

ANATOMICAL STUDY OF THE STEM AND LEAF OF *LIPPIA NODIFLORA* MICH

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(Received February 26, 1963)

The macro and microscopic characters of the leaf and stem of *Lippia nodiflora* Mich, have been described. The young stem is characterized by the presence of collenchyma bands at the ridges while in the older stem the collenchyma encircles the entire cortex. The most distinguishing character of the stem is the presence of unicellular two-armed trichomes on it. The leaf is isobilateral but the palisade cells towards the upper epidermis are longer than those towards the lower epidermis. The characteristic feature of the leaf is the presence of large water-storage cells beneath the epidermis and in between the palisade cells. Trichomes resembling the stem trichomes are also present.

Introduction

Lippia nodiflora Mich (Vern-Bukan) belongs to the family *Verbenaceae*. It occurs in all the tropical and warm temperate regions of the world. It is also widely distributed throughout Indo-Pakistan sub-continent and Ceylon. The whole plant is valued as a diuretic. Infusions of leaves and tender stalks are given to children and to women after delivery. It is also reported to be useful in the treatment of diseases of heart, asthma and bronchitis. It is made into a poultice and used as a maturant for boils.¹ It is also used as a demulcent and as a cooling drug. Two crystalline glycosides and a number of sugars have been isolated from the plant.²

As no detailed anatomical studies have been carried out on this plant so far, the present work was undertaken.

Botanical Description

Lippia nodiflora is a small, prostrate, perennial, much-branched herb, extending from 6" to 30", often rooting from the nodes, making large patches. Stem quadrangular, leaves subsessile, cunnate and spatulate, serrate at the broad tip, base attenuate and entire, about one inch in length, opposite and decussate. Flowers pink or white, crowded in axillary, long stalked, oblong head; peduncle is 1"-3", with very rarely another from the opposite axil. Bracts 1/10", ovate, shortly acute. Calyx minute, divided into two, hairy. Corolla 1/20" to 1/8"; tube, slender; mouth, 2-lipped, the lower lip being rather longer. Stamens four, in unequal pairs. Fruit dry, minute, separating into 2-1 seeded pyrenes.^{3,4}

Materials and Methods

The material was collected from the experimental farm of North Regional Laboratories, P.C.S.I.R., Peshawar. The identity of the material was checked by comparing with the authentic herbarium specimens available in these Labora-

tories. Pieces of stems and leaves were fixed in F.A.A. for microtome sectioning. The material was then dehydrated by normal butyl alcohol and ethyl alcohol, and the paraffin embedding was done according to Zirkle's method as given by Youngken.⁵ The microtome sections were stained with safranin and fast green. Jeffrey's method⁶ was employed for the maceration of the tissues. Fresh hand sections were cut for the various microchemical tests as given by Johansen and E. Gurr.^{6,7} The uniform powdered material was obtained by sifting it through a No. 60 sieve and studied after clearing in 50% chloral hydrate. Cell measurements were taken with the help of an eye piece micrometer.

Description of the Stem

Macroscopic Characters.—The stem is green and quadrangular and its diameter varies from a few m.m. to 5 m.m. A transversely cut surface of the stem presents a greenish appearance. The amount of xylem as indicated by its creamy appearance comprises about two thirds of the entire diameter of the stem. The odour is slight and the taste is bitter and acrid. (Fig. 1).

Microscopic Characters.—The stem is quadrangular in outline and a large number of two-armed trichomes are present on the surface. The stem is encircled by a single-layered epidermis, the cells of which are cuticularized and are rectangular in shape. The continuity of the epidermis is broken by the presence of stomata in it. The epidermal cells measure 12-28-40 μ in length and 24-32-45 μ in breadth. In the young stem, beneath the epidermis occurs a band of collenchymatous cells in the region of the ridges. In the older shoots the collenchyma cells form a continuous ring round the cortex (Fig. 2). In the fresh hand section the cell walls of collenchyma are shining while in a stained slide this layer can be differentiated by its deep stain. Below the collenchyma layer is the cortex, the cells of which are thin-walled. Cortical cells in the transverse section measure about 21-38-46 μ , while in the tangential

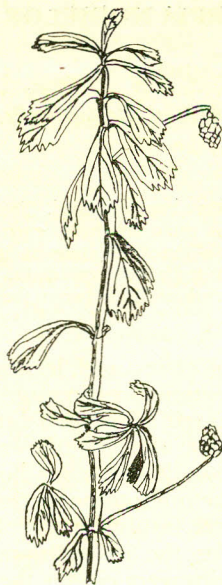


Fig. 1.—Shoot of *Lippia nodiflora*.

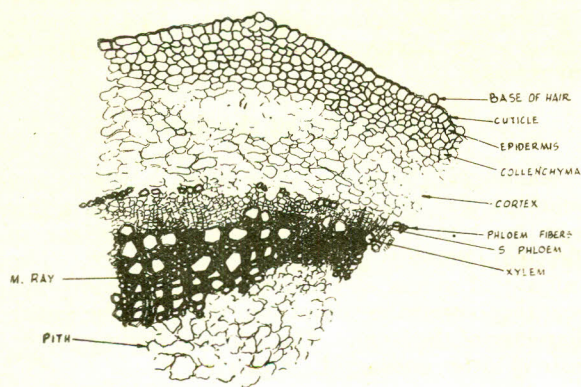


Fig. 2.—T. S. *Lippia nodiflora* stem.

section, they measure about $46-104-163\mu$. The cells contain large amount of starch and calcium oxalate crystals.

A single-layered endodermis delimits the cortical region from the vascular region in the very young stem but later on the endodermis is ruptured due to secondary growth and is not visible. Below the cortical region is the vascular region which occurs in the form of a cylinder. Only a limited amount of secondary growth takes place through the activity of the vascular cambium. The primary phloem elements are later transformed into phloem fibres which are scattered in the peripheral region of the vascular region. The secondary phloem formed through the activity of the vascular

cambium is 5 to 6 layers in thickness and it completely encircles the xylem elements. The phloem elements are composed of sieve tubes, companion cells, phloem parenchyma, phloem rays and phloem fibres.

The xylem as mentioned previously occupies about one third of the entire diameter of the stem. It is in the form of a cylinder traversed by uniseriate, biseriate, and multiseriate rays, but commonly the rays are biseriate. The secondary xylem formed through the activity of vascular cambium is composed of vessels, tracheids, fibres, xylem parenchyma and ray cells. The vessels are generally of medium size. The vessel thickening may be scalariform and pitted. Pitting is of the bordered type. The vessels measure about $139-368-693\mu$ in length and $13-31-54\mu$ in diameter. Tracheids vary in their shape. The thickenings are generally pitted but a few tracheids with spiral thickenings are also present. These measure about $185-310-570\mu$ in length and $6-13-17\mu$ in diameter. Fibres with thick walls and some with pits are also seen in the xylem and measure about $231-1016-1994\mu$ in length and $5-8-12\mu$ in diameter. Pits in the ray and wood parenchyma are similar. Xylem parenchyma cells measure about $46-90-168\mu$ in length and $8-12-15\mu$ in breadth. (Fig. 3).

The xylem cylinder encircles the pith which is parenchymatous and thin-walled. Pith cells measure about $17-42-103\mu$ in diameter. Pith cells also contain starch.

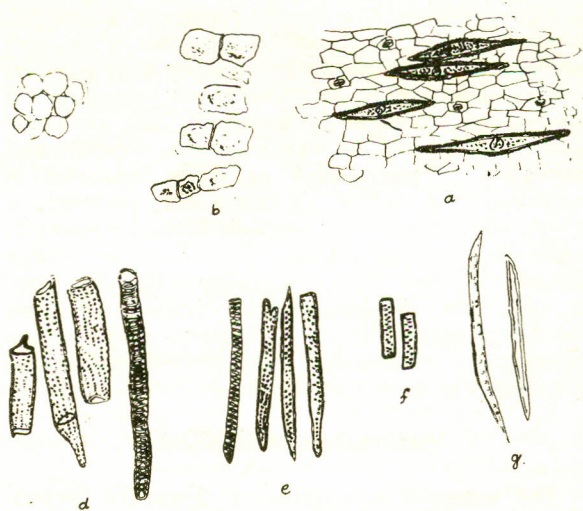


Fig. 3.—Macerate of *Lippia nodiflora* stem. (a, Epidermis with trichomes; b, Cortex cells; c, Pith cells; d, Vessels; e, Tracheids; f, Xylem parenchyma; g, Fibres).

Description of the Leaf

Macroscopic Characters.—The leaves are arranged in opposite and decussate manner on the nodes and leaves are numerous, nearly without stalks, obovate, and 1-2.5 cm. long with blunt or rounded tip and wedge shaped base; the margin on the upper half is sharply toothed. Stomata are present on both the sides. Large number of unicellular, two-armed trichomes are present on the surface. The odour is slight and the taste bitter and acrid.

Microscopic Characters.—Transverse section of the leaf shows a thin layer of cuticle and bases of trichomes on both the sides. The internal structure of the leaf is isobilateral. The epidermal cells are almost rectangular in shape and measure about $20-32-56\mu$ in length and $15-28-32\mu$ in breadth. Stomata are seen on both the sides of the leaf. The most characteristic feature of the leaf is the presence of a large number of unicellular, two armed trichomes on the epidermis. Another important character of the leaf is the presence of group of large water storage cells in between the palisade parenchyma of the upper epidermis. Below the epidermis is the palisade parenchyma, the cells of which are filled with chloroplasts. The palisade parenchyma occurs in two to three layers. These cells measure about $13-25-38\mu$ in length and $8-12-15\mu$ in breadth. The palisade parenchyma of the upper side differ from the palisade of the lower side in that it is longer (Fig. 4).

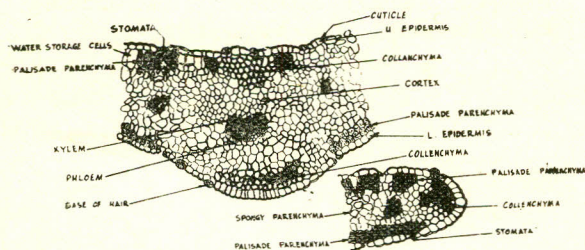


Fig. 4.—T.S. of *Lippia nodiflora* leaf.

In the midrib region of the leaf is a band of collenchymatous cells towards the upper as well as the lower epidermis. A collenchyma band is also seen towards the extreme end of the lamina. Below this collenchyma is the cortex whose cells are parenchymatous. Spongy parenchyma occurs below the palisade parenchyma. These cells are also parenchymatous and some air spaces are present in between. Some cells of spongy parenchyma have small chloroplasts and have a few

starch grains. Most of the cells are without any cell contents except for cytoplasm etc.

In the midrib region there is a crescent-shaped vascular bundle which does not show any secondary growth. The bundle is also not surrounded by any vascular sheath but it is embedded in the cortical cells. The xylem elements occur towards the upper side of the leaf while the phloem elements occur on the lower side of the leaf. The xylem consists of a few annular, spiral and scalariform vessels, tracheids, fibres and xylem parenchyma. Vessels measure about $108-416-526\mu$ in length and $10-15-17\mu$ in diameter. Tracheids may also have annular, spiral, and scalariform thickenings and measure about $250-495-724\mu$ in length and $8-12-15\mu$ in breadth. The fibres are long, pointed, thick-walled cells measuring about $354-447-893\mu$ in length (Fig. 5). The phloem elements are composed of sieve tubes and phloem parenchyma.

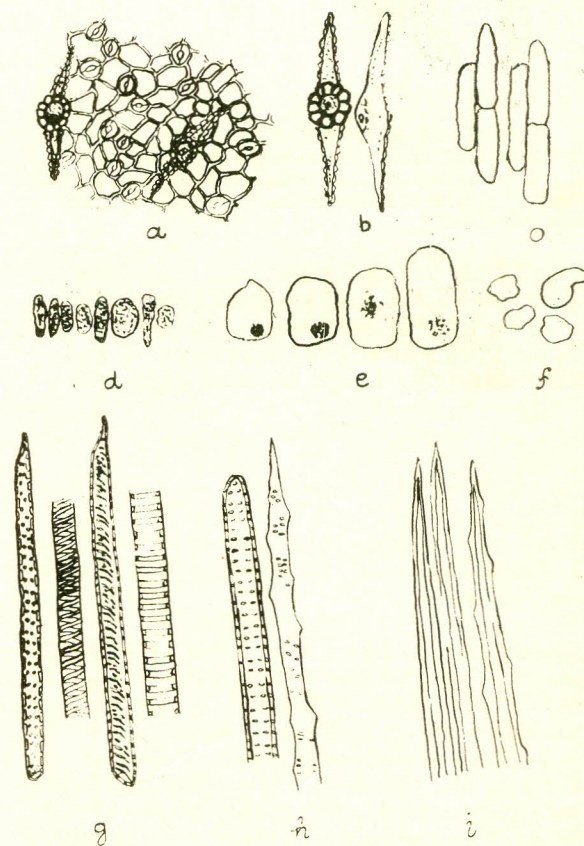


Fig. 5.—Macerate of *Lippia nodiflora* leaf. (a, Epidermis with stomata and trichomes; b, Trichomes; c, Cortical cells; d, Palisade cells; e, water storage cells; f, Spongy cells; g, Vessels; h, Tracheids; i, Fibres.

Powdered Shoot

The powder is green in colour with slight odour and bitter taste. The following structures are visible under microscope (Fig. 6):

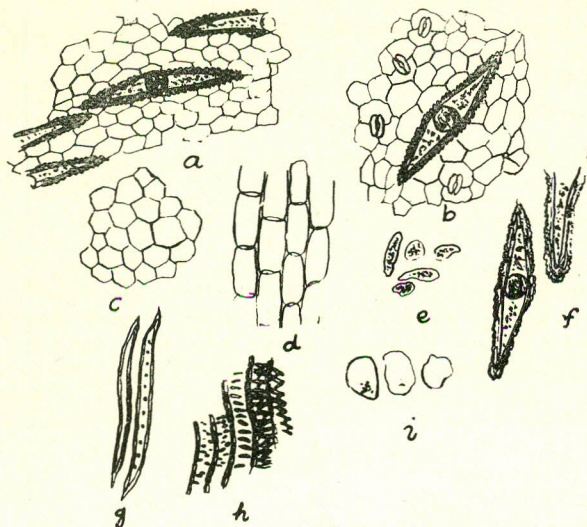


Fig. 6.—Powdered Shoot of *Lippia nodiflora* (a, Stem epidermis; b, Leaf epidermis; c, Cortical cells; d, Cortical cells; d, (longitudinally), e, Palisade cells; f, Broken trichomes; g, Fibres; h, Vessels and Tracheids; i, Water storage cells of leaf.)

(i) Epidermis of the stem with trichomes; (ii) Epidermis of the leaf with trichomes; (iii) Cortical cells; (iv) Palisade cells; (v) Broken parts of trichomes; (vi) Fibres; (vii) Broken vessels and tracheids; and (viii) Large water storage cells

Microchemical Tests.—Microchemical analysis of the stem indicates the presence of starch in the cells of the cortex and pith. Calcium oxalate crystals are also present in the cortex. The stem gave negative tests for the alkaloids. The leaf also shows the presence of starch in the palisade parenchyma and calcium oxalate in the midrib region.

Acknowledgements.—Thanks are due to Dr. S.A. Warsi, Director, North Regional Laboratories, P.C.S.I.R., Peshawar for his encouragement and interest in the present work.

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