C H A P T E R - XX

(Fig. 1 - 13 : Merremia emarginata Hallier.)

Fig. 1 - Entire plant. x 1.

Fig. 2 - T.s. lamina. x 450.

Fig. 3 - 3A - Upper epidermis with stomata. x 450.

3B - Lower epidermis with stomata. x 450.

Fig. 4 - T.s. midrib. x 450.

Fig. 5 - T.s. petiole (diagrammatic). x 60.

Fig. 6 - Trichomes. x 80.

Fig. 7 - T.s. young stem (diagrammatic). x 70.

Fig. 8 - T.s. old stem (diagrammatic). x 70.

Fig. 9 - A & B - T.s. stem. x 450.

Fig. 10. - T.s. old stem. x 450.

Fig. 11 - Stem maceration - showing trachedial vessesl. x 350.

Fig. 12 - T.s. root, showing tetrarch stele. x 730.

Fig. 13 - T.s. root, showing triarch stele. x 730.

x-x-x-x-x-x-x

A.Sp. - air space; Cb. - cambium; Ck. - cork; Col. - collenchyma; Cor. - cortex; Ep. - epidermis; Ep_1 - upper epidermis; Ep_2 - Lower epidermis; Fl. - flower; Ft. - fruit; G.H. -glandular trichome; Pal - palisade; Ph. - phloem; Phg. - phellogen; P.Ph. - perimedullary phloem; P.fb. - pericyclic fibres; Sp. - spongy; TV. - trachedial vessel; Xy. - xylem.

the adaxial side while the third, which is very big, forms an arc on the abaxial side. Clusters of calcium oxalate crystals are present in phloem parenchyma and in the parenchymatous cells of the cortex (Fig.5).

Stem (Plate XX) : Young stem and branches show the presence of large air spaces in the cortex and the pith (Figs. 7 and 8). Epidermis develops a thin layer of cuticle and bears a few long and two-celled trichomes, as in case of the petiole; the basal cell is smaller and having a thicker cell wall than the terminal cell (Fig. 6) A few of these trichomes are septate and measure 161-265-328 μ in length. 2-3 hypodermal layers of parenchymatous cortex contain chloroplasts. Secondary growth sets in at a very early stage and is of adaptive type (Figs.7 and 8)

Phellogen develops just below the epidermis in the old stem (Fig. 9a). Cork cells are rectangular and lignified. There are about 3-4 layers of radially arranged cork cells, the upper one or two layers of which mostly get collapsed. Endodermis gets greatly elongated tangentially due to secondary growth and its radial walls are thickened (Fig. 10). Small groups of pericyclic fibres are present around the conducting tissues.

Phloem consists of sieve tubes, companion cells and phloem parenchyma. Cells of the sieve tubes

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are more or less of the same size as those of phloem parenchyma (Fig. 9b). 1-2 big resin ducts are observed in this tissue. Primary phloem cells are not distinguishable. Cambium consists of 2-3 layers of distinct cells (Fig. 9b).

Xylem is comparatively a broader zone and consists mainly of vessels, tracheids, a few fibres and xylem parenchyma. Vessels occur singly or in groups of two. A few of them show tylosis. In macerated material both the vessels and the tracheids show circular elliptical bordered pits (Fig. 11). A few of the vessels are trachedial vessels in that they show opening on lateral walls (Fig.11). Medullary rays with radially elongated parenchymatous cells traverse the conducting tissues and may be uniseriate to triseriate. Perimedullary phloem is present below the primary xylem groups on the periphery of pith (Fig. 10).

Central pith is parenchymatous. A number of small resin ducts are present on the periphery. Clusters of calcium exalate crystals are present in the parenchymatous cells of the cortex, phloem and pith (Figs. 9a and 9b). Crystals in the cortex are very big and measure from 19-50 μ in diameter while those of the phloem are smaller and measure from 11.5 - 19 μ in diameter. In old stem starch is present in the cells of the cortex, phloem parenchyma and secondary medullary rays. The grains are simple or compound and measure 2.7-54-10-8 μ in diameter.

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Hypocotyl (Plate XVIII, 2): The change in the vascular tissue from root to stem is accompanied by multiplication of vascular tissues and forking, rotation and fusion of strands. In this case, the changeover from root to stem is of Mirabilis type (Eames and MacDaniel, 1947).

T A B L E - 26

Measurements of stem elements

Elements	Length	Width
Vessel	158 - 266 - 405 μ	29.6-66.6-88.4 μ
Tracheid	126 - 267 - 350 μ	11-19-22 μ

Root: Young roots show triarch or tetrarch stele (Plate, XX, 12 & 13). Adventitious roots which arise from the pericyclic region of the stem and branches do not come out obliquely but travel through the cortex for a distance. This can be observed from the transverse section of the rhizome cut transversely (Plate XVIII, 3). Young roots show the presence of air spaces in the cortex (Plate XX, 12).

Old root shows secondary growth. Air spaces are also present in the cortical region (Plate XVIII, 3). Pericycle and endodermis are obscure. Phloem consists of sieve tubes, companion cells and pholem parenchyma. As in the case of stem, the cells of the sieve tubes are as

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big and phloem parenchyma. Cambium consists of 1-2 layers of distinct cells. The central exarch xylem varies from triarch to hexarch (Plate XVIII, 3). Xylem consists of the central solid core and consists mainly of vessels and tracheids. A few fibres and xylem parenchyma are also present. Vessels are mostly solitary and along with tracheids show circular or ellipsoidal bordered pits. Uniseriate to triseriate medullary rays traverse the conducting tissue.

Cells of the phelloderm and phloem parenchyma show the presence of big and small clusters of calcium oxalate crystals respectively. In old root, few starch grains are found in phloem parenchyma and medullary rays. The starch grains are simple or compound and measure 2.7-5.4-11 μ in diameter.

T A B L E - 27

Measurements of root elements

Elements	Length	Width
Vessel	217-359-501 μ	38-57-103 μ
Tracheid	74-93-118 μ	38-37-50 μ

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Microchemical tests:

Phenols and tannins :

(1) Water extract of the powdered drug gives dirty white to brownish precipitates with lead acetate, thus indicating the presence of mucilage and glycosides. The same extract with $FeCl_3$ solution gives brownish to reddish precipitates, thus indicating the probable presence of phenols and tannins.

(2) Resin: With Sudan III, resin is stained golden yellow to pinkish. Resin is present more in the pith of the stem and the midrib.

(3) Reducing sugars and glycosides: The powdered drug was extracted with hot water. The concentrated brown extract showed the presence of reducing sugars and glycosides (Basu and Rakhit, 1957).

(4) Mucilage : With ruthenium red, no pink colour was noticed indicating its absence.

(5) Sterol : Alcoholic filtrate of the powdered drug was evaporated and the residue dissolved in chloroform. The resultant solution showed positive tests for sterol with Liebermann Burchard (Allen, 1948) and Hesse's tests (Resenthaler, 1930).

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(6) Alkaloids: Powdered drug was extracted with ammoniacal chloroform and the extract evaporated. The residue was dissolved in dilute HCl. The resultant solution did not answer any of the tests for alkaloids.

Pharmacological investigations of

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Preparation of aqueous extract :

The combined filtrates after repeated digestion of 40 mesh powder with hot water were concentrated under reduced pressure. A weighed quantity of the resinous residue obtained was dissolved in water and the pH of the solution was adjusted between 6 and 7.

Preparation of the fractions 'A' and 'B' :

50 g. of the powder was completely exhausted in soxhlet apparatus with alcohol. The extract was concentrated under reduced pressure which gave a resinous mass. This was treated with ether. The matter left over was concentrated under reduced pressure to get fraction 'A'. The ether layer was shaken with 1% ammonia and the ammoniacal layer removed and concentrated under reduced pressure to get fraction 'B'.

Experimental :

The concentrated residue of fraction 'A' was dissolved in 70% alcohol so as to represent 2 g. of the drug in 1 c.c. of the extract. Water extract as well as

Fig.1

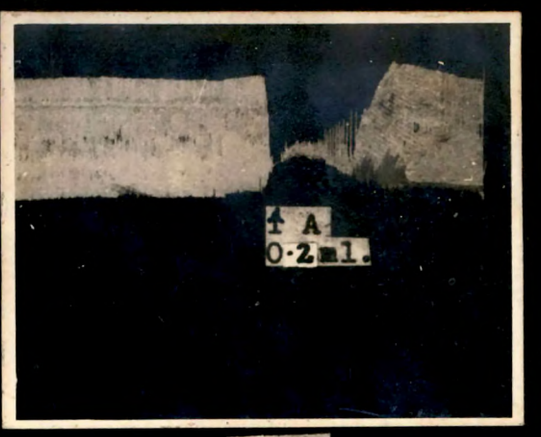

Fig.2

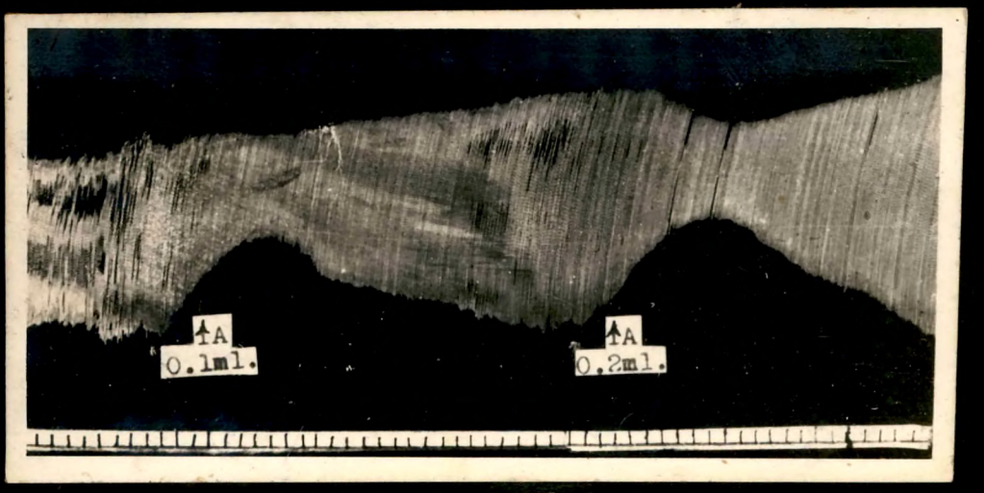

Fig.3

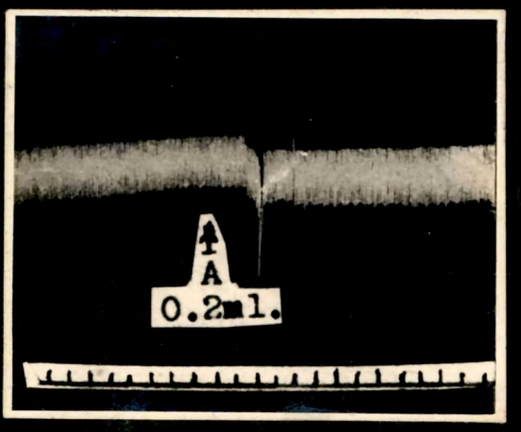

Fig.4

P L A T E - XXI

(Figs. 1 - 4 : Merremia emarginata Hallier)

Fig. 1 - General behaviour on healthy albino rats.

Fig. 2 - Effect on frog's heart.

Fig. 3 - Effect on rabbit's heart.

Fig. 4 - Effect on respiration of rabbit

A - alcoholic extract.

fraction 'B' was found to be less active and are not represented here. Pharmacological studies of fraction 'A' have been made which are as follows (Plate XXI and XXII).

Effect on general behaviour of healthy albino rats (Plate XXI,1):

Different doses of alcoholic extract were injected to male healthy albino rats weighing 150-200 g. and observed for 4-5 hours. Alcoholic extract in a dose of 0.25 ml/100 g. has shown complete depression (Fig. 1). Figure 1 also shows the control with 70% alcohol.

Effect on isolated frog's heart (Plate XXI, 2):

Frog's heart was isolated, perfused with Cyme's canula and kept at constant pressure. The effect of 0.1 ml. and 0.2 ml. of fraction 'A' showed the reversible depression of the heart.

Effect on rabbit's heart and coronary vessels (Plate XXI,3) :

The effect of 0.1 ml. and 0.2 ml. of fraction 'A' showed the reversible inhibitory effect on the isolated rabbit's heart attached to Langendroff's heart perfusion assembly and perfused with Ringer's solution at 37°.

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The effect on the coronary vessels were studied by counting the number of drops of perfusate coming out through coronary vessels per 30 seconds in the above experiment. Fraction 'A' decreased the outflow by 36.4% on injecting 0.1 ml. of the dose.

Effect on respiration of rabbit (Plate XXI, 4) :

Effect of fraction 'A' was also studied on respiration of rabbit. The respiration records were made as per Burn (1952). Fraction 'A' in a dose of 0.2 ml. was found to relax the muscle.

Similarly, uterine horn of healthy vergin albino rat was suspended in oxygenated Dale's solution at 35°. Fraction 'A' upto a dose of 0.4 ml. was found to have no effect.

Spasmolytic effect was further studied on barium chloride, histamine acid phosphate and acetylcholine induced spasm.

Action on barium chloride induced spasm (Plate XXII, 6):

Guinea-pig's ileum was suspended in well oxygenated Tyrode's solution at 37°, in a mammalian organ bath (25 ml. capacity). Each one of the contractions (B) in the figure was due to 1 ml. of barium chloride solution. Effects of 0.2 ml. and 0.3 ml. of fraction 'A' (B + A) in the

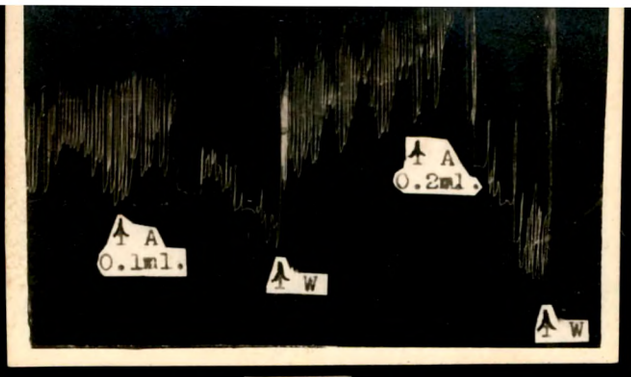

A
0.2ml.
A
0.1ml.
W
W

Fig.5

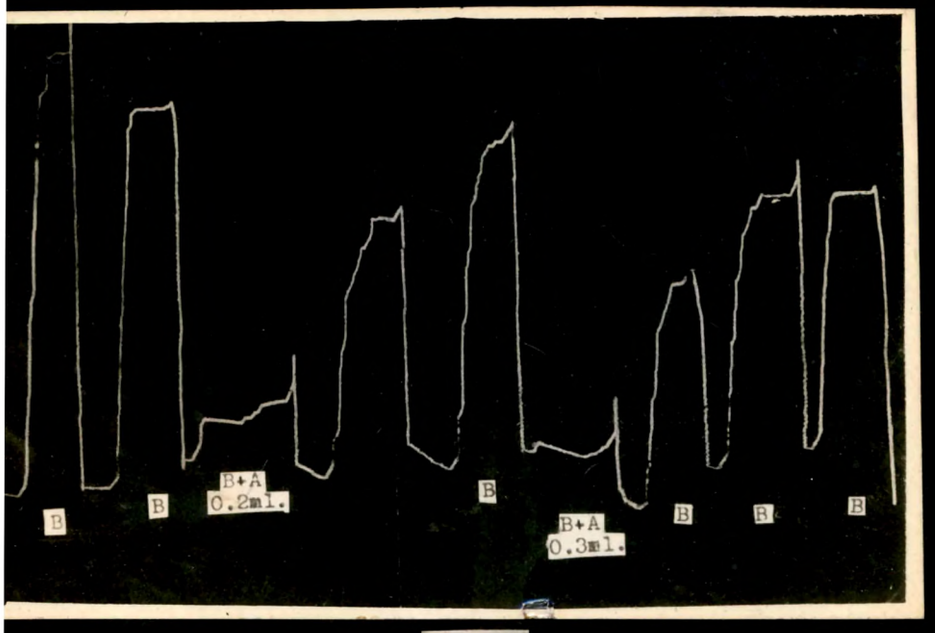
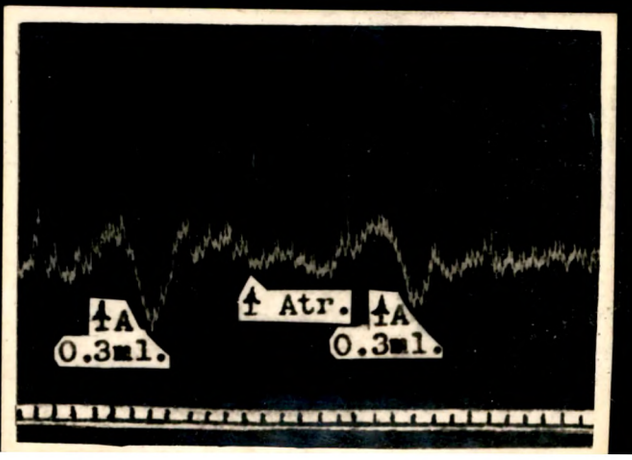

B
B
B+A
0.2ml.
B
B+A
0.3ml.
B
B
B

Fig.6


H
H+A
H
H
0.2ml.


A
0.3ml.
Atr.
A
0.3ml.

P L A T E - XXII

(Figs. 5 - 8 : *Merremia exarginata* Hallier)

Fig. 5 - Effect on rat's duodenum.

Fig. 6 - Antibariumeffect on guinea pig's ileum.

Fig. 7 - Antihistaminic effect on guinea pig's ileum.

Fig. 8 - Action of blood pressure on cat.

A - alcoholic extract;

W - washing;

H - Histaminic acid phosphate;

B - Barium chloride;

Atr. - Atropine sulphate.

figure showed antibarium activity. 0.3 ml. of the fraction 'A' almost completely antagonised the effect of 1 mg. barium chloride.

Action on histamine induced spasm of guinea-pig's ileum (Plate XXII, 7):

Guinea-pig's ileum was suspended in well oxygenated Tyrode's solution at 37° in a mammalian organ bath of 25 ml. capacity. All contractions (H) in the figure are of 1 : 50 millions of histamine acid phosphate. Effects of 0.1 ml. and 0.2 ml. of fraction 'A' (B + A) in the figure showed antihistaminic property. 0.2 ml. of fraction 'A' caused about 55% reduction of contraction due to 1 : 50 millions of histamine acid phosphate.

Action on acetylcholine induced spasm of rat's ileum:

Action of fraction 'A' on acetylcholine induced spasm was studied on rat's ileum suspended in Tyrode's solution at 37° in a mammalian organ bath of 25 ml. capacity. A dose upto 0.4 ml. of fraction 'A' was found to have no marked effect on acetylcholine induced spasm (1 : 5 million).

Action on blood pressure of cat (Plate XXII, 8):

Healthy cats of either sex were anaesthetised with chloralose 80 mg./kg. given intramuscularly. The blood

pressure was recorded as usual and the drug was injected through the femoral vein. Fraction 'A' caused transient fall of the blood pressure as shown in the figure. Atropinaisation (2 mg./kg. atropine sulphate) did not cause any alteration with the effect of fraction 'A'.

Action on rectus abdominis muscle of frog:

Rectus abdominis muscle of frog was suspended in a simple organ bath of 25 ml. capacity containing well aerated frog's Ringer's solution at room temperature. Fraction 'A' upto a dose of 0.5 ml. had neither shown any effect nor changed acetylcholine induced spasm.

D I S C U S S I O N

Merremia emarginata and Centella asiatica closely resemble each other morphologically. They belong to different families and naturally differ from each other anatomically. M. emarginata grows in a more moist environment which can be ascertained by the presence of large air spaces in cortex and pith of stem and root. The important differences in their morphological and microscopical features which may help one to distinguish these two species are therefore as follows :-

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T A B L E - 28

Merremia emarginata	Centella asiatica
LEAF	
Dimensions: 1.3-3.5 cm. x 1.2-5 cm.	Dimensions: 0.65-3.1 cm. x 0.65-3.1
Lower surface of leaf bears soft hairs on prominent veins	Hairs absent
Leaves exstipulate	Leaves have adnate stipules
HISTOLOGICAL LEAF	
Stomata rubiaceous	Stomata both rubiaceous and caryophyllaceous
Typical glandular hairs on both surfaces and long two celled hairs on lower surface of young leaves	Hairs absent
Cuticle well developed and occurs as prominent striations	Cuticle not so well developed and occurs as faint striations
Cluster of calcium oxalate crystals found in mesophyll	Rosettes of calcium oxalate crystals are found in mesophyll
Vascular bundle of midrib bicollateral	Vascular bundle of midrib collateral

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Merremia emarginata	Centella asiatica
Collenchyma present only below the upper epidermis in midrib Palisade ratio: 19-23.5	Collenchyma present below both upper and lower epidermis in midrib Palisade ratio: 3.25-5.75
	PETIOLE
Groove present on adaxial side	Has a characteristic outline due to the sides projecting out from adaxial groove
Epidermis bears many long and two-celled hairs	Hairs are absent
Collenchymatous hypodermis forms a closed ring	Collenchymatous hypodermis does not form a closed ring; it is absent on sides of projecting arms
Three vascular bundles present, two near the adaxial groove are smaller than the third which is bigger and forms an arc in the middle near dorsal side	Seven vascular bundles are present; two located in the projecting arms and rest form an arc in the middle near abaxial side.
Calcium oxalate cluster crystals present in cortical and phloem parenchyma	Rosette crystals of calcium oxalate present in cortical parenchyma only

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Merremia emarginata	Centella asiatica
	STEM
Hairs present on young stem	Hairs absent
Hypodermis parenchymatous	Hypodermis collenchymatous
Large air spaces present in cortex and pith	Small intercellular spaces present in cortex and pith only
Secondary growth is present	Secondary growth absent
Lignified cork cells, phellogen and phelloderm present	Phellogen not developed
Endodermis in old stem greatly elongated tangentially	Endodermis not distinguished
Small groups of pericyclic fibres absent	Pericyclic fibres absent

Pharmacologically, the drug possesses general depressent action on rats. The alcoholic extract of the drug is cardio inhibitory and causes coronary constriction in rabbit. It possesses a hypotensive effect which is not affected by atrophine sulphate. It is also found to exhibit antibarium and antihistaminic effects but no marked antiacetylcholine effect. Thus, the effect of the drug is more musculotropic than neurotropic.

Merremia emarginata Wight

S U M M A R Y

The morphological and histological characters of the different parts of the plant, M.emarginata have been described. The plant is popularly called as 'Brahmi' and is sold in bazars of Saurashtra and North Gujarat as such. The leaf has a bicollateral vascular bundle and possesses both glandular as well as two-celled covering trichomes. Palisade ratio is high (19.0-23.5). In the petiole, clusters of calcium oxalate crystals are present in the parenchymatous cells of the cortex, phloem and pith. Large air spaces are present in the cortex and pith of the stem and only cortex in root. Secondary growth is present. Microchemical tests with the powdered drug reveal the presence of resin, glycoside, reducing sugar and starch; no alkaloid is present.

Alcoholic extract of the entire plant is generally found to be more active than the aqueous extract. It possesses depressive action on albino rats. It also shows reversible inhibitory effect on frog's heart. The extract possesses spasmolytic action on the smooth muscles of guinea-pig. It exhibits antihistaminic and antibarium activities on guinea-pig's ileum and rat's duodenum respectively; antiacetylcholine effect is not marked when rested on rat's duodenum. It also shows hypotensive effect which is not affected by atropine sulphate. The extract has no effect on rat's uterus and rectus abdominis muscle of frog.