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Morphological and anatomical studies of the stem of *Pachypodium lamerei* Drake, family Apocynaceae, cultivated in Egypt

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Abstract
Family Apocynaceae is rich in many secondary metabolites with important biological and economic values. One of these plants belongs to Apocynaceae is *Pachypodium lamerei* Drake. The available literature showed a study that described the micromorphological characters of the spines and stem of *P. namaquanum*. The present study examines various standardized parameters as morphological and histological characters which could be helpful in authentication of the stem of *P. lamerei* Drake.

Keywords: *Pachypodium lamerei* Drake, Apocynaceae, stem, morphological, anatomical.

1. Introduction
Family Apocynaceae is rich in many secondary metabolites with important biological and economic values viz.; triterpenes, cardenolides, sterols, saponins, and alkaloids [1-4]. One of these plants belongs to Apocynaceae is *Pachypodium lamerei* Drake. Although, *P. lamerei* Drake is native to Madagascar [5], lately it is frequently produced as a commercial ornamental plant around the world [6]. Reviewing the available botanical literature, two literatures are available on *P. namaquanum*, viz, general morphological and micromorphological features [7] in addition to the anatomical characters of the spines and stem [8]. The present study examines various standardized parameters as morphological and histological characters, which could be helpful in authentication of the stem of *P. lamerei* Drake.

2. Taxonomy
Pachypodium lamerei Drake belongs to [9, 10]: **Kingdom:** Plantae, **Subkingdom:** Viridiplantae, **Infrakingdom:** Steptophyta, **Division:** Tracheophyta, **Subdivision:** Spermatophytina, **Infradivision:** Angiospermae, **Class:** Magnoliopsida, **Suborder:** Asteranae, **Order:** Gentianales, **Family:** Apocynaceae, **Subfamily:** Apocynoidae, **Tribe:** Malouetieae, **Genus:** *Pachypodium* Lindl. and **Species:** *P. lamerei* Drake.

3. Materials and Methods
3.1 Plant material
The plant of *P. lamerei* Drake were collected in May 2010. Agr. Eng./Tereez Labib (the consultant of plant taxonomy at the Ministry of Agriculture and ex. director of El-Orman Botanical Garden, Giza, Egypt) identified the plant. A voucher specimen was kept in the Herbarium of Pharmacognosy Dept., Faculty of Pharmacy, Minia University, Minia, Egypt. The number of the voucher specimen is (Mn-Ph-Cog-006). The plant material used for botanical study was taken from the fresh samples of stems of *P. lamerei*, as well as from the samples preserved in alcohol (70%)-glycerine-water (1:1:1). The stems of the plant were left for air-drying in shade, reduced to powder appropriate for microscopical inspection and stored in well-closed containers.

3.2 Preparation of samples for microscopical examination
Chloral hydrate, iodine, phloroglucinol, concentrated hydrochloric acid, safranin and light green were used for preparation and dying the plant sections and the powder.

3.3 Microscopic studies
Surface preparations, transverse sections (T.S.) as well as the powder of the leaves were used for observation of various microscopic features**.** Microscopic studies were done by using

microscope with camera, Leica® (Germany) and 8 megapixels digital camera, Samsung (Korea).

4. Results and discussion

4.1 Microscopical characters

P. lamerei has a stout, fleshy, spiny stem resembling that of a cactus [Figures **1** and **2**]. The stem surface is covered with fleshy protuberances arranged spirally [Figure **2**]. At the apex of the stem, each protuberance is seen to be situated in the axil of a leaf. When the leaf fall off, its scar is carried up by the protuberance by subsequent growth at the base of that protuberance [Figure **2**] [7, 8]. Each protuberance ends in three hard and sharp thorns which bend downwords. The two lateral spines are longer than the median one [Figure **2**]. The function of the spines is to store water for the formation of flowers and to reflect the intense sunlight away from the stem. Near the stem base, the spines fall off and their function is probably performed by a thick covering of cork [7, 8]. The stem measures (30-100 cm) in length and (3-6 cm) in diameter. The spines are (1-2.5 cm long) and (1-2 mm wide).

Fig 1: A photo of *Pachypodium lamerei* Drake.

Fig 2: Protuberances of the stem bearing spines and the scars of the leaves (x 0.56).

4.2 Microscopical characters

A transverse section of the stem is nearly circular in outline with projecting spines from the protuberances [Figure **3**]. The stem is mainly composed of parenchymatous ground tissue thus the thickness of the stem is mainly due to bulky pith. The pith consists of large-celled, water-storing parenchyma, in which the vascular bundles and laticiferous elements run in all directions [8]. Different sections were made in different parts of the stem to clarify the anatomical structure of the stem and to identify the differences between these different parts of the stem [Figure **4**].

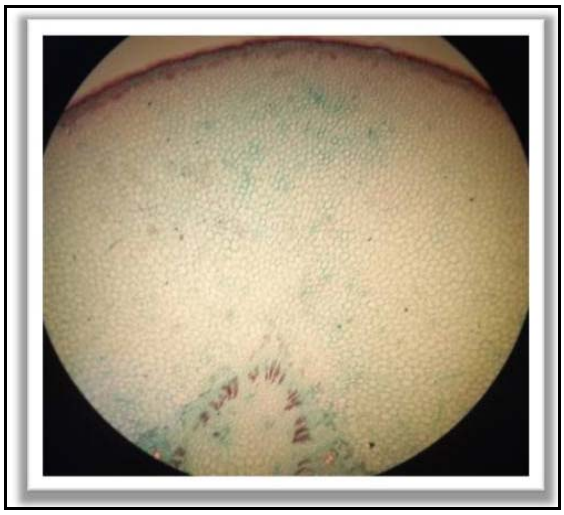

Fig 3: Diagrammatic T.S. of the stem nearly circular in outline with projecting spines from the protuberances (x 15).

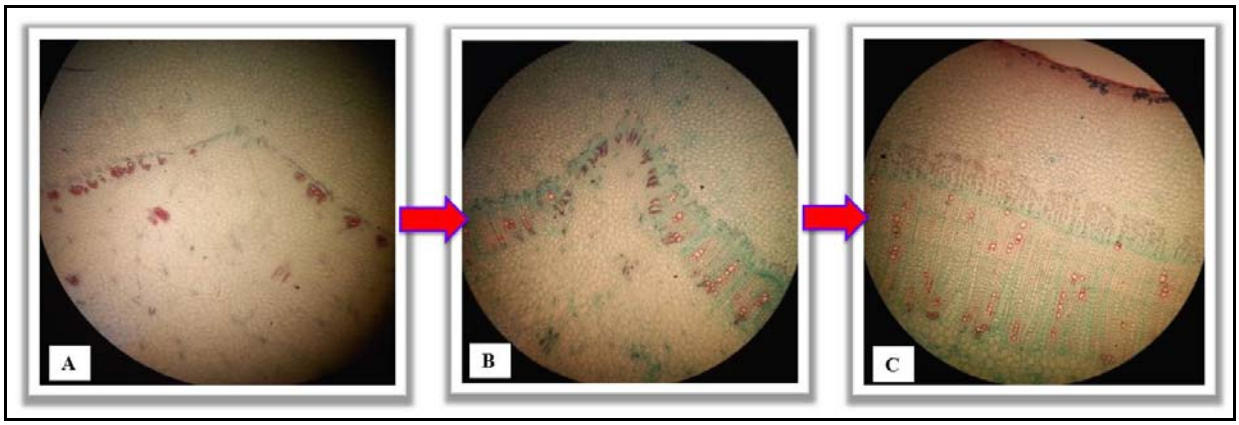

Fig 4: Diagrammatic T.S. of the stem; **A**-Upper part, **B**-Middle part and **C**-Lower part (x 10.6).

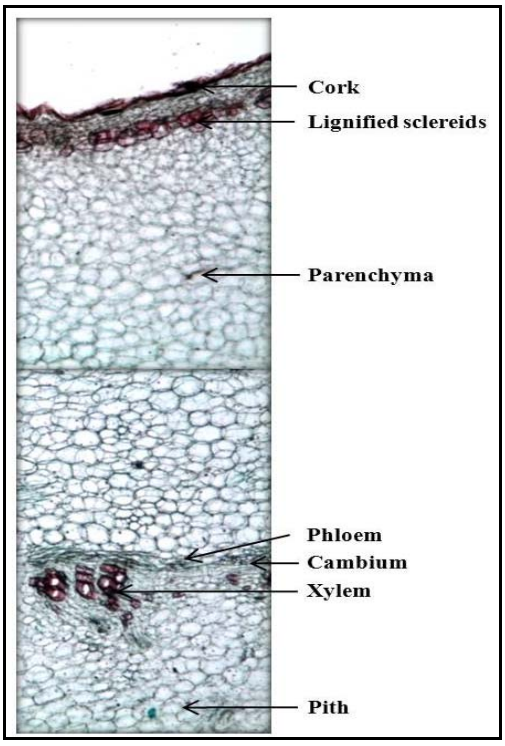

Fig 5: Detailed T.S. of the upper part of the stem (x 40).

4.2.1 Upper part of the stem

A section in the upper part of the stem reveals the presence of a small layer of cork cells surrounding the major proportion of the transverse section of the stem followed by a secondary cortex. The pericycle is parenchymatous and indistinguishable. The vascular bundles are bicollateral and form acute angles in some places. Moreover, scattered auxiliary vascular bundles are present in the ground tissue.

4.2.1.1 The cork

It is formed of 2-3 layers of thin walled polygonal cells [Figures **5** and **6B**]. The cork cells appear in surface view as brown polygonal cells with isodiametric walls as shown in the powder [Figure **12A**].

4.2.1.2 The secondary cortex

It is formed of an outer zone of collenchyma followed by an inner zone of parenchyma. The collenchyma layer is arranged in 2-4 rows of thin walled rounded to oval cells with no intercellular spaces. It shows small lignified sclereids [Figures **5** and **6B**] which become more elongated and aggregated near the zones of spines [Figure **6B**]. Prisms and clusters of calcium oxalate (Caox) and numerous starch granules are also observed in the collenchyma layer [Figure **6B**].

The parenchyma layer is formed of thin walled large, rounded to oval cells with intercellular spaces. Parenchyma cells are filled with numerous rounded starch granules [Figure **6B**] which are simple or aggregated in very large numbers, with a central dashed or cleft hilum and invisible striations as shown in the powder [Figure **12C**]. It is worthy mentioned that,

lignified sclereids are present in large numbers in the parenchyma layer at the positions of the formation of spines [Figure **6A**]. The endodermis is indistinguishable.

4.2.1.3 The vascular tissue
The vascular bundles are bicollateral with intraxylary phloem. The presence of the intraxylary phloem is very characteristic for most of the genera of Apocynaceae [8].

4.2.1.3.1 The pericycle
It is parenchymatous and indistinguishable.

4.2.1.3.2 The secondary phloem
It is narrow and composed of soft cellulosic elements; sieve tubes, companion cells and phloem parenchyma transversed by broad medullary rays. The phloem parenchyma cells are sub-rectangular to polygonal in shape. The medullary rays are uniseriate or biseriate consisting of elongated parenchyma cells [Figure **6D**].

4.2.1.3.3 The cambium
It is formed of about 3-5 rows of thin walled, cellulosic and meristematic cells [Figure **6D**].

4.2.1.3.4 The xylem
It forms a relatively narrow zone composed of lignified xylem vessels, wood fibers and wood parenchyma [Figure **6D**]. The wood fibers are slightly lignified, fusiform with wide lumen and tapering ends as shown in the powder [Figure **12D**].

4.2.1.4 The pith
It comprises the majority of the sector [Figure **4**]. It is formed of large, nearly rounded, water storing, thin walled parenchyma cells containing numerous starch granules, prisms of calcium oxalate and scattered accessory vascular bundles running in all directions[8] [Figures **6E** and **6F**].

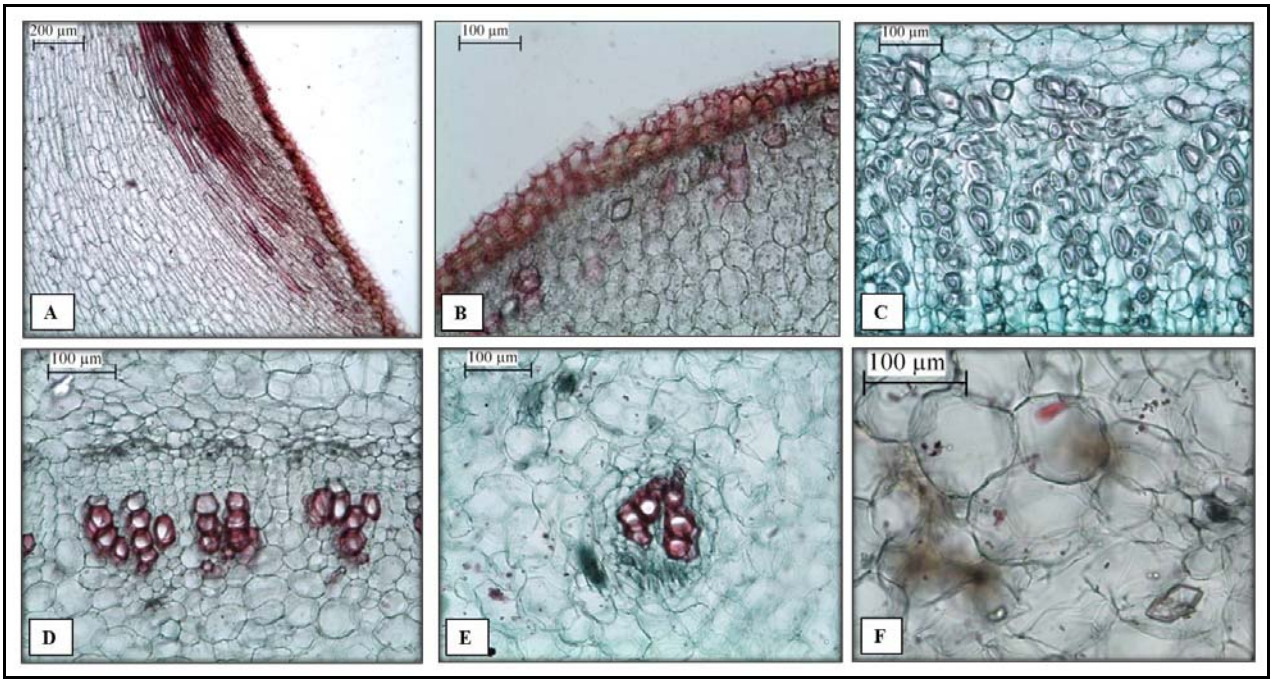

Fig 6: Characteristic microscopical features of the stem; **A-**Elongation of the lignified sclereids at the sites of spines, **B-**Collenchyma cells of the cortex showing lignified sclereids prisms and clusters of Caox, **C-**Laticiferous vessels, **D-**T.S. in the upper part of stem showing the vascular tissue, **E-**Solitary vascular bundles in the pith and **F-**Parenchyma cells of the pith containing prims of Caox. (All x100 except **A** x 40).

4.2.2 The middle part of the stem
A transverse section of the middle part of the stem is nearly circular in the outline with projecting spines from the protuberances [Figure **7**]. It is larger than the upper part of the stem in diameter. The cork is formed and surrounds the whole sector but it appears exfoliated in many areas [Figures **7** and **8**]. The secondary cortex consists of an outer zone of collenchyma cells arranged in 2-4 rows. Many lignified sclereids are observed in the stem's collenchyma either single or in groups [Figures **7** and **8**]. The collenchyma layer is followed by a wide zone of parenchyma. Both the collenchyma and parenchyma layers contain numerous starch granules [Figure **8**]. The pericycle is indistinguishable.

What characterize the transverse section of the middle part of the stem is the appearance of laticiferous vessels at the vascular tissue zone [8] [Figures **8** and **9A**]. The vascular tissue is wider than that present in the upper part of the stem [Figures **8** and **9A**].
The phloem is composed of sieve tubes, companion cells and phloem parenchyma. The cambium is formed of 3-6 rows of thin walled, cellulosic, meristematic cells [Figure **9A**].
The secondary xylem forms a wide zone consisting of xylem vessels, wood parenchyma and wood fibers [Figures **9A** and **9B**]. The xylem vessels are lignified, mainly spiral and reticulate as shown in the powder [Figures **12E** and **12F**]. Wood fibers are elongated, lignified with relatively wide lumena and tapering ends as shown in the powder [Figure **12D**].
The wood parenchyma cells are slightly lignified with pitted walls [Figures **9A** and **9B**]. The secondary xylem is traversed by elongated, non-lignified, biseriate to triseriate medullary rays [Figure **8** and **9**].
The pith is relatively narrower than that of the upper part of the stem. It is formed of large, nearly rounded parenchyma cells containing starch granules and showing accessory

vascular bundles [Figure **7**].

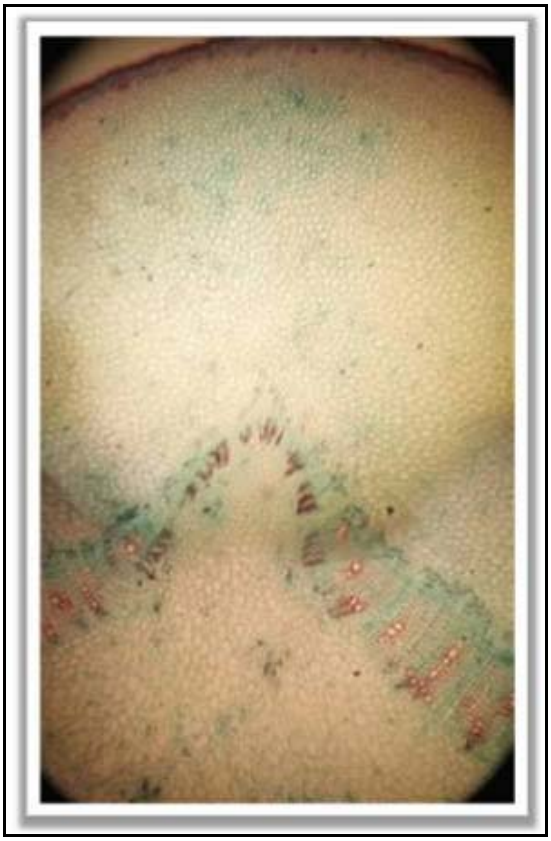

Fig 7: Diagrammatic T.S. of the middle part of the stem (x 23).

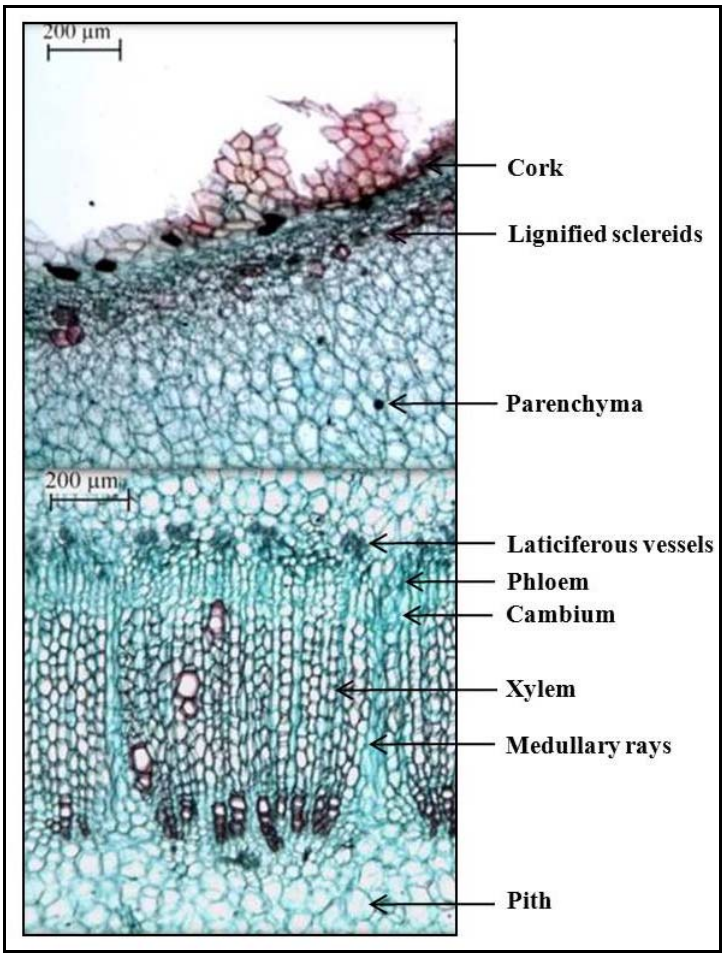

Fig 8: Detailed T.S. of the middle part of the stem (x 40).

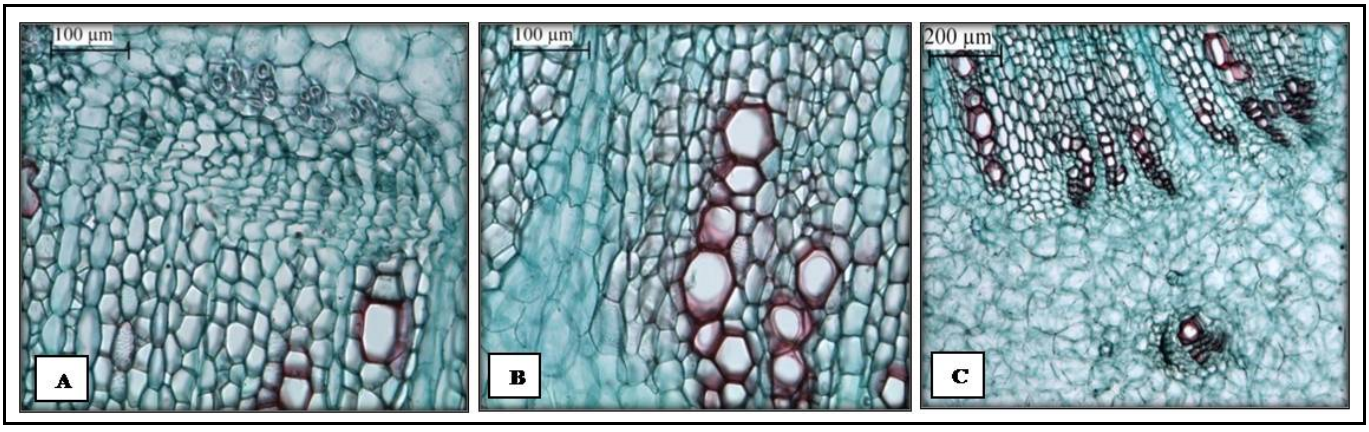

Fig 9: Detailed T.S. of the middle part of the stem showing; **A-**The laticiferous vessels and the vascular tissue, **B-**The xylem vessels, wood parenchyma and medullary rays and **C-**The accessory vascular bundles in the pith. (All x 100 except **C** x 40).

4.2.3 The lower part of the stem

The transverse section of the lower part of the stem largely resembles that of the middle part of the stem. The cork is more exfoliated [Figure **10**]. The cortex constitutes a smaller zone than the middle part. It contains prisms of calcium oxalate and starch granules [Figures **10** and **11**].

4.2.4 The powder of the stem

It is brownish yellow in color showing a hard texture with a faint odor and a slightly bitter taste.

The elements of the powdered stem are shown in [Figure **12**].

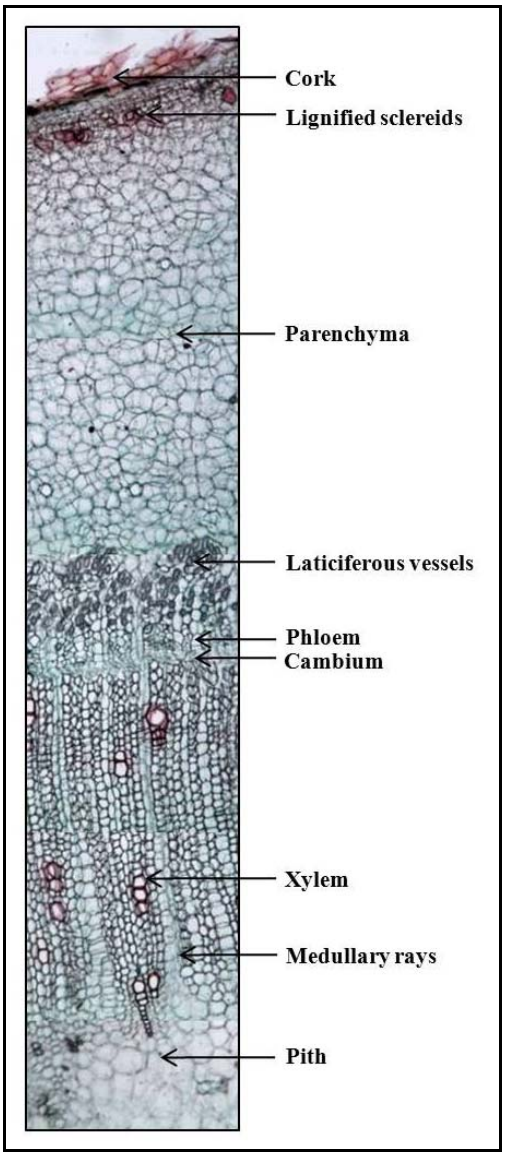

Fig 10: Detailed T.S. of the lower part of the stem (x 40).

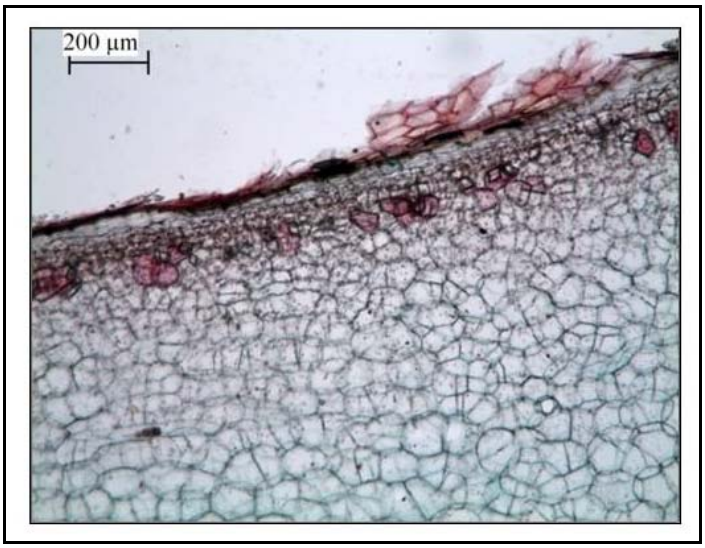

Fig 11: Detailed T.S. of the cortex of the lower part of the stem showing lignified sclereids, starch granules and prisms of Caox (x 40).

Table 1: Microscopical dimensions of different structures of the stem of *P. lamerei* (µm).

Item	Length	Width	Height	Diameter
Cork	36-73-91	36-45-55	5-11-22	
Cortex parenchyma				57-79-86
Sclereids	46-50-88	34-50-81		
Laticiferous vessels	387-419-452			25-33-50
Prisms of Caox	27-36-45			
Cluster crystals of Caox				93-64-43
Starch granules				11-14-18
Wood parenchyma	38-47-63	22-28-31		
Xylem vessels				19-27-31
Wood fibers	177-183-190	10-11-12		
Medullary rays	120-80-60	40-50-70		
Pith parenchyma				80-110-130

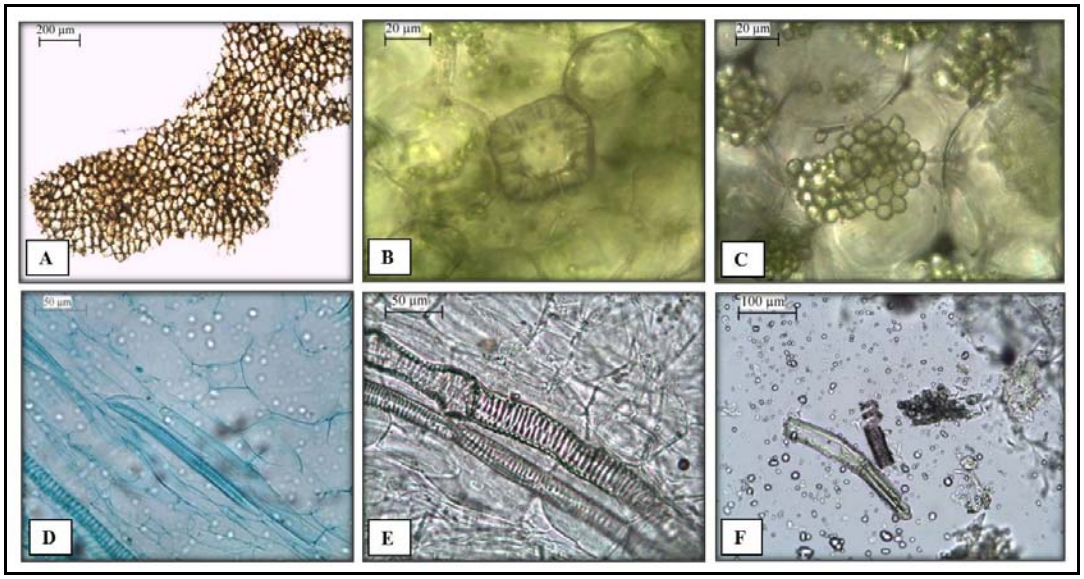

Fig 12: The stem powder; **A-**Cork cells (x 40), **B-**Lignified sclereids (x 400), **C-**Starch granules (x 400), **D-**Wood fibres (x 200), **E-**Reticulate xylem vessels (x 200) and **F-**Spiral xylem vessels (x 100).

5. Conclusion

From over present study entitled "Morphological and Anatomical Studies of the Stem of *Pachypodium lamerei* Drake, Family Apocynaceae, Cultivated in Egypt", the botanical identification could be useful in authentication of the stem. From the taxonomical point of view, it can be used for further pharmacognostical and pharmacological investigations of the plant.

6. Acknowledgement

We specially wish to express our sincere thanks and gratitude to Dr./ Ahmed M. F. Mohamed (Botany & Microbiology Department, Faculty of Science, Assiut University, Assiut, Egypt) for the identification of the botanical characters of the stem.

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