

CHEMICAL INVESTIGATION OF PETROLEUM ETHER EXTRACT AND BOTANICAL STUDY OF *AMMANNIA AURICULATA* WILLD

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ABSTRACT

The pet. ether fraction of *Ammannia auriculata* Willd. afforded three major compounds identified as pentacosanol (1), β -sitosterol (2) and betulinic acid (3). They were identified through analysis of their different physico-chemical data. The macro- and micro-morphological characters of the different parts of *Ammannia auriculata* Willd. (family Lythraceae) have been studied to identify the titled plant in both entire and powdered form.

Keywords: pentacosanol, β -sitosterol, betulinic acid, micro-morphology, *Ammannia auriculata*, lythraceae.

INTRODUCTION

Family Lythraceae (العائلة الخنثائية) includes about 27 genera and up to 600 species of herbs, shrubs, and trees. The family members are widely distributed, but most of them are abundant in the American tropics (Lawrence, 1969; Bailey, 1975 and Bolous, 2000). The family belongs to Division, spermatophytæ; Phylum, angiospermae; Subphylum, Dicotyledons; Order, myrtiflorae (Lawrence, 1969; Benson, 1976; and Samuel *et al.*, 1986).

Genus *Ammannia* which comprises 25 species mostly grow in wet places. This genus is normally occur in ponds and rice fields in Egypt, tropical Africa, West Asia to India, China, Australia, central and South America. The plant usually forms fruits in August to December (Bolous, 2000).

From the environmental point of view, *Ammannia* causes a problem to farmers in the rice fields so, its identification is important.

Moreover, different medical purposes; antiuro-lithic, analgesic, antipyretic and diuretic activities were reported for *Ammannia* species (Prasad *et al.*, 1994, Joanoferc *et al.*, 2003 and Dhanapal *et al.*, 2004). In addition, useful compounds that could be used for treatment of many diseases e. g. betulinic acid which is used in treating cancer and β -sitosterol which is used as anti-inflammatory were reported in *Ammannia* (Thakkar *et al.*, 1986).

In the course of our study concerning the chemical contents of *Ammannia* species, several natural products were isolated from *Ammannia auriculata* Willd. for the first time such as pentacyclic triterpenes, flavonoids and tannins. So, this study is believed to has environmental and medicinal value.

This study also includes the macro- and micro- morphological characters of *Ammannia auriculata* Willd. for its identification in entire and powdered form.

Plant samples:

Plant material of *Ammannia auriculata* Willd. was collected during the flowering to fruiting stages, on October 2006, from rice fields in Mansoura vicinity, Egypt. The identity of the plant was confirmed by Prof. Dr. Ibrahim Mashaly, Plant Ecology, Faculty of Science, Mansoura University, Egypt. Voucher specimens have been deposited at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University.

Experimental:

The powdered-aerial parts (570 gm) were extracted with methanol. The solvent was evaporated to dryness in a rotary evaporator at 450C. The residue was suspended in distilled water and then partitioned with petroleum ether. The petroleum ether fraction was evaporated to yield the pet. ether residue weighed (15gm), it was applied as a band onto the top of a silica gel column and eluted with hexane then ethyl acetate/ hexane mixture, increasing polarity, (0 - 35% v/v). Fractions of 50 ml were collected and monitored by TLC using ethyl acetate/ hexane (30:70) as developing system and vanillin / H₂SO₄ acid as spray reagent. Similar fractions were pooled together and purified to yield compound 1 (eluted by 7% mixture, 25mg), compound 2 (eluted by 10% mixture, 200mg) and compound 3 (eluted by 15% mixture, 50mg).

Small pieces of the different parts of *Ammannia auriculata* Willd. were fixed in formalin-acetic acid-alcohol mixture (90:5:5 v/v), dehydrated in ethanol and embedded in paraffin wax (m.p. 50-54°C) then cooled and sectioned at 15-20 µm by a rotary microtome, dewaxed in xylene. The powder of each organ (root, stem, leaf and fruit) and the sections were

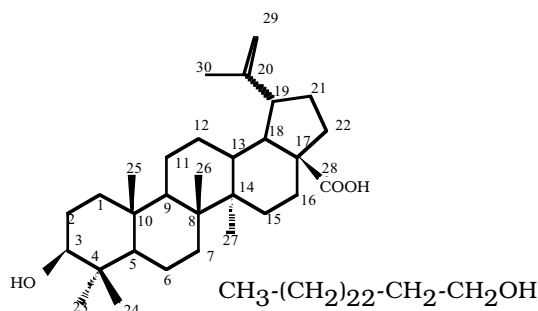
gently warmed with chloral hydrate stained by phloroglucinol/ HCl and preserved using glycerin. Other samples were mounted with distilled water to examine starch granules and calcium oxalate crystals. Photo images for the sections and isolated elements were captured using a digital camera.

RESULTS AND DISCUSSIONS

Column chromatography of the pet. ether fraction afforded three major compounds. They were identified as pentacosanol (1), β-sitosterol (2) and betulinic acid (3) by co-chromatography with authentic samples and comparison of their IR, MS, ¹³C and ¹H NMR spectral data with those reported for similar compounds (Aguirre et al., 2006 and Wang et al., 2007).

Pentacosanol (1), mp. 75°. EI-MS m/z 392 (M+Na+H). ¹H-NMR (CDCl₃, 500 MHz, δppm): 3.64 (t, *J* = 6.1 Hz, H-1), 1.56 (m, H-2), 1.24 (H-4 - H-24) and 0.87 (t, *J* = 6.8 Hz). ¹³C-NMR (CDCl₃, δppm): 63.2 (C-1), 32.9 (C-2), 32 (C-3), 22.8-29.8 (C-4 - C-24) and 14.24 (C-25). **β-Sitosterol (2)**, m.p. 138-139°. IR (KBr, ν_{max}) 3423, 2940, 2867, 1639, 1461 and 1376 cm⁻¹. **Betulinic acid (3)**, m.p. 291-293°. HRESI-MS m/z 534.4958 (M+2K). ¹H-NMR (CDCl₃, 500 MHz, δppm): 3.16 (dd, *J* = 4.86, 11.4, H-3), 0.67 (H-5), 1.6 (m, H-12), 2.2 (ddd, *J* = 3.3, 12.6, H-13), 2.25, 1.2 (H-16), 1.43 (m, H-18), 2.9 (m, H-19), 1.96 (m, 21β, 22β), 0.96 (s, H-23), 0.75 (s, H-24), 0.82 (s, H-25), 0.93 (s, H-26), 0.97 (s, H-27), 4.6 (d-like, *J* = 2, 29β) and 4.7 (d-like, *J* = 1.85, 29α). ¹³C-NMR (CDCl₃, δppm): 38.7 (C-1), 27.4 (C-2), 79 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 34.37 (C-7), 40.73 (C-8), 50.5 (C-9), 37.2 (C-10), 20.8 (C-11), 25.54 (C-12), 38.4 (C-13), 42.4 (C-14), 30.5 (C-15), 32.1 (C-16), 56.2 (C-17), 49.3 (C-18), 46.9 (C-19), 150.4 (C-20),

29.7 (C-21), 37 (C-22), 28 (C-23), 15.3 (C-24), 16 (C-25), 16.1 (C-26), 14.7 (C-27), 179.6 (C-28), 109.7 (C-29) and 19.4 (C-30).



Betulinic acid (3) Pentacosanol (1)

Macro-morphology:

Ammannia auriculata Willd., syn. *Ammannia arenaria* Kunth. (Bolous, 2000) belongs to family Lythraceae. The plant is erect annual herb with four-angular narrowly winged branched stem carrying green cauline leaves and axillary dichasial cyme inflorescence, as well as globose dehiscent capsules and well-developed tap-root system (plate 1). The plant usually starts flowering and fruiting in August to December.

The root is cylindrical tap root, highly branched towards the end making a dense tuft with several rootlets. The outer surface of root is rough and covered with a yellowish brown scaly layer. It measures 12-17 cm long and 3 mm diameter. The root is odorless and having astringent taste.

The leaf is simple, green in color, exstipulate, 1.5-7.5 cm long and 3-15 mm wide, linear-lanceolate in shape, entire margin, auriculate at the base and has acute apex. It is one-nerved and the midrib is prominent on the lower surface and grooved on the upper surface. It is odorless and tasteless.

The stem is light green in color, erect, branched, four-angular, narrowly winged and

measuring 15-60 cm high. The leaves are arranged in an opposite decussate sequences. The internodes are of 2-3.5 cm in length. The fresh stem is flexible but the dry has a fibrous fracture.

The inflorescence is pedicellate and composed of axillary dichasial cyme inflorescence arranged on a racemose rachis in opposite decussate branching.

The flowers are 3-12 dense in axillary cymes, the pedicel measuring 0.5-3 mm long, the peduncle measuring 0.5-1.5 cm long. The calyx is membranous; it is green in color, measuring 1-1.5 mm long, campanulate with triangular four sepals.

The corolla consists of four pink, obovate petals measuring 1.2-1.5 mm long and 1.2-1.3 mm wide. The androecium consists of 4-8 long filaments included stamens. The gynoecium is composed of three free carpels forming 3 locules. This lies within the reported gynoecea for *Ammannia*; 2-6 carpels, 2-6 locules (Bolous,2000). Ovary is superior. Each locule contains several anatropous ovules arranged on axial placentation. The ovary measures 1-1.5 mm in length. The style is measuring 1.2-1.8 mm long with capitate stigma.

The capsules are globose, membranous and purple in color enclosed by the persistent calyx segments and measuring 1.8-2.5 mm long and 1.5-3 mm width at the middle. It encompasses 3 locules, each locule is filled with numerous tiny seeds. The style is persistent in the fruiting stage with capitate stigma, measuring 0.1-0.3 mm high. The Seeds are golden-brown, obovoid, exalbuminous with straight embryo measuring about 1mm long.

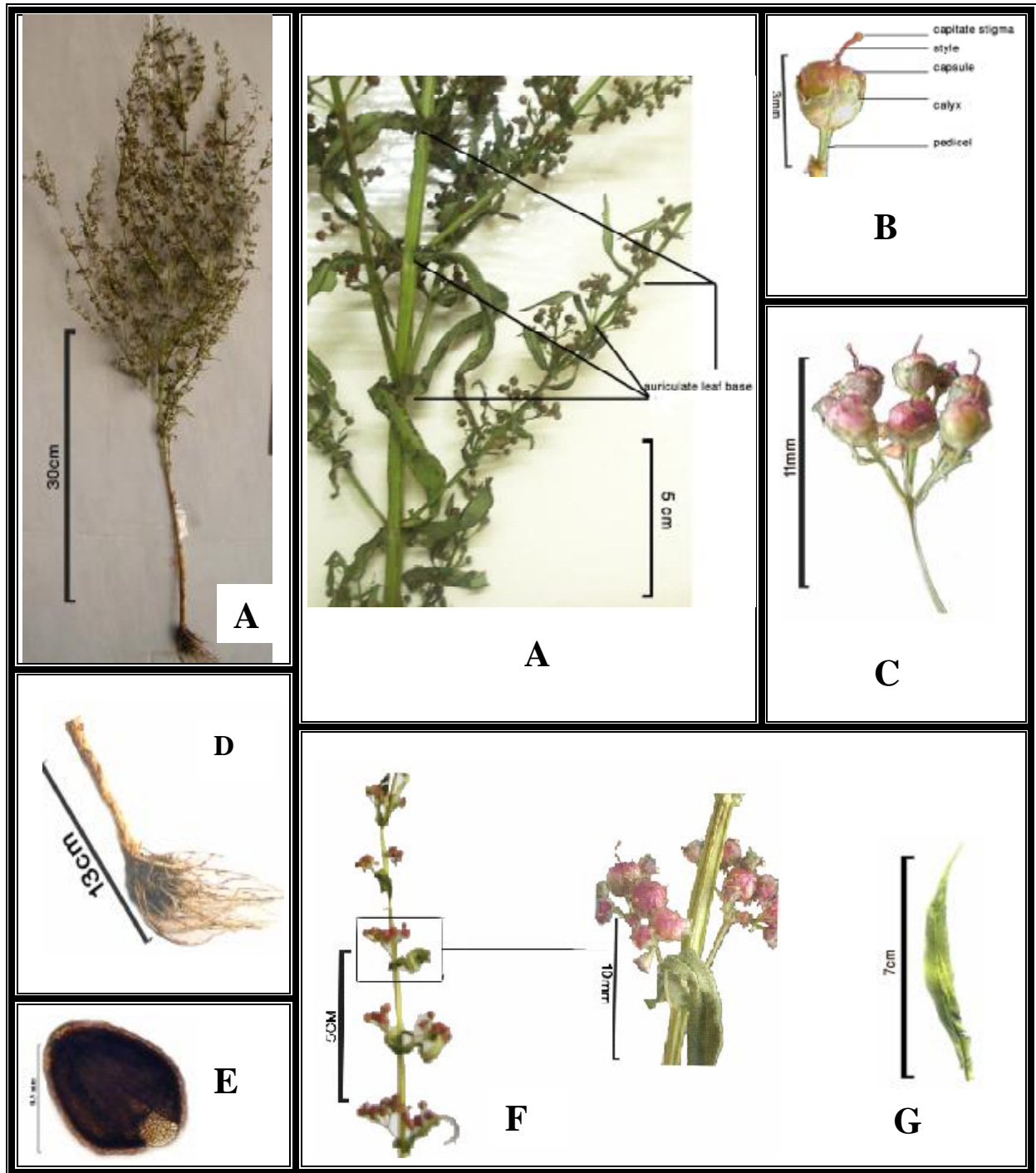


Plate 1: Macro-morphology of *Ammannia auriculata* Willd. (Whole plant and different parts) colored photos.

A: Whole plant,

B: capsule,

C: Inflorescence,

D: Tap-root,

E: tiny seed,

F: winged stem, inflorescence and the leaf,

G: leaf.

Micro-morphology**A- Anatomy of the leaf:**

The transverse section in the leaf of *Ammannia auriculata* Willd. (fig. 1 and plates 2A, 3A) is convex on the lower surface and grooved on the upper surface at the midrib region. The rest of the lamina is a ribbon-shaped. The lamina reveals a heterogeneous, dorsiventral mesophyll interrupted in the midrib region by cortical and vascular tissues. The upper and the lower epidermises are glabrous, but carrying papillae at the margin. The stomata are present on both sides. The lamina shows a columnar palisade layer beneath the upper epidermis and 3-4 layers of parenchymatous spongy tissue. The midrib region shows a crescent-shaped large central bicollateral vascular bundle. Endodermis and pericycle are not differentiated.

Upper epidermis is formed of polygonal, tabular cells with slightly thick cellulose slightly wavy anticlinal walls and covered with smooth to warty cuticle. The epidermal cells are frequently displaced with large isodiametric to elongated mucilaginous cells measuring 36.06-52.4-59.01 μ length 19.67-27.86-36.06 μ width and 28-32-40 μ height. The epidermal cells measure 38.4-49.8-61.5 μ length, 15.4-26.5-30.7 μ width and 16-20.8-28 μ height. Both upper and lower epidermises have anomocytic stomata without any trichomes.

The lower epidermis is formed of polygonal to isodiametric cells with thick cellulose slightly wavy anticlinal walls. The epidermal cells are covered with thick warty cuticle. They measure 30.8-43.8-53.84 μ length, 15.38-25.38-38.46 μ width and 15.38-23.85-27 μ height.

The palisade is formed of one layer of compact cylindrical, columnar, thin walled cells containing chloroplasts; they measure 40-53.2-64 μ in length and 8-13.6-16 μ in width. The spongy tissue is formed of 3-4 layers of polyhedral nearly rounded chlorenchymatous cells with thin cellulose walls and measure 15.38-22.3-30.7 μ in diameter. The spongy parenchyma contains crystals of solitary calcium oxalate clusters.

The cortical tissue shows upper and lower parenchyma of 3-4 and 5-7 layers, respectively. The palisade is interrupted in midrib region by parenchymatous cells. The cortical parenchyma consists of polygonal cells, measuring 23.1-29.2-38.46 μ in diameter, with thin cellulose walls and small triangular intercellular spaces. They contain solitary crystals of calcium oxalate clusters measuring 31-55.8-69.2 μ in diameter. The central region is occupied by bicollateral vascular bundle. The vascular strands consist of several rows of lignified xylem elements with outer and inner batches of phloem.

Xylem is composed of lignified, annular and spiral vessels measuring 15.38-30.7-34.6 μ in diameter. Fibers are few with more or less tapering ends, with moderate thick lignified wall. They measure 995-1010-1024 μ length and 15.38-21.5-23.1 μ width. Xylem also contains elongated lignified wood parenchyma.

The phloem is formed of small cells with thin cellulose walls and hardly differentiated into sieve tubes, companion cells and phloem parenchyma.

The powdered leaf is dark green in color,

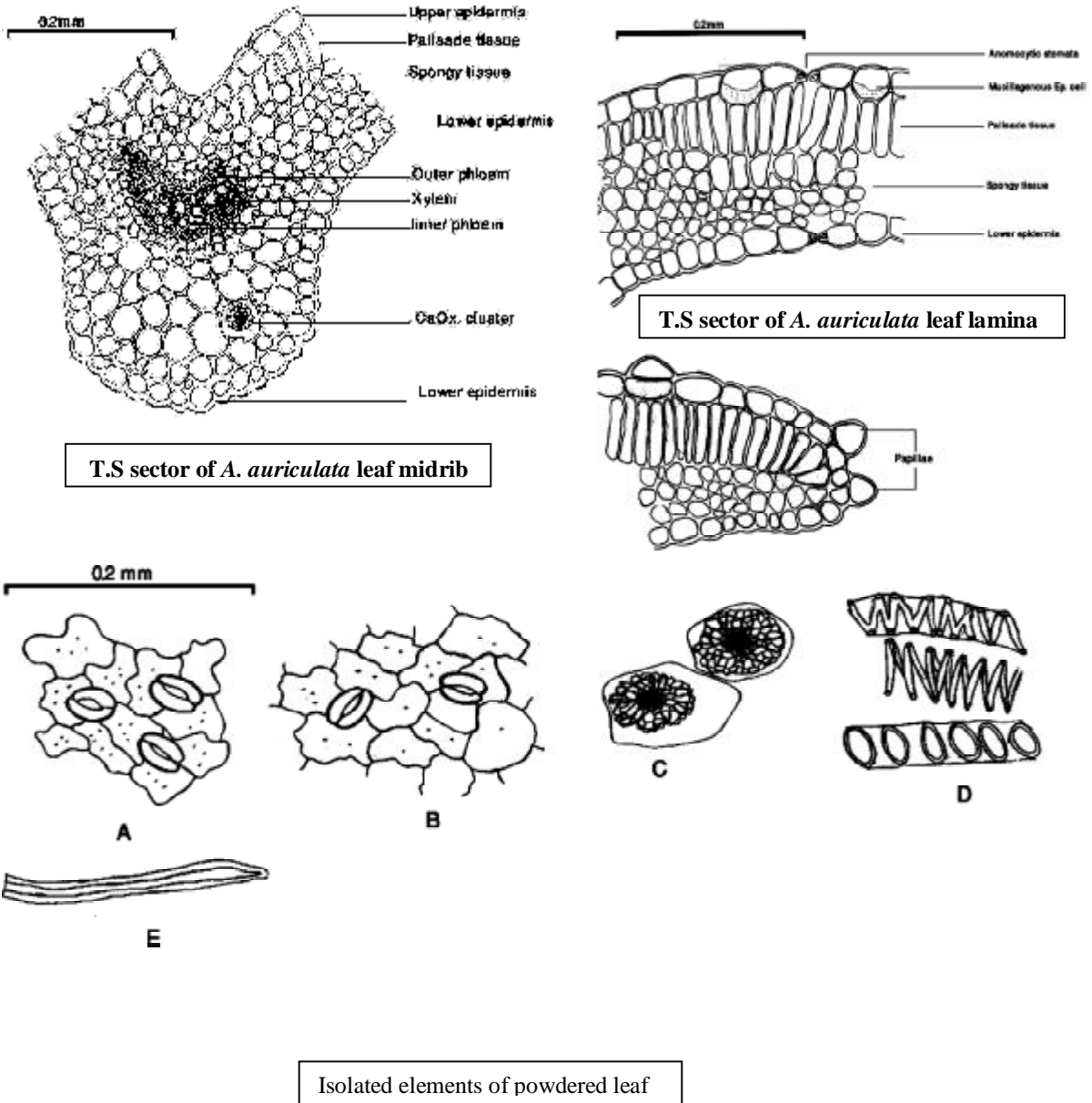


Figure 1 : Detailed transverse section of *Ammannia auriculata* Willd. leaf and its powder.

- A:** lower epidermis, **B:** upper epidermis, **C:** CaOx. Clusters,
D: spiral and reticulate xylem vessels, **E:** isolated fiber.

salty in taste and odorless. It is characterized microscopically by the presence of fragments of epidermis with polygonal, slightly wavy anticlinal walls and covered with warty cuticle and showing anomocytic stomata, other fragments of lignified annular and spiral xylem vessels, fragments of the mesophyll parenchymatous cells showing palisade and spongy tissue with cluster crystals of calcium oxalate and fragments of the fibers of the vascular bundle with moderately thick lignified walls and more or less tapering ends.

B- Anatomy of the stem

The transverse section of the stem (fig. 2 and plates 2C, 3C) is almost quadrangular, each angle extend to form bi-forked wing. It is formed of an epidermis surrounding a cortex formed of several layers of chlorenchyma. The pericycle consists of isolated patches of fibers. The vascular system is formed of a bicollateral vascular bundle. The pith is wide. Cluster crystals of calcium oxalate are scattered in the pith and cortex parenchyma.

The epidermis consists of tabular polygonal, slightly elongated to isodiametric cells, with straight anticlinal walls. The cells are covered with thin striated cuticle. The epidermal cells measure 45.16-77.4-148.4 μ length, 25.8-33.5-38.7 μ width and 11.4-18.3-22.8 μ height. Anomocytic stomata are generally few. Trichomes are absent.

Cortex is formed of several layers of thin-walled chlorenchyma of more or less rounded cells. Air spaces are distributed through out the cortical tissue that previously reported in genus *Ammannia* (Metcalfe *et al.*, 1972), cluster crystals of calcium oxalate are scattered in the cortical parenchyma. The isolated patches

of pericycle consist of non-lignified fibers. The fibers have a wide lumen, moderately thick walls with acute tapering ends. The phloem is formed of small cells irregular in outline with thin cellulosic walls and hardly differentiated into sieve tubes, companion cells and phloem parenchyma.

Xylem consists of lignified vessels, tracheids, fibers and parenchyma. The vessels are spiral, annular, pitted and measure 11.5-25-32 μ diameter. Wood fibers are lignified with tapering ends and measure 13.33-20.6-33.3 μ diameter. The wood parenchyma consists of axially elongated cells with pitted and lignified walls. Small patches of intraxylary phloem are present.

The pith consists of more or less rounded parenchymatous cells, with thin cellulosic walls, showing intercellular spaces. The cells measure 27.36-43.80-61.10 μ diameter. They contain starch granules that measure 8.42 - 10-13.2 μ diameter and scattered cluster crystals of calcium oxalate that measure 31-55.8-69.2 μ in diameter.

The powdered stem is yellowish green in color, odorless and having a slight salty taste. It is characterized microscopically by the presence of fragments of the epidermis consisting of polygonal to axially elongated cells with straight anticlinal walls and covered with thick striated cuticle and anomocytic stomata, fragments of the lignified spiral, annular and pitted xylem vessels, fragments of polygonal parenchymatous cells of the pith containing starch granules with thin cellulosic walls, fragments of non lignified pericyclic fibers, scattered cortical chlorenchymatous cells and fragments of cortical tissue containing calcium oxalate clusters.

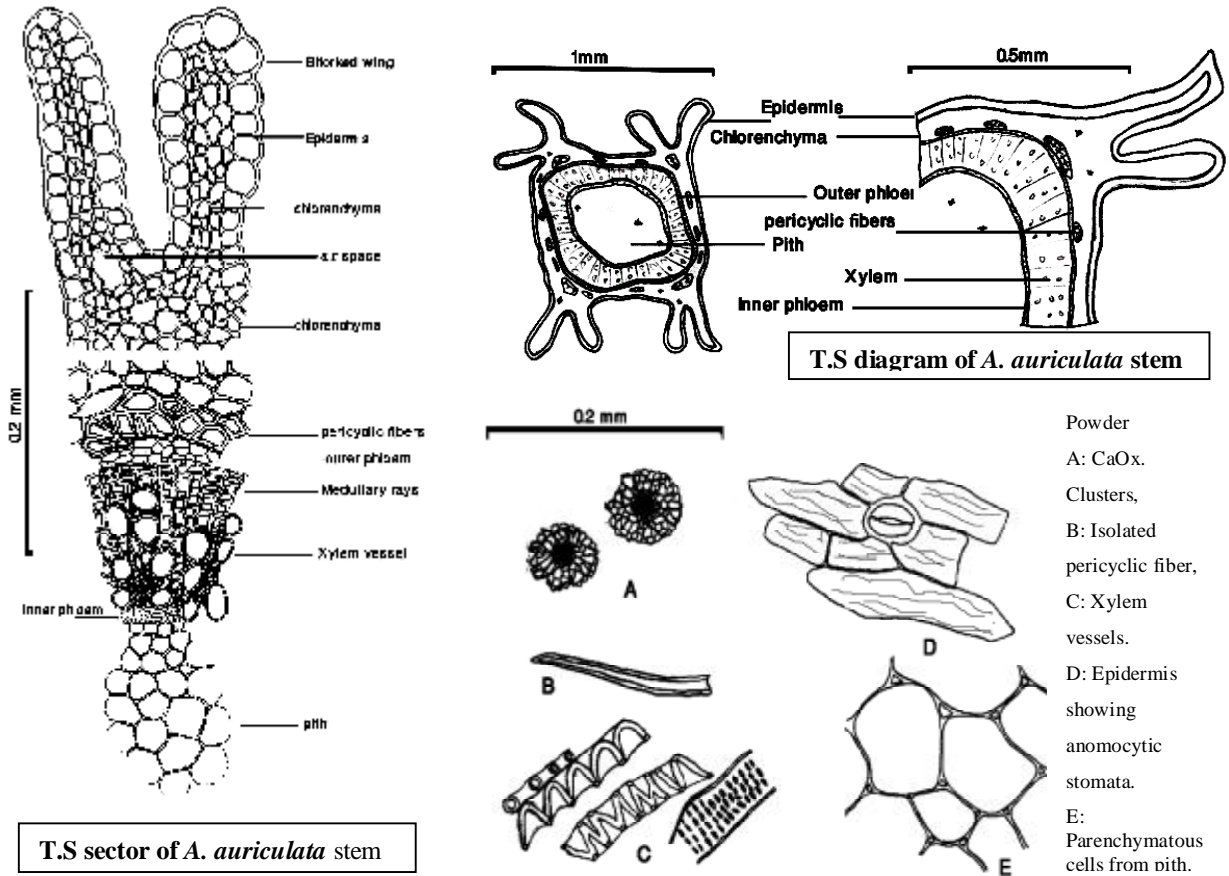


Figure 2 : Detailed and diagrammatic transverse section and powdered stem of *Ammannia auriculata* Willd.

C- Anatomy of the root

The transverse section of the root (fig. 3 and plates 2B, 3B) is circular in outline, showing a narrow cork separated from a wide vascular tissue by phylloclerm. The central vascular bundle consists of a wide ring of xylem and a peripheral narrow band of phloem. The xylem constitutes about three-quarter of the diameter of the sector.

The cork cells are arranged in 2-4 rows of polygonal to rectangular tangentially elongated cells with moderately thick suberized walls, measuring 30-~~43~~-55 μ length, 10-~~16~~-20 μ width and 7.41-11.85-18.52 μ height. The cork cambium is developed at the outer periphery of the phloem; accordingly, the primary cortex is pushed outward forming lacerated tissue containing solitary calcium oxalate clusters.

The secondary cortex (phelloderm) consists of thin walled tangentially elongated polyhedral parenchymatous cells measuring 11.1-23.7-33.3 μ length and 7.4-11.85-18.52 μ width and containing solitary calcium oxalate clusters.

The vascular bundle is arranged in the form of a ring as a result of secondary growth. An outer phloem is followed by inner secondary xylem. The center of the root is occupied

by compressed primary xylem. The vascular tissue is traversed by funnel-shaped medullary rays.

The phloem is composed of 3-4 layers of thin walled cellulosic cells, irregular in outline, and appear more or less compressed. They are hardly differentiated into sieve tubes and companion cells. The cambium is hardly distinguished. The secondary xylem occupies about three-quarters of the diameter of the sector followed by compressed primary xylem inward. It is composed of lignified elements; bordered pitted vessels, fibers, as well as lignified wood parenchyma. The vessels are wide have thick lignified walls, they measure 22.2-33.3-44.4 μ diameter. The fibers are lignified with tapering ends and measure 10-15-26.6 μ diameter. The medullary rays are funnel-shaped; consist of biserriate rows of rectangular lignified parenchyma.

The powdered root is yellowish in color, salty in taste and odorless. It is characterized microscopically by the presence of fragments of the cork cells with moderately thick suberized walls, fragments of the secondary cortical parenchyma containing calcium oxalate clusters, fragments of lignified bordered pitted xylem vessels, fragments of xylem fibers and solitary calcium oxalate clusters.

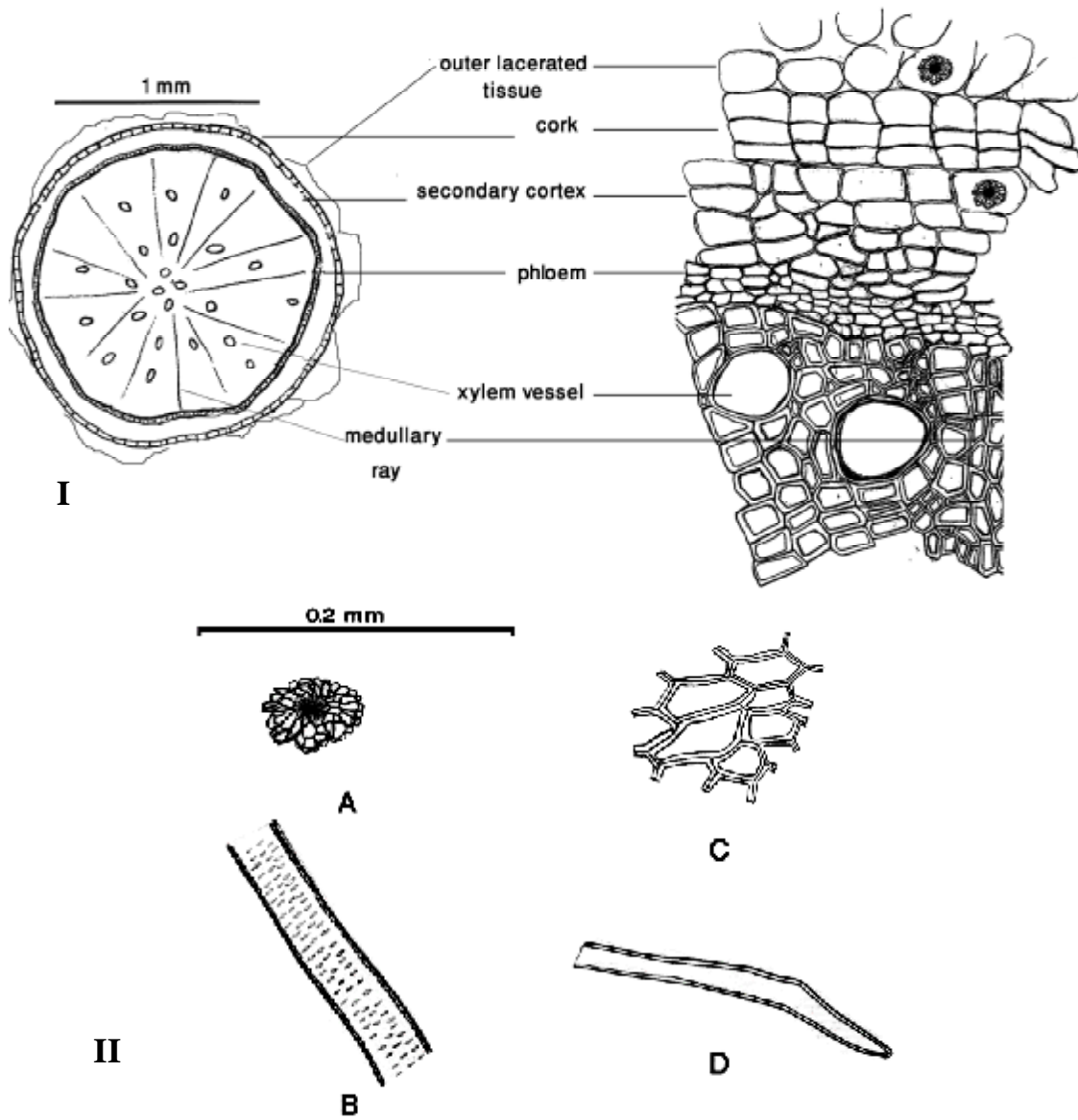


Figure 3 : I- Detailed diagrammatic transverse section in *Ammannia auriculata* Willd. root
II- Powder: **A:** Ca Ox. Clusters, **B:** Xylem vessel, **C:** Cork cells, **D:** Isolated fiber.

D- Anatomy of the fruit

A transverse cut in the fruit capsule (fig. 4 and plate 3D) is more or less oval in outline. It shows tri-locular capsule and each locule is filled with numerous tiny seeds.

The pedicel

The transverse section of the pedicel is circular to quadrangular in outline. It has wing-like extension at two adjacent angles. The different tissues of the cortex, patches of pericyclic fibers and a bicollateral vascular bundle are similar to those of the stem, except for the inner phloem which is being relatively wider and narrow pith.

The calyx the transverse section of the thin membranous calyx revealed that it consists of thin walled rounded to polygonal parenchymatous cells filled with calcium oxalate clusters. The cells are measuring 26.6-37.3-46.6 μ diameter.

The pericarp is membranous. The epicarp is similar to endocarp; consist of one layer of polyhedral parenchymatous cells with thin

cellulosic walls and the epicarp cells are covered smooth cuticle. They measure 13.63-19.1-22.7 μ length and 27.4- 33.7-50 μ width.

The mesocarp

It is formed of moderately thick walled polyhedral parenchymatous cells. They measure 18.2-24.5-31.82 μ length and 10-14.1-18.18 μ width. It contains granular reddish-brown colored substances.

The placenta is formed of polygonal parenchymatous cells with thin cellulosic walls. It contains starch granules.

The powdered fruit is reddish brown in color, tasteless, and odorless. It is characterized microscopically by the presence of fragments of the epicarp showing polyhedral, thin cellulosic cells, fragments of the parenchymatous cells of the mesocarp showing moderately thick walls, containing reddish brown granular contents, fragments of the endocarp, numerous tiny reddish brown seeds, fragments of calyx cells containing calcium oxalate clusters and dark brown capitate stigma.

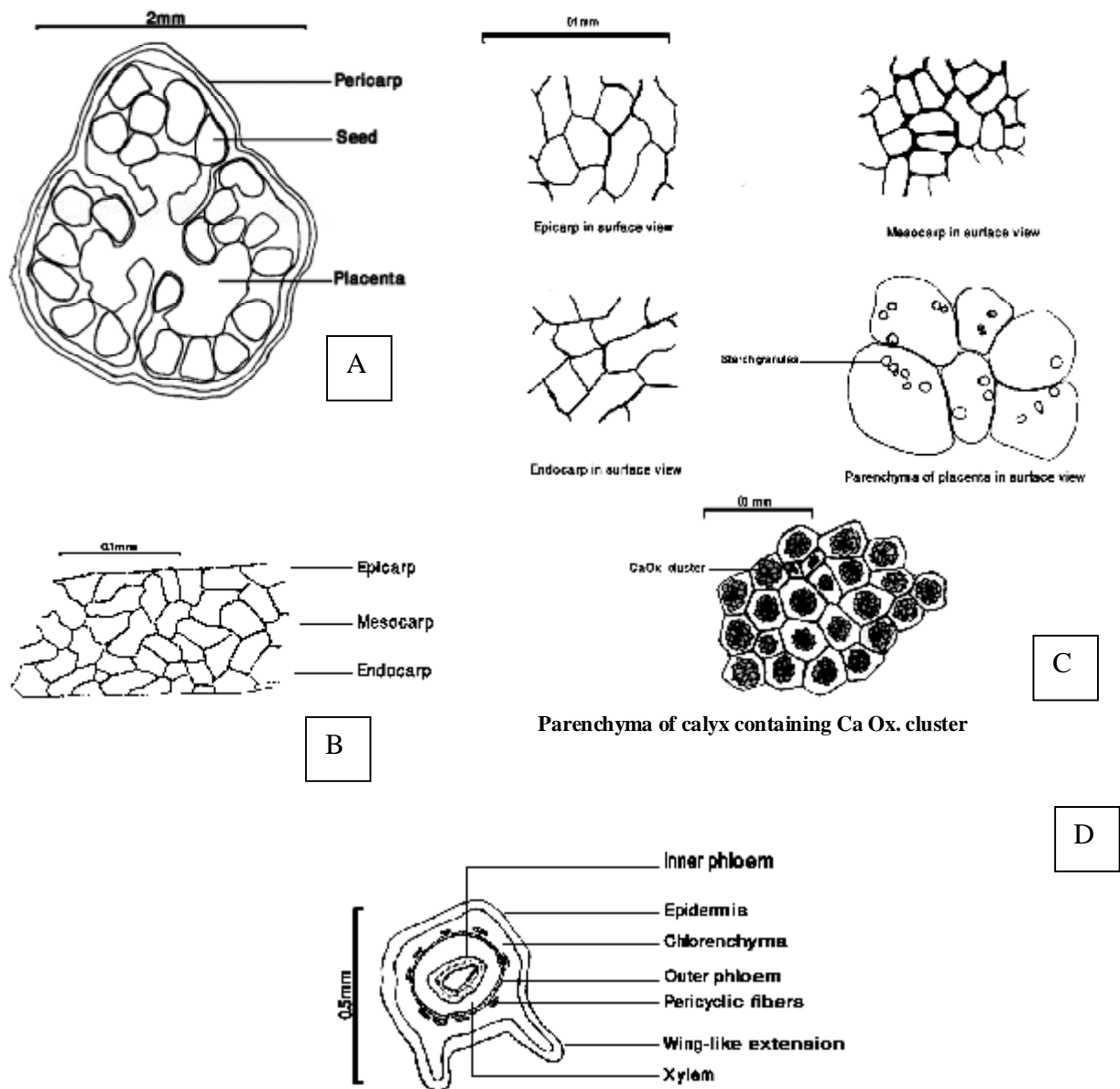
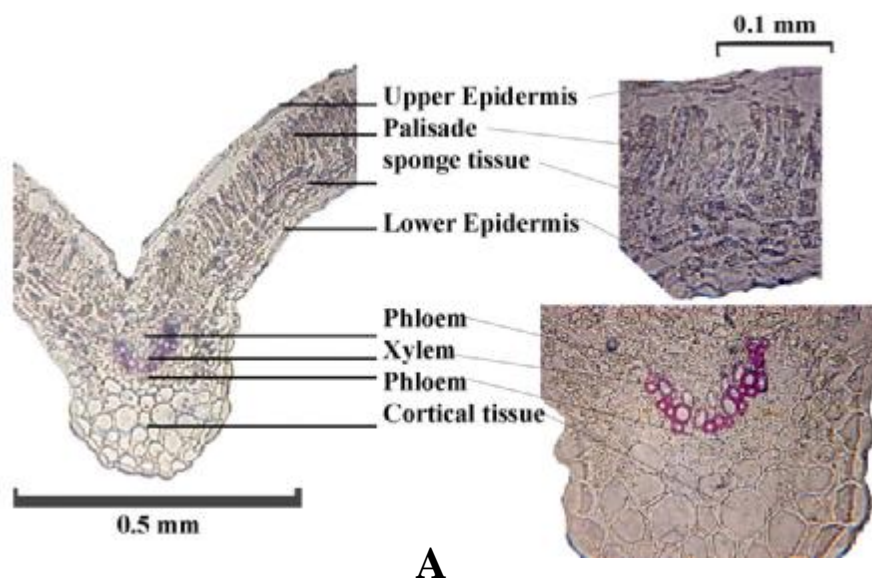
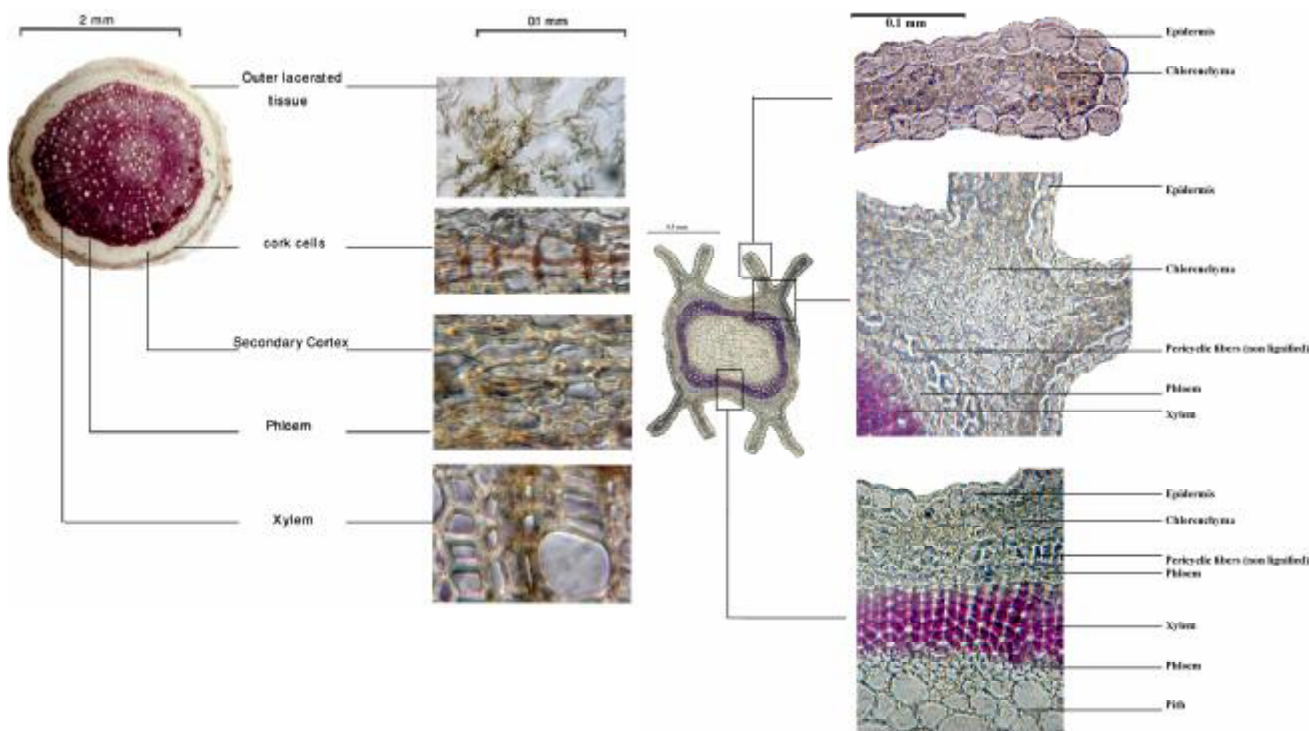


Figure 4 : **A:** Diagrammatic T. S. in capsule, **B:** T. S. sector in pericarp, **C:** elements of fruit powder, **D:** Diagrammatic T.S. in pedicel.



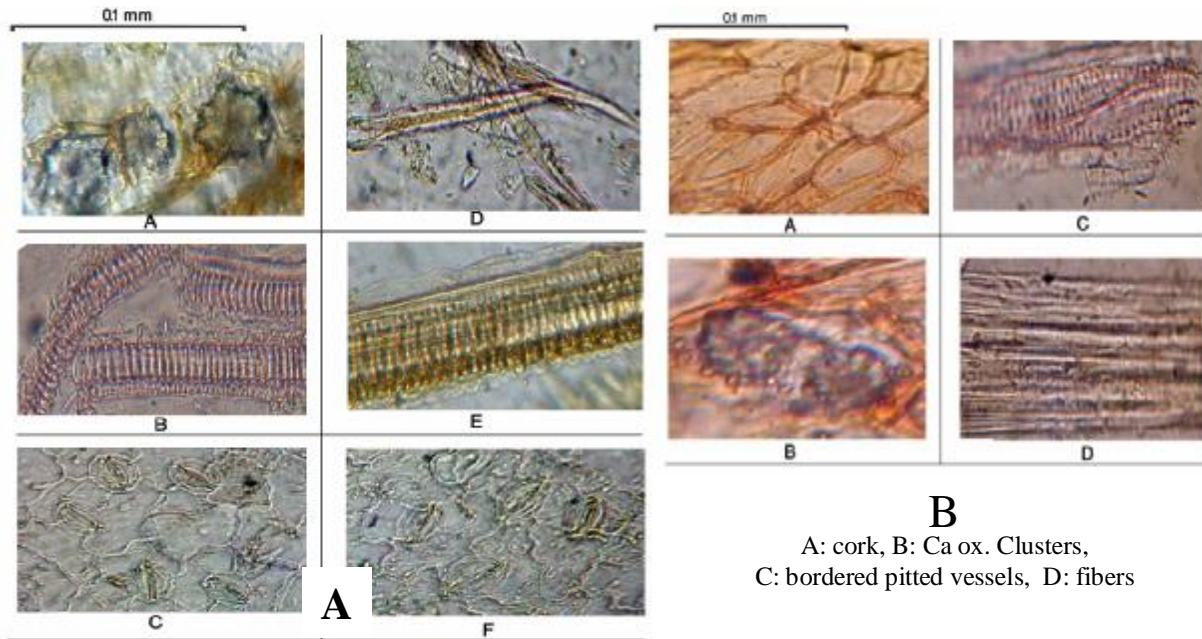
A



B

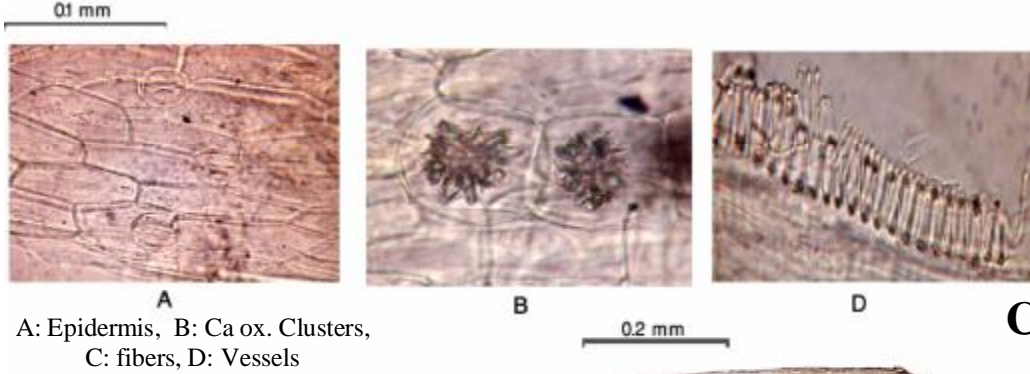
C

Plate 2 : Colored photos for **A** : T.S of the leaf, **B** : T.S of the root, **C** : T.S of stem.

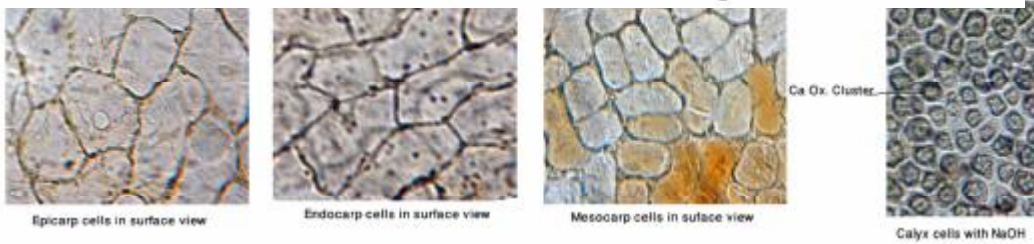


B
 A: cork, B: Ca ox. Clusters,
 C: bordered pitted vessels, D: fibers

A: Ca ox. Clusters, B, E: Vessels,
 C: upper epidermis, D: fibers, F: lower epidermis



A: Epidermis, B: Ca ox. Clusters,
 C: fibers, D: Vessels



Capitate stigma

D

Plate 3 : Colored photos for **A:** powdered leaf, **B:** powdered root, **C:** powdered stem, **D:** powdered fruit.

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Received on 13 / 12 / 2009

الملخص العربى

فحص كيميائى لخلاصة الإثير البترولى ودراسة نباتية لنبات الأمانيا أوركيولاتا وبلد

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لقد تم فصل مركبات البنثاكوزانول والبيتاسيتوسيتترول وحمض البتيولينك من خلاصة الإثير البترولى لنبات الأمانيا أوركيولاتا وتم التعرف على هذه المركبات بالطرق الفيزيوكيميائية المتبعة فى هذا الصدد، ولهذا فقد أجريت دراسة عيانية ومجهرية لأوراق وسيقان وجذور وثمار نبات الأمانيا أوركيولاتا بغرض التعرف عليه فى صورته الكاملة أو على هيئة مسحوق، ونظراً لأهمية التعرف على نبات الأمانيا أوركيولاتا لما يسببه من متاعب لمزارعى الأرز فإن هذا البحث سوف يساعد على التعرف على هذا النبات ومن ثم التخلص منه، بالإضافة إلى ذلك فإن هذا النبات يمكن إستخدامه لأغراض طبية مثل إذابة حصوات الكلى، ولذلك فإن هذه الدراسة لها أهمية على المستويين البيئى والطبى.

**CHEMICAL INVESTIGATION OF PETROLEUM ETHER EXTRACT
AND BOTANICAL STUDY OF AMMANIA AURICULATA WILLD**

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Reprint

from

Journal of Environmental Sciences, 2010; Vol. 39, No. 3

