IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. III (Jul – Aug 2017), PP 60-68
www.iosrjournals.org

Studies on Micromorphological Taxonomic Variations in *Abutilon* Species of Indian Thar Desert

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Abstract: *Micromorphological taxonomic variations of leaves in three common species of genus Abutilon Mill.viz. Abutilon indicum(Linn.) Sweet,Abutilon pannosum (Forst.f.) Schlect. and Abutilon ramosum (Cav.) Guill. and Perr. in Indian Thar desert were studied and its taxonomic relevance for identification and delineation of species were evaluated. Leaves were amphistomatic and amphitrichomic while stomata and trichomes were more concentrated on abaxial leaf surface. Both anomocytic and anisocytic stomata were reported. Epidermal cells on adaxial leaf surface were polygonal with straight wall pattern while on abaxial leaf surface irregular shaped epidermal cells with undulating wall pattern were observed. Morphologically six diverse types of trichomes were recognized from both adaxial and abaxial leaf surfaces. These were placed under two basic types-eglandular and glandular trichomes. Further three subtypes of eglandular trichomes; simple unicellular, bifurcated and stellate trichomes and three subtypes of glandular trichomes; stalked capitate glands, multicellular flask shape glands and disk shape peltate glands were reported. Present study supports the view that leaf micromorphological features can be used as an important supportive taxonomic character for identification and delineation of species in its vegetative state in all seasons throughout the year.*

Keywords: *Taxonomic variations, Leaf micromorphology, Abutilon, Trichomes, Peltate glands, Bifurcated trichome.*

Date of Submission: 08-07-2017 Date of acceptance: 26-07-2017

I. Introduction

The great Indian Thar desert lies between 24º to 28º N latitude and 68º to 71º E longitude across four states in India- Rajasthan, Haryana, Punjab and Gujarat. It covers approximately 10 per cent of total geographic area of India. Rajasthan is the second largest state of India. It covers 60 per cent area of Indian Thar desert.Climate of the Thar desert is characterized by extreme of temperature, severe drought accompanied by high wind velocity, low relative humidity and scanty precipitation. It is one of the smallest and heavily populated deserts of the world. The area of this region is rich in biodiversity and habitat diversity as variations in geographical conditions provide an extreme habitat for wide range of flora of Bryophytes, Pteridophytes, single Gymnosperm-*Ephedra foliate* and Angiosperms.

The genus *Abutilon* Mill.[1,2]is one of the largest and most difficult genera of family Malvaceae[3,4].The genus comprises approximately 200 recognised species distributed in tropical and subtropical regions of the earth[5].Total 18 species has been reported from India[6,7]. From Indian Thar desert 6 species of *Abutilon* has been reported[8]. This genus differs from other closely related generas of Malvaceae in having tri to multiseeded mericarp, lack of an epicalyx and dorsal wings in mericarps and presence of an endoglossum[9]. Itis an erect, annual or perennial herb or shrubs and even small trees. The plant has high medicinal value, its roots, leaves, flowers and seeds are documented to possess medicinal properties due to presence of various phytoconstituents. Traditionally it is used for curing leprosy, diabetes, jaundice, piles, ulcers, bronchitis, diarrhoea, inflammation of bladder etc.[10,11].Fibre obtained from the plant could be used as a substitute for jute[12]. Plant morphology has served largely for systematics, on the basis of morphological characteristics plants can be grouped into different systematic subunits [13].Traditional identification of plants is heavily based on reproductive structures, specifically on flower and fruit characters which are not available in all the seasons throughout the year so it is very difficult and cumbersome to identify a plant in absence of flower and fruit in its vegetative state. Micromorphological characters including leaf epidermal cells, stomata, glandular and eglandular trichomes have assumed great taxonomic significance as a viable taxonomic marker. In this regard good attempts have been made [14-16]. In Indian Thar Desert various species of *Abutilon* grows a plenty in wild state and can be seen everywhere along the road side and in open field. Despite of its ease of availability and high medicinal value no revisionary work has been carried out yet on its morphological parameters. A comprehensive report on the quantitative characteristics of micromorphological features of leaf

epidermis was totally missing from the taxonomic literature of the species of this genus available in the Indian Thar Desert. Therefore there was an urgent need of critical revisionary taxonomic treatment of this genus. Present work was aimed to study micromorphological taxonomic variations in some species of *Abutilon viz. Abutilon indicum*(Linn.)Sweet, *Abutilon pannosum* (Forst.f.)Schlect and *Abutilon ramosum*(Cav.)Guill.and Perr. and to recognize taxonomic importance of these characters in identification and delineation of species throughout the year in all the seasons.

II. Materials and Methods:

Fresh plant samples were collected by the field surveys of different localities of Jodhpur, Jaisalmer and Bikaner. The plant specimens were brought to the laboratory in polythene bags and preserved in pressed form in plant press. Herbarium specimens were prepared and voucher specimen were deposited in the Herbarium of Department of Botany, Jai Narain Vyas University, Jodhpur (Rajasthan)for further study.

Field studies included growth characters, habit, habitat, colour of stem, height of plant, opening of flower etc. For micromorphological investigations, medium sized portions of mature fully expanded leaves were selected. Epidermal peels were taken out manually by placing the leaf blade portion to be studied on clean glass slide. The epidermis above the desired leaf surface was scrapped off carefully with the help of sharp razor blade and continuously washed with aid of soft caramel brush till the clearance of epidermal peel. The epidermal peel so obtained was stained in one per cent saffranine for two to four minutes and rinsed carefully to remove excess stain and finally mounted in 10 per cent glycerine.

Stomatal index was calculated using following equation:

$$\textbf{S.I.} = \frac{S}{E+S} \times 100$$

Where:

S.I. = Stomatal index

S. = Number of stomata per unit area of leaf epidermis.

E. = Number of epidermal cells in the same unit area of leaf epidermis.

Photograph were taken with microscope attached with Nikon FX-35A Japan camera. All measurements were taken with aid of an ocular micrometer and converted by the ocular constant with respect to the power under which they were taken.

III. Results And Discussion

Total three species of *Abutilon* were examined including *Abutilon indicum*, *Abutilon pannosum* and *Abutilon ramosum.* Both adaxial and abaxial leaf surfaces were investigated. The results obtained in this study were presented in **table.1** and **table.2** and **figures 1,2and 3**. Leaves of all species were amphistomatic and amphitrichomic with more concentrated stomatas on abaxial leaf surface.

Epidermal cells:

The epidermal cells on adaxial leaf surface were polygonal in shape with straight wall pattern**(Fig.1A,2A,3A)** while on abaxial leaf surfaces cell shape was irregular with undulating wall pattern in all three species studied**(Fig;1B,2B,3B).**However degree of undulation was higher in *Abutilon ramosum* abaxial leaf surface**(Fig;3B).** There was no much variations observed in epidermal cell size among all three species.

Stomata:

Presence of stomata were observed on both surfaces of leaf but number of stomata were more on abaxial leaf surface. Both anisocytic and anomocytic types of stomata were observed in three species. In *Abutilon indicum* one anomalous type of contiguous stomata **(Fig; 1C)** were reported additionally. Highest stomatal index was reported in adaxial leaf surface of *Abutilon pannosum* **(18.83±1.49 µm)** and the lowest in *Abutilon indicum* **(8.7±2.8µm).** Guard cell length of both leaf surfaces of *Abutilon pannosum* were comparatively higher**(25.77± 1.17 µm of adaxial surface),(24.72±1.96 µm of abaxial surface)** while other two species showed less variations in guard cell length**(21.04 ± 1.17 µm)** both adaxially and abaxially.

Trichomes:

In *Abutilon*, distinguishing characters which were important taxonomically for identification and delineation of plant species were trichomes micromorphology. *Abutilon* species were amphitrichomic. On abaxial leaf surface trichomes were so densely arranged that it covered the epidermal surface completely. Total six diverse types of trichomes were observed in all three species investigated which were placed under two categories eglandular and glandular trichomes and these were simple unicellular trichome, Bifurcated or forked

trichomes and multicellular stellate trichomes under eglandular trichome and peltate glands, capitate glands and flask shaped glands under glandular trichomes. Although morphologically all types of trichomes were almost similar with slight variations in flask shaped trichomes but there was considerable difference in distribution, number of ray cells, relative arm length and width in stellate trichomes among all the species studied. In *Abutilon indicum* and *Abutilon pannosum* number of ray cells observed were 3-11**(Fig; 1F, 2E)** while in *Abutilon ramosum* it was 3-8**(Fig;3E).** Trichomes of *Abutilon pannosum* were distinct in having thickest ray cells in its stellate trichomes (**24.19±.98 µm)**, bifurcated trichomes **(12.62 ±.98 µm)** and simple trichomes**(14.20 ±.64 µm)**. Dimorphic stellate trichomes,one with shorter ray cells and another with very long ray cells were observed in *Abutilon indicum***(Fig;1G**) and *Abutilon pannosum***(Fig;2F)**.In *Abutilon pannosu*m ray cells were comparatively longer **(416 ± 100.98µm)** than in *Abutilon indicum* **(102.12 ± 9.55 µm).** In both the species, trichomes were arranged in multiple layers sheathing the epidermal cells completely. In *Abutilon ramosum* only single type of stellate trichomes with uniform arm length **(Fig;3G)** were observed and simple unicellular trichomes observed in *Abutilon ramosum* were longest and higher in density on both leaf surfaces. Capitate glands and peltate glands were observed in all the species showed little variations in size. Peltate glands were disk shaped and surrounded by epidermal cell sheath **(Fig; 1I, 2H,3I,3J),** capitate glands were divided into multicellular stalk and bulbous head can be seen clearly near the margins of leaf veins and veinlets**(Fig;1J,2I,3K).** Flask shaped glandular trichomes were multicellular and uniseriate with capitate head. In *Abutilon ramosum* these flask shaped glands were divided into 3-5 celled basal swallon portion and 2-4 celled prominent neck like portion **(Fig;3H)** whereas, in *Abutilon indicum* and *Abutilon pannosum* multicellular flask shaped glands were composed of 8-12 cells that were slightly dilated at basal portion with gradual narrowing upwards**.(Fig;1J and 2I).**

Implications of foliar micromorphological features has been emphasized by many researchers[17-20].Critical studies on leaf micromorphological features are very useful when integrated with morphological data for identification and delineation of any plant species[21]. Present study elucidated the micromorphological features of three species of *Abutilon* namely *Abutilon indicum, Abutilon pannosum,* and *Abutilon ramosum.* Although foliar micromorphological features of some species of *Abutilon* has been studied previously.However its Indian counterpart from Thar desert area showed some micromorphological variations both qualitatively and quantitatively. Three species of *Abutilon* under study were amphistomatic and amphitrichomic with stomata and trichomes were more concentrated on abaxial leaf surface, One interesting character observed in *Abutilon pannosum* was that, the stomata were more concentrated on adaxial leaf surface with larger guard cells area. Both anisocytic and anomocytic types of stomata observed in all species. Most species can have more than one type of stomata [22]. As all three species have both type of stomata so the observed character was of less taxonomic interest however, in *Abutilon indicum* one anomalous type of stomata called contiguous stomata has been reported on abaxial leaf surface. The variation observed could be due to environmental factor or pollution as plant collected along the road side. Stomatal index calculated was highest for *Abutilon pannosum* adaxial leaf surface. As value of guard cell area and stomatal index was independent on the size of leaf surface in different environment and thus is a reliable factor for identification [23]. Epidermal cell shape was polygonal with straight wall pattern on adaxial leaf surface while irregular shaped and undulating wall pattern on abaxial leaf surface.Undulating wall pattern was frequently more pronounced in shade than in sun morphotypes[24] . In *Abutilon ramosum* cell wall pattern was highly undulating suggesting that shade morphotype, as these plants were collected from shady areas mostly under the shade of big trees. Although some variations observed in epidermis and stomatal features, but these variations were of little taxonomic importance to delimit different taxa alone but in integration with foliar trichome micromorphology, these characters could provide a great deal of taxonomic evidence and can be used for identification and delineation of species. The foliar trichomes possess noticeable diversity in *Abutilon* species. Total six different types of eglandular and glandular trichomes namely simple unicellular trichomes, bifurcate or forked trichomes, stellate trichomes, peltate glands, flask shaped glands and capitate glands were observed in all three species studied. However, variations were observed in their relative length and thickness and number of ray cells. Earlier only unicellular simple and stellate trichomes were described in *Abutilon indicum*[25]. In present study glandular trichomes were also reported from this plant. Trichome micromorphological features regarded as very useful taxonomic character within the family Malvaceae.[26-29]. Presence of stellate trichomes was characteristic feature of entire family of Malvaceae. [30,31]. In *Abutilon indicum* and *Abutilon pannosum* number of ray cells recorded were 3 -9 which were different from previous record of presence of more than 12 ray cells[32].Multicellular trichomes with 2-8 arms described as stellate trichomes, In *Hibiscus rosasinensis* and *Pavonia*2-8 armed and 5-12 armed stellate trichomes were reported respectively[33,34]. Further 2 branched trichomes treated as a separate class and stellate trichomes were classified on the basis of number of ray cells as 3-9 branched or more as stellate trichomes [35]. In present study, same classification was followed and two armed trichomes were described here as bifurcated or forked trichomes. Flask shaped glandular trichome reported in all the three species were quantitatively different. In *Abutilon ramosum* flask shaped glands were thickest followed by *Abutilon*

pannosum and *Abutilon indicum*. Presences of various flask shaped trichomes were also reported in *Abutilo*n species and *Hibiscu*s species of Pakistan [36]. Flask shaped trichomes were uniseriate and multicellular (3-8 celled) described as type three which was reported for first time in all the three species. In present work dimorphic stellate trichomes were observed in both *Abutilon indicum* and *Abutilon pannosum*. Similar types of stellate trichomes were previously reported in *Althea rosea* and *Althea laveteraeflora* [37].

IV. Conclusion

The present study indicated that there were considerable taxonomic micromorphological variations observed in stomatas and trichomes of the leaf of *Abutilon* species such as stomatal index,anomalous stomata, undulating subsidiary cells and epidermal cells, different types of eglandular and glandular trichomes etc. These variations were found to be significant from taxonomic point of view and can be used to delineate species in its vegetative state.

Acknowledgement

Authors are thankful to the University Grant Commission, New Delhi for providing financial assistance in form of scholarship to one of the author and through CAS programme in the Department of Botany, Jai Narain Vyas University, Jodhpur (Rajasthan) India.

Table 1. Micromorphological features of leaf epidermis of *Abutilon* species

S.No.	Taxa investigated	Leaf surface	No.of stomata(Mean)	Guard cell length(Mean±SE)	Guard cell width(Mean±SE)	Subsidiary cell length(Mean±SE)	Subsidiary cell width(Mean±SE)	Epidermal cell shape and wall pattern	Epidermal cell No.(Mean)	Epidermal cell length(Mean±SE)	Epidermal cell width(Mean±SE)	Stomatal index(Mean±SE)
1.	*Abutilon indicum*	Adaxial	4	21.64±1.27	14.30±0.63	21.89±0.641	12.96±1.37	Polygonal with straight wall pattern	33.4	31.03±1.53	16.85±1.34	8.76±.288
		Abaxial	9	21.64±1.27	6.32±0.64	25.69±0.66	11.57±0.64	Irregular with undulating wall pattern	43.6	41.02±3.38	17.36±0.64	16.70±0.87
2.	*Abutilon pannosum*	Adaxial	10	25.77±1.17	7.08±0	42.36±5.90	14.69±1.26	Polygonal with straight wall pattern	43.6	43.65±3.06	19.98±1.34	18.83±1.49
		Abaxial	8.6	24.72±1.96	5.70±0.66	34.5±2.45	11.92±2.45	Irregular with undulating wall pattern	64.4	35.14±2.19	21.64±1.86	12.63±0.47
3.	*Abutilon ramosum*	Adaxial	6.8	21.64±0	7.08±0	23.84±1.73	18.52±0.77	Polygonal with straight wall pattern	68.8	42.08±2.10	20.84±1.76	13.86±1.26
		Abaxial	9.4	21.64±0	7.08±0	27.86±0.61	12.27±1.66	Irregular with undulating wall pattern	44.6	42.08±3.10	16.33±0.98	17.37±0.63

Note; Result based on mean of 3 replicates

+Table 2. Trichomes characteristics of *Abutilon* species.

S.No.	Taxa investigated	Leaf surface	Simple trichome (Mean±SE)µm		Forked trichome (Mean±SE) µm		Stellate trichome (Mean±SE) µm			Flask shape trichome (Mean±SE) µm		Peltate gland (Mean±SE) µm		Capitate gland (Mean±SE) µm	
			Length	Width	Length	Width	Length	Width	No.of ray cells	Length	Width	Length	Width	Length	Width
1.	*Abutilon indicum*	Adaxial	45.76±1.78	6.31±0.64	53.12±6.19	6.31±0.64	102.12±9.55	7.89±0	3-9	116.77±21.89	9.99±0.52	21.04±1.16	17.35±1.34	20.5±0.98	15.2±0.98
		Abaxial	217.0±10.95	11.68±.61	64.6±6.37	6.31±0.64	288.60±54.26	5.26±0		127.29±7.64	13.67±.53	21.04±1.18	16.83±.644	28.93±2.35	20.51±0.98
2.	*Abutilon pannosum*	Adaxial	142.02±10.15	11.57±0.64	169.37±10.06	12.62±0.98	416.61±100.98	24.19±0.98	3-9	128.34±15.81	11.57±0.64	25.24±1.96	29.98±1.58	27.8±0.64	25.77±0.98
		Abaxial	132.02±21.32	14.20±0.64	190.98±17.20	9.99±0.30	643.80±95.48	14.20±1.34		145.70±5.36	11.57±1.34	28.46±1.48	23.14±0.98	26.82±0.98	21.04±1.18
3.	*Abutilon ramosum*	Adaxial	181.47±14.04	11.57±0.64	155.69±23.93	8.41±0.52	224.22±21.75	11.52±0.64	3-7	137.28±10.56	23.14±2.26	25.77±1.28	20.51±1.53	32.61±0.64	23.67±1.44
		Abaxial	209.87±13.82	10.52±0	176.21±7.89	10.52±0	244.2±9.92	7.89±0		184.10±10.25	15.25±8.98	19.46±1.34	15.78±1.18	26.3±1.18	21.56±0.53

Note; Result based on mean of three replicates

1.*Abutilon indicum* growing in field.

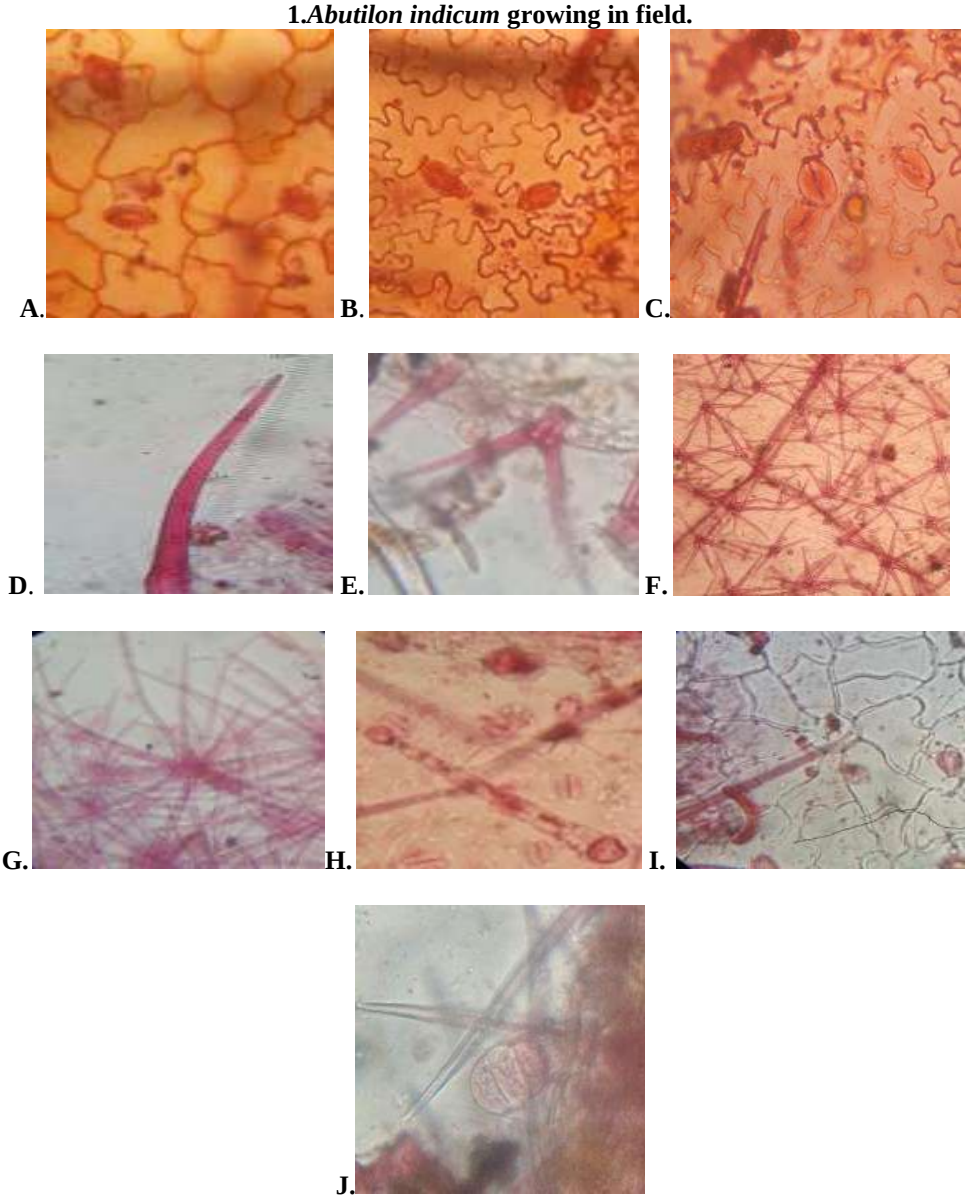

Figure 1.*Abutilon indicum*(Leaf micromorphological features)**:A;** Stomata with polygonal epidermal cells on adaxial leaf surface. **B;** Stomata with irregular epidermal cells on abaxial leaf surface. **C;** Contigous stomata sharing common guard cell on abaxial leaf surface. **D;** Simple unicellular trichomes(adaxial leaf surface). **E;**Bifurcated or Forked trichomes(adaxial leaf surface). **F;** Stellate trichomes on adaxial leaf surface. **G;** Stellate trichomes with long ray cell on abaxial leaf surface. **H;** Multicellular flask shaped glands. **I;** Disk shaped peltate glands. **J;** Capitate glands.

2. *Abutilon pannosum* growing in field

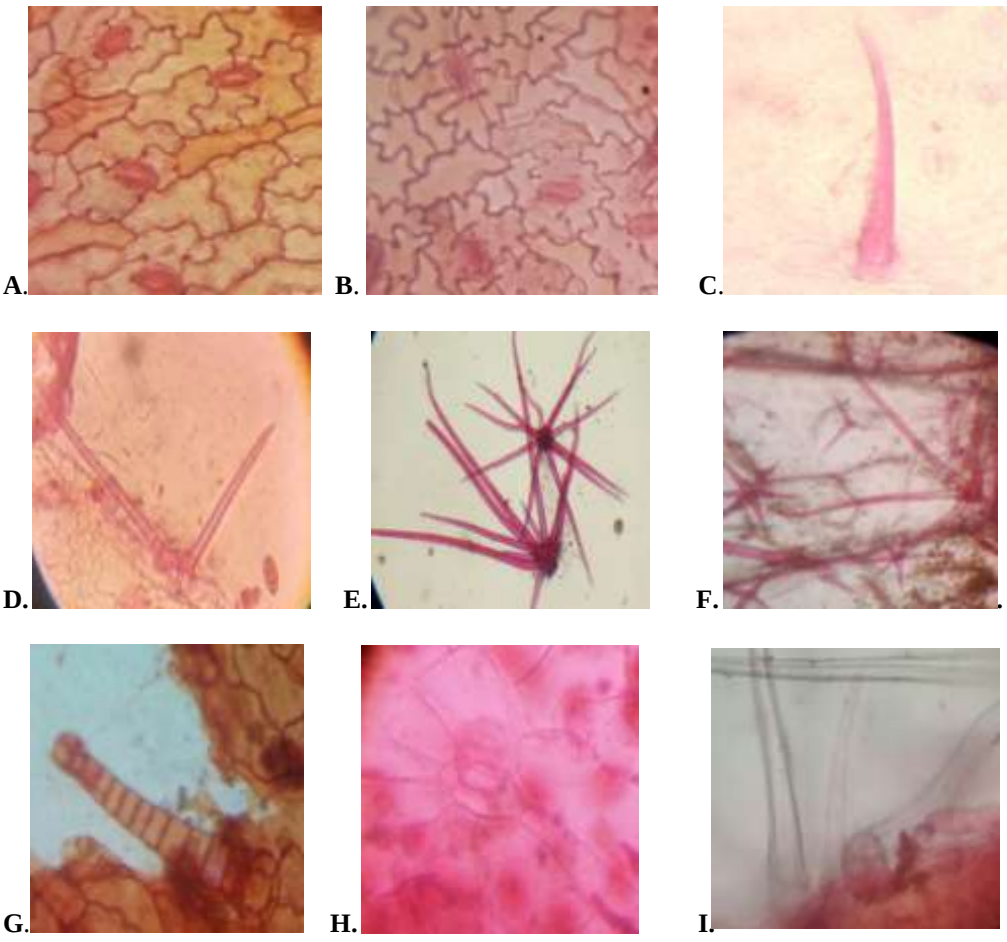

Figure2. *Abutilon pannosum* (Leaf micromorphological features**):A;** Stomata with polygonal epidermal cells on adaxial leaf surface. **B;**Stomata with irregular epidermal cells on abaxial leaf surface. **C;** Unicellular trichomes **D;** Forked trichomes **E;**Stellate trichomes **F;**Stellate trichomes with long ray cells on adaxial leaf surface **G;** Multicellular flask shaped glands **H;** Disk shaped peltate glands **I;** Capitate glands.

3.*Abutilon ramosum* growing in field

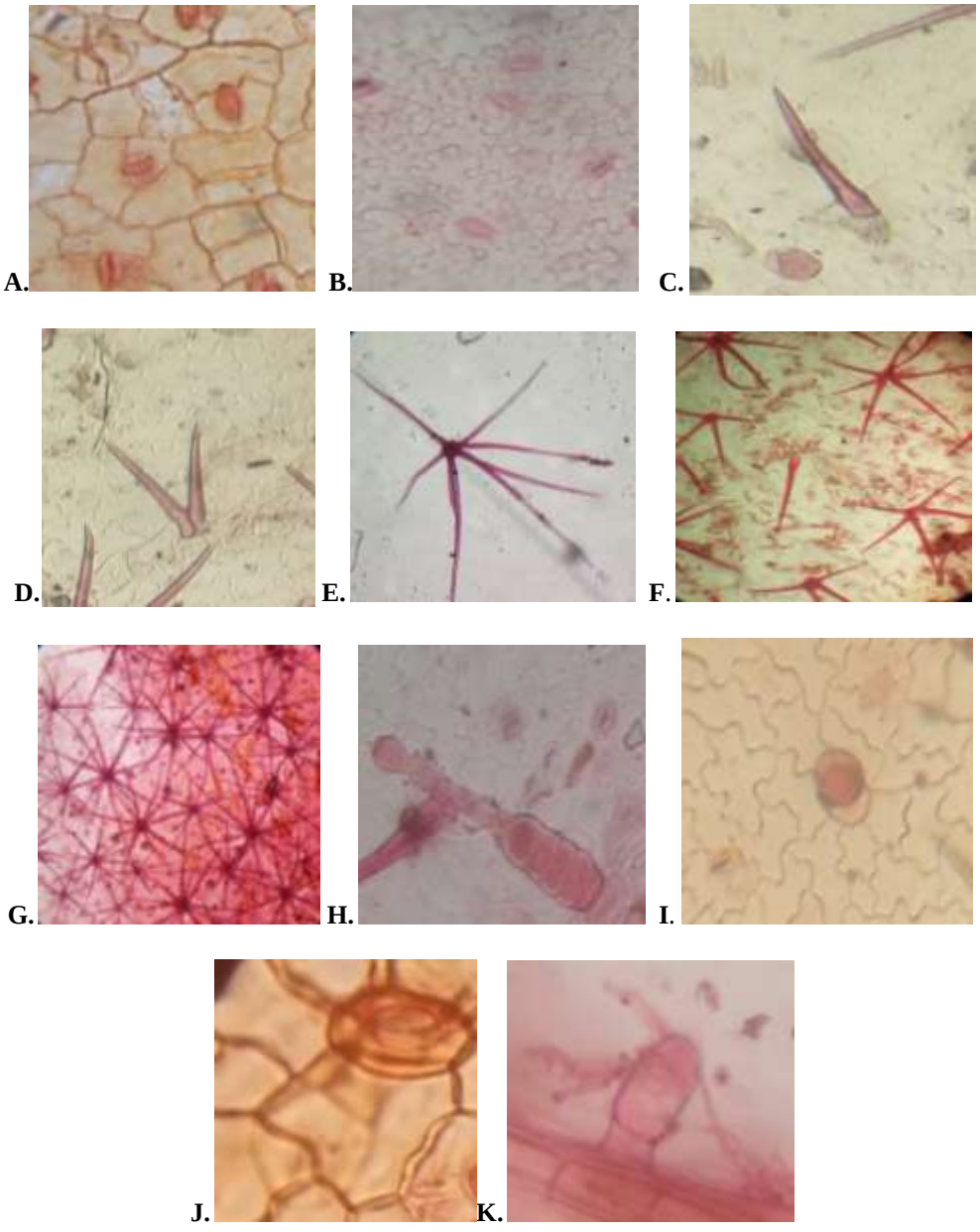

Figure3. *Abutilon ramosum* (Leaf micromorphological features)**:A;**Stomata and polygonal epidermal cells on adaxial leaf surface. **B;** Stomata and irregular epidermal cells on abaxial leaf surface. **C;** Unicellular trichomes **D;**Bifurcated trichomes **E;**Stellate trichomes **F;**Stellate trichomes on adaxial leaf surface **G;** Stellate trichomes on abaxial leaf surface **H;** Flask shaped glands **I,J;** Disk shaped peltate glands. **K;** Capitate glands.

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IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Ilham Bano. "Studies on Micromorphological Taxonomic Variations in Abutilon Species of Indian Thar Desert." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 12.4 (2017): 60-68.