STUDY OF MORPHOLOGY, ANATOMY, ANDPHYTOCHEMISTRY IN *Euphorbia milii*ch. MAHARASHTRA STATE OF INDIA

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ABSTRACT

The present work includes the study of Morphology, Anatomy, vessel elements which may be useful for the identification of a particular plants drug because in it drug the entire of various fragments of tissue are present. Many anatomists have contributed on the various aspect of vessel elements several workers studed the structure of vessel element. Woodworth (1935) Studied it in passifloraceae ; Chedle (1942,1955) and Fahn (1954a) in monocotyledons ; Cheadle and Kosakai (1973,1974,1975,1976) in Junicales, Hypoloytrieae and Alstroemeriales throughout the 20th century certain workers studied the dimension of vessel element and its importance in the phylogeny. They are chalk and chattaway (1934,1935) Scholander (1958). Zimmermann and Ayodeji (1981). The study of Euphorbia was carried out by Metcalf and chalk (1950) Solereder(1908) in the form of books Morphotaxonomy, Pharmacognosy and Biology of some Euphorbias (Kadam 1995) Chlorophyll concentration and free amino acide of some plants (Maclachalam 1963) Survey of Indian medicinal plant for saponin, alkaloids and flavonoids (Kapooretal, 1969) General Laboratory Manual in Biochemistry.

Keywords: *Morphology, Anatomy, vessel elements, E. milli, phylogeny.*

Introduction

Scientific categorization is firmly related with the government assistance of society as it relates the protection of biodiversity and maintainable use of plant assets and natural administration. Scientific categorization or plant precise is part of plant science which manages the course of action portrayal, terminology and transformative status of different plant bunches including variety and speciation. The term Taxonomy was instituted by A.P. de Candolle (1813). Ordered examination and information collection can be completed in fields, research center, gardens, libraries, herbarium and utilizing PC which depends on specific standards, as ? uniform arrangement of articulation, all around represented characters, portrayal, distinguishing proof, characterization and phylogenetic angles, delimitation of plant gatherings, phonetic (similitudes of the aggregate of the life form) and cladistics scientific categorization (transformative highlights), International code of Botanical Nomenclature (ICBN) and as all-encompassing science. There are four ordered basic parts which improve on the interaction of distinguishing proof up to species level. These parts are recognizable proof, portrayal, arrangement and naming. The point of order is to mastermind huge number of plants in an improved on way for viable correspondence and comprehension. The rule rank of characterization is division, class, request, family, sort and species. Depiction is an exact technique for correspondence about plants which gives portrayal, recognizable proof, grouping and phylogeny. Terminology is naming the plants in logical way. A definitive objective of terminology is to give a right name to every taxon. ICBN follow certain standards to administer entire technique of terminology which are (a) Botanical classification is autonomous of Zoological classification (b) The utilization of names of scientific categorization is controlled through terminology type (c) Nomenclature of scientific classification depends on need of distribution (d) Each scientific categorization with a specific circumscription, position and rank can bear just one right name (e) Scientific name are treated as Latin , paying little mind to their determination (f) Rule of Nomenclature are retro-dynamic except if explicitly restricted. To furnish a right name of taxon with the assistance of an example, there is a legitimate gadget known as embodiment which includes ?types. The rule need of name doesn't have any significant bearing to the names of taxa over the names of family. Need of classification of vascular plants starts with the distribution of

species plantarum by Linnaeus (May 01, 1753). The term species name is inadequate without alluding to conventional name which is particular Latinized thing or a word treated as thing and consistently composed with an underlying capital letter. A total herbal name of a plant should be trailed by the third component for example the name of the creator who initially portrayed the plant. ID is to decide right name and position for example. There are sure ordered organizations which help to recognize right plant arrangement, phylogeny, greenery and vegetation; these are Herbarium, Botanical Gardens, Botanical Survey of India and Taxonomic Literature. Along these lines, scientific categorization is a unique science as one of the most seasoned just as late trains of natural science which in alternate manner is gathering of the information through organized course of action of plants.

Review of Literature

(Jayaraman 1981) Ash & Sillca Contents and Silica depositional patterns have been determined for selected tissue of 19 different species of plants (lanning FC Elewuterium L.N.) Lactiferous plants as a source of rubber and hydrocarbon (Krishnamachary B.) Effect of auto exhaust pollution at Byculla on the leaf anotomy of some weeds (Salgare S.A., Iyer M.P. 1991) 92 terms relating to the epidermal tissue is defined under the following heads. Epidermal cell complex, stomatal complex trichome complex and miscellaneous (Shanmukhrao S. 1987) Eleven type of hairs were recorded in 10 species of the tribe crotonae (Euphorbiaceae) in the heading of structure and distribution of foliar hair in the tribe crotoneae (Shastry AVVS kannabiran B 1994) A preliminary report on anti-eosinophila effect of *Euphobiamilli in bronchian asthma* (Khare M.L. saxena R. C. 2000) Changes in amino acid and protein levels in the leaves of the Euphorbia (B.S. Afria& D. Mukherjee1981) Classification and distribution of laticifer inbearing plants (David 1872, Hanstein, 1864 : Hartig 1962 Mayur 1905) Isolation of terpenoids and steroid from several Euphorbiaceae plants (Tabakar and Matsunga S.2000) Preliminary phytochemical studies on some medicinal plants(Sundaresan V : De Britto A.J. 2000)

The studies in the vegetative anatomy have considerable scope, many authors contributed their lion's share in this field. The important ones are solerdor (1908), Sinnott (1914), Haberlandt (1914), Metcalfe and Chalk (1950) Deshpande and Avtar Singh (1967), Shah (1968) Inamdar and Murthy (1977,1978), Nishion, Eisho (1978),Bhatt and singh (1979), BirBahadur, Narsaiah and Farooqui (1985), Carlquist (1992) Bakale and Sharma (1982) Anilkumar and Rao (1984).

Identification of powder's and extracts is also progressing well in some of the recently established laboratories. The main center of this activity is FRLHRT

Banglore, TBGRI, Thiruwanantpuram NGCPR, Pune uttam Vanaushadhi Sanshodhan sanstha Mumbai, AyurvedRasashala Pune; Banras, Hydrabad and few others. These center have devoloped analytical methods for identification of whole plants as well as their parts Asnokan and Jayaraman (1998) Chowdharyetal (1998) Alametal (1998) Waradpande (1995) have done a commendable work in standardization of certain plants drugs. Recent publication by Kulkarni&Apte (2000) is very helpful in understanding the research methodology for student.

The above work present a brief account of pertinentresearch on the Experimental taxonomic studies in genus *Euphorbia* (Euphobiaceae) from Marathwada. " It will be seen that although the Genus has been exhaustively studied for their chemical content, Still it does leaves considerable scope for further studied to confirm repeat, modify or add to the observation and deductions.

Intensive studies on morphology, histology, histochemistry and physical studies of vegetative of the species would be very revealing and significant in evaluation of Genus *Euphorbia* L. It is therefore with such a background that this investigation was carried out.

The Genus Euphorbia L is large one and the plants which are easily available were collected from different localities in different seasons. The observation variouscharacters of studied plants suggest the availability and exploitation

of species from genus in the systematic position and differentiation between species.

Materials and Methods

The information regarding to Genus Euphorbia L. used in the region was collected from various sources such as tribal Hakims, Vaidyas,Street Vendors and local people. During the first phase, the collections of species of *Euphorbia* were made by visits to various localities from Marathwada and its adjoining regions. Several tours were arranged to collect plant species in different seasons Specimens collected from different localities were dried with help of blotter method and made into herbarium specimens and deposited in the herbarium of Department of the Botany, ShriShivajicollegeParbhani as voucher specimens for ready reference. Specimens were identified with the help of floras and difficult ones were referred to the experts for correct valid identify. The fresh material of specimen's and their parts used in anatomy were collected from the field and preserved in FAA (100 % alcohol 70 CC, Acetic acid 25 CC and formalin 5 CC) for microscopic studies.

1. For gross anatomical studies of root, stem and leaves free hand section were taken with the help of blade (microtome for leaves) and sections were stained by using double stained differential staining technique. Illustrations of internal structures were drawn with the help of Camera Lucida using Indian ink.
2. For leaf architecture the leaves were cleaned by immersing in 10-20% aqueous sodium trichloroacetic acid and phenol solution 2.1 and stained with Kores stamp pad purple ink (rao et al., 1980) and micro photographs were taken with the help of Asia Pentax camera.
3. For dermal studies peals from fresh preserved leaf materials were taken and stained it in 1 % Safranin, line drawings and microphotographs were taken. The stomatal index was calculated by using following equation (salisbury, 1927 and 1932)

$$sl = \frac{S}{E+S} \times 100$$

Where

'S' is the number of stomata per unit area,

'E' is the number of epidermal cell in the same area

Stomatal index have been calculated out of an average of 10 readings.

4. For study of vessels the preserved material were made into small pieces and boiled and cooled repeatedly until free from the air. A macerated fluid was prepared by taking aqueous chromic acid (as per Jeffrey's). The pieces of wood were kept in the fluid for 24 hours and after 24 hours the material was crushed with the help of glass rod and washed with distilled water to remove excess stain. The material was stained in 1 % saffranin for 6 hours and microscopic observations. The camera Lucida of the vessels were drawn by taking measurements the illustrations were drawn with India ink and microphotographs were taken wherever possible.

Observations

The various characters of vessel elements viz,.size wall thickening, shape, tail and characters of perforation plate like number, orientation and shape were studies. A survey of about 30-50 vessel elements of stem was carried out.

The range of length and width of vessel elements was determined by the measurement of 20-25 vessel elements and were classified as per the classification given by Radlford et al. (1974). This is reproduced here for perusal's

A.	Extremely short	Less than 175 um
B.	Very short	175 to 250 um
C.	Moderately short	251 to 350 um
D.	Medium size	351 to 800 um
E.	Moderately Long	801 to 1100 um
F.	Very Long	over 1900 um

5. The micro-chemical tests were performed as per (johansens, 1940 and Gurr, 1965) and results were tabulated for ready reference.
6. For determination of ash value and percentage extractives methods were used as recommended by Anonymous (1966, 1973). All the observations were statistically analyzed using suitable methods.(Freud, 1977).

Determination of Ash-values

1. Preparation of Ash

10g of drug was incinerated in a silica crucible over the burner. The charred material was

heated in a muffle furnance for six hours at 600-650 C. The ash formed was white and free from carbon. It was then cooled and weighed on the ash less filter paper.

2. Determination of Acid Insoluble Ash

The ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. Insoluble matter was collected in the crucible or ash less filter paper and washed with hot water, ignited and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.

3.Determination of Water-Soluble ash

The ash was boiled for 5minutes with 25 ml. of distilled water. Insoluble matter was collected in a crucible of ashless filter paper and washed with hot water, ignited and weighed. Weighed.Of the insoluble matter was substracted from the weight of ash. The difference in weight represents the water-soluble ash. Percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of Water soluble extractives

10 g of air dried drug (Coarsely powered) was macerated with in a closed flask, for 24 hours, shaking frequently. Solution was filtered and 25 ml of filtrate was evaporated in a tarred flat bottom shallow dish, further dried at 100 c. The percentage of water soluble extractive was calculated with reference to air dried drug.

Determination of Alcohol Soluble Extractive

10 g of air dried (Coarsely powered) was soaked with 100 ml of alcohol in a closed flask for 24 hours with frequent shaking. It was filtered rapidly, taking precautions against loss of alcohol. 25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100 c and weighted.

The percentage of alcohol soluble extractive was calculated with reference to the air dried drug.

Determination of ether soluble extract

10 g of air dried drug, (coarsely powered) was macerated with 100 ml of ether in a closed flask for twenty four hour with frequent shaking. It was filtered rapidly, taking precaution against loss of ether.25 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100 c and weighed . The percentage of ether soluble extractive was calculated with reference to the air dried drug.

Histochemistry

For the histochemical studies free hand sections of the organs to be studied, were taken and treated with the respective reagents to localize components, viz. Starch, proteins, tannins, fat, alkaloids, calcium, lignin, silicon, and resin in the tissues (Jhohanson, 140). The tests employed are as follow.

Result and Discussion

Morphology of *Euphorbia milli* Ch.

des. Moulins in Bull. Hist. nat. Soc. Bordeaux 1:27.30. Pl. 1.1826; croizat in J. Arn.Arb. 21:506. 1940; Naik, fl. Osmanabad 301.1979.*E.splendens*Boj. ex Hook. In Bot. Mag. t. 2902.1829; Cooke, Fl. Pres. Bombay 3:66 (BSI reprint 1958).

Woody, somewhat scandent shrub, stem terete, much branched, and numerous long straight sharp thorne, leaves mostly on young shoots, alternate, oblong spathulate, tricucoronate 6 x 1-3 cm, narrowed at base; entire, undulate, obtuse, glabrous on both the surfaces, petiole short or obscure. Cyathia in long peduncle dichotomous Cyme, each closely subtended by two, broad ovate or sub orbicular, bright red bracts, 1-1.5 cm across, fruit not seen.

Grown in pots.

Fls– More or less throughout the year.

Gross Anatomy of *Euphorbia milii*ch(plate No 1)

Transverse section of root

T.S. of root is irregularly circular in C.S. periderm consist of 2-4 layered elongated, thin walled phellem. Phelloderm is followed by thin walled parenchymatous, Cortex are situated in this cortex cells crystal shaped calcium oxalates are found this cortex is followed by small layer of sec phloem bellow to this layer the sec xylem are intermixed with medullary rays.

Transverse section of stem. :- (plate No 1)

It is circular in C.S. epidermis is made from thick walled parenchyma covered with thick waxy cuticle. It is followed by a large zone of cortical parenchyma these cells are filled with crystals of calcium oxalate and palisade assimilatory tissue. Bellow to this layer a small layer of compactly arranged cells

followed by sec xylem with medullary rays, and at the center large pith is situated.

Transverse section of leaf :- (plate No 2 a)
T.S. of leaf is plano convex in structure, the upper and lower epidermis is single layered, made from slightly thicken upper epidermal cells than lower ones, palisade tissue are situated bellow the upper epidermis filled with chlorophylls, the spongy parenchyma above, lower epidermal part is parenchymatous sheath are at the mid rib reason, At the center single collateral and open type vascular bundle is situated. It is surrounded by parenchymatous sheath.

Stomata :- (plate No 2b)
The leaves are amphistomaticsometimes different type of stomata are found on lower side and upper side on lower side cruciferous type of stomata found. The stomatal index 15-32. The average size of stomata is 21.6 x 10 mu.

Leaf architechture :- (plate No 2c)
Type of venation is pinnate, cemptodromousbrochidodromous reticulate. Areolation'squadrangular, veinlet's are branched, tip of veinlet's are spiral oval and elongated.

Vessel element of *Euphorbia milii*ch :- (plate No 1)
Vessel element of root
Dimensions
Extremely short (class A) very short (class B) moderately short (class C) vessel were observed. The frequency of moderately short (class C) was higher (50.20). The extremely short (class A) vessel were less (23.53) frequency the average diameter of vessel element is 18.1 mu.

Lateral wall thickening
Spiral and pitted thickening were common. Among pits the irregular and alternate piting was common.

Tail
Vessel element with long pointed tail and long blunt, tail, short blunt tail were observed.

Perforation Plate
In the vessel only simple perforation plates were present.

Orientation
The vessels with oblique and transverse peroration were observed.

Shape of perforation plate
More commonly vessels have oval or lenticular perforation plates.

Root Fibers
The length of root fiber is 370-492 mu and the average length is 432 mu. the diameter of fiber is between 18.1-27 mu. and the average diameter is 19.8 mu all the fibers are pointed at both the ends.

Tracheid's
The length of tracheid element 280-355 mu.and average length of tracheid's element is 306 mu. The diameter of tracheid element is 18-27 mu. andaverage diameter is about 18 mu. the shape of tracheid element is spindle shaped.

Vessel element of stem (plate No 1 (d)
Dimensions
Extremely short (class A) very short (class B) moderately short (class C) vessels were observed. The frequency of moderately short vessel was higher (70.07) and Extremely short vessel shows less frequency (12.33).

Shape
The shape of vessels element is cylindrical, linear.

Lateral wall thickening
Simple pitted thickening were common, pits alternate, sometime irregular.

Tail
Tail with long blunt, short blunt, short pointed were commonly observed.

Perforation plate
In the vessel, only simple perforation plates were observed.

Orientation
The vessel with oblique and transverse perforation plates wereobserved.

Shape of perforation plate
More commonly vessels have perforation plate oval in shape.

Stem Fiber
The length of stem fiber is between 300-410 mu and the average length is 360 mu. the diameter of fiber is between 0.9-18 mu. and

average diameter is 12 mu. all the fibers are pointed at both the ends.

Tracheid's

The length of tracheid element is in between 410-515 mu. theaverage diameter is between 484 mu. The diameter of tracheid is between 18-20.5 mu, and average diameter is between 19 mu, all the tracheid are spindle shaped.

Determination of ash value in root, stem and leavesof *Euphorbiamilii*Ch

In the leaves total ash contain was highest (9.75) in percentage as compare to root (6.35) the and stem (7.12) it shows lowest percentage in root (6.35) as compare to root and leaves.

In the root water soluble ash was highest in percentage (4.80) as compare to the stem (3.80) and leaves (4.72) it is lowest in stem (3.80) as compare to root and leaves.

In the root acid insoluble ash was higher (3.44)in percentage as compare to the stem (2.94) and leaves(2.89) it is lowest in the leaves (2.89) compare to the root(3.44) and stem (2.94).

Quantitative estimation of total carbohydrate amino acid and determination of lipid in root, stem and leaves of *Euphorbia milii* Ch

In the leaves total carbohydrate was higher(2.75) in percentage as compare to the stem (2.70) and root (2.55) it is lowest percentage in stem (2.70) as compare to the root and stem.

In the stem total amino acids was higher(3.19) in percentage as compare to the root (2.90) and leaves (1.55) it is lowest in the leaves(1.55) as compare to the stem and leaves.

In the stem total lipids are higher (0.67) in percentage as compare to the root (0.45) and leaves (0.57) it is lowest in root (0.45) compare to root and leaves.

Photo spectrometry of Tannin& resin in the root stem& leaves ofEuphorbia milii Ch

In the root the optical density of resin was higher (1.36) as compare to stem (1.20) and leaves (1.09) it is lowest in the leaves of (1.09) compare to root and stem

In the root the optical density of tannin was higher (0.055) as compare to the stem (0.034) and leaves (0.015) it is lowest in leaves (0.015) compare to the root and stem *Euphorbia milii*Ch.

Conclusion

Flower containing Cyathia in long peduncle dichotomous Cyme, each closely subtended by two, broad ovate or sub orbicular, In T.S Root phellem. Phelloderm is followed by thin walled parenchymatous and in stem epidermis is made from thick walled parenchyma covered with thick waxy cuticle, In leaf palisade tissue are situated bellow the upper epidermis filled with chlorophylls, the spongy parenchyma above.

Anatomically the leaves are amphistomatic sometimes different type of stomata are found on lower side and upper side on lower side cruciferous type of stomata. Venation is pinnate, cemptodromousbrochidodromous reticulate. Areolation's quadrangular, veinlet's are branched, tip of veinlet's are spiral oval and elongated.Vessel element of rootThe frequency is moderately short (class C) was higher.In stem

The frequency of moderately short vessel was higher (70.07). Spiral and pitted thickening were common in Root and in stem Simple pitted thickening were common. In root vessels have oval or lenticular perforation plates. In stem vessel with oblique and transverse perforation plates were observed.The length of root fiber in root is 370-492 mu and in stem is between 300-410 mu. The length of tracheid element 280-355 mu.And in stem it is about 410-515 mu.

Cyathia characters of E. milli shows clear difference of it than other species, Anatomical comparative study of root and stem the anatomical characteristics shows difference between them in majority of aspects.ash value and in root, stem and leaves of *Euphorbia milii*Ch. Also shows great variation. In ash value and Quantitative estimation of total carbohydrate amino acid and determination of lipid in root, stem and leaves of *Euphorbia milii*Ch. also shows great variation.

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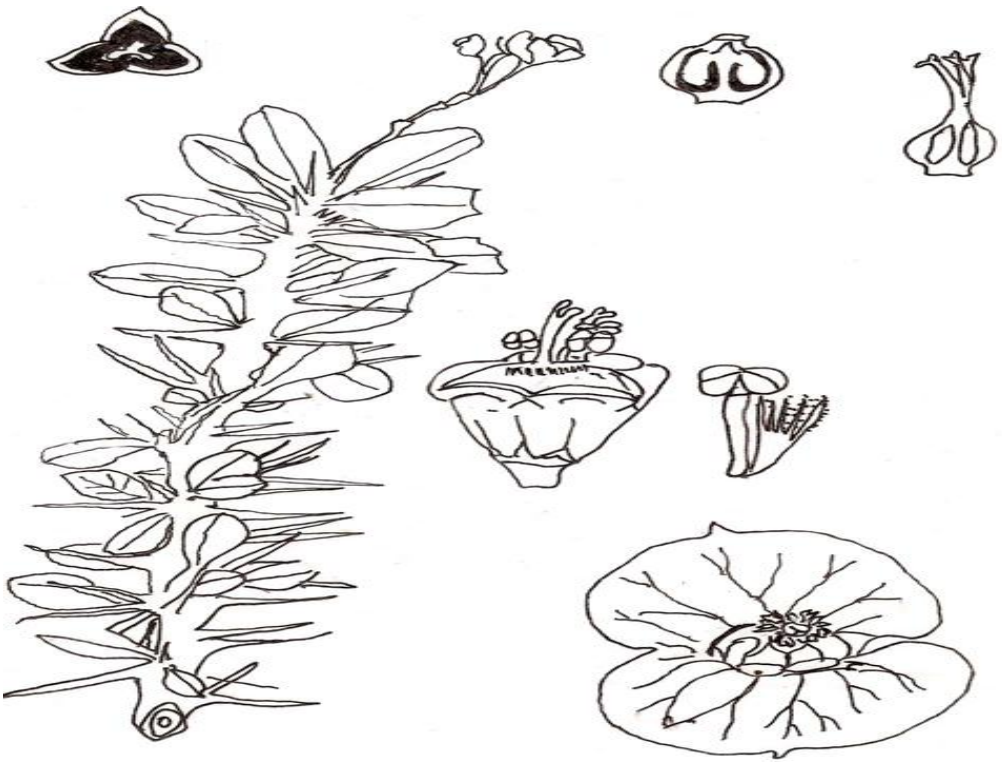
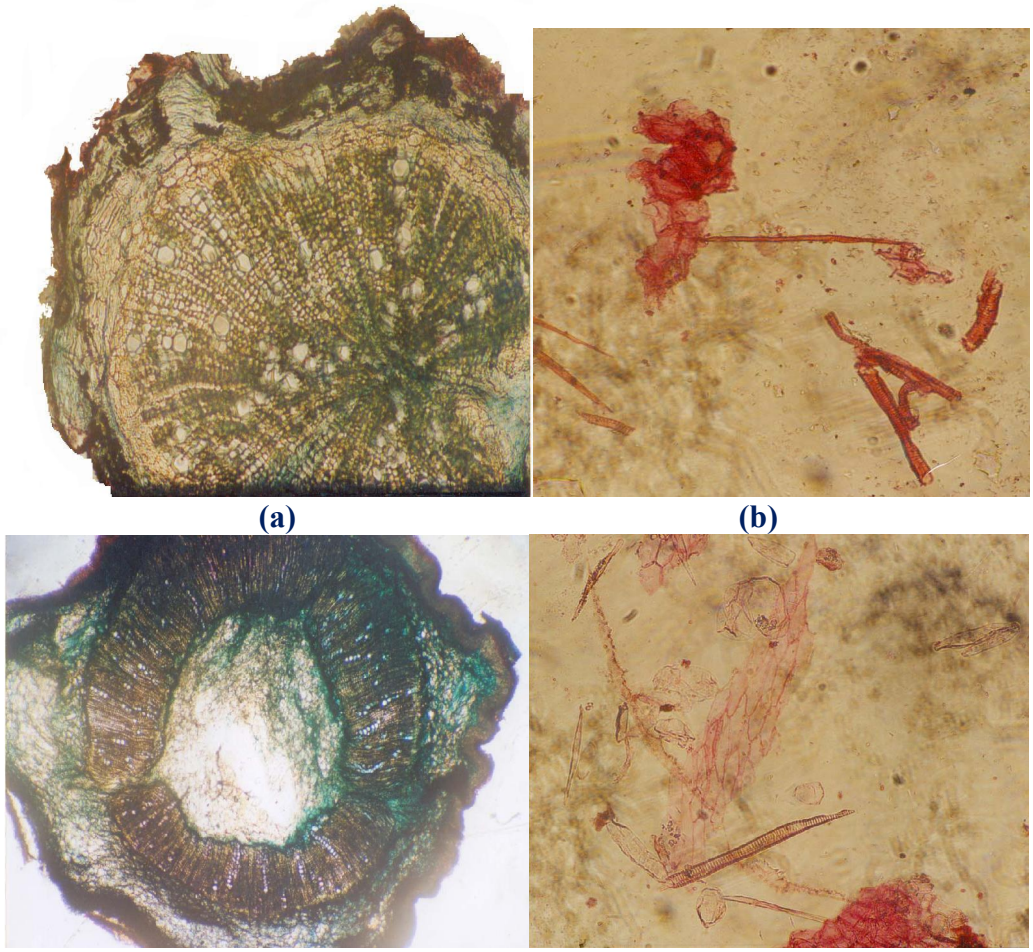


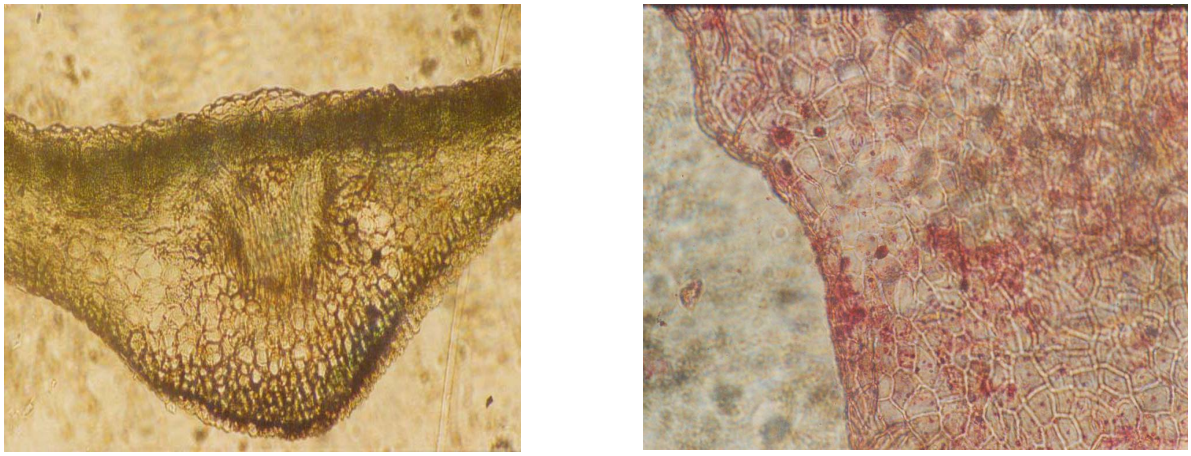
Line drawing of *Euphorbia Milii*Ch.showing Habit, Cyathia , Ovary L.S. Ovary T.S, Stamen, Pistil



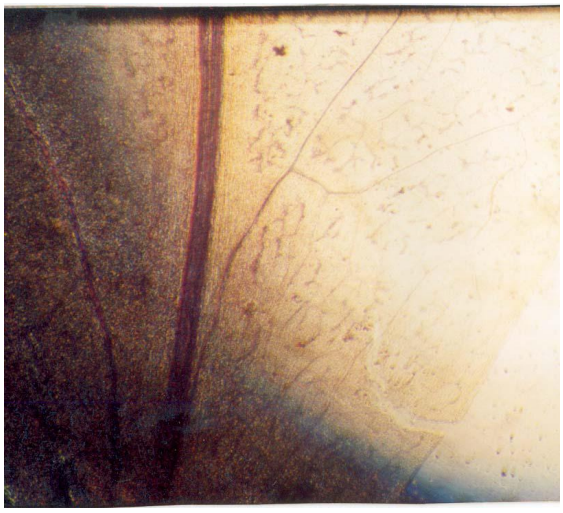
(a) **(b)**

(c) **(d)**

Plate 01- a T.S of Root, b Vessel element of Root, c T.S of Stem, d Vessel element of Stem.



(a) **(b)**



(c)

Plate 02- a) T.S. of Leaf, b) Stomata, c) Leaf architechture.

Classification (After radfordetal) and ralative frequency (%) of different classes of vessel element in the root and stem of *Euphoribamilii*ch.

Table No. 01 Vessel element of E.millich-des-moulis in Bull Root.

Class A		**Class B**		**Class C**	
Percentage(%)	Range of length (mu)	Percentage(%)	Range of length (mu)	Percentage(%)	Range of length (mu)
23.53	120 to 162	26.27	200 to 216	50.20	252 to 324

Table No. 01 :- vessel element of E.miliich-des-moulls in Bull. stem.

Class A		**Class B**		**Class C**	
Percentage(%)	Range of length (mu)	Percentage(%)	Range of length (mu)	Percentage(%)	Range of length (mu)
12.33	144 to 171	17.60	**180 to 225**	70.07	**234 to 288**