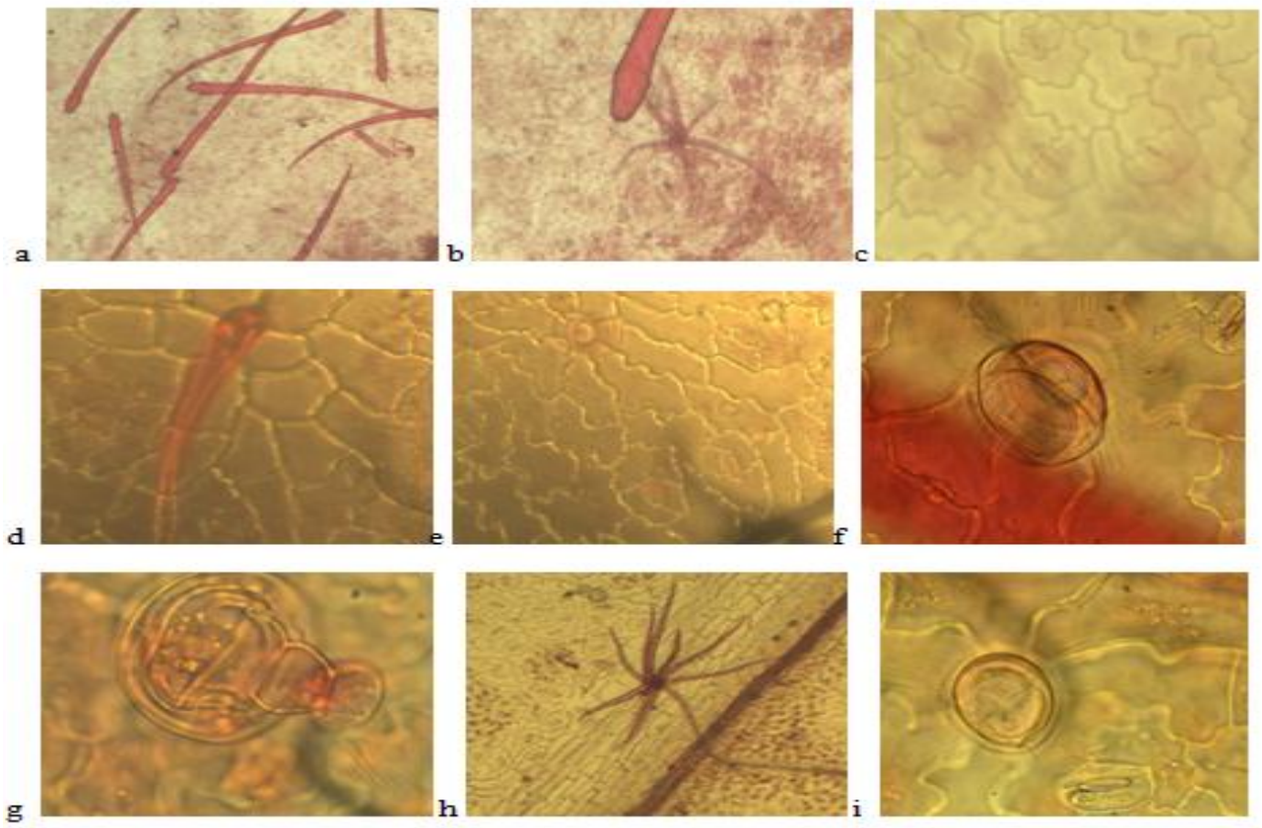

Plate-2. ***Sida alba*** **Linn.**(Adaxial and Abaxial Features). **Adaxial features** 2a: Unicellular trichomes (x40) 2b: Stellate trichomes(x100). 2c: Anisocytic stomata (x400). 2d: Basal cells of unicellular trichome (x400). 2e: Epidermal cells (x400) 2f: A hydropoten cell (x1000). **Abaxial features 2**g: Glandular trichome (x1000) 2h: Stellate trichome (x100) 2i: Hydropoten cell (x1000).

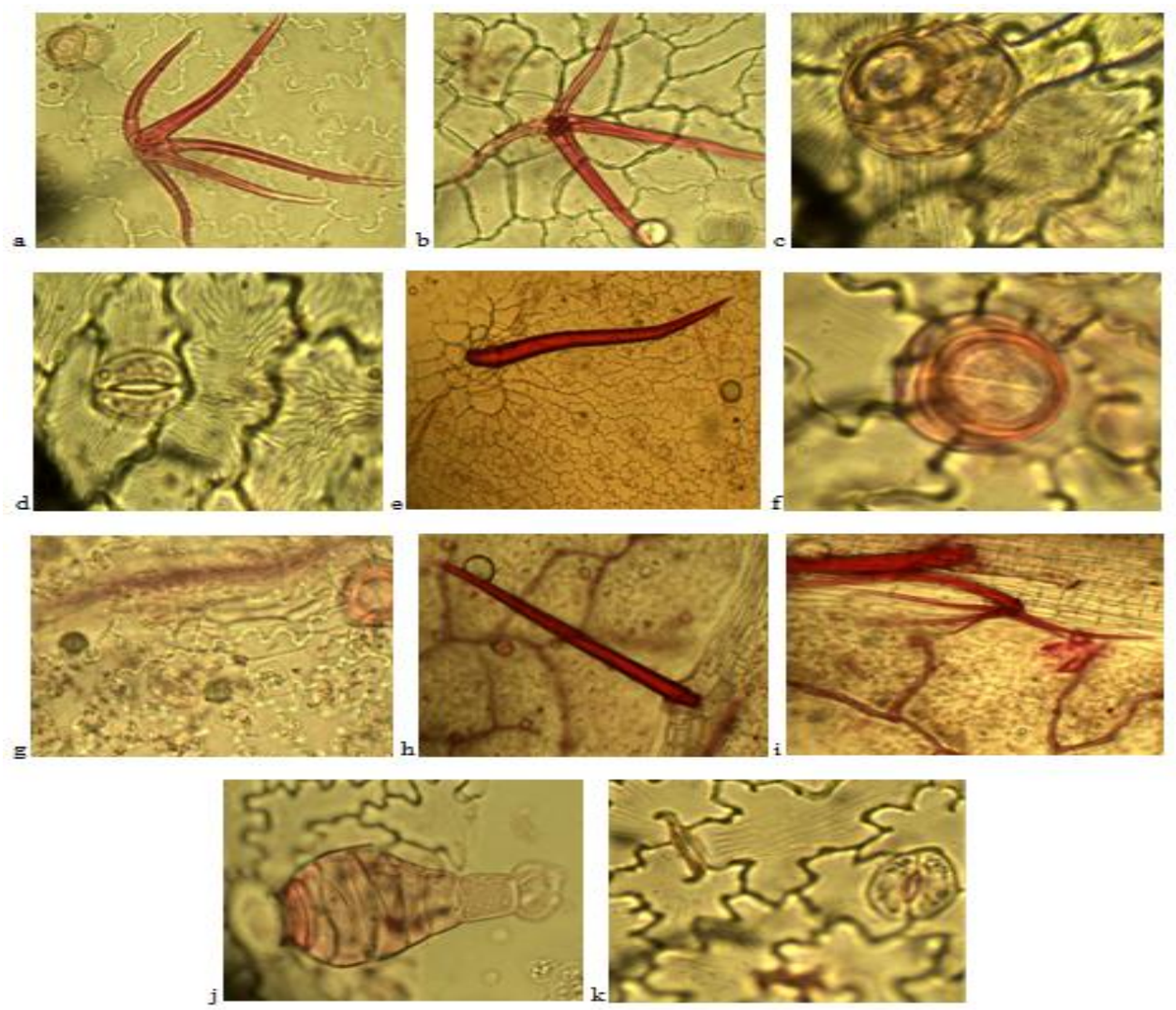

Plate-3. ***Sida corymbosa*** R.E.Fries(Adaxial and Abaxial Features). **Adaxial features** 3a:Five-armed trichome (x100). 3b: Four- armed trichome (x100). 3c: Hydropoten cell (x1000) 3d: Striae (x400). 3e: Unicellular trichome (x100). **Abaxial features** 3f: Hydropoten cell(x1000). 3g: Druses (x400) 3h: Unicellular trichome (x100). 3i:Stellate trichome (x100) 3j: Glandular trichome (x400). 3k: Anomalous stomata (x400).

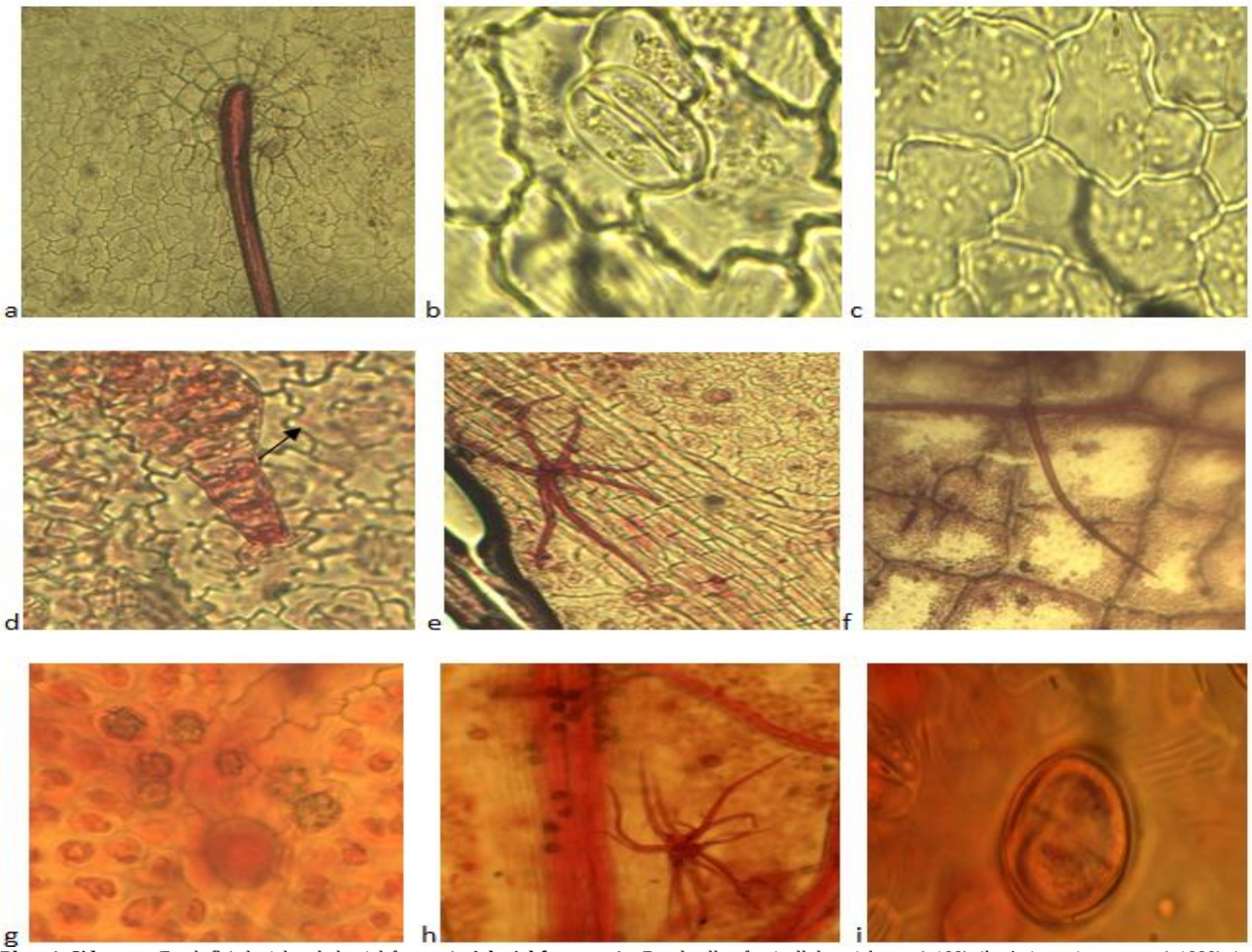

Plate-4. ***Sida ovata*** Forsk fl.(adaxial and abaxial features). **Adaxial features 4**a: Basal cells of unicellular trichome (x100) 4b: Anisocytic stomata (x1000) 4c: Epidermal cells (x1000). **Abaxial features** 4d: Glandular trichome (x400), 4e: Stellate trichome (x100), 4f: Unicellular trichome (x100) 4g: Druses (x400) 4h: Stellate trichome (x100) 4i: Hydropoten cell (x1000)

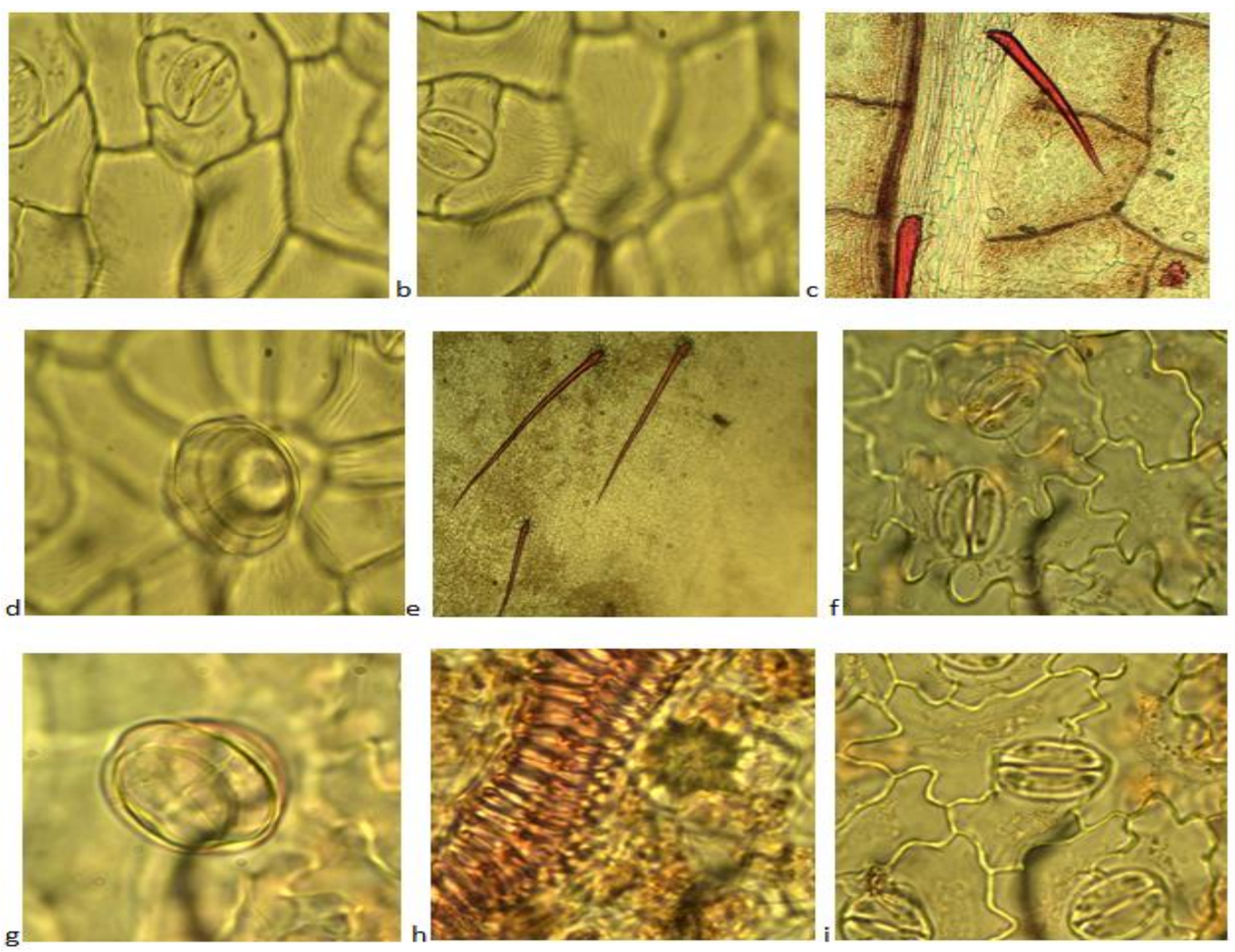

Plate-5. ***Sida scabrida*** Wight & Arn.(Adaxial and Abaxial Features). **Adaxial features** 5a: Anisocytic stomata (x1000) 5b: Striae (x1000) 5c: Hydropoten cell (x1000) 5d: Unicellular trichome (x40). **Abaxial features 5**e: Unicellular trichomes (x100) 5f: Undulating cell walls with shared subsidiary cell (x1000). 5g: Hydropoten cell (x1000) 5h: A Druse (X1000). 5i: Anisocytic stomata (x400)

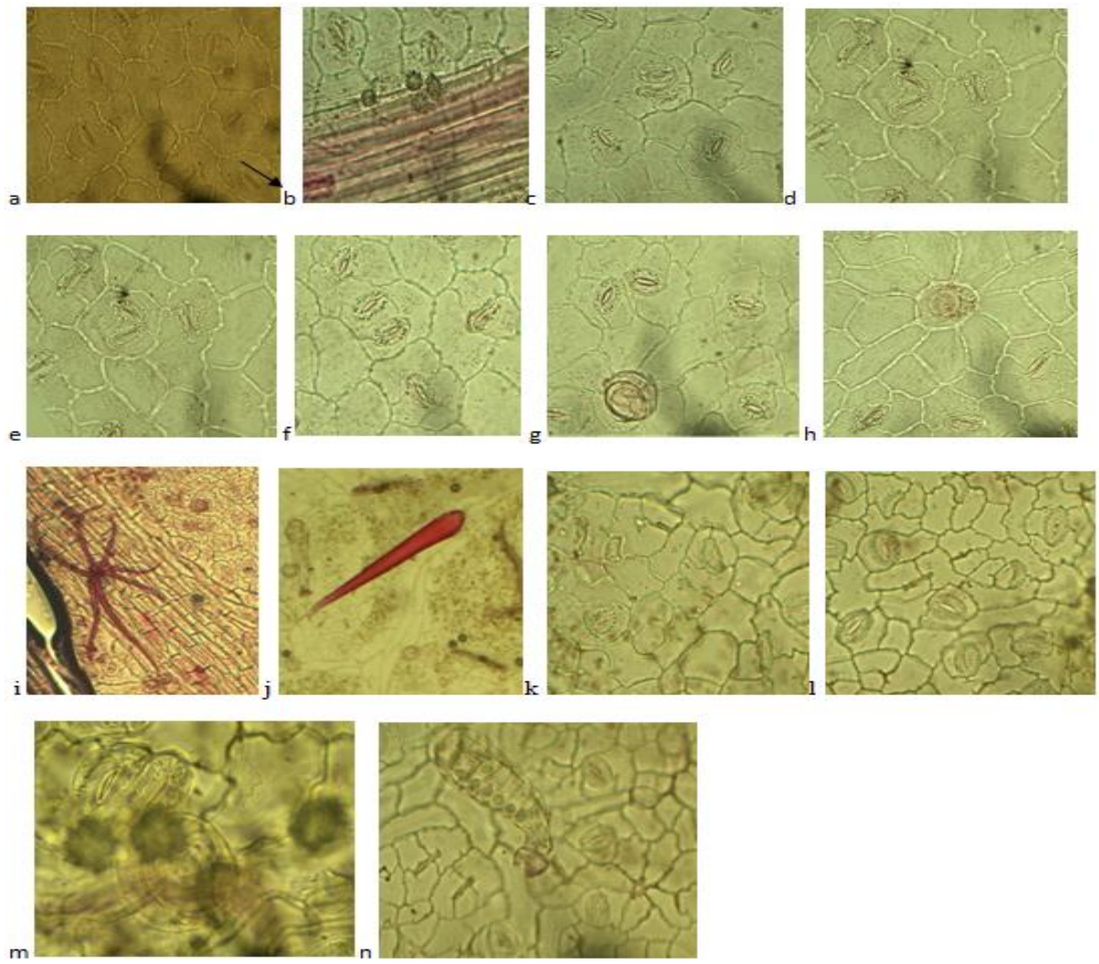

Plate-6. *Sida stipulata* Car.(adaxial and abaxial features). **Adaxial features**-6a: Anisocytic stomata (x400) 6b: Druses (x400) 6c,d,e &f: Twin stomata (x400) 6g:Stomata with shared subsidiary cell (x400) 6h: Basal cells of trichome (x400). **Abaxial features**- 6i: Stellate trichome (x100) 6j: Unicellular trichome (x100) 6k,l: Anomalous stomata (x400) 6m: Hydropoten cell, twin stomata and druses(x1000) 6n: Glandular trichome and stoma without guard cells (x400)

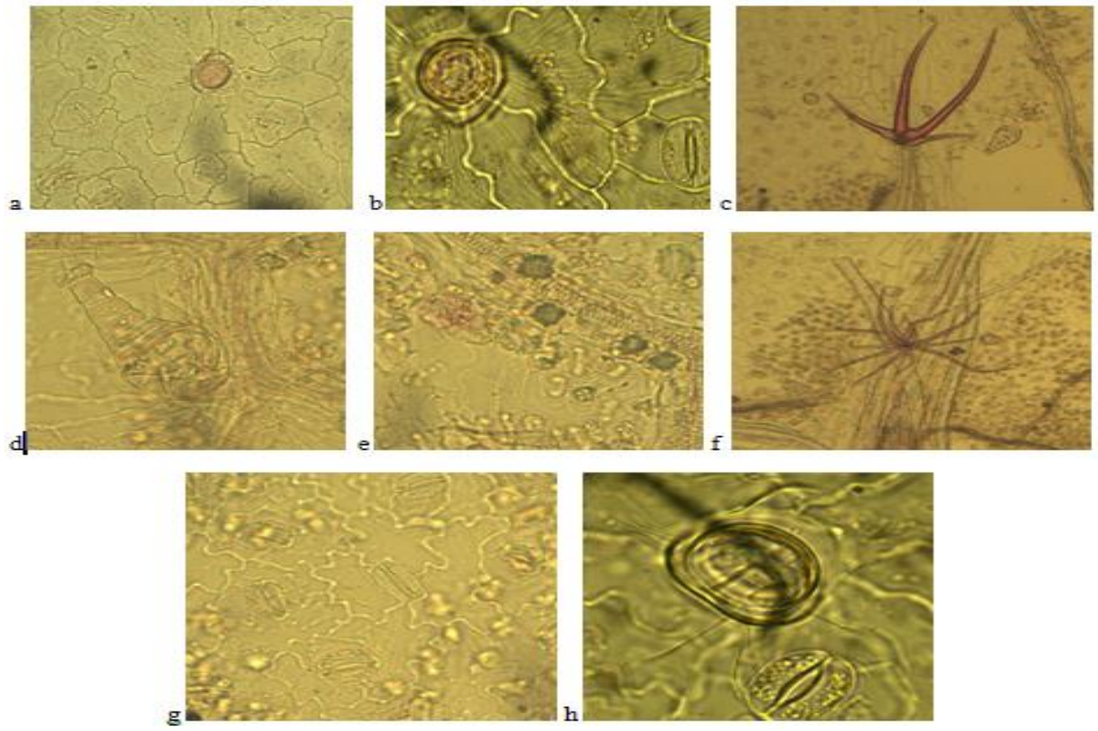

Plate-7. *Sida urens* Linn.(adaxial and abaxial features). **Adaxial features**- 7a: Basal cells of the hydropoten (x400) 7b: Hydropoten cell (x100). **Abaxial features**- 7c: Four-armed trichome (x100) 7d: Glandular trichome (x400)7e: Druses (x400) 7f: Stellate trichome (x100) 7g: Anisocytic stomata (x400) 7h: Hydropoten cell (x1000)

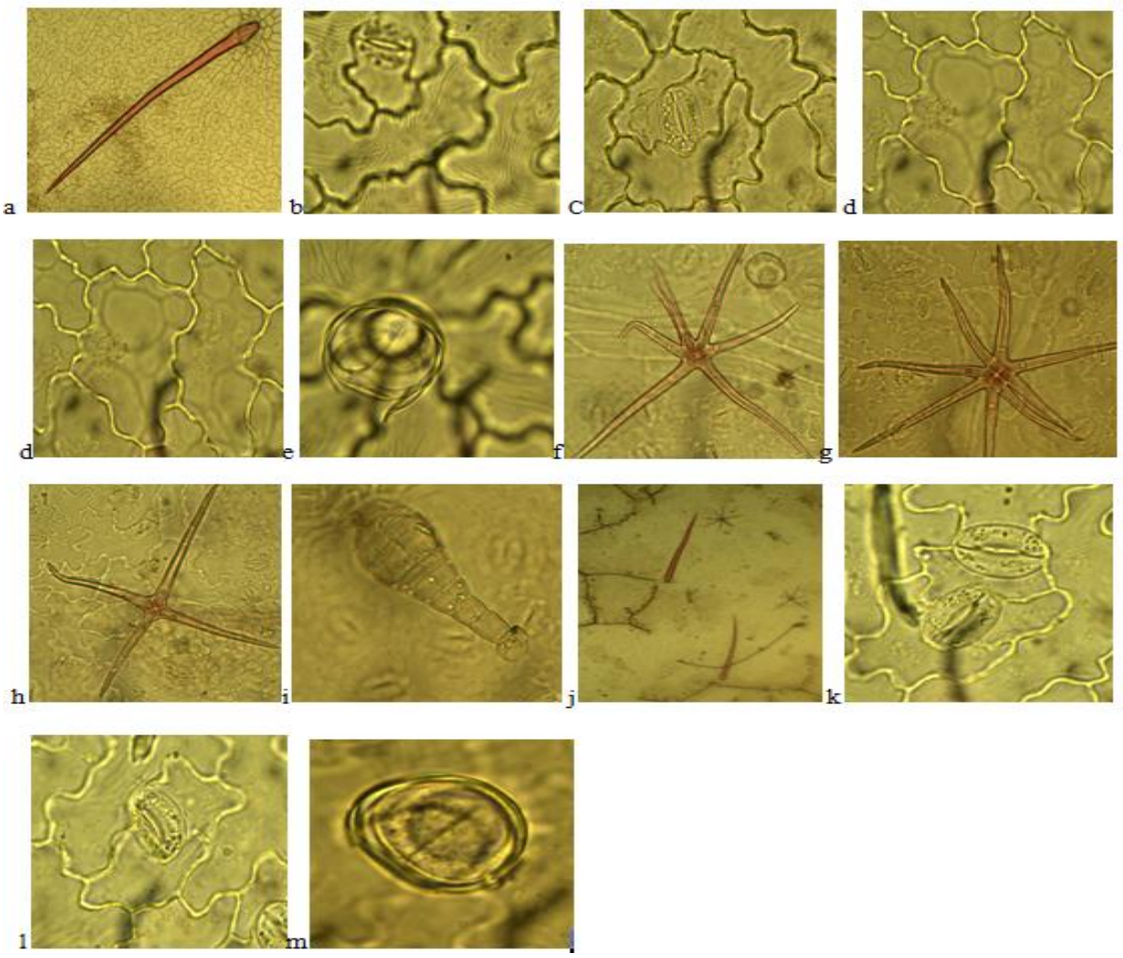

Plate-8. *Sida* sp. (Adaxial and Abaxial features) **Adaxial Features** 8a: Unicellular trichome (x100). 8b: Striae (x1000). 8c: Anisocytic stoma (x1000). 8d: Epidermal cells (x1000). 8e: Hydropoten cell and striae (x1000). **Abaxial features** 8f & g: Stellate trichomes (x400). 8h: Four-armed trichome (x400)8i: Glandular trichome (x400) 8j: Unicellular trichomes(x40) 8k: Stomata with shared subsidiary cell (x1000) 8l: Under developed guard cell (x1000)8m: Hydropoten cell (x1000)

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