

Morphological, anatomical and chemical studies of the species of *Euphorbia* Linn.

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The present study enumerates the morphological, anatomical and chemical parameters for speciation of closely related species of the genus *Euphorbia* viz. *E. prostrata* Ait., *E. microphylla* Heyne and *E. thymifolia* Linn. The species show close morphological similarities and create confusion in identification and speciation. The morphological features include shape, apex, base, midrib, venation, colour of glands, presence or absence of hairs. SEM study of stem, leaves, capsules and seed, cuticular observations and chemical analysis for the extraction of different organic constituents were carried out.

Key-words – *Euphorbia*, Speciation, Morphology, Anatomy, Pigments, SEM.

INTRODUCTION

THE genus *Euphorbia* Linn. is highly versatile as it is distributed throughout the world in tropical, subtropical and temperate belts. Gaucher (1902), Croizat (1940), Perry (1943), Kapil (1959), Webster (1957, 1975), Biscose (1910), Chaghtai Garg (1979), Panigrahi and Prasad (1966), Sehgal and Paliwal (1975), Raju and Rao (1977), Huft (1982), Binoj Kumar and Gopalan (1998) have contributed to the taxonomy of the genus *Euphorbia* Linn.

Various phytochemicals such as diterpenoids, triterpenoids, flavonoids, anthocyanins, sterols, fatty acids etc. have been isolated from *Euphorbia* species. The pharmacological and antimicrobial activities have also been reported (Evans & Kinghorn 1977, Singla & Pathak 1990, Holden & Mahlberg 1992). Extensive analysis of closely related species of *Euphorbia* have been undertaken from morphological, anatomical and chemotaxonomical points of view to differentiate the species.

Specimens of *Euphorbia prostrata* Ait (red and green forms), *E. microphylla* Heyne and *E. thymifolia* Linn., were collected from banks of Yamuna at Delhi, hills of Rishikesh, Srinagar, plains of Haridwar, Lucknow and Ghats of Mumbai and Goa. *E. prostrata* (red forms) is distributed as weed in dry habitats, *E. prostrata* (green) is restricted to the moist sandy habitat, *E. microphylla* is found near the lakes,

ponds and on river banks and *E. thymifolia* is very less in number and restricted to the dry sandy habitat. The specimens are deposited in the Herbarium of National Botanical Research Institute, Lucknow.

Morphological Observation

Prostrate laticiferous herbs, common as weed (Text figures 1, 3, 5). Stem slender, prostrate, divaricately branched, more or less hairy/glabrous. Leaves generally opposite, sometimes alternate. Stipules small or absent. Inflorescence solitary or in small axillary cymes.

Involucre often 4-5 lobed, at tip bearing 1-5 glands (Pl. 2, fig. 2) alternating with the lobes, glands often with a petaloid limb, bracteoles present. Male flowers stalked, stamen without floral envelope. Female flowers ovary 3-celled exerted stalk in the centre of the involucre, style 3, free or connate, entire or two lobed or 2b-fid. Capsule two valved, 1-2 seeded cocci separating from a persisting axis. Seeds laterally attached at or above the middle of the cell with or without an aril or thickening at the hylum, carunculate and embryo straight.

The '*Euphorbia* species complex' includes those species having prostrate habit. In this complex the speciation was completely based on presence or absence of petaloid glands of involucre. Capsule glabrous (*E. microphylla*), capsule pubescent at the

Table 1. Showing the morphological features of *Euphorbia* species

Species	Morphological Features								
	Leaves	Stem	Capsule	Glands	Stipules	Seed	Inflorescence	Veination Type	Trichomes
<i>E. prostrata</i> (Red form)	Obovate, dentate margin at apical region	A few hairs present	Hairs only at cocci	4-red coloured	Hairy	4-angled	Cythium	1.3.1.3	Absent
<i>E. prostrata</i> (Green form)	Obovate, dentate margin, nerves prominent	Hairs absent	Pubescent only at the angle of cocci	4-green coloured	Hairy	4-angled	Cythium	1.3.1.3	Absent
<i>E. thymifolia</i>	Obovate or elliptic-oblong, dentate margin, nerves obscure	Hairy throughout (pubescent)	Hairy, obtusely keeled pubescent all over	3-4 light green, minute glands	Hairy fimbriate	4-angled	Cythium	1.3.1.3	Present
<i>E. microphylla</i>	Cordate, opposite, entire, notched at apex	Glabrus, slender	Glabrus, keeled, short deeply 2-fid	5-purple coloured	Broad acreate with small quadrangular limb	4-angled, obtusely quadrangular	Cythium	1.3.1.3	Absent

angles of cocci and glabrous elsewhere (*E. prostrata*). Leaves crenulate; capsule pubescent or hirsute all over; seed furrowed (*E. thymifolia*). The morphological, anatomical and chemical observations on these three closely related species of *Euphorbia* are given in Tables 1,2.

The complex shows presence of accumulated group of tracheidal elements and xerophytism; and the sheath around the veins (Pl 3, figs. 1,2,4). The three veins strands (Tri-veined) entering the petiole or at the base of leaf in all the species of *Euphorbia* complexes, but in *Euphorbia hirta* tri-veined and bi-veined type of habit found in two colour forms. The plasticity of venation is observed in the form of areole size, number of vein ending and their tips per areole.

SEM Observation

The scanning electron micrograph of stem (Pl. 1, figs. 1, 2, 3) shows fibrous cellular outline but in *E. thymifolia*, the fibres are densely packed and forming elongated cells. The cells join end to end in *E. thymifolia* and in *E. microphylla* cells are barrel shaped and their end walls show thick deposition of wax. In *E. prostrata* the surface cells of the stem are small, square and thin walled which joins end to end. *E. thymifolia* contains uniseriate trichomes all over the stem but it is lacking in *E. prostrata* and *E. microphylla*.

The surface structure of leaf (Pl. 1 figs. 4, 5, 6) shows differences in cellular outline. Trichomes are present in *Euphorbia thymifolia* but absent in *E. microphylla* and a few are present in *E. prostrata*.

PLATE 1

1. Hairy stem surface structures of *E. thymifolia* x 200.
2. Stem surface details of *E. prostrata* x 200.
3. Glabrous stem surface of *E. microphylla* x 200.
4. Hairy adaxial surface of *E. thymifolia* x 200.
5. Glabrous adaxial leaf surface of *E. prostrata* x 200.
6. Glabrous adaxial leaf surface of *E. microphylla* x 200.
7. Adaxial leaf surface structure of *E. thymifolia* x 2500.
8. Adaxial leaf surface of *E. prostrata* x 2500.
9. Adaxial leaf surface details of *E. microphylla* x 2500.
10. Seed surface details in close up view of *E. thymifolia* x 1000.
11. Seed surface structure in close up view of *E. prostrata* x 500.
12. Seed surface structure in close up of *E. thymifolia* x 500.
13. Complete seed surface structure of *E. thymifolia* x 150.
14. Complete seed surface structure of *E. prostrata* x 110.
15. Complete seed surface structure of *E. microphylla* x 110.

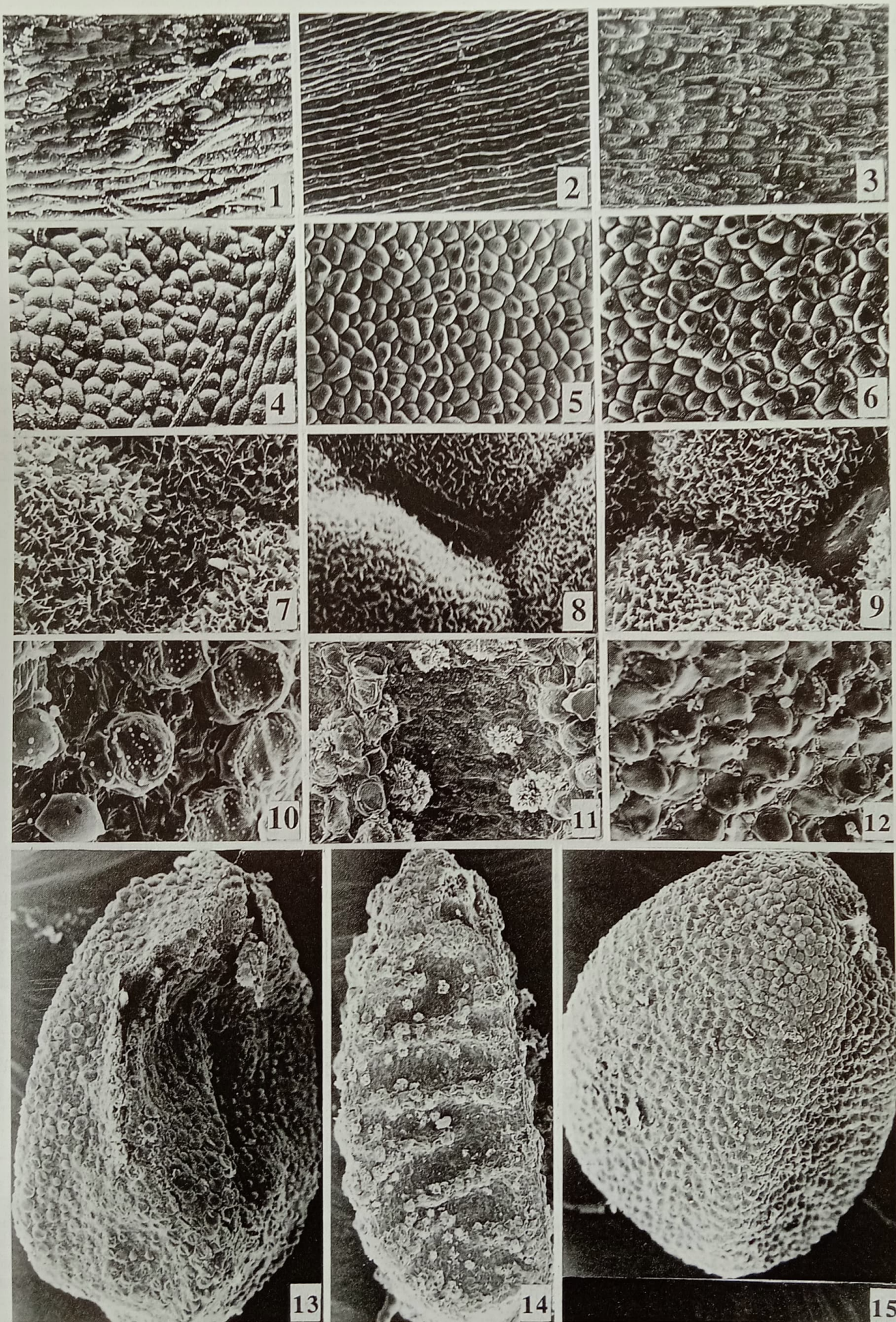


PLATE I

Table 2. Showing the cuticular & chemical features of *Euphorbia* species

Species	Cuticular Features			Chemical Features					
	Stomata	Stomata/sq. mm	Stomatal index	Sample No.	Chl-a	Chl-b	Total	Carotenes (mg/kg)	Sugar
<i>E. prostrata</i> (Red form)	Anamocytic	164	13.2	E1-A	273.38	245.04	518.42	320.00	Fructose
				E1-B	294.11	284.14	571.25	384.00	absent
<i>E. prostrata</i> (Green form)	Anamocytic	170	15.1	E2-A	404.93	411.76	816.69	312.00	Fructose
				E2-B	382.67	386.92	769.59	318.00	absent
<i>E. thymifolia</i>	Anamocytic	250	26.3	E5-A	629.075	539.45	1168.53	105.00	Fructose
				E5-B	629.076	534.45	1168.53	Nil	present
<i>E. microphylla</i>	Anamocytic	178	20.2	E6-A	755.4	805.2	1560.6	115.00	Fructose
				E6-B	755.4	805.2	1560.6	12.00	present

The outer periclinal wall of the cells of the upper epidermis is slightly convex and shows polygonal cells. Stomata are present on both the surfaces of leaf (Pl. 1, figs. 7, 8, 9). Stomata on upper surface are comparatively smaller and lesser in number than the same on lower surface. Trichomes are present throughout on both the surfaces in *E. thymifolia* but rarely on the lower surface in *E. prostrata* and totally absent in *E. microphylla*. Wax deposition is thicker on upper surface than on the lower surface. Papillae are absent; stomata are elliptical, irregularly distributed. Lower epidermal cells polygonal, stomata sunken type. Peristomatal rims not clear. In *E. thymifolia*, the cells are polygonal, thick walled and compactly arranged. In *E. microphylla* cells are heragonal or polygonal and loosely arranged. The wax deposition is comparatively lesser in *E. microphylla* than in *E. prostrata* and *E. thymifolia*. The uniseriate hairs are seen only in *E. thymifolia*.

The seed of *E. thymifolia* is elliptic in shape and

densely covered by measles like ornamentation throughout the seed surface. The seed of *E. microphylla* is rounded and measles like ornamentation is densely packed than that of *E. thymifolia*. The seed of *E. prostrata* is tongue shaped having ridges and furrows, pointed apex and a few measles like ornamentation throughout the surface seed. The close up view of *E. thymifolia* shows balloons like rusted surface while in *E. prostrata* it is small and globular. In *E. microphylla* it is papillate in outline.

The capsule of *E. thymifolia* (Pl. 2, figs. 1, 2, 3) is covered throughout by an uniseriate hairs. The hairy structure emerges from a deeper zone of tissue. In case of *E. prostrata* capsule (Pl. 2, figs. 4, 5, 6,) bears uniseriate hairs only on the cocci which emerge from bulbus stock cell. The wax deposition is interrupted in linear fashion in both the species. In *E. microphylla* the capsule (Pl. 2, figs. 7, 9) is glabrous; size is bigger than that of *E. thymifolia* and *E. prostrata*. The cells of *E. microphylla* are hexagonal

PLATE 2

1. Capsule cellular details and arrangement of trichomes of *E. thymifolia* x200. 2. Close up view of trichomes showing wax patterns of *E. thymifolia* x500. 3. Surface overview of capsule of *E. thymifolia* x80. 4. Capsule close up view of cocci and arrangement of hair of *E. prostrata* x200. 5. Capsule close up view of *E. prostrata* showing wax arrangement on hair and its bulbous base x500. 6. Surface overview of capsule of *E. prostrata* x50. 7. Cellular details glabrous capsule of *E. microphylla*'s x500. 8. Cyathium inflorescence of *E. microphylla* showing glands and point of emergence of female flower. (9) Glabrous capsule of *E. microphylla* showing surface overview x41.

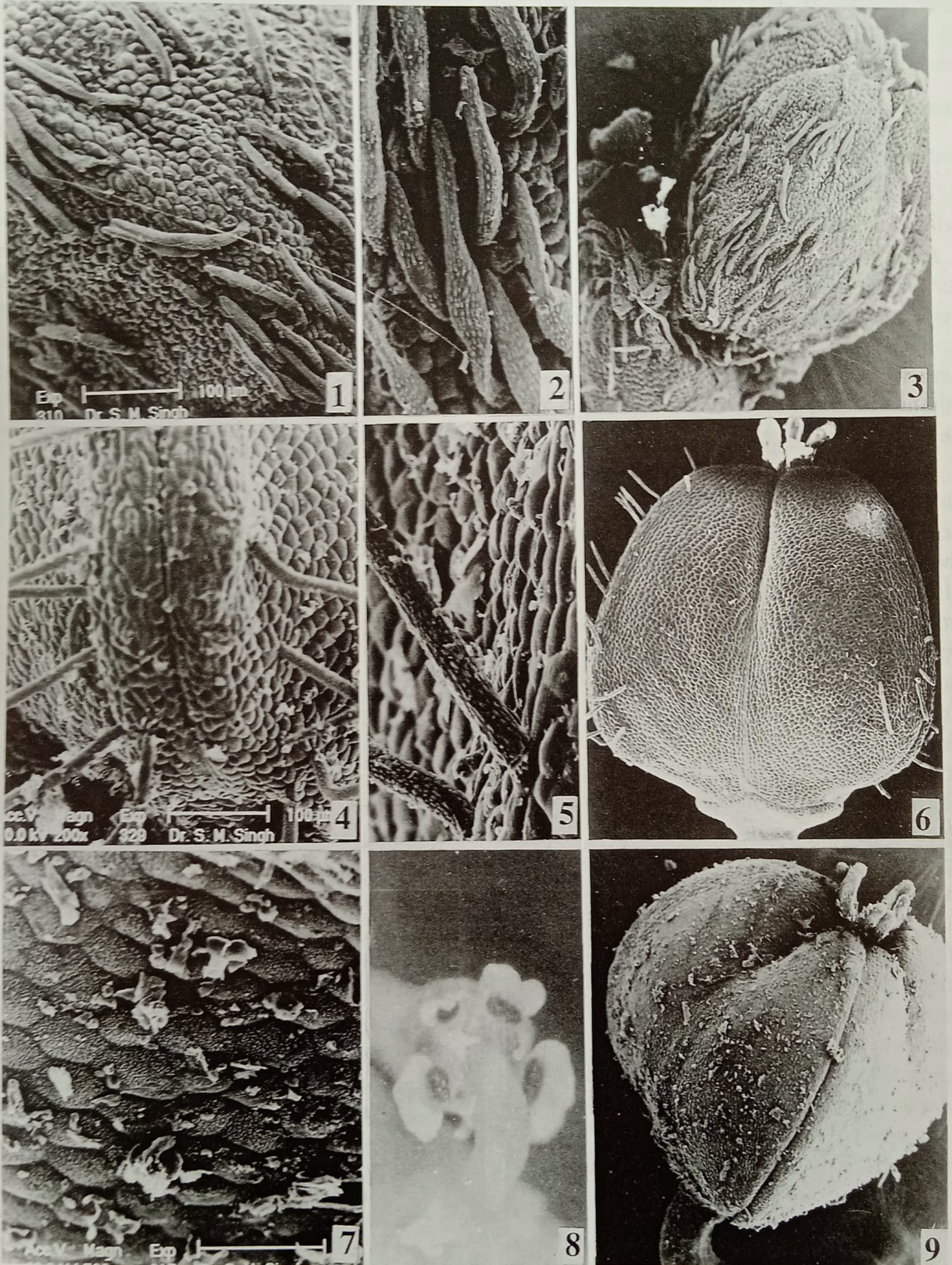


PLATE 2

and arranged end to end, but in case of *E. thymifolia* and *E. prostrata* they are globular, pentagonal and loosely arranged.

Anatomical Observation

Leaf and Stem

Cell walls range from straight (*E. thymifolia*) to variously arched (*E. prostrata*) and undulated types (*E. microphylla*). (Text fig. 2, 4, 6). The *Euphorbia* species complex shows anamocytic stomata which are irregularly distributed, the trichomes present in *E. thymifolia* are uniseriate with tapering apex. Mode of stomatal development is generally uniform; variations in the mature organization are produced at a later stage due to spatial readjustments.

In general, in all the species of *Euphorbia* complex (Pl. 3 fig. 6, 8, 9); epidermal cells are slightly thicker than those of internal cells. Stomata are sunken type. Hypodermis present on the surfaces of leaf. The palisade and spongy parenchyma cells are present throughout except at median portion. At the midrib the upper epidermis has 2-3 layers of ground parenchyma but at lower epidermal side 6-7 layers of cells surround the vascular bundles. The ground parenchyma cells are large, more or less cubical, sometimes transversely stretched. Palisade cells are chlorophyllous and form a photosynthetic layer. The hypodermis is a diagnostic tool for the species. The upper hypodermal palisade layer is comparatively thick walled and continuous in *E. prostrata* and *E. thymifolia* but it is slightly thin walled in *E. microphylla*. In *E. prostrata* the cells are comparatively smaller than *E. thymifolia*. The palisade tissue generally consists of vertically elongated, cylindrical cells, two to three times as high as wide, compacted in varying degree. Spongy cells are loose and rounded in shape. The intercellular space system of spongy tissue is in connection with external atmosphere via stomata and substomatal chambers.

The vascular bundle is mesarch (Table 1).

The stem anatomy (Pl. 3 figs. 3,5,7) shows an outer epidermal layer which is consist of barrel shaped cells in *E. prostrata* and *E. microphylla* but irregular in *E. thymifolia*. A thick cuticular layer above the epidermis is found in *E. prostrata* and *E. thymifolia* while in case of *E. microphylla* it is thin. Stomata are rarely seen in young stem. Cortex is generally wide, 6-8 layer in thickness, parenchymatous, subepidermal cells are smaller than central cells. The central cells are associated with small intercellular spaces. The cortical cells are comparatively thick walled in *E. prostrata* and *E. thymifolia* than in *E. microphylla*. The cortical layers surround central vascular bundles and pith in all the species. The normal vascular bundles show six vessels and 20 rows of xylem in *E. microphylla* and *E. prostrata* but xylem vessels are thicker in *E. microphylla*. In *E. thymifolia* vessels are five with 20- rows of xylem and phloem, number of xylem and phloem increases near the branch. Vascular bundle endarch.

Chemical Analysis

The pigments of *Euphorbia* species complex include chlorophyllarotene. The chlorophyll is of two types viz. chlorophyll-a and chlorophyll-b. The chl-a ranges from 273-404 mg/kg. in *E. prostrata*, 629.075 mg/kg. in *E. thymifolia* and 755.4 mg/kg. in *E. microphylla*. The chl-b ranges from 245.04-411.76 mg/kg. in *E. prostrata*, 539.45 mg/kg. in *E. thymifolia* and 805.2 mg/kg. in *E. microphylla*. The carotene ranges from 320-384 mg/kg. in *E. prostrata*, 105 mg/kg. in *E. thymifolia* and 115-120 mg/kg. in *E. microphylla*.

The quantitative variation in amount of pigment molecules in *Euphorbia* species complex shows that their basal metabolic rates (BMR) are different. The species lack vigour constituent except in *E. microphylla* (Table 2).

PLATE 3



1. A part of whole mount leaf of *E. microphylla* x40 2. A part of whole mount leaf of *E. prostrata* x40. 3. Stem anatomy of *E. thymifolia* x200 4. A part of whole mount leaf of *E. thymifolia* x40 5. Stem anatomy of *E. prostrata* x200 6. Leaf anatomy of *E. thymifolia* x400 7. Stem anatomy of *E. microphylla* x200. 8. Leaf anatomy at median portion of *E. microphylla* x40.

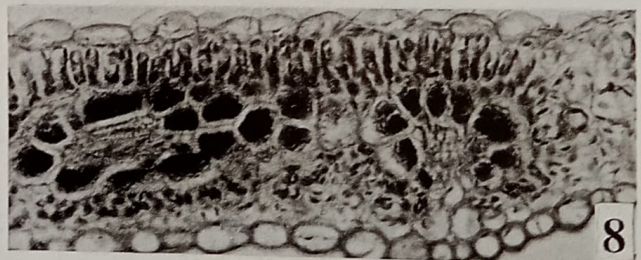
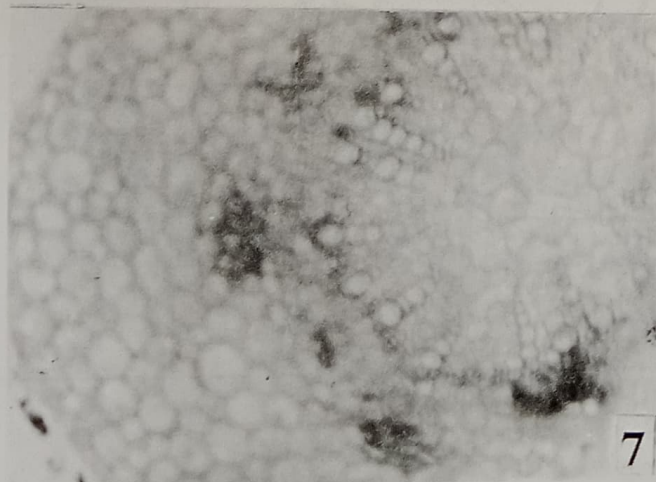
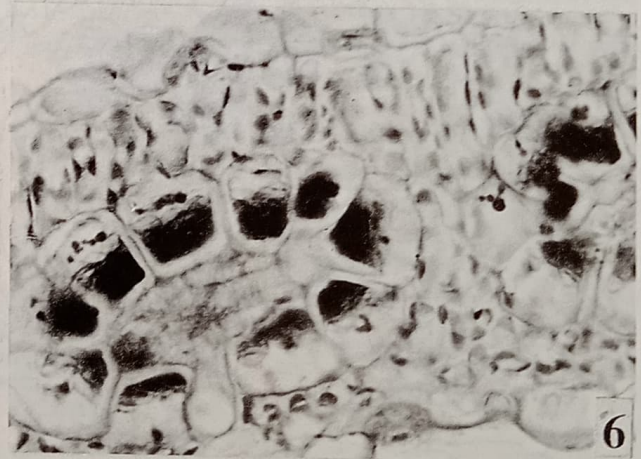
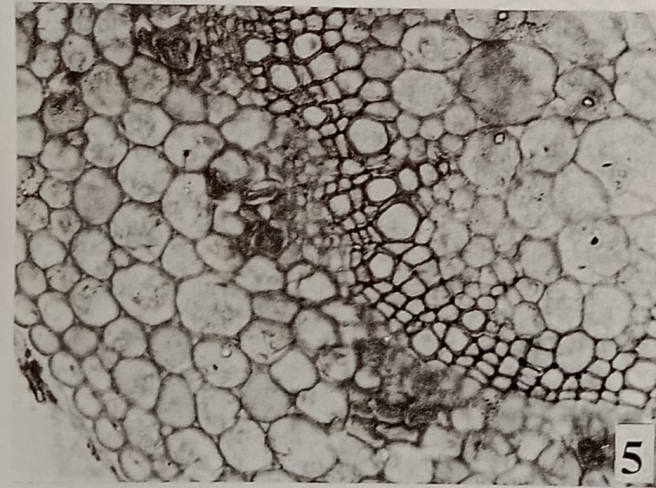
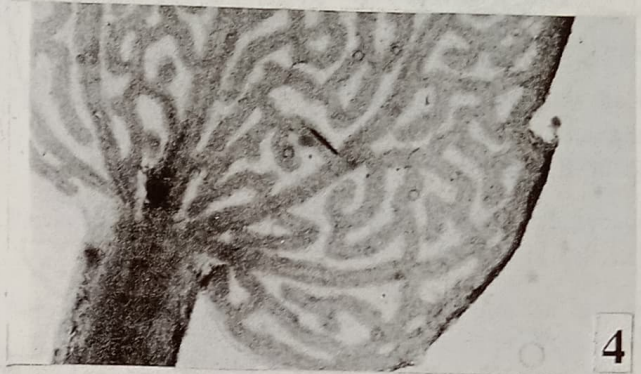
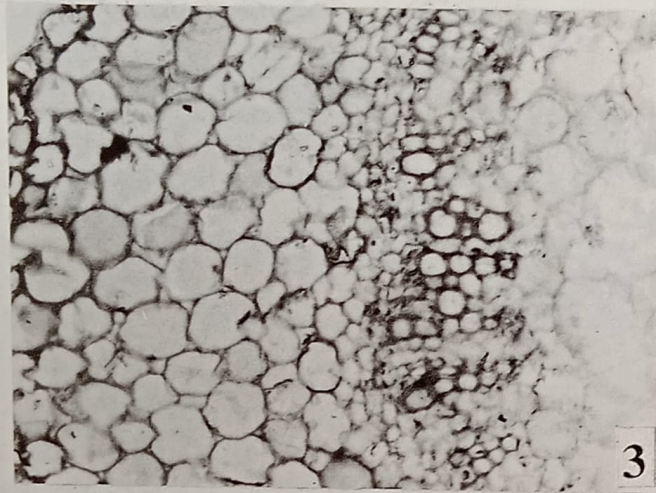


PLATE 3

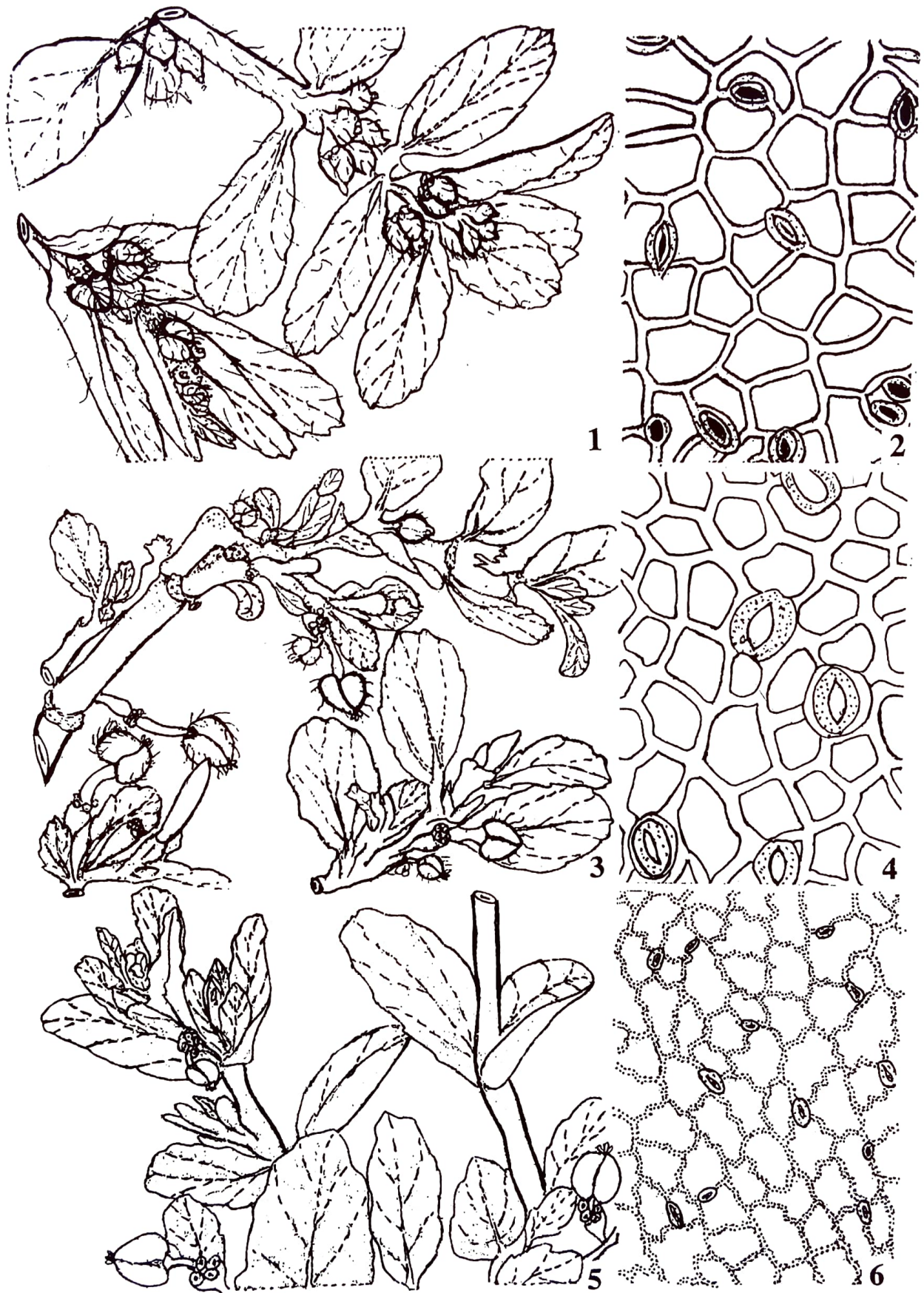


PLATE 4

1. Morphological features of *E. thymifolia*. 2. Cuticular feature of *E. thymifolia* x400. 3. Morphological features of *E. prostrata*. 4. Cuticular features of *E. prostrata* x400. 5. Morphological features of *E. microphylla*. (6) Cuticular features of *E. microphylla*.

Morphological, anatomical and chemical studies on different species of *Euphorbia* show distinct features and suggest that *Euphorbia prostrata*, *E. microphylla* and, *E. thymifolia*. are natural compilation of the subsection Chamaesyceae (section Anisophyllum) of the family Euphorbiaceae.

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