

Microscopical Studies on the leaf and petiole of *Vernonia amygdalina* Del.

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ABSTRACT

*Detailed microscopical studies of the leaf and petiole of *Vernonia amygdalina* Del. (Asteraceae) which is a Sudanese folkloric medicine were carried out. It had been found that there were anomocytic stomata, the stomatal sizes and indices were larger on the abaxial surface. There were many epidermal hairs of different types; unicellular, multicellular, glandular and non-glandular hairs the heads of the glandular hairs appeared bi-lobed. The mean palisade ratio was found to be four. The scanning electron microscopy of the epidermal surfaces revealed that the amount of wax was large on the adaxial surface whereas the number of stomata was more on the abaxial surface. The vascular bundle region of the leaf was divided into four bundles separated by biserriate rays of parenchyma. The powder of the dry leaves of *V. amygdalina* was dark green in color with faint smell, calcium oxalate crystals were scattered, small veins appeared in longitudinal views with different types of vessel thickening. The transverse section of the petiole showed that the vascular bundles were arranged in a circle, each vascular bundle was preceded by pericyclic fibers.*

Key words: *Vernonia amygdalina*, Asteraceae, leaf, petiole, epidermis.

INTRODUCTION

This study is aimed to provide valuable and reliable illustrated anatomical descriptions of the leaf and petiole of *Vernonia amygdalina* Del. This plant was selected for its great importance in Sudanese folkloric medicine and for proper authentication of crude drug material which is standard of safety and quality to be maintained. The literature survey revealed that no microscopical studies were carried on it in the Sudan. The family Asteraceae is the largest family of the vascular plants, includes 950 genera and 20.000 species. Economically the family is of considerable importance, it includes sources of food to man but many members are noxious weeds and others are used to a limited extent in medicinal or patented preparations [1]. According to [2] the family was divided into 8 tribes: *Cichorieae*, *Heliantheae*, *Astereae*, *Inuleae*, *Cynareae*, *Vernonieae*, *Eupatorieae* and *Senecioneae*. *Vernonia* is a genus of about 1000 species some species are edible and of economic value. They are known for having intense purple flowers. *Vernonia amygdalina* is a small shrub grows in tropical Africa. It is commonly

called bitter leaf because of its bitter taste. The leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. In the wild, chimpanzee have been observed to ingest the leaves when suffering from parasitic infections [3].

MATERIALS AND METHODS

The plant material *Vernonia amygdalina* Del. was obtained from a material deposited at Medicinal and Aromatic Plant Research Institute Herbarium in 1998.

1. Preparation of Temporary Slides:

i) Epidermal tissue system: [4]

The epidermal samples were taken from the dried herbarium specimens, the foliar leaves were revived in boiling water with the addition of a detergent for 25-40 minutes and soaked in distilled water to allow the leaves to expand properly then the epidermal tissues were gently removed with a razor blade. The epidermal peels were mounted in 10% aqueous glycerin and examined. The stomatal index was calculated using the formula $(S/E+S) \times 100$ whereas S: number of stomata in an area and E: number of epidermal cells in the same area.

Abaxial and adaxial leaf surfaces were examined using scanning electron microscopy techniques. Observations were taken at 400 and 4000 magnifications. The specimens were mounted on circular stubs with double-sided tape, coated with gold to a thickness of 20 to 25nm. and examined on a Cambridge "Stereos can" MK 11A SEM at an acceleration potential of 10KV. Selected areas were photographed.

ii) Powders of the dry plants:

The powders of the dry leaves were cleared in chloral hydrate solution, mounted in 10% aqueous glycerin, covered with a cover-slip and examined to outline the diagnostic features of the leaves in the powdered conditions.

iii) Palisade ratio:

Segments of 3-4 mm from the studied plant leaves were soaked in equal amounts of chloral hydrate and phenol in test tubes, boiled in a water bath for about 15-20 minutes, mounted in the same reagent and examined. The palisade ratio, which is the number of palisade cells beneath one epidermal cell, was calculated. Four readings were taken and then the average value was calculated.

2. Preparation of Permanent Slides: [5]

The leaves were segmented and fixed for at least 72 hours using the standard fixative (FAA), formaldehyde- glacial acetic acid – 70% ethyl alcohol (5:5: 90 v/v), washed with distilled water, dehydrated using serial concentrations of ethyl alcohol, cleared using a mixture of 1:1 cedar wood oil: absolute alcohol, into pure cedar wood oil followed by a mixture of cedar wood oil and xylene and finally left overnight in pure xylene. Wax embedding was carried out in an oven adjusted at 60°C. Soft tissues were sectioned; sections were collected on glass slides. The slides were left overnight on a hot plate to give maximum expansions of the tissues. Dewaxing of soft tissues was done by immersing the slides with their sections in pure xylene. The sections were then dehydrated by transferring them into series of ethyl alcohol concentrations and stained by flooding them with safranin and fast green stains, mounted in a drop of Canada balsam, covered with a cover slip and left to dry in an oven adjusted at 60° C for at least three days. The prepared slides were examined under the microscope, the eyepiece lens was (x10) whereas the objective

lenses were (x4, x10 and x25), measurements and drawings were made for the temporary slides using the drawing tube fitted in the microscope. The prepared slides were photographed using (Leitz Dialux 20) microscope fitted with (Wild PMPS II) camera, using Kodak colored films 36 ExP. 24 x 36mm ISO 100/210. The microscopical characters of the plant species studied were outlined. The results were presented quantitatively and qualitatively. The quantitative characters of the leaves were presented using tables whereas the qualitative characters were represented using micrograms and photomicrographs.

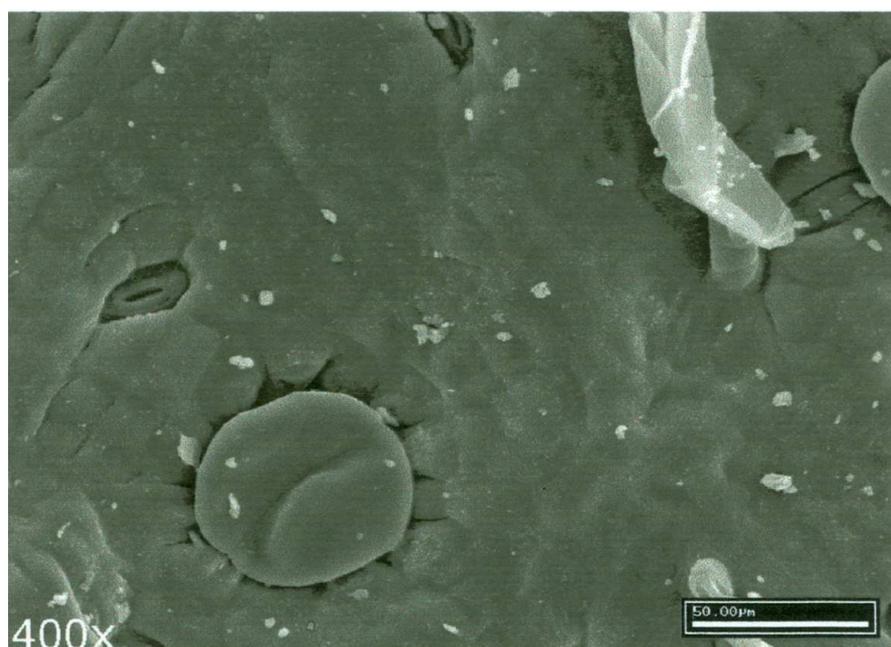
RESULTS

Epidermal surfaces of the leaves

The epidermal cells were tetragonal, pentagonal and hexagonal in surface views, the sizes of the epidermal cells in the abaxial surface was found to be larger than those on the adaxial surface (table 1). The stomata were anomocytic, the stomatal sizes and indices were larger on the abaxial surface. There were many epidermal hairs of different types; unicellular, multicellular, glandular and non-glandular hairs the heads of the glandular hairs appeared bi-lobed the glands were about 73.75 μ m in diameter. The calcium oxalate crystals were large and with different shapes and sizes. The mean palisade ratio was found to be four. The scanning electron microscopy of the epidermal surfaces of the leaf of *V.amygdalina* revealed the presence of large quantities of wax forming rod-shape above the epidermal cells of abaxial surface and smooth dense lumps of wax covering the adaxial epidermal cells. The stomata appeared sunken with radiating striae and stomatal rims raised into funnel like structures with long and narrow aperture. The epidermal hairs were of glandular types with clear bi-lobed glandular heads (plate 1). The amount of wax was found to be larger on the adaxial surface whereas the number of stomata was more on the abaxial surface.

Surface	Epidermal cell size (um)	Stonatal type	Stomatal size (um)	Stomatal index	Epidermal hair length (um)	Epidermal hair density	Crystal size(um)
Adaxial	15.0 x 18.41	anomocytic	14.8x21.27	18.75	158.3	Dense	37.5
abaxial	16.15 x 19.38	anomocytic	17.57x23.12	19.60	255.1	v. dense	40.7

Table 1: microscopical characters of the leaf epidermis of *V. amygdalina*



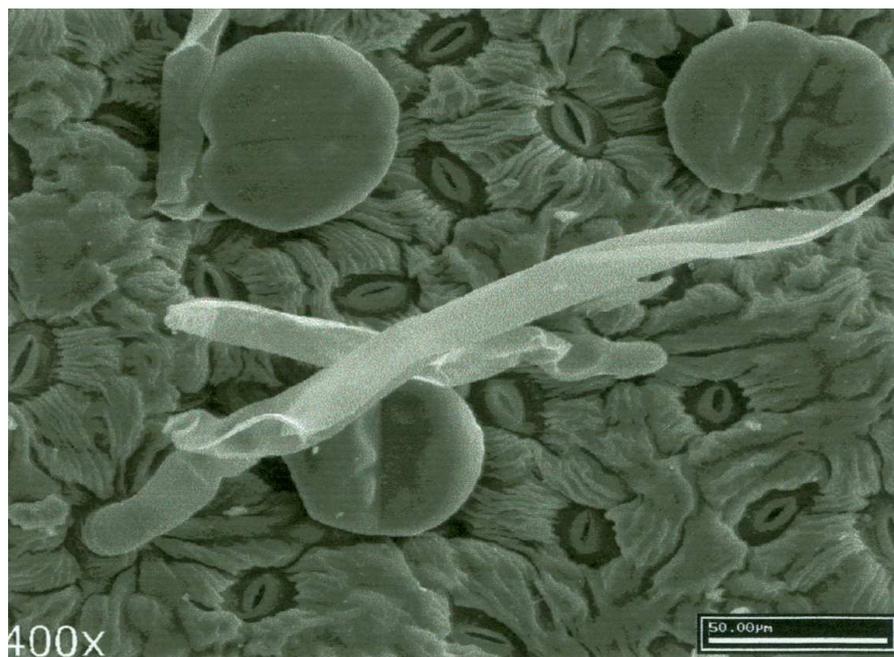


Plate 1: Scanning electron micrographs of the epidermis of the leaf of *V. amygdalina* above adaxial and below abaxial.

Diagnostic features of the powder of the dry leaves

The powder of the dry leaves of *V. amygdalina* is dark green in color with faint smell. Microscopically (Fig. 2), it includes numerous glandular hairs with bi-lobed heads and non-glandular hairs of different lengths. Calcium oxalate crystals are scattered. Small veins appeared in longitudinal views with different types of vessel thickening. Hexagonal and pentagonal epidermal cells appeared in surface view including anomocytic stomata, multicellular hairs and bi-lobed glandular heads. Parenchyma cells are found in the section in different shapes and sizes.

Transverse sections:

The Leaf:

The upper epidermis was a single layer of barrel-shaped cells. The epidermal cells were covered by a thick cuticle about 6.46μ thick, sunken stomata were found between the epidermal cells (plate 2A). Epidermal hairs were found, some of them were sunken and sessile. A narrow hypodermal layer was found. The dorsiventral mesophyll consisted of two layers of cylindrical palisade cells and 4-5 layers of spongy parenchyma with small intercellular spaces. The lower epidermis was similar to the upper epidermis in structure but the number of stomata was more and the cuticle was thicker. Small lateral veins appeared in longitudinal views in the leaf lamina. In the midrib region; the upper epidermis was papillose, followed by five layers of collenchyma and a wide region of different sizes of parenchyma cells. Many multicellular hairs were found and they were of different lengths. The vascular bundle region was divided into four bundles separated by biserrate rays of parenchyma (plate 2B). Each vascular bundle protected by an upper and a lower patch of sclerenchyma cells. A wide phloem region was found towards the lower epidermis protected by thick sclerenchyma cells. The xylem was formed of vessels arranged into 5-8 rows of vessels, in each row there were 2-6 vessels. The parenchyma cells below the vascular bundle were formed of 3-5 layers large cells and they were containing calcium oxalate druses. They were followed by collenchyma cells.

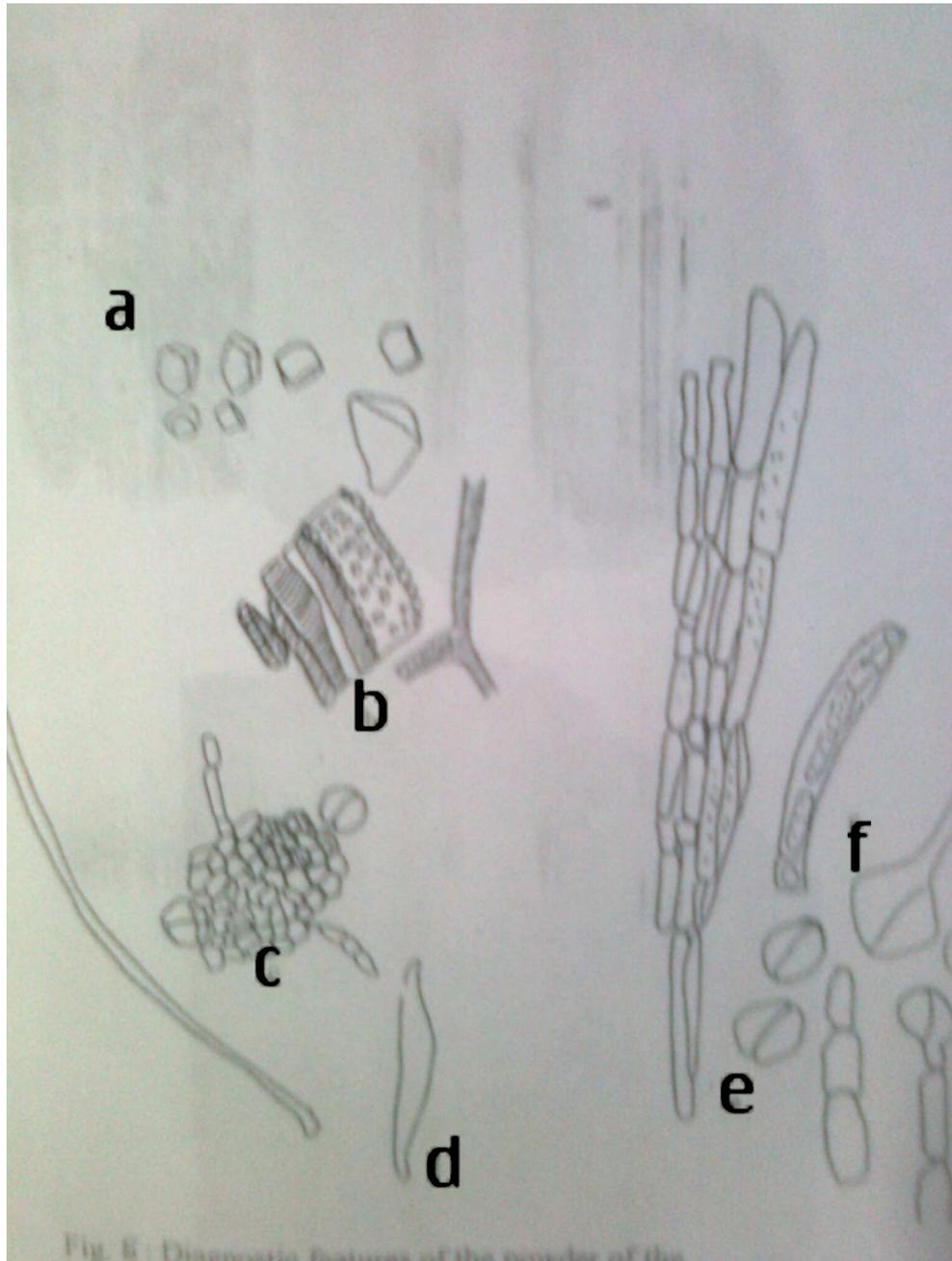


Fig. 1: Diagnostic features of the powder of the

Fig 1 : Diagnostic characters of the powder of the dry leaf of *V. amygdalina*
a. calcium oxalate crystals b. veins in longitudinal section c. epidermal cells in surface view d. epidermal hairs e. Parenchyma cells f. multi cellular epidermal hairs.

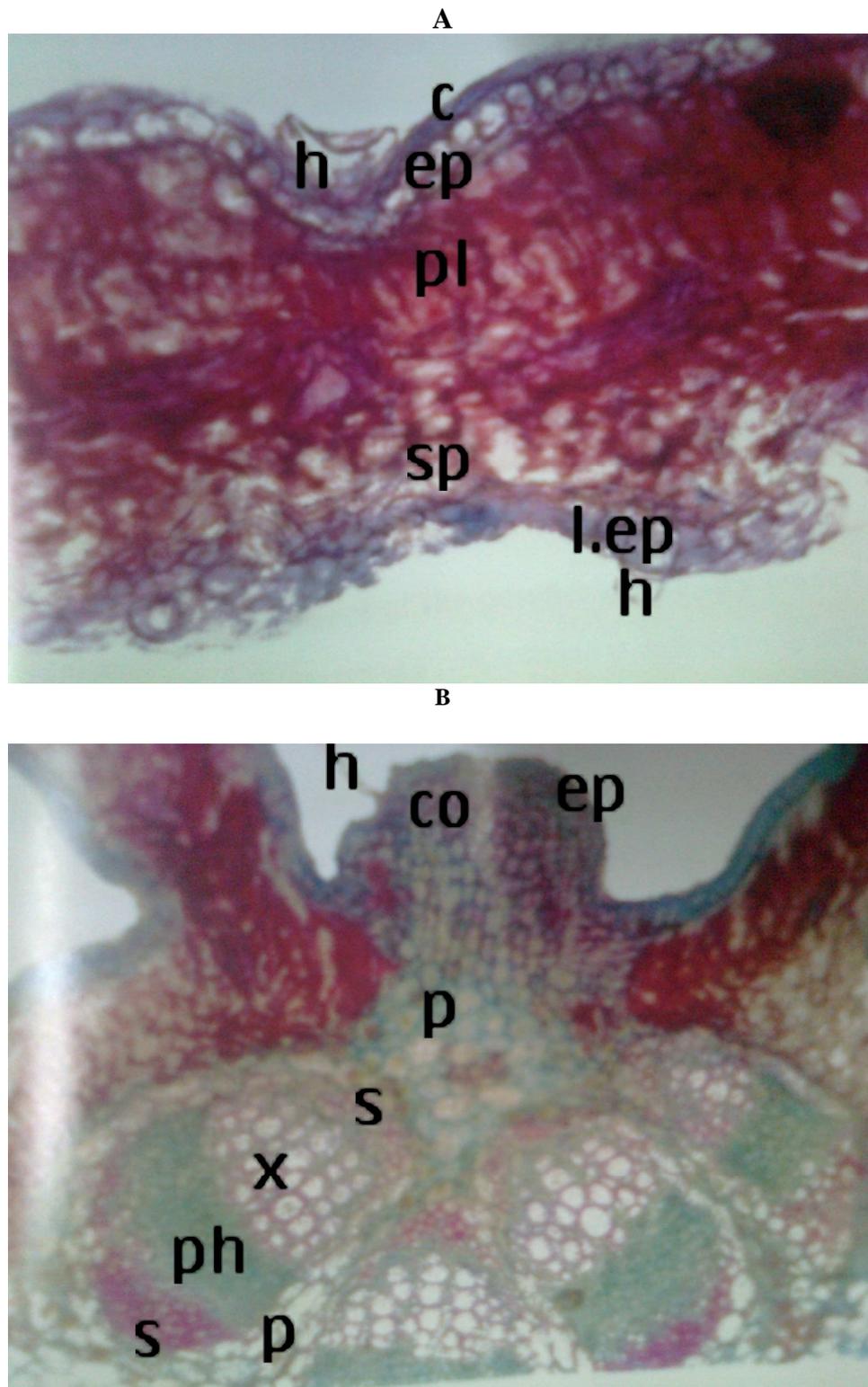


Plate 2: Transverse sections of the leaf of *Vernonia amygdalina*

A. Leaf lamina (x250) B. Midrib region (x100)

c. cortex, co. collenchyma, ep. Epidermis, h. hair, l.ep. Lower epidermis, p. parenchyma, pl. palisade layer, s. sclerenchyma, sp. Spongi parenchyma, x. xylem.

The petiole:

The general structure of the transverse section of the petiole (plate 3A) appeared circular. The outermost layer is formed of one layer of epidermis with some hairs followed by 4 layers of collenchyma and parenchyma cells. The vascular bundles are arranged in a circle, some of the bundles are separated by uni or biseriate parenchyma cells and each vascular bundle is preceded by pericyclic fibers. The phloem region is formed of primary and secondary phloem and they are followed by the xylem which is formed of secondary xylem vessels separated by xylem sclerenchyma and few primary xylem vessels separated by xylem parenchyma (plate 3B). The pith is a wide region of thickened parenchyma cells.

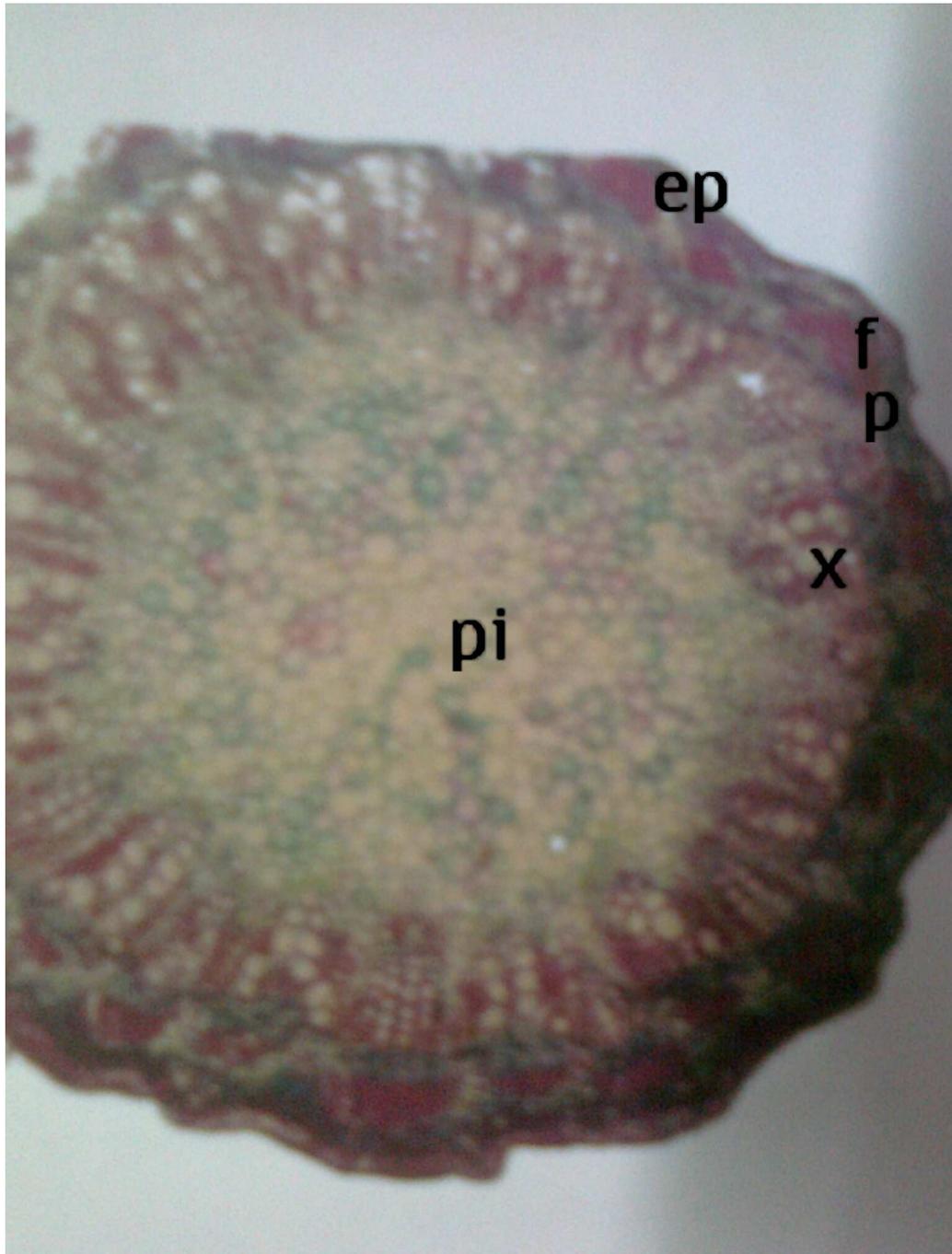


Plate 3: Transverse sections of the petiole of *V. amygdalina* X40
ep. Epidermis, f. fiber, p. phloem, pi. Pith, x. xylem.

DISCUSSION

The anatomy of the leaf and petiole of *Vernonia amygdalina* is studied in detail. Epidermal hairs are found to be of different types; they are simple, unicellular, multicellular, glandular and non-glandular hairs. The glandular hairs are provided with bi-lobed heads. The adaxial epidermis is covered by a thick cuticle and the stomata are sunken. These two features are adaptations to minimize the loss of water as the plant is found in relatively dry places. The stomatal type is anomocytic similar to what is mentioned by [6], they also reported the anisocytic stomata to be present in the family *Compositae*. The anomocytic type of stomata was also reported by [7]. Scanning electron microscopy of the leaf surface proved the presence of sunken stomata, glandular hair with bi-lobed heads, sessile glandular hairs and it also showed the presence of rod-shaped wax.

The palisade ratio of this species is found to be four. The vascular bundle of the leaf is found to be divided into four bundles by parenchyma cells. [6] reported that the vascular bundles of the veins frequently provided with a distinct sheath of parenchyma. The vascular bundles are surrounded on the upper and the lower sides by sclerenchyma cells. This feature was not mentioned for the family but it is known that sclerenchyma cells provide support and protection to the plant.

The petiole is found to process collateral vascular bundles covered by pericyclic fiber strands. [6] stated that the petiole of the genus *Vernonia* exhibit in transverse sections a ring of collateral vascular bundles each of which is accompanied in the pericyclic region by a large strand of fibers. No hypodermis is found in the petiole but [6] stated that the hypodermis is recorded below the upper epidermis of some species of the genus *Vernonia*. They also reported that the petiole of the material examined at Kew, generally showing simple arc of separate bundles. In this study the petiole is provided with a continuous circle of bundles, this is the type of secondary thickening occurred in this plant.

CONCLUSION

These microscopical characters for *Vernonia amygdalina* leaf and petiole could serve useful in the identification of this plant species. The palisade ratio had been calculated for the first time, the bi-lobed glands of the glandular hairs represent an important diagnostic character for this species.

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