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BOTANICAL AND GENETIC CHARACTERIZATION OF *FICUS CYATHISTIPULA* WARB. GROWING IN EGYPT

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ABSTRACT: *Ficus cyathistipula* Warb. growing in Egypt is an evergreen tree, reaching up to six meters high. The macro- and micromorphological characters of the stem, leaf and fruit of the plant have been studied with the aim to find the diagnostic features and pointed towards their identification in both entire and powdered forms: partly united, leafy and conspicuous stipules, numerous calcium oxalate crystals, collateral vascular bundles with group of intraxylary phloem, mucilage and tannin cells, laticiferous vessels were the most significant. A total of 60 RAPD fragments have been recorded when DNA of the plant under investigation was amplified using eleven decamer primers designed for the genetic characterization. Furthermore, proximate analysis for the leaves was carried out to facilitate the detection of the quality and uniformity of the plant where the results (g %) showed a total ash (13.06), acid insoluble ash (0.58), water soluble ash (12.39), crude fiber (38) and moisture content (47.5).

INTRODUCTION: Family Moraceae is known as the mulberry family or the fig family. It comprises about 40 genera and 1000 species. They are deciduous or evergreen, monoecious or dioecious shrubs, trees, or rarely herbs with milky latex [1]. Some *Ficus* species are grown for their edible fruits referred to as figs, while others for providing shade and as ornamental plants, all of them possess a white to yellowish sap known as latex [2]. *Ficus* comprises about 800 species of deciduous or evergreen trees, shrubs, herbs, climbers and creepers [3, 4].

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Dioecious trees are with male and gall flowers separated from female flowers in different receptacles. The female flowers with short style get consumed by their inhabitant wasp larvae. The long styled female flowers usually do not harbour wasps but produce seeds. In unisexual species, male trees produce no seeds as all female flowers are short-styled and infested by wasp larvae. The wasps entering the female tree syconia are unable to lay eggs as all the flowers are long styled. Thus, the female trees produce seeds [5]. The fruit is produced by the union of the cymose inflorescence to form a hollow, fleshy axis bearing the flowers on its inner surface. The young fruit is rich in latex, while no latex is found in the mature ones and the fleshy axis is filled with sugar [6].

Traditionally, certain *Ficus* species were reported to have hypotensive, antidiabetic, mild laxative, antirheumatic and digestive activities. They have

been used as anti-inflammatory in urinary tract infections and as anthelmintic against intestinal parasites [7]. Medicinally, genus *Ficus* is also well documented for its anti-oxidant, antihepatotoxic, antimicrobial and anticancer activities [8]. *Ficus cyathistipula* Warb. is an ornamental tree widely spread in Tropical Africa [9]. The genus *Ficus* is countries distinguished by its characteristic fruit [10]. Herbal drugs play an important role in health care programs in the developing country, however these drugs aren't accepted because of the lack of standardization, the achievement of the process of standardization can be done by pharmacognostic studies [11]. A literature survey indicated that no data is available on the anatomical studies on the plant under investigation. This study includes the morphological and the anatomical features, as well as the DNA fingerprinting of the plant. Proximate analysis was also done which could be useful criteria in quality control for the identity and purity of the leaves of the plant.

MATERIALS AND METHODS:

Plant material:

Samples of *F.cyathistipula* Warb. were collected during the years 2011-2012 in the fruiting stage from El-Orman Botanical Garden, Giza, Egypt.

Botanical study:

Specimens for morphological studies were dried according to standard herbarium techniques and voucher samples (No. 13.5.2013) were deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. Anatomical investigations were performed on cross-sections of the fresh samples of the young and old stems, the leaf and the fruit and on air-dried finely powdered samples. The photographs were taken by a Leica DFC500 digital camera.

Genetic profiling:

DNA fingerprinting:

Entire fresh leaves of the plant under investigation were freeze-dried and ground to fine powder under liquid nitrogen prior to DNA isolation [12].

DNA extraction:

The frozen leaves (100 mg) were powdered in liquid nitrogen, lysed with 400µl of lysis buffer AP1. Cell debris, proteins and polysaccharides were precipitated by 130µl of buffer AP2. 1.5 volumes of binding buffer AP3/E were added to promote binding of DNA. Contaminants were removed by two wash steps. Pure DNA was eluted in small volume of low-salt buffer or water [13].

Oligonucleotide primers:

A set of 11 primers were used for RAPD analysis: TTCCGAACCC (OPA-15), GACCGCTTGT (OPA-17), CAATCGCCGT (OPA-11), CCACAGCAGT (OPB-18), TTCGAGCCAG (OPC-01), GTCCCGACGA (OPC-07), GTTGCCAGCC (OPC-19), AATCGGGCTG (OPA-04), GTGATCGCAG (OPA-10), CAGCACCCAC (OPA-13), GTTGCGATCC (OPA-20).

Polymerase Chain Reaction (PCR):

The amplification reactions in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2mM dNTPs, 1 µM primer F, 1µM primer R, 1 U Taq DNA polymerase and 25 ŋg template DNA were carried out following a thermal cyclic program. PCR amplification was done in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to complete 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

Gel electrophoresis and staining:

Amplified products were analysed by electrophoresis on 1.5% agarose gel and stained with ethidium bromide. A molecular size marker of 1 Kbp was used as standard marker.

Proximate analysis:

Determination of moisture and ash contents:

Proximate analysis of the leaves was carried out by adopting the procedures of the Association of Official Analytical Chemists (A.O.A.C., 2000) [14]. The determined analytical standards included the total ash, acid- and water-insoluble ashes, crude-fiber and moisture contents. All values were determined gravimetrically and expressed as % w/w with respect to the air-dried material.

RESULTS AND DISCUSSION:

Macromorphological characteristics:

Ficus cyathistipula Warb. is an evergreen, medium-sized tree. It reaches up to 6 m high and about 28 cm in diameter. The fruiting period is from September to December. **Fig. 1** showed **the stem;** the main stem (trunk) is hard, solid, cylindrical, erect with thick brown cork showing longitudinal fissures and transverse cracks (**Fig. 1A and B**). The trunk measures up to 6m in height and about 22-28 cm in diameter. The old branches are cylindrical, grayish brown in color showing rough longitudinally striated surface. The young branches are green in color, 11-15 cm in height and 0.5-1 cm in diameter, cylindrical showing longitudinal striations and break with fibrous fracture. **The leaf** (**Fig. 1B-D**): the leaves are simple, cauline, spirally arranged, green in color, the upper surface is darker than the lower surface, stipulate, glossy, petiolate and leathery. The leaf lamina is obovate to oblanceolate in shape, up to 13-22 cm length and 7.5-8.5 cm width, with entire margin, acuminate apex and symmetric base. The surface is glabrous, showing reticulate pinnate venation with the midrib prominent to the lower surface. Lateral veins are 5-8 pairs, widely spaced, anastomosing near the margin.

The stipules are partly united, leafy, conspicuous and persistent up to 2 cm (**Fig. 1E**). The petiole is short, measures 1.5-4 cm in length and 3-4 mm in diameter, pale green, glabrous and cylindrical. **The Fruit** (**Fig. 1F and G**)**:** the figs (syconia) are axillary, solitary, in pairs or in threes, pedunculate, globose to obovoid, rough or warty to touch, green in color, and measures up to 3.5 cm in diameter. The length of the peduncle reaches 2-2.5 cm and 0.2-0.3 cm in diameter. Transverse and longitudinal cuts showed hollow and fleshy axis bearing flowers on its inner surface (**Fig. 1H and I**).

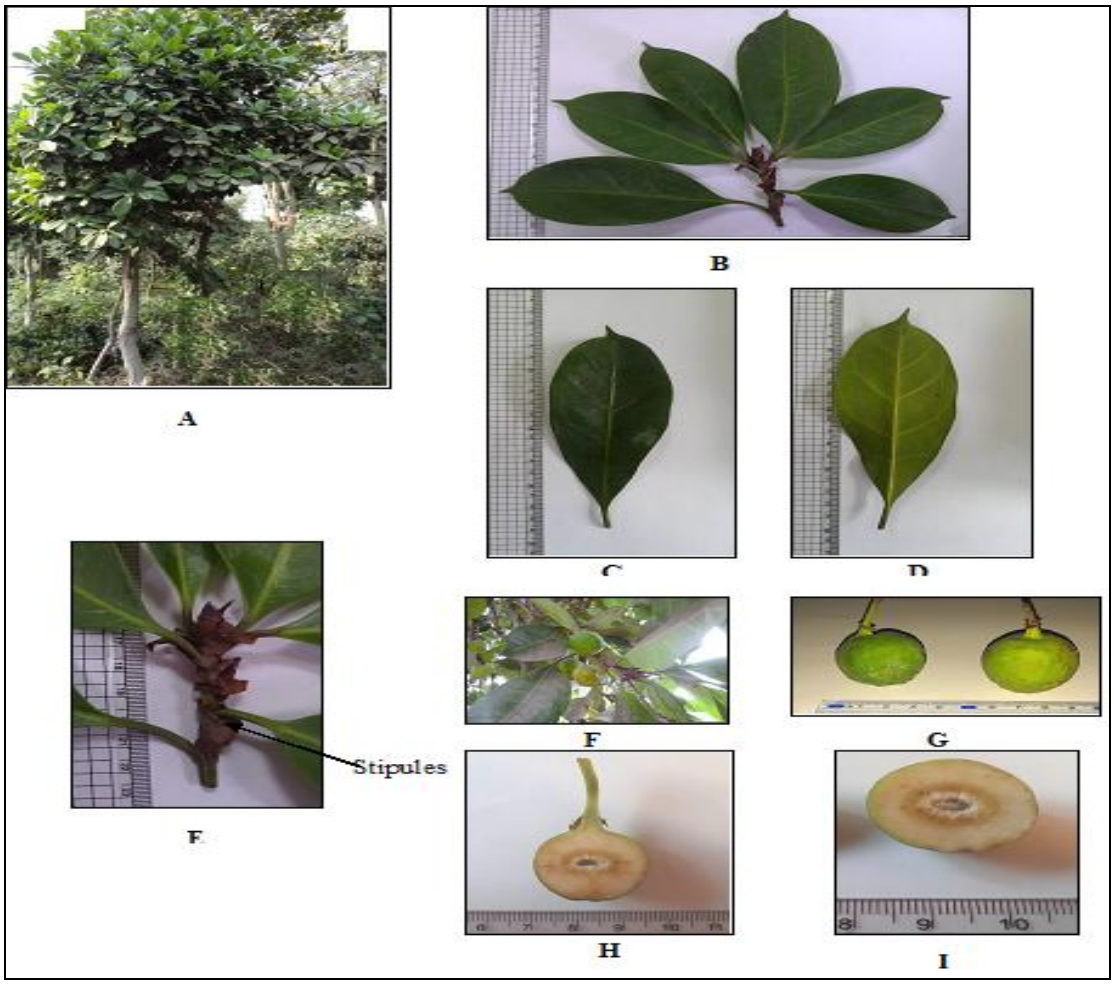

FIG.1: PHOTOGRAPHS OF THE TREE, BRANCH, LEAVES AND FRUITS OF *FICUS CYATHISTIPULA* WARB. (A) Photograph of the tree (X 0.008). (B) Branch bearing the leaves (X 0.157). (C) Upper surface of the leaf (X 0.143). (D) Lower surface of the leaf (X 0.156). (E) Stipules (X 0.37). (F) Fruiting branch (X 0.069). (G) Entire fig (X 0.342). (H) L.cut in fig (X 0.375). (I) T.cut in fig (X 1.04).

Micromorphological characteristics:

The young stem A transverse section in young stem as shown in **Fig. 2**, is more or less circular in outline. It shows an epidermis followed by a parenchymatous hypodermis then the cortex. The endodermis is indistinguishable. The pericycle is formed of parenchyma interrupted by groups of lignified fibres surrounding an almost complete ring of vascular tissue traversed by uni to pleuriseriate medullary rays and enclosing a wide parenchymatous pith with numerous scattered prisms, cluster crystals of calcium oxalate crystals and starch granules. **The epidermis** (**Fig. 2A-C** and **4**): It consists of polygonal, isodiametric or axially elongated, rectangular thick-walled cells having straight anticlinal walls covered with smooth cuticle. Some epidermal cells contain prisms and cluster crystals of calcium oxalate. Rare stomata of anomocytic type. Trichomes are absent. **The hypodermis** (**Fig. 2A** and **B**): The hypodermis consists of 1-2 rows of thin-walled parenchyma cells showing prisms, cluster of calcium oxalate crystals. Some cells contain mucilage (stained red with ruthenium red T.S.).

The cortex (**Fig. 2A** and **B**): It is formed of several rows; the first row consists of parenchyma cells, each showing a prism of calcium oxalate crystal, followed by 2-3 rows of sclereids of nearly polygonal, isodiametric or axially elongated cells rectangular in shape with pitted lignified walls and wide lumina followed by 7-8 rows of collenchyma and several rows of parenchyma cells abutting the lower epidermis. Some parenchyma cells contain starch granules (stained blue with iodine T.S.), mucilage (stained red with ruthenium red T.S.) and show large intercellular spaces. The endodermis is indistinct.

The pericycle (Fig. 2A, B and **4**): It consists of groups of lignified fibres interrupted by parenchyma showing prisms or cluster crystals of calcium oxalate. The fibres are fusiform in shape with straight and/or undulated walls, narrow or wide lumina and rounded apices. **The vascular tissue** (**Fig. 2A, B** and **4**): It is formed of an almost complete ring of collateral vascular bundle, separated by cambium which is almost indistinguishable and traversed by uni to pleuriseriate medullary rays showing prisms of calcium oxalate crystals. The phloem consists of soft tissues of sieve tubes, companion cells and thin walled phloem parenchyma containing prisms or cluster crystals of calcium oxalate with no fibres. Laticiferous vessels are present in the phloem region (stained brown with iodine T.S.). The xylem is formed of a continuous ring of lignified spiral and annular vessels enclosing central parenchyma that contain groups of intraxylary phloem. Wood fibres are short with straight lignified wall, wide lumina and rounded apices. Wood parenchyma consist of cells with pitted lignified walls. The cambium is almost indistinguishable.

The pith (**Fig. 2A** and **B):** The pith is relatively wide, consists of large rounded parenchymatous cells with thick, lignified, pitted walls showing prisms and cluster crystals of calcium oxalate, tannin cells (stained green with ferric chloride) and starch granules (stained blue with iodine T.S).

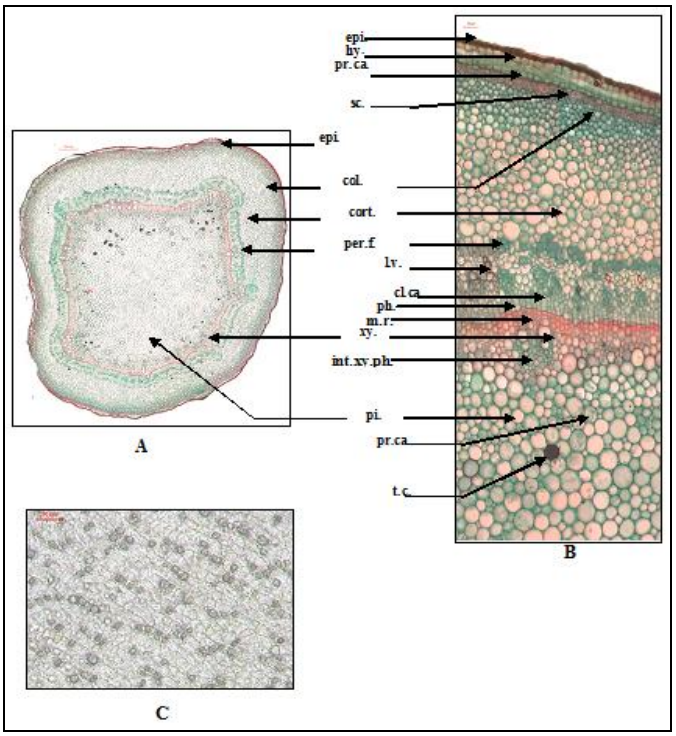

FIG.2: MICROMORPHOLOGY OF THE YOUNG STEM OF *FICUS CYATHISTIPULA* WARB. A) Low power of the young stem (X 5). (B) High power of the young stem (X 50). (C) Surface preparation of the young stem (X 116.6).cl.ca., cluster of calcium oxalate; col., collenchyma; cort., cortex; epi., epidermis; hy., hypodermis; int.xy.ph., intraxylary phloem; l.v., laticiferous vessel; m.r., medullary ray; per.f., pericyclic fibre; ph., phloem; pi., pith; pr.ca., prism of calcium oxalate; sc., sclereid; t.c., tannin cell; xy.v., xylem vessel.

The old stem: A transverse section in the old stem as shown in **Fig. 3**, closely resembles that of the young stem except in the following:

1) Presence of cork (**Fig. 3C**) that consists of 4-6 rows of brown tangentially elongated, radially arranged cells having thick straight suberized walls, followed by 1-2 rows of flattened parenchyma cells of the secondary cortex.
2) The vascular tissue is wider. It is formed of a continuous ring of collateral vascular bundle, separated by cambium that is formed of 2-3 rows of tangentially elongated thin walled cellulosic cells and traversed by straight uni- to pleuriseriate medullary rays showing prisms of calcium oxalate crystals. The xylem is formed of lignified spiral, annular and pitted vessels.
3) The pith is narrower.

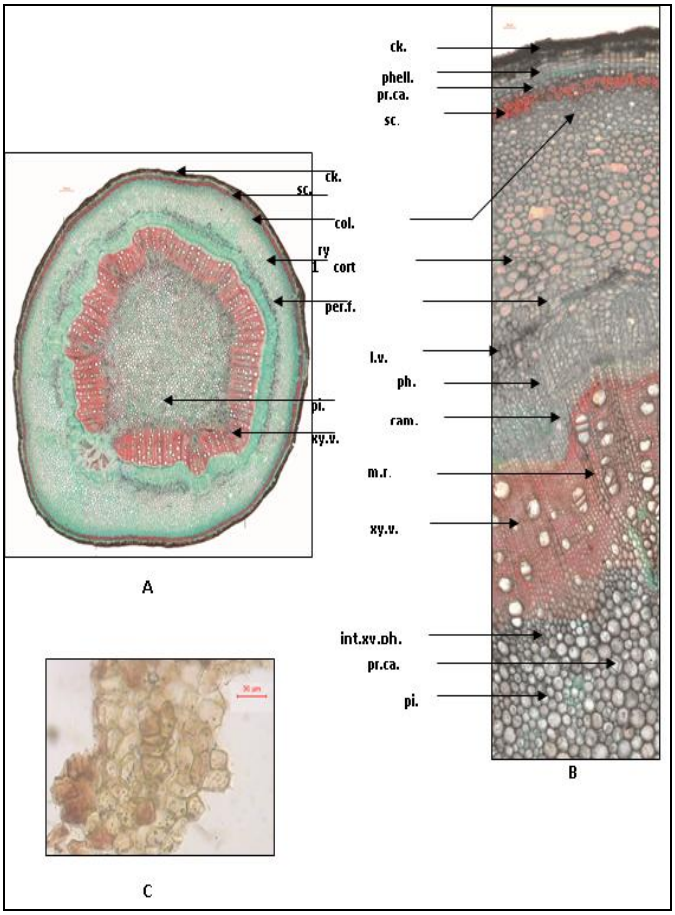

FIG.3: MICROMORPHOLOGY OF THE OLD STEM OF *FICUS CYATHISTIPULA* WARB. (A) Low power of the old stem (X 10). (B) High power of the old stem (X 50). (C) Surface view of the cork (X 150). ck., cork; ck.cam., cork cambium; col., collenchyma; 1ry cort., primary cortex; l.v., laticiferous vessel; m.r., medullary ray; per.f., pericyclic fibre; phell., phelloderm; ph., phloem; pi., pith; pr.ca., prism of calcium oxalate; sc., sclereid; xy.v., xylem vessel.

Powdered stem is illustrated in **Fig. 4**: The powder is yellowish-brown in color, odorless with astringent taste. It is characterized microscopically by the following features:

1) Fragments of epidermal cells polygonal, isodiametric or axially elongated with straight thick anticlinal walls and showing prisms and cluster of calcium oxalate crystals. Rare anomocytic stomata are present.
2) Fragments of thick walled cork cells showing brownish contents.
3) Fragments of lignified pericyclic fibres with straight or undulated walls showing narrow or wide lumina and acute apices.
4) Numerous sclereids of polygonal, isodiametric or axially elongated, rectangular cells with thick, pitted lignified walls.

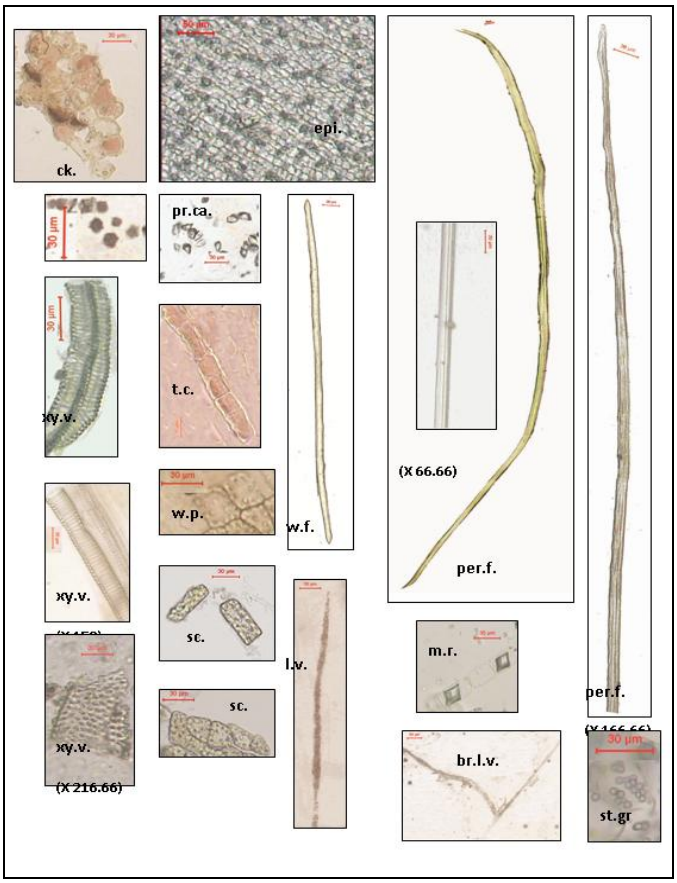

FIG.4: POWDERED STEM. br.l.v., branched laticiferous vessel (X 100); ck., cork (X 116.6) cl.ca., cluster of calcium oxalate (X 200) ; ep., epidermal cell (X 150) ; l.v., laticiferous vessel (X 150); m.r., medullary ray (X 100); per.f., pericyclic fibre; pr.ca., prism of calcium oxalate (X 100); st.gr., starch granule (X 400); sc., sclereid (X 133.33); t.c., tannin cell (X 66.67); w.f., wood fibre (X 66.67); w.p., wood parenchyma (X 166.67); xy.v., xylem vessel (X 66.67).

5) Fragments of lignified xylem vessels having spiral, annular and pitted thickenings.
6) Numerous scattered prisms and cluster crystals of calcium oxalate.
7) Fragments of wood parenchyma having pitted lignified walls.
8) Fragments of medullary rays showing scattered prisms of calcium oxalate.
9) Fragments of lignified wood fibres with straight walls having wide lumina and more or less rounded apices.
10) Fragments of parenchyma containing tannins cells.
11) Parenchyma cells containing starch granules.

The leaf: A transverse section through the leaf lamina and midrib shows upper and lower epidermises enclosing in between dorsiventral mesophyll as presented in **Fig. 5**. The mesophyll is composed of 1-3 rows of palisade cells on the upper side. The palisade is continuous over the midrib region. There is a hypodermis of parenchyma cells abutting the upper epidermis. The spongy tissues are loosely arranged. The midrib is prominent on the lower surface and nearly flat on the upper one and exhibits a main crescent-shaped collateral vascular bundle accompanied by several inverted smaller ones enclosing central parenchyma that contains groups of intraxylary phloem. The cortical tissue is represented by 2-3 rows of sclereids of nearly polygonal, rectangular, isodiametric to slightly elongated in shape with lignified walls and wide lumina abutting the lower epidermis followed by collenchyma of 14-17 rows abutting the lower epidermis and followed by parenchyma. The vascular system is surrounded by a pericycle formed of groups of lignified fibres interrupted by parenchyma.

The epidermis (**Fig. 5A and B**): The upper epidermal cells in surface view (**Fig. 7A**) are polygonal, nearly isodiametric, with straight, thick anticlinal walls, covered with smooth cuticle. Stomata and trichomes are absent. The upper epidermis shows radiating cells (5-6 cells) surrounding the site of cystolith. The lower epidermal cells (**Fig. 7B** and **C**) are similar in shape to the upper surface and characterized by sunken anomocytic type surrounded by 4-6 subsidiary cells. Some epidermal cells show cluster or prisms of calcium oxalate crystals. The neural epidermal cells (**Fig. 7D and F**) are polygonal, isodiametric to slightly elongated. Trichomes and stomata are absent from both neural epidermises.

The hypodermis (**Fig. 5B, C** and **D**): It consists of 2-5 rows of polygonal small parenchyma cells situated just beneath the upper epidermis. Some hypodermal cells show prisms, cluster of calcium oxalate crystals and a large stalked elongated cystolith of calcium carbonate (dissolved in HCL with effervescence) (**Fig. 5D**). Some hypodermal cells contain mucilage (stained red with ruthenium red T.S.). **The mesophyll** (**Fig. 5C and D**): It shows a layer of palisade abutting the upper hypodermis and continuous along the midrib region. The palisade cells are arranged in 1-3 rows; they are columnar, cylindrical and thin-walled cells containing chloroplast. The spongy tissues are loosely arranged.

The midrib (**Fig. 5B**): The cortical tissue of the midrib consists of 2-3 rows of sclereids of polygonal, nearly isodiametric rectangular cells with lignified walls and wide lumina abutting the lower epidermis, then 14-17 rows of collenchymatous cells. This is followed by more or less rounded, thin-walled parenchymatous cells. Some parenchyma cells contain tannin (stained green with ferric chloride T.S.), prisms and cluster crystals of calcium oxalate and mucilage (stained red with ruthenium red T.S.).

The vascular tissue: consists of a collateral crescent vascular bundle separated by cambium, traversed by uni- and biseriate medullary rays and surrounded by groups of lignified pericyclic fibres interrupted by parenchymatous cells. The vascular tissue is accompanied by several inverted smaller ones enclosing central parenchyma that contain groups of intraxylary phloem. Laticiferous vessels are observed (stained brown with iodine T.S.) in the phloem region. The phloem composed of soft phloem elements and traversed by uni and biseriate medullary rays consisting of thin-walled parenchymatous, somewhat radially elongated cells. The xylem composed of lignified vessels, wood fibres and wood parenchyma and is traversed

by uni and biseriate medullary rays containing prisms crystals of calcium oxalate. The xylem vessels are arranged in radial rows and show spiral and annular thickenings.

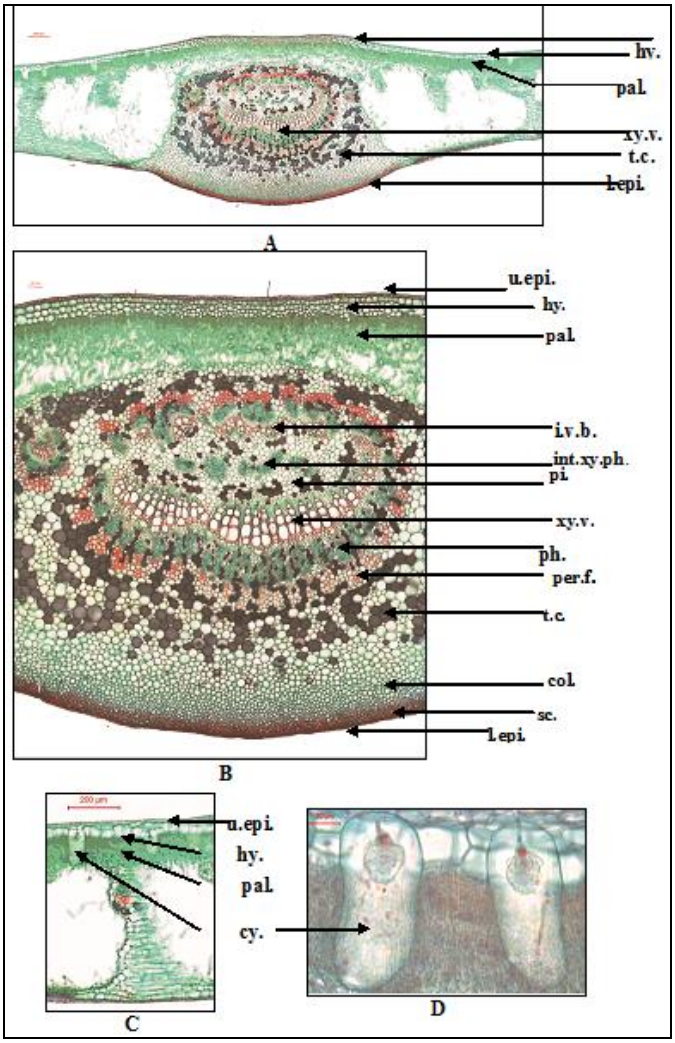

FIG.5: MICROMORPHOLOGY OF THE LEAF OF *FICUS CYATHISTIPULA* WARB. (A) Low power view of the leaf (X 15). (B) High power view of the leaf (X 40). (C) High power view of the leaf lamina (X 32.5). (D) Cystolith (X 150). col., collenchyma; hy., hypodermis; i.v.b., inverted vascular bundle; int.xy.ph., intra xylary phloem; l.epi., lower epidermis; pal., palisade; per.f., pericyclic fibre; ph., phloem; pi., pith; sc. sclereid; t.c.; tannin cells; u.epi., upper epidermis; xy.v., xylem vessel.

The petiole: A transverse section in the petiole as shown in **Fig. 6** is more or less circular in outline. **The epidermis** (**Fig. 6A, B and 7D**): It is formed of polygonal, isodiametric cells with straight thick anticlinal walls and covered with smooth cuticle showing prisms and cluster of calcium oxalate crystals, devoid of stomata and trichomes. **The hypodermis** (**Fig. 6A and B**): It is formed of one row of thin-walled parenchyma cells. Some hypodermal cells show prisms and cluster of calcium oxalate crystals and mucilage. **The cortex** (**Fig. 6A** and **B**): It is formed of several layers, the first 1-2 rows are polygonal nearly isodiametric sclereids with pitted lignified walls and wide lumina, followed by 12-14 rows of collencyma, followed by several rows of parenchyma cells with scattered cluster and prismatic of calcium oxalate crystals. The endodermis is indistinct. **The pericycle** (**Fig. 6A** and **B**): It is parenchymatous. Vascular bundle is crescent-shaped, collateral accompanied by inverted smaller ones enclosing central parenchyma that contains groups of intraxylary phloem. The xylem is traversed by uni to pleuriseriate medullary rays showing prismatic crystals of calcium oxalate, arranged in radial rows showing spiral and annular thickenings. The phloem is formed of thin-walled cellulosic, soft phloem elements.

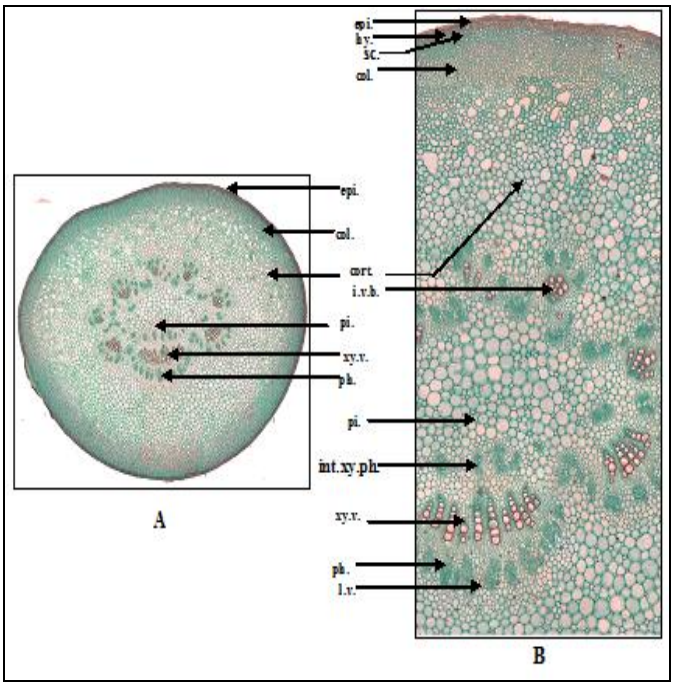

FIG. 6: MICROMORPHOLOGY OF THE PETIOLE OF *FICUS CYATHISTIPULA* WARB. (A) Low power view of the petiole (X 10). (B) High power view of the petiole (X 16.6). col., collenchyma, cort., cortex; epi., epidermis; hy., hypodermis; i.v.b., inverted vascular bundle; l.v., laticferous vessel; int.xy.ph., intraxylary phloem phloem; pi., pith; ph., phloem; sc., sclereid; xy.v., xylem vessel.

Powdered leaf:

The powder is green in color with characteristic odor and astringent taste. It is microscopically characterized by the following features, as illustrated in **Fig. 7**

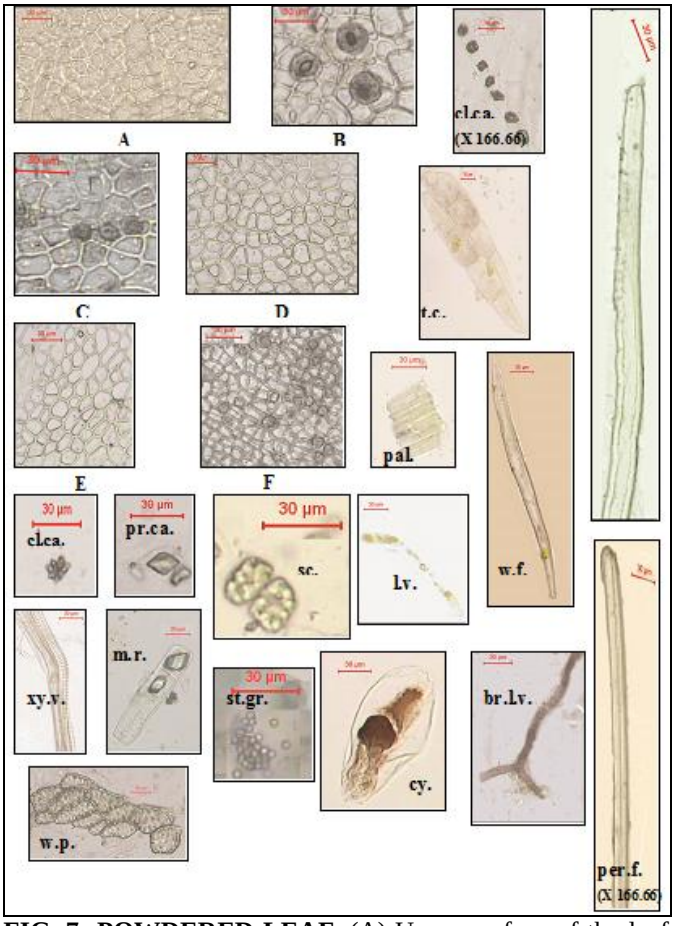

FIG. 7: POWDERED LEAF. (A) Upper surface of the leaf (X 133.33). (B) Lower surface of the leaf (X 166.66). (C) Lower surface of the leaf (X 200). (D) Upper neural epidermis (X 133.33). (E) Lower neural epidermis(X 133.33). (F) Petiole epidermis (X 150). br.l.v., branched laticiferous vessel (X 116.66)); cl.ca., cluster of calcium oxalate; cy., cystolith (X 133.33); l.v., laticiferous vessel (X 100); m.r., medullary ray (X 100); pal., palisade (X 150); per.f., pericyclic fibre; pr.ca., prism of calcium oxalate (X 216.6); sc., sclereid of the petiole (X 333.33); st.gr., starch granule (X 283.33); t.c., tannin cell (X 66.67); w.f., wood fibre (X 100); w.p., wood parenchyma (X 83.33); xy.v., xylem vessel (X 100).

1) Fragments of the upper epidermis of the lamina showing polygonal, nearly isodiametric, with anticlinal walls, covered with thick, smooth cuticle and devoid of stomata. The upper epidermis shows radiating cells (5-6 cells) surrounding the site of cystolith.
2) Fragments of the lower epidermis of the lamina which are closely similar to the upper being characterized by anomocytic type surrounded by 4-6 subsidiary cells.
3) Fragments of the neural epidermis showing polygonal, axially elongated cells.
4) Fragments of the palisade cells which are columnar and thin-walled.
5) Fragments of xylem vessels with spiral and annular thickenings.
6) Fragments of medullary rays showing prisms of calcium oxalate crystals. Cluster crystals and prisms of calcium oxalate scattered in parenchymatous cell.
7) Cluster crystals and prisms of calcium oxalate scattered in parenchymatous cells.
8) Fragments of lignified pericyclic fibres with straight and undulated walls, narrow or wide lumina and rounded apices.
9) Fragments showing lignified wood parenchyma which are thick, pitted and with lignified walls.
10) Branched and simple laticiferous vessels.
11) Cystolith of calcium carbonate.
12) Fragments of epidermal cells of petiole showing polygonal nearly isodiametric with thick straight anticlinal walls covered with smooth cuticle and devoid of stomata showing cluster and prisms crystals of calcium oxalate.
13) Fragments of sclereids which are polygonal, nearly isodiametric rectangular cells with thick lignified walls.
14) Fragments of wood fibres with straight walls, wide lumina and rounded apices.
15) Parenchyma cells containing starch granules.

The fruit: A transverse section in the fruit is more or less circular in outline, as presented in **Fig. 8**. It shows epidermis followed by wide parenchymatous cortical tissue which is traversed by some collateral vascular bundles, laticiferous vessels and scattered tannin cells. Also, prisms and cluster crystals of calcium oxalate are scattered in the cortical tissue. **The epidermis** (**Fig. 8A, B** and **10A**): It consists of polygonal, isodiametric or axially elongated cells with straight thick anticlinal walls, covered with smooth cuticle showing rare sunken anomocytic stomata. The trichomes are non-glandular, unicellular, thick-walled with acute apex and wide lumen.

The wide parenchyma (**Fig. 8A-D**): The parenchyma are made up of large, irregular cells forming the great part of the receptacle showing

cluster and prisms of calcium oxalate and interspersed with numerous laticiferous vessels (**Fig. 8C**) (stained brown with iodine T.S.), tannin cells (stained green with ferric chloride solution), mucilage cells (stained red with ruthenium red T.S.) and collateral vascular bundles (**Fig. 8D**).

The fruit stalk*:* A transverse section in the fruit stalk appears in **Fig. 9** more or less circular in outline and is nearly similar to that of the stem with the following differences:

1) The epidermal cells are polygonal, axially elongated (**Fig.10B**) with straight anticlinal walls showing trichomes which are non-glandular, unicellular, thick-walled with acute apices and wide lumina followed by the cortex, the first rows showed some scattered polygonal, isodiametric rectangular lignified sclereids with wide lumen.
2) No pericyclic fibres.

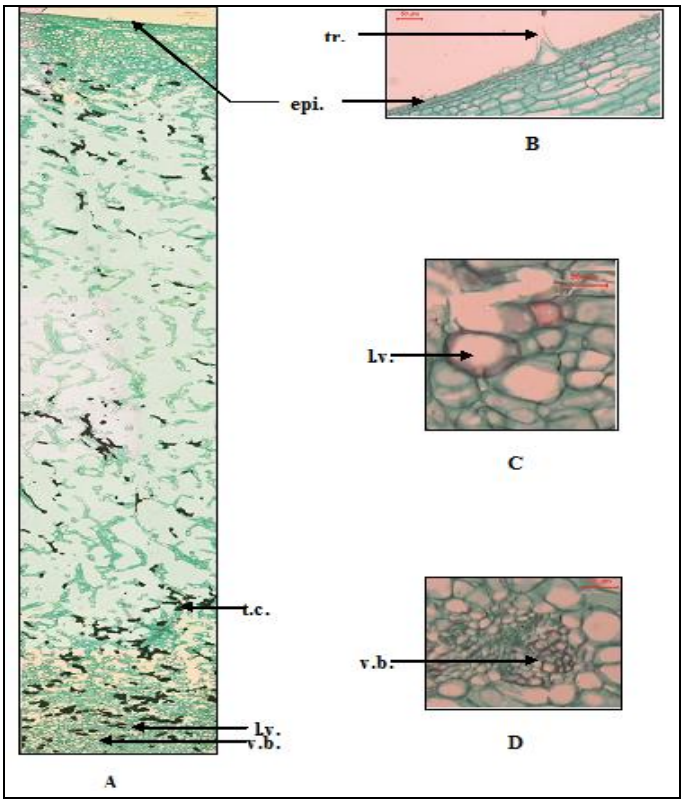

FIG.8: MICROMORPHOLOGY OF THE FRUIT OF *FICUS CYATHISTIPULA* WARB. (A) Low power view of the fruit (X 20). (B) Part of the high power view showing the epidermis and the trichome (X 60). (C) Part of the high power view showing laticiferous vessels (X 216.6). (D) Part of the high power view showing vascular bundle (X 166.66). epi., epidermis; l.v., laticiferous vessel; t.c., tannin cell; tr., trichome; v.b., vascular bundle.

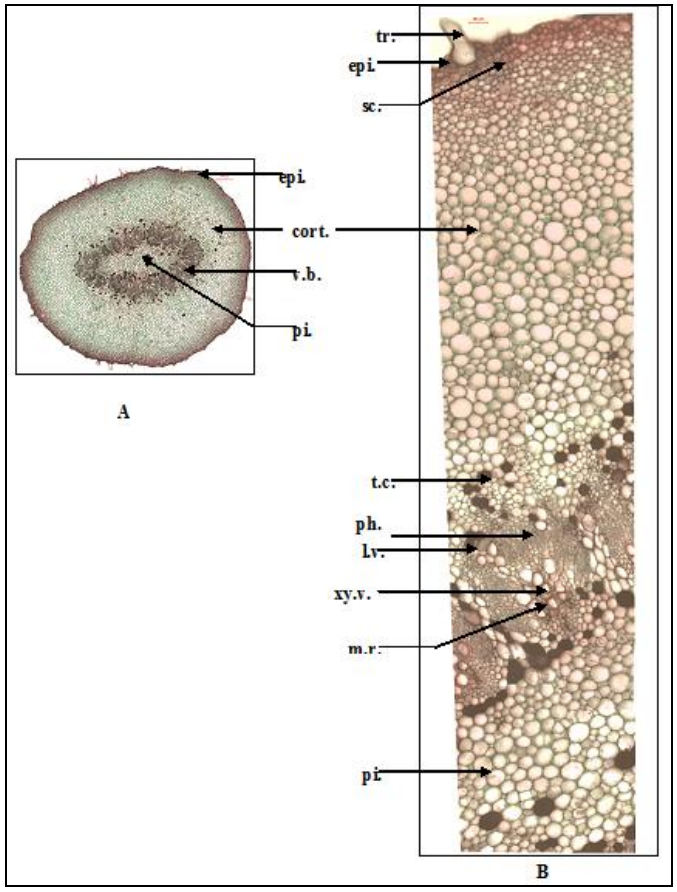

FIG.9: MICROMORPHOLOGY OF THE FRUIT OF *FICUS CYATHISTIPULA* WARB (A) Low power view of the stalk (X 12.5). (B) High power view of the stalk (X 83.33). cort., cortex; epi., epidermis; l.v., laticiferous vessel; m.r., medullary ray; ph.,phloem; pi., pith; sc., sclereid; t.c., tannin cell; tr.,trichome; v.b., vascular bundle; xy.v., xylem vessel..

Powdered fruit: The powder is pale green in color with characteristic odor and astringent mucilaginous taste. It is microscopically characterized by the following features, as illustrated in **Fig. 10**

1) Fragments of the epidermis of the fruit showing polygonal, isodiametric or axially elongated cells with straight thick anticlinal walls, covered with smooth cuticle showing rare sunken anomocytic stomata. The trichomes are non-glandular, unicellular, and thick-walled with acute apices and wide lumina.
2) Fragments of xylem vessels with spiral, annular and pitted thickenings.
3) Fragments of medullary rays.

4) Cluster crystals and prisms of calcium oxalate scattered in parenchymatous cells.
5) Branched and simple laticiferous vessels.
6) Fragments of sclereids of the stalk which are polygonal isodiametric with thick lignified walls.
7) Parenchyma cells containing starch granules.
8) Fragments of parenchyma containing tannin cells.
9) Fragments of the epidermal cells of the stalk showing polygonal axially elongated cells.
10) Non-glandular, unicellular, thick-walled trichomes with acute apices and wide lumina.

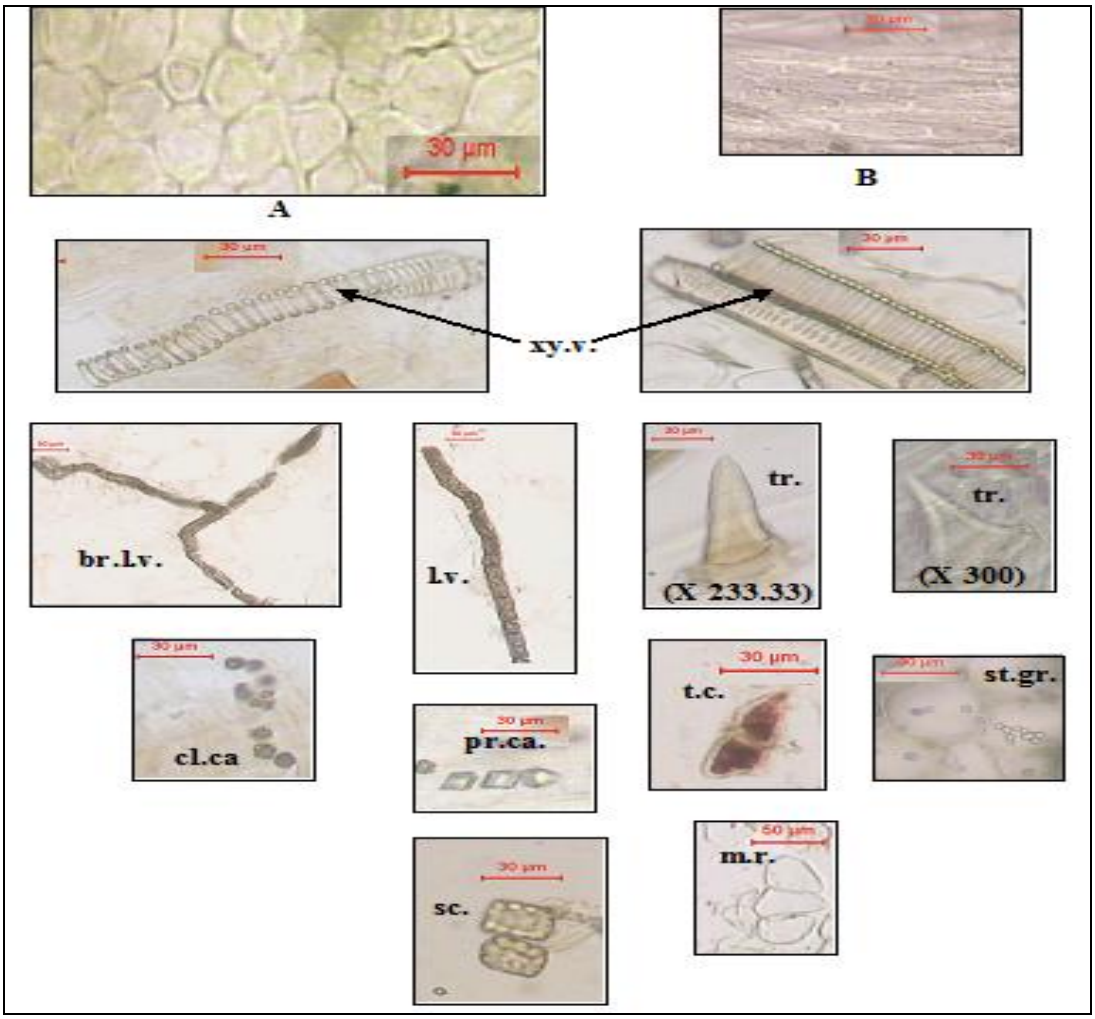

FIG.10: POWDERED FRUIT. (A) epidermis of the fruit (X 483.33).(B) epidermis of the stalk (X 333.33). br.l.v., branched laticiferous vessel (X 150); cl.ca., cluster of calcium oxalate (X 333.33); epi., epidermal cell of the fruit (X 433.33); l.v., laticiferous vessel (X 150); m.r., medullary ray (X 180); pr.ca., prism of calcium oxalate (X 316.66); sc., sclereid (X 333.33), st.ep., stalk epidermis (X 333.33); st.gr., starch granule (X 333.33); t.c., tannin cell (X 416.66); tr., trichome; xy.v., xylem vessel (X 333.33)..

The microscopical measurements of the different elements in microns are listed in **Table 1**.

TABLE 1: MICROSCOPICAL MEASUREMENTS OF THE ELEMENTS OF THE LEAF, STEM AND FRUIT OF *FICUS CYATHISTIPULA* WARB. (IN MICRONS)

Element	Length	Width	Diameter	Height
1- The Stem				
Cork	15-20-22	20-22-30		20-23-30
Epidermal cells	9-12-18	6-15-18		19-26-33
Calcium oxalate cluster			5-7-9	

Calcium oxalate prism	9-12-15	9-12-15		
Pericyclic fibres	800-1400-1950	12-15-30		
Wood fibres	600-750-900	10-12-15		
Wood parenchyma	23-25-30	13-20-23		
Xylem vessels			24-33-68	
Tannin cells	30-48-54	21-24-30		
Laticiferous vessels			5-10-13	
Branched laticiferous vessels			6-9-12	
Medullary rays	30-35-42	28-30-35		
Sclereids	50-52-55	15-21-25		
Starch granules			2-3-5	
2- The leaf				
Upper epidermis	16-18-23	12-14-19		6-12-24
Lower epidermis	12-21-25	12-16-18		6-12-24
Upper neural epidermis	7-9-13	5-8-9		6-12-24
Lower neural epidermis	15-25-30	10-12-17		6-12-24
Petiole epidermis	5-11-15	7-11-14		10-20-30
Palisade cells	38-41-43	4-7-8		
Stomata	20-23-25	20-23-25		
Calcium oxalate prismatic	8-11-13	5-10-19		
Calcium oxalate cluster			9-12-15	
Medullary rays	36-51-60	17-24-30		
Pericyclic fibres	276-456-500	13-16-18		
Wood fibres	270-312-360	6-15-18		
Wood parenchyma	22-30-45	45-60-75		
Xylem vessels			6-9-12	
Tannin cells	70-80-90	25-45-50		
Laticiferous vessels			6-9-12	
Branched laticiferous vessels			5-7-10	
Sclereids	18-20-24	13-15-16		
Cystolith	132-192-214	64-72-90		
Starch granules			2-4-5	
3- The fruit				
Epidermal cells	9-12-14	14-20-23		5-10-15
Stalk epidermis	25-33-45	4-9-12		6-10-13
Calcium oxalate prisms	9-11-13	12-15-18		
Calcium oxalate cluster				6-7-9
Xylem vessels			15-18-23	
Laticiferous vessels			20-22-27	
Branched laticiferous vessels			16-19-22	
Trichomes	96-98-101	24-27-31		
Starch granules			1-3-5	
Tannin cells	20-22-25	15-17-22		
Medullary rays	17-22-36	33-37-50		
Sclereids	24-27-30	12-18-21		

Genetic profiling:

DNA fingerprinting:

The banding profile produced by the eleven decamer primers used in RAPD analysis of *Ficus cyathistipula* Warb. showed distinguishable bands and illustrated in **Fig. 11** and **Table 2**. A total of 60 different RAPD fragments have been recorded showing 8 bands by each of OPA-11 and OPA-04 primers ranging from (1.25-0.28 Kbp) and (2-0.3 Kbp), respectively, 7 bands by OPC-07 primer ranging from (1.1- 0.42 Kbp), 6 bands by each of OPA-15 and OPA-17 primers ranging from (1.25-0.42 Kbp) and (1.5-0.28 Kbp), respectively, 5 bands by each of OPB-18 and OPC-19 primers ranging from (1.25- 0.45 Kbp) and (1.6-0.55 Kbp), respectively, 4 bands by each of OPC-01, OPA-13 and OPA-20 primers ranging from (1- 0.4 Kbp), (1.1-0.58 Kbp) and (0.7-0.23 Kbp), respectively and 3 bands by OPA-10 primer (1.5-0.7 Kbp). The analysis of RAPD data, under the experimental conditions, can thus be helpful in the control analysis for differentiation and quality assurance of the plant.

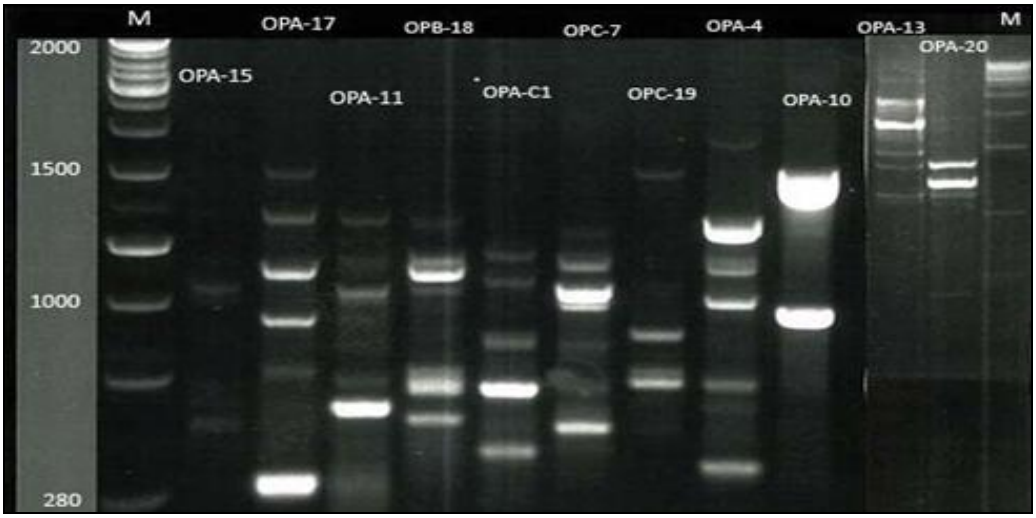

FIG.11: THE RAPD ELECTROPHORETIC PROFILE OF *FICUS CYATHISTIPULA* WARB. GENERATED BY ELEVEN DECAMER PRIMERS

Proximate analysis:

The results (g % w/w) of proximate analysis of *Ficus cyathistipula* showed a total ash (13.06), acid insoluble ash (0.58), water soluble ash (12.39), crude fiber (38) and moisture content (47.5).

TABLE 2: MOLECULAR SIZE IN BASE PAIRS OF AMPLIFIED DNA FRAGMENTS PRODUCED BY ELEVEN DECAMER PRIMERS IN *FICUS CYATHISTIPULA* WARB.

Molecular size (bp)	OPA-15	OPA-17	OPA-11	OPB-18	OPC-01	OPC-07	OPC-19	OPA-04	OPA-10	OPA-13	OPA-20
1800	-	-	-	-	-	-	-	-	-	-	-
1700	-	-	-	-	-	-	-	-	-	-	-
1600	-	-	-	-	-	-	+	-	-	-	-
1500	-	+	-	-	-	-	-	-	+	-	-
1400	-	-	-	-	-	-	-	-	-	-	-
1300	-	-	-	-	-	-	-	-	+	-	-
1250	+	+	+	+	-	-	-	-	-	-	-
1200	-	-	-	-	-	-	-	+	-	-	-
1100	-	-	-	-	-	+	-	-	-	+	-
1000	-	-	+	-	+	-	-	+	-	-	-
950	+	-	-	+	-	-	-	+	-	-	-
900	-	+	+	+	-	+	-	-	-	-	-
850	-	-	-	-	+	-	-	-	-	-	-
830	-	-	-	-	-	-	-	-	-	-	-
800	+	-	+	-	-	+	+	+	-	+	-
750	-	-	-	-	-	-	-	-	-	+	-
700	-	+	+	-	-	+	+	-	+	-	+
600	-	-	-	-	+	+	-	-	-	-	+
580	-	-	-	-	-	-	+	-	-	+	
550	-	+	-	-	-	-	+	+	-	-	+
520	-	-	+	+	-	-	-	-	-	-	-
500	+	-	-	+	-	+	-	-	-	-	-
470	-	-	+	-	-	-	-	-	-	-	-
460	+	-	-	-	-	-	-	+	-	-	-
450	-	-	-	-	-	-	-	-	-	-	-
420	+	-	-	-	-	+	-	-	-	-	-
400	-	-	-	-	+	-	-	-	-	-	-
300	-	-	-	-	-	-	-	+	-	-	-
280	-	+	+	-	-	-	-	-	-	-	-
230	-	-	-	-	-	-	-	-	-	-	+
Total	**6**	**6**	**8**	**5**	**4**	**7**	**5**	**8**	**3**	**4**	**4**

(+) and (-) = presence and absence of bands, respectively.

CONCLUSION: A study on *Ficus cyathistipula* Warb. growing in Egypt was performed: It included the botanical identification, the genetic characterization and the determination of total ash, acid insoluble ash, water soluble ash, crude fiber and moisture content. This is the first report on authentication and quality control of the plant.

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