ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 11, pp.74998-75002, November, 2018

DOI: https://doi.org/10.24941/ijcr.33004.11.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

FOLIAR EPIDERMAL STUDIES OF SOME MEDICINALLY IMPORTANT PLANTS OF SCROPHULARIACEAE

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ARTICLE INFO

Article History:
Received 06th August, 2018
Received in revised form
14th September, 2018
Accepted 26th October, 2018
Published online 29th November, 2018

Key Words:

Foliar epidermis, Scrophulariaceae, stomata, trichomes, *Cymbalaria muralis*, *Kickxia ramosissima*, *Linaria dalmatica*.

ABSTRACT

The family Scrophulariaceae includes several ornamentals and plants with medicinal importance. In the present investigation foliar epidermal characteristics of three plants useful in ethnic/folk medicine - *Cymbalaria muralis*, *Kickxia ramosissima* (both used in diabetes treatment) and *Linaria dalmatica* (used to stimulate liver function) are studied and their salient epidermal features of leaf are presented in detail here. Sinuous epidermal anticlinal walls, anomocytic and anisocytic stomata, cuticular ledges, peristomatal rims, polar nodules are the characters commonly shared by all the three members. Leaves are hypoamphistomatic with heteromorphic stomata (in shape) in *C. muralis*, *K. ramosissima*; amphistomatic in *L. dalmatica* and atrichous in *C. muralis* and *L. dalmatica*. Stomatal groups, stomatal chains, contiguous stomata and long stalked (3 - celled) glandular trichomes with 4 - celled globose head are the exclusive features found in *K. ramosissima*.

***Citation: Lahari P. S. and Hanumantha Rao, B.*.. 2018.** "Foliar epidermal studies of some medicinally important plants of scrophulariaceae", *International Journal of Current Research*, 10, (11), 74998-75002.

INTRODUCTION

The family Scrophulariaceae comprises of 220 genera and 3000 species (Willis, 1973; Richardson, 1993). The family includes several ornamentals and medicinally important plants belonging to the genera - *Digitalis*, *Bacopa*, *Euphrasia*, *Scoparia*, *Limnophila, Russelia*, *Verbascum*, *Lindenbergia*, *Torenia*, *Anticharis*, *Antirrhinum*, *Cymbalaria*, *Linaria*, *Kickxia* and *Schweinfurthia*. The importance of foliar epidermal features in pharmacognistic studies has been revealed through some of the earlier works in this family by Santhan (2014); Syed Sabiha (2015); Sutar Sangeetha and Dharasurkar (2015); Manasa Ranjan Mishra *et al.* (2012); Acharya Rabinarayan *et al.* (2012); Sandhya *et al.* (2011); Emam *et al.* (2016); ElzbietaWeryszko-Chmielewska *et al.* (2010). In the present investigation characteristics of the foliar epidermis like epidermal cell complex, costal cells, stomatal complex and trichome complex of *Cymbalaria muralis* P.Gaertn., B. Mey. and Scherb., *Kickxia ramosissima* (Wall.) Janch. and *Linaria dalmatica* (L.) Mill of the tribe Antirrhineae (Wettstein, 1897) have been studied in detail and are presented here. *Cymbalaria muralis* and *Kickxia ramosissima* are mainly useful in the treatment of diabetes and *Linaria dalmatica* to stimulate the liver function.

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MATERIALS AND METHODS

Leaf materials of *Cymbalaria muralis* were collected from Herbarium, Department of Botany, Andhra University, Visakhapatnam (Voucher specimen no. 1393); *Kickxia ramosissima* from BSI, Coimbatore (Voucher specimen no. 15902) and *Linaria dalmatica* from BSI, Kolkata (Voucher specimen no. 159). Epidermal scrapes were prepared by following Theunissen's technique (1989). The material was pretreated in Glycerin (97%) and Ethanol (1:1) for 24hours. A portion of the leaf to be examined was placed on the slide with the epidermis to be investigated facing downwards. The specimen was covered with a thin layer of Glycerin that acts as a lubricant. The unwanted epidermis was gradually scrapped away using a razor blade to expose the mesophyll. A piece of the specimen measuring 5-10 mm was placed in 60% solution of Nitric acid in a35 ml test tube and warmed over a flame, agitating the test tube gently for 2 to 3 minutes. The mesophyll turned yellow, disintegrated and thus liberating the remaining epidermal layer. The acid was decanted and the isolated epidermis was rinsed 4-5 times with distilled water. The peels were stained in 1% aqueous solution of Safranine and mounted in Glycerin. The Stomatal Index (S.I) was calculated by following Salisbury's method (1927). Epidermal Cell Frequency, Stomatal Frequency and Stomatal Index have been calculated out of 10 readings. The photographs were taken by using RM-600 (Radical) monocular microscope fitted with MD 35 digital eyepiece, 640×480 pixel USB to Dell laptop.

Terms used for describing epidermal cells, stomata and trichomes are of Metcalfe and Chalk (1950, 1979), Inamdar *et al.* (1986), Raja Shanmukha Rao (1987), Prabhakar and Leelavathi (1989) and Prabhakar (2004).

RESULTS AND DISCUSSION

Cuticle: A very thin cuticle is present over the epidermal cells of *Cymbalaria muralis*, *Linaria dalmatica* and adaxial surface of *Kickxia ramosissima*. It is moderately thick in the abaxial surface of *Kickxia ramosissima*. Presence of conspicuous cuticular striations was observed over the periclinal and anticlinal walls of the epidermal cells in the abaxial surface of *Kickxia ramosissima* (Fig. 13). The surface of the epidermal cells is smooth, without any ornamentation in *C. muralis*, *L. dalmatica* and adaxial surface of *K. ramosissima*.

Epidermal Cell Complex

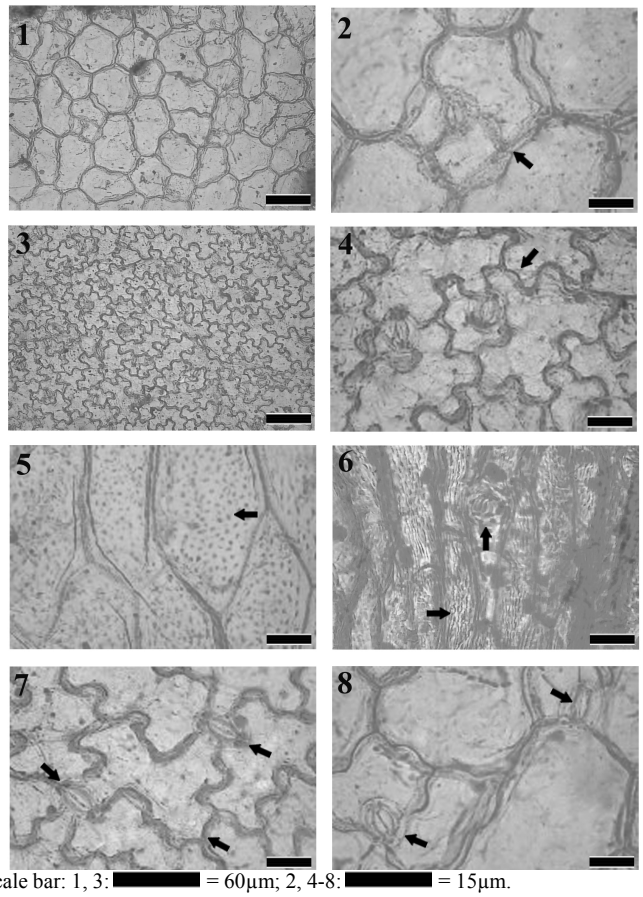
Epidermal cells: The epidermal cells exhibit variation in their shape, nature of anticlinal walls, orientation and size (Fig. 1, 3, 10, 12, 23, 25). The mature epidermal cells are irregular to polygonal and aniso to isodiametric in the adaxial surface of *C. muralis* (Figs. 2), *L. dalmatica* (Figs. 24). These are irregular, anisodiametric in the abaxial surface of *C. muralis* (Figs. 4), *L. dalmatica* (Fig. 26), and both the surfaces of *K. ramosissima* (Figs. 11, 13). The anticlinal walls in the adaxial surface are straight, curved to undulate in *C. muralis* (Fig. 2); wavy to undulate in *L. dalmatica* (Fig. 24) and these cells are sinuous with 'U' and 'V' shaped sinuses in the abaxial surface of *C. muralis* (Fig. 4) and both the surfaces of *K. ramosissima* (Figs. 11, 13); undulate to sinuous with 'U' shaped sinuses in the abaxial surface of *L. dalmatica* (Fig. 26). Anticlinal walls are straight (upper epidermis) and sinuous nature (lower epidermis) were earlier reported in *Antirrhinum majus* by Nyawuame and Gill (1993).

The epidermal cell frequency per sq. mm. in the adaxial surface ranges from 169 to 259 in *C. muralis*; 477 to 636 in *K. ramosissima*; 217 to 289 in *L. dalmatica*. In the abaxial surface it ranges from 187 to 241 in *C. muralis*; 278 to 411 in *K. ramosissima*; 96 to 247 in *L. dalmatica*. The average epidermal cell frequency per sq. mm. in the adaxial surface is 208 in *C. muralis*; 555 in *K. ramosissima*; 251 in *L. dalmatica*. In the abaxial surface it is 209 in *C. muralis*; 335 in *K. ramosissima*; 189 in *L. dalmatica*. Thus the average epidermal cell frequency per sq. mm. varies between 208 (smallest) as in *C. muralis*, 555(largest) as in *K. ramosissima* in the adaxial surface and 189 (smallest) as in *L. dalmatica* and 335 (largest) as in *K. ramosissima* in the abaxial surface. The size of the epidermal cells in the adaxial surface ranges from 58.5×58.5 µm to 121.5×90 µm in *C. muralis*; 60×45 µm to 117×72 µm in *K. ramosissima*; 58.5×31.5 µm to 108×58.5 µm in *L. dalmatica*. In the abaxial surface it ranges from 76.5×27 µm to 135×94.5 µm in *C. muralis*; 63×33 µm to 135×66 µm in *K. ramosissima*; 90×40.5 µm to 121.5×99 µm in *L. dalmatica*. In the adaxial surface, the average epidermal cell size in µm is 99.9×79.65 in *C. muralis*; 78.9×53.4 in *K. ramosissima*; 78.3×47.7 in *L. dalmatica*. In the abaxial surface it is 103.95×65.25 in *C. muralis*; 93.6×51 in *K. ramosissima*; 104.4×67.5 in *L. dalmatica*. In the adaxial surface the average epidermal cell size is smallest in *L. dalmatica* 78.3×47.7 µm and largest in *C. muralis* 99.9×79.65 µm. In the abaxial surface, the average size is smallest in *K. ramosissima* 93.6×51 µm and largest in *L. dalmatica* 104.4×67.5 µm.

Costal cells: The costal cells are distinct. The cells are parallely arranged (Figs. 5, 6, 14, 15, 27, 28). These cells are elongated, polygonal in the adaxial surface of *C. muralis* (Fig. 5) and *K. ramosissima* (Fig. 14); elongated, polygonal, rectangular in both the surfaces of *L. dalmatica* (Figs. 27, 28) and in the abaxial surface of *C. muralis* (Fig. 6); elongated, irregular in the abaxial surface of *K. ramosissima* (Fig. 15). The sidewalls are straight to curved in both the surfaces of *C. muralis* (Figs. 5, 6) and in the abaxial surface of *L. dalmatica* (Fig. 28). In *K. ramosissima* these cells are curved to wavy in the adaxial surface (Fig. 14) and curved to sinuous in the abaxial surface (Fig. 15). Cuticular striations over the periclinal and anticlinal walls of the costal cells are observed in both the surfaces of *K. ramosissima* (Figs. 14, 15) and in the abaxial surface of *C. muralis* (Fig. 6). Cuticular ornamentation over costal cell walls is observed in the adaxial surface of *C. muralis* (Fig. 5).

Stomatal Complex: The leaves are hypoamphistomatic in *C. muralis*, *K. ramosissima*, (Figs. 1, 2, 3, 4, 10, 11, 12, 13) and amphistomatic in *L. dalmatica*. The stomata are variously oriented, usually scattered and are distributed all over the lamina (Figs. 1, 3, 10, 12, 23, 25). The stomatal type is anisocytic in both the surfaces of *C. muralis*; *K. ramosissima* and *L. dalmatica* (Figs. 2, 4, 11, 13, 17, 20, 24, 30, 32). The anomocytic stomata are found to be distributed along with anisocytic in both the surfaces of *L. dalmatica* (Figs. 29, 30); in the adaxial surface of *K. ramosissima* (Fig. 16) and in the abaxial surface of *C. muralis* (Fig. 7). Hypostomatic leaves with anisocytic stomata were reported in *Antirrhinum majus* by Nyawuame and Gill (1993). Stomata are oval shaped in both the surfaces of *C. muralis* (Figs. 8, 9); abaxial surface of *K. ramosissima* (Fig. 17); *L. dalmatica* (Fig. 31). Elliptic shaped stomata are present in *K. ramosissima* (Figs. 17). Circular shaped stomata are also observed in the abaxial surface of *C. muralis* (Fig. 9); *K. ramosissima* (Fig. 17); *L. dalmatica* (Fig. 31). Thus the stomata are heteromorphic with oval, elliptic and circular shapes, in the abaxial surface of *K. ramosissima* (Fig. 17).

Cuticular ledges around the stomatal pores are observed in both the surfaces of *C. muralis* (Figs. 8, 9), *L. dalmatica* (Figs. 29, 31) and in the abaxial surface of *K. ramosissima* (Fig. 18). Peristomatal rim around the stomata is noticed in the adaxial surface of *C. muralis* (Fig. 8) and in the abaxial surface of *K. ramosissima* (Fig. 19), *L. dalmatica* (Fig. 31). Polar nodules are observed in both the surfaces of *L. dalmatica* (Figs. 24, 31) and in the abaxial surface of C. *muralis* (Fig. 7); *K. ramosissima* (Fig. 19). Stomata with unequal guard cells are noticed in the adaxial surface of *K. ramosissima* (Fig. 20) and abaxial surface of *L. dalmatica* (Fig. 30). Superposed type of contiguous stomata observed in the adaxial surface of *K. ramosissima* (Fig. 21). In addition to these, presence of common subsidiary cells is noticed in the abaxial surface of *L. dalmatica* (Fig. 32). Stomatal groups and stomatal chains are observed in the abaxial surface of *K. ramosissima* (Figs. 18, 19). The stomatal frequency per sq. mm. in the adaxial surface ranges from12 to 18 in *C. muralis*; 13 to 40 in *K. ramosissima*; 66 to 102 in *L. dalmatica*. In the abaxial surface it ranges from 72 to 133 in *C. muralis*; 106 to 265 in *K. ramosissima*; 90 to 139 in *L. dalmatica*. The average stomatal frequency per sq. mm. in the adaxial surface is 14 in *C. muralis*; 24 in *K. ramosissima*; 88 in *L. dalmatica*. In the abaxial surface it is 109 in *C. muralis*; 173 in *K. ramosissima*; 107 in *L. dalmatica*.

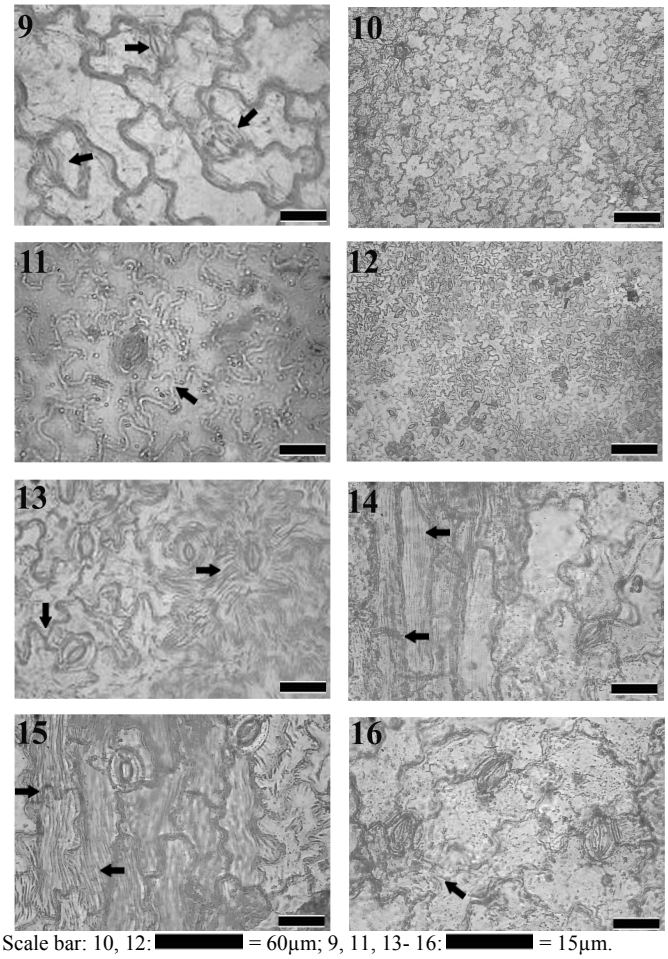


Scale bar: 1, 3: [redacted] = 60µm; 2, 4-8: [redacted] = 15µm.

Figures 1 - 8: Foliar epidermis of *Cymbalaria muralis*. 1, 2, 5, 8: Upper; 3, 4, 6, 7: Lower

1. Epidermal cells with polygonal to irregular outline and straight, curved to undulate anticlinal walls,
2. Epidermal cells with polygonal to irregular outline and curved to undulate anticlinal walls and anisocytic stomata,
3. Epidermal cells and stomata,
4. Epidermal cells with irregular outline, sinuous anticlinal walls with 'U' and 'V' shaped sinuses and anisocytic stomata,
5. Elongated, polygonal costal cells with parallel arrangement, straight to curved side walls and cuticular ornamentation over costal cells,
6. Elongated, polygonal, rectangular costal cells with parallel arrangement, straight to curved side walls, cuticular striations over costal cell walls and stomata in costal region,
7. Anomocytic stomata and stomata with polar nodules,
8. Oval shaped stomata with cuticular ledges and peristomatal rim.

Thus the average stomatal frequency per sq. mm. varies between 14 as in *C. muralis* and 83 as in *L. dalmatica* in the adaxial surface and 107 as in *L. dalmatica* and 173 as in *K. ramosissima* in the abaxial surface. Ormrod and Renney (1968), earlier reported a mean frequency of stomata as 2/mm² in the upper surface and 188/mm² in the lower surface of *L. vulgaris*. Nyawuame and Gill (1993) recorded a mean frequency of 8.1/mm² in *Antirrhinum majus*. The Stomatal Index in the adaxial surface is 6.31 in *C. muralis*; 4.15 in *K. ramosissima*; 24.85 in *L. dalmatica*. In the abaxial surface it is 34.28 in *C. muralis*; 34.06 in *K. ramosissima*; 36.15 in *L. dalmatica*. Thus the Stomatal Index is lowest in *K. ramosissima* (4.15), highest in *L. dalmatica* (24.85) in the adaxial surface and it is smallest in *K. ramosissima* (34.06) highest in *L. dalmatica* (36.15) in the abaxial surface. The size of the stomata in the adaxial surface ranges from 18×13.5 µm to 36×31.5 µm in *C. muralis*; 24×18 to 33×27 µm in *K. ramosissima*; 22.5×18 to 36×22.5 µm in *L. dalmatica*. In the abaxial surface it ranges from 27×27 to 49.5×40.5 µm in *C. muralis*; 24×21 to 33×30 µm in *K. ramosissima*; 18×13.5 to 31×27 µm in *L. dalmatica*.



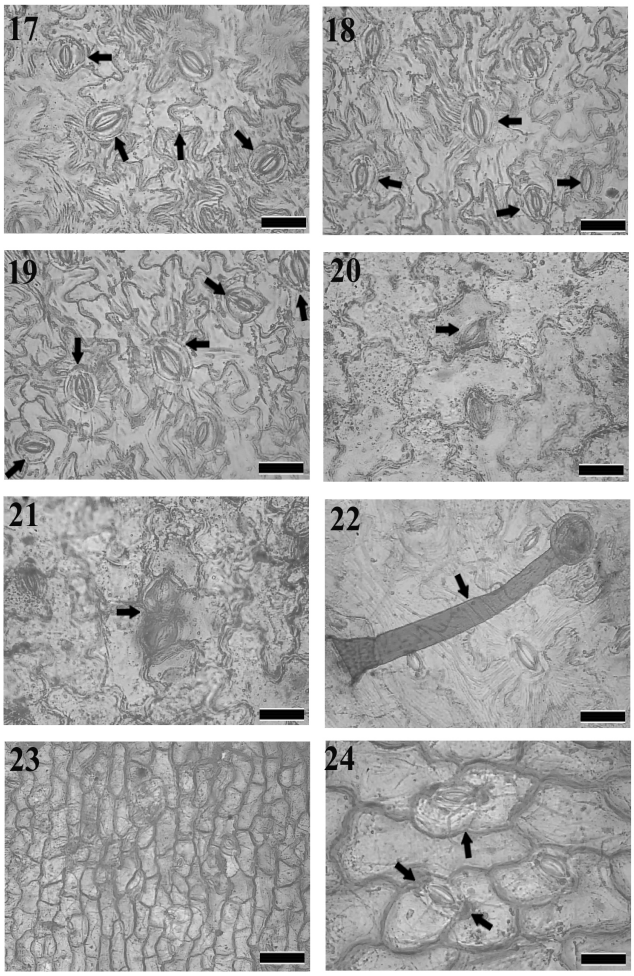
Scale bar: 10, 12: [redacted] = 60µm; 9, 11, 13- 16: [redacted] = 15µm.

Figures 9: Foliar epidermis of *Cymbalaria muralis*; 10 - 16: *Kickxia ramosissima*. 9, 12, 13, 15: Lower; 10, 11, 14, 16: Upper.

9. Oval, circular shaped stomata and stomata with cuticular ledges,
10. Epidermal cells and stomata,
11. Epidermal cells with irregular outline, sinuous anticlinal walls with 'U' and 'V' shaped sinuses and anisocytic stomata,
12. Epidermal cells and stomata,
13. Epidermal cells with irregular outline, sinuous anticlinal walls with 'U' and 'V' shaped sinuses, anisocytic stomata and cuticular striations around stomata,
14. Elongated, polygonal costal cells with parallel arrangement, curved to wavy side walls, stomata in costal region and cuticular striations over periclinal and anticlinal walls of costal cells,
15. Elongated, irregular costal cells with parallel arrangement, undulate to sinuous side walls, cuticular striations over anticlinal and periclinal walls of costal cells and stomata in costal region,
16. Anomocytic stomata.

The average size of the stomata in the adaxial surface is 26.6×20.7 µm in *C. muralis*; 30×22.8 µm in *K. ramosissima*; 28.8×19.35 µm in *L. dalmatica*. In the abaxial surface it is 37.8×32.4 µm in *C. muralis*; 28.2×25.5 µm in *K. ramosissima*; 26.5×18.45 µm in *L. dalmatica*. Ormrod and Renney (1968) reported 34µm as the mean stomatal length of both surfaces in *L. vulgaris*, while Nyawuame and Gill (1993) reported 28.2/17.3 µm as the mean stomatal length and breadth respectively in *A. majus*. Thus the average size of the stomata in µm varies between 26.6×20.7 µm (smallest) as in *C. muralis* and 30×22.8 µm (largest) as in *K. ramosissima* in the adaxial surface and 26.5×18.45 (smallest) as in *L. dalmatica* and 37.8×32.4 (largest) as in *C. muralis* in the abaxial surface.

Trichome Complex: Leaves are atrichous in *C. muralis*, *L. dalmatica* and amphitrichous in *K. ramosissima*. Only glandular trichomes are present in *K. ramosissima*.

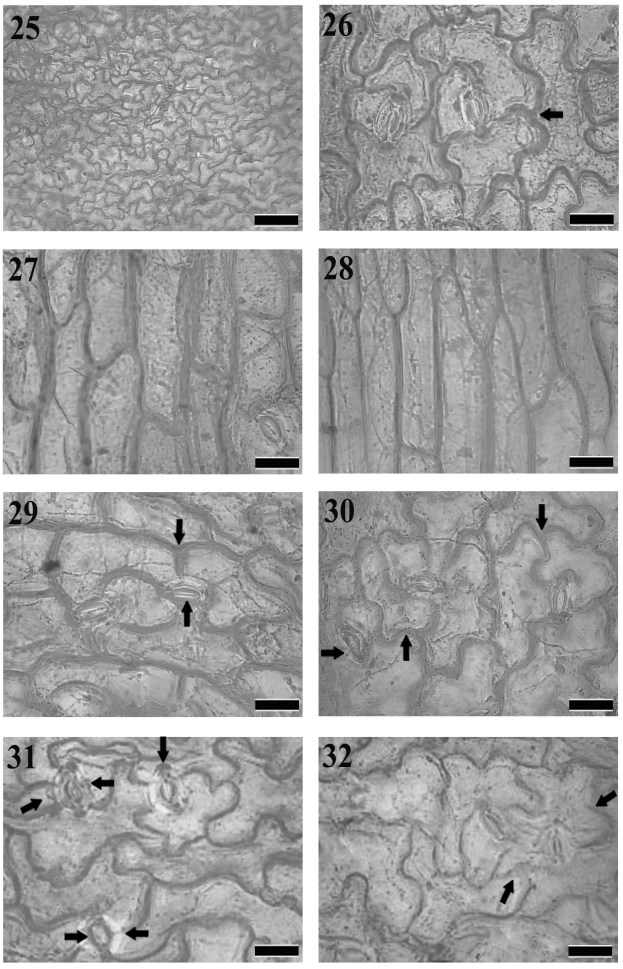


Scale bar: 17 - 22, 24: = 15µm; 23: = 60µm.

Figures 17- 22: Foliar epidermis of *Kickxia ramosissima*; 23, 24: *Linaria dalmatica* 17 - 19, 22: Lower; 20, 21, 23, 24: Upper.

17. Anisocytic stomata and heteromorphic stomata in shape (oval, elliptic, circular),
18. Stomata with cuticular ledges and large, small stomata, stomatal group,
19. Stomatal chain (oblique) and stomata with polar nodules and peristomatal rim,
20. Stomata with unequal guard cells,
21. Contiguous stomata (superposed pairing),
22. Glandular trichome with 3-celled, uniseriate stalk and globose head in costal region,
23. Epidermal cells and stomata,
24. Epidermal cells with polygonal to irregular outline, wavy to undulate anticlinal walls and anisocytic stomata with polar nodules.

Glandular trichomes: These are found distributed in both the surfaces of the leaf. The glandular trichomes are simple, multicellular and uniseriate. The foot of the trichome is embedded in the epidermis. Body is distinguishable in to a stalk and head. The stalk is long, 3-celled, usually constricted at cross walls and 4-celled with globose head (Fig. 22). Among the members of this tribe, presence of glandular hairs with unicellular or uniseriate stalks of varying length and heads usually composed of 1-4 cells was recorded earlier in the species of *Antirrhinum*, *Cymbalaria*, *Linaria* and *Nemesia* (see, Metcalfe and Chalk, 1950). Harkiss (1971) also reported the presence of glandular trichomes over the leaf epidermises of *Antirrhinum majus*.



Scale bar: 25: = 60µm; 26 - 32: = 15µm.

Figures 25 - 32: Foliar epidermis of *Linaria dalmatica*. 25, 26, 28, 30 - 32: Lower; 27, 29: Upper.

25. Epidermal cells and stomata,
26. Epidermal cells with irregular outline, undulate to sinuous anticlinal walls with 'U' shaped sinuses and anisocytic stomata,
27. Elongated, polygonal to rectangular costal cells with parallel arrangement and curved to straight side walls,
28. Elongated, polygonal to rectangular costal cells with parallel arrangement and straight to curved side walls,
29. Elliptic shaped, anomocytic stomata with cuticular ledges,
30. Anisocytic, anomocytic stomata and stomata with unequal guard cells,
31. Oval, circular shaped stomata with cuticular ledges, polar nodules and peristomatal rim,
32. Anisocytic stomata with common subsidiary cell.

Conclusion

Foliar epidermal features are proved to be useful in taxonomic studies and also in pharmacognistic evaluations. The details of epidermal cell complex, stomatal complex and trichome complex of three medicinally important plants of Scrophulariaceae - *Cymbalaria muralis*, *Kickxia ramosissima* and *Linaria dalmatica* are presented above. The results obtained now may be used further to develop characteristics of authenticity of these medicinal plants.

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