Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2016; 8(8): 1135-1149

ISSN- 0975 1556

Research Article

Comparative Botanical Study and DNA Finger Printing of Certain Eugenia Species Growing in Egypt

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Available Online:10th August, 2016

ABSTRACT

Objective: to discriminate between six Eugenia species growing in Egypt based on their micro-morphological and genetic characterization.

Methods: For establishment of different botanical and genetic criteria, this study presents a comparative investigation of the botanical features of the leaves and stems through microscopically investigation of the prepared entire, transverse sections and isolated elements of these organs. Furthermore, the DNA of the six Eugenia species was extracted from leaf samples and Inter Simple Sequence Repeat (ISSR) analysis using six primers of arbitrary sequences.

Results: Comparative botanical characters of the different organs were identified. On the other hand, the total number of amplified product was 68 fragments, which were generated by the six primers; the primer HB-09 recorded 55.5% as the highest percentage of polymorphism. As well as, the primer HB-44 recorded 54.5% polymorphism. The primer HB-15 recorded the lowest percentage of polymorphism (10%) and the highest degree of similarity (90%).

Conclusion: For the present study, microscopical characters, as well as, DNA fingerprinting can be considered as the identifying parameters to authenticate and differentiate between the six *Eugenia* species under study.

Keywords: *Eugenia* species, Myrtaceae, Botanical profiling, DNA fingerprinting, ISSR.

INTRODUCTION

Family Myrtaceae (Myrtle family) is the ninth largest flowering plant family; it comprises about 130-150 genera and 3800-5500 species of trees and shrubs[1, 2]. It is economically important in the production of timber, gums, essential oils, fruits and spices as it contains many commonly cultivated ornamentals[3]. *Eugenia* is a genus of flowering plants in the myrtle family Myrtaceae. It has a worldwide distribution in tropical and subtropical regions. All species are woody evergreen trees and shrubs. Several are grown as ornamental plants, eaten fresh or used in jams and jellies[4]. Several species belonging to the Myrtle family and species *Eugenia* are used as medicinal plants in Paraguay. The herbalist recommended a water decoction of *E. uniflora* L. as a diuretic and antihypertensive, to lower cholesterol and uric acid, to lose weight, and to act as a digestive and astringent. Self-medication with the plant is very common. *E. uniflora* L. has been identified as one of the species called Nangapiry in Paraguayan folklore[5]. *E. jambos*, also had a long history of use in Indian traditional folk medicine for the treatment of numerous ailments. The fruit has been used as a tonic for the brain and liver and as a diuretic. The flowers are believed to reduce fever, and the seeds were used to treat diarrhea, dysentery and catarrh. In South-American cultures, the seeds have additionally been used as an anesthetic and recent studies have shown *E. jambos* extracts to have a similar analgesic efficacy to morphine in rats[6]. In Indian traditional medicinal systems, *E. jambos* leaves were also used as a diuretic, an expectorant in the treatment of rheumatism; to treat sore eyes; and as a febrifuge. Bark of the *E. jambos* tree is used to treat asthma, bronchitis and hoarseness. Cuban healers have also used the root to treat epilepsy. A decoction of the astringent bark of *E. aquea* was used for a local application on thrush[7]. However, *Eugenia* have been studied for their biological and medicinal activities such as antibacterial[8, 9], anti-inflammatory[10, 11], antidiabetic[12, 13], cytotoxic activity[14] and antioxidant[15]. All the previously mentioned therapeutic activities are due to the high content of secondary metabolites, especially, the volatile oils[16, 17], flavonoids[18], phenolic acids[19], fatty acids[20], triterpenes, coumarins, tannins[21, 22] and anthocyanin pigments[23]. The authentication of the botanical identity of the herbal material has to be confirmed by genetic analysis as the genetic makeup of herbal species is independent of their physical form, physiological and external conditions such as temperature, soil, humidity or rainfall. Analysis of well-characterized marker compounds, through deoxyribonucleic acid (DNA), is now the most popular method for the identification of herbal materials[24]. Combining the use of DNA and chemical fingerprinting will be an effective tool in authentication and quality control of plant drugs. DNA fingerprinting refers to the use

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of techniques based on polymerase chain reaction (PCR) - a system for the amplification of DNA - to reveal the specific DNA profile for a particular organism which is as unique as a fingerprint. Similarity of DNA fingerprints depends only on genetic closeness of the tested samples that it can distinguish plants from different families, genera, species and even cultivars[25]. Inter Simple Sequence Repeat (ISSR) technique provides an approach to find polymorphisms within species, or genetic differences between species. It is based on the principle of Polymerase Chain Reaction (PCR) that utilizes a single random oligonucleotide primer of arbitrary sequence to amplify genomic DNA taken as a template[26]. The amplified fragments are separated on an agarose gel by electrophoresis to generate the DNA profiles. Hence, the objective of this work was targeted towards discrimination between six *Eugenia* species growing in Egypt through an establishment of different botanical and genetic criteria.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Eugenia javanica* Lam., *Eugenia uniflora* L. and *Eugenia aquea* Burm. F. were collected from EL-Zohreya Garden, *Eugenia supra-axillaris* Spring ex Mart. and *Eugenia jambolana* Lam., were collected from the Zoo, in May (spring) 2013. Prof. Dr. Wafaa M. Amer Botany Department Faculty of Science authenticated the plant. A voucher specimen (2-6-2014) of each species was deposited at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Botanical profiling

Photographs for macro and micro-morphological study were taken using Casio digital camera and Leica DFC500 digital camera, respectively. Samples of the leaves, petioles and stems were separated and examined either fresh or after keeping in ethanol (70%) containing 5% glycerol, as well as the fresh leaves and stems were boiled with KOH for preparation of isolated elements.

DNA extraction

DNA was extracted from 10 g of leaf tissue in 1.5 ml microfuge tubes using the DNA extraction method described by Williams *et al*.[27]

Oligonucleotide primers

Six primers, purchased from Operon Technologies Inc. (Almeda, California, USA), were used for Inter Simple Sequence Repeat (ISSR) analysis, with the following sequences: HB-44: 5 CTC TCT CTC TCT CTC TTG 3, HB-09: 5` GTG TGT GTG TGT GC 3`, HB-11: 5` GTG TGT GTG TGT TGT CC3`, HB-12: 5 CAC CAC CAC GC 3, HB-13: 5` GAG GAG GAG GC 3`, HB-15: 5´ GTG GTG GTG GC 3`.

Polymerase chain reaction (PCR)

PCR amplification was conducted with 25 µl of reaction mixture containing 1% Triton 10-X reaction buffer (100 mMolTris–HCl (pH = 8.3), 500 mMolKCl, 0.01% (w/v) gelatin), 2.0 µl MgCl2 (25 mMol), 2.5 µl of each dNTP (2 mMol), 3 µl primer, 0.3 µl of Taq polymerase (Promega), and 2.5 µl of genomic DNA and completed to volume with distilled water. The reaction mixture was overlaid with two drops of mineral oil. The amplification reaction was carried out in a Thermocycler Perkin–Elmer Cetus 480 (Warrington, UK). The thermo cycler was programmed for one cycle of 5 min initial strand separation at 94 °C and for 40 cycles each 1 min at 94 °C for denaturation, 1 min primer annealing at 36 °C, a 7 min primer elongation at 72 °C, followed by one cycle of final primer extension at 72 °C for 10 min.

Gel electrophoresis and staining

PCR products were separated in 1.4% agarose gel by electrophoresis in TE buffer (10 mMolTris–HC1, 1.0 mMolEDTA, pH = 8.0) with a constant power of 100 V for about 3 h. The products were stained with ethidium bromide and then visualized and photographed under UV light using Bio-Rad Gel Doc-2000 (UK).

RESULTS AND DISCUSSION

Botanical profiling

Macromorphological study of different Eugenia species

Macromorphology of Eugenia jambolana L. (Fig. 1)

It is a large evergreen tree. Its height ranged from 3 to 3.5 m, sometimes, it attains to 10 m. The bark is pale brown, slightly rough on old stems. The stem and old branches are hard, solid, and cylindrical with rough surface and fine longitudinal fissures. The young branches are flexible and green in color. The leaves are opposite, simple with entire margin, elliptical to broadly oblong in shape with acute to acuminate apex and symmetric base. The surface of the leaf is smooth and glossy. The texture of the leaf is leathery. The leaves are 14-19 cm long, 4.5-5 cm width, with pinnate reticulate venation, aromatic when crushed. The petiole is cylindrical measuring 1 to 1.5 cm length and 2 to 3 mm diameter. The fruits are variable in size, measuring 2.5 cm long, ellipsoid or oblong in shape. They are green in color when young, but when ripen, the fruits turn into purple color. The fruit contains one large seed, purple in color. The comparison between macrmorphological features of the six species were tabulated in Table (1). The figures of other species were illustrated from Fig. (2) to Fig. (6).

Micromorphological study of different Eugenia species

Micromorphological study of Eugenia jambolana

The Leaf- Fig. (7)

The Epidermis Fig. (9 A, B)

Upper epidermis: It is formed of polygonal cells with thick cellulosic, beaded, wavy anticlinal walls, they are covered by thin striated cuticle and stomata are absent. Lower epidermis: It is similar to the upper epidermis but smaller in size, the cells have thick beaded anticlinal walls, less wavy than upper epidermis. The stomata are of paracytic type and rarely of anisocytic type.

The Mesophyll: Fig. (7)

The mesophyll is dorsiventral, being differentiated into disconnected upper palisade and spongy tissue. The palisade consists of 2-3 rows of cylindrical, columnar, thin walled cells usually having straight walls and containing chloroplasts, closely packed perpendicular to the upper epidermis and is discontinued in the midrib region by parenchymatous tissue. A spongy tissue is formed of 7 to 10 rows of elongated or rounded shaped parenchyma cells, having many intercellular spaces.

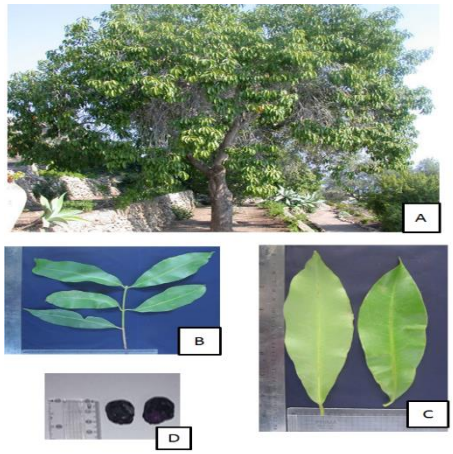

Figure 1: A: The tree of *E. jambolana* (X=0.02), B: The aerial part showing the stem and leaves (X= 0.13), C: The upper and lower surface of the leaf (X= 0.22), D: The fruit of *E. jambolana* (X= 0.43).

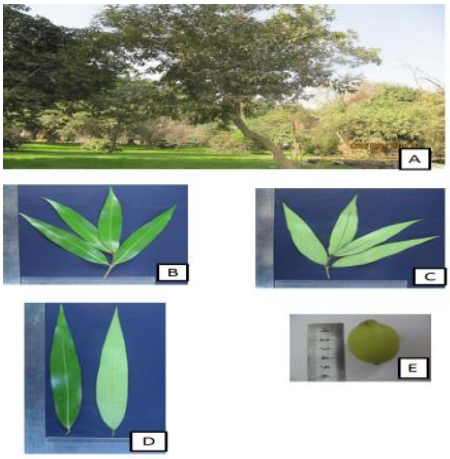

Figure 2: A: The tree of *E. jambos* (X=0.017), B: The aerial part showing the stem and upper surface of leaves (X= 0.17), C: The aerial part showing the stem and lower surface of leaves (X= 0.17), D: The upper and lower surface of the leaf (X= 0.3), E: The fruit of *E. jambos* (X= 0.3).

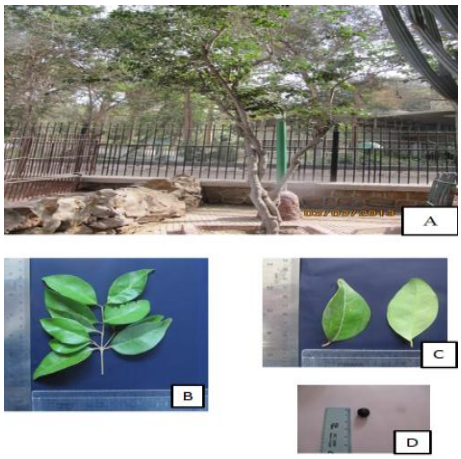

Figure 3: A: The tree of *E. supra-axillaris (*X= 0.025), B: The aerial part showing the stem and leaves (X= 0.3), C: The upper and lower surface of the leaf (X= 0.3), D: The fruit of *E. supra-axillaris* (X= 0.5).

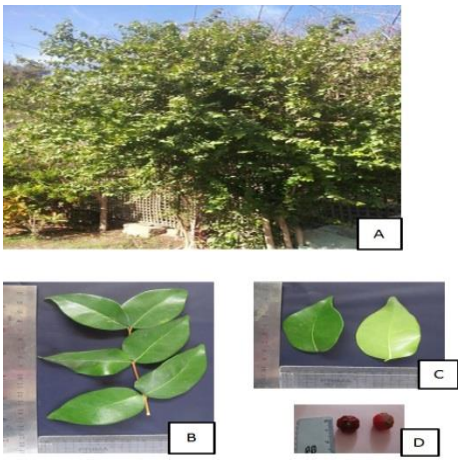

Figure 4: A: The tree of *E. uniflora* (X=0.045), B: The aerial part showing the stem and leaves (X= 0.18), C: The upper and lower surface of the leaf (X= 0.26), D: The fruit of *E. uniflora* (X= 0.5).

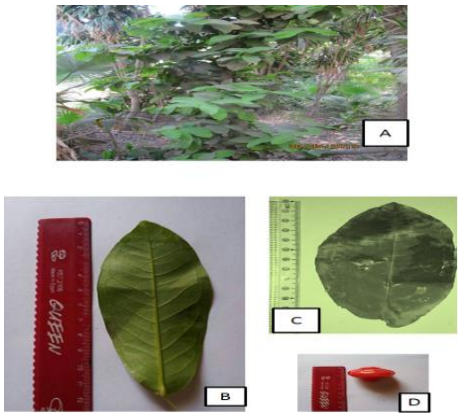

Figure 5: A: The tree of *E. aquea* (X=0.02), B: The lower surface of the leaf (X= 0.5), C: The upper surface of the leaf (X= 0.29), D: The fruit of *E. aquea* (X= 0.3).

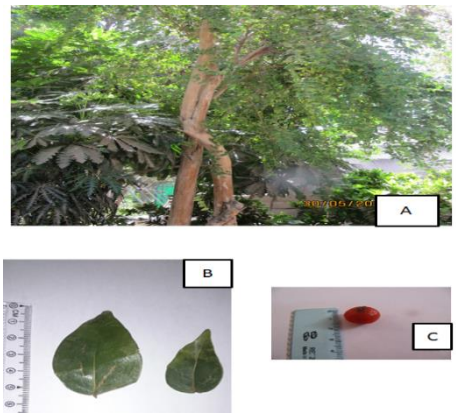

Figure 6: A: The tree of *E. javanica* (X=0.03), B: The upper and lower surface of the leaf (X= 0.32), C: The fruit of *E. javanica* (X= 0.5).

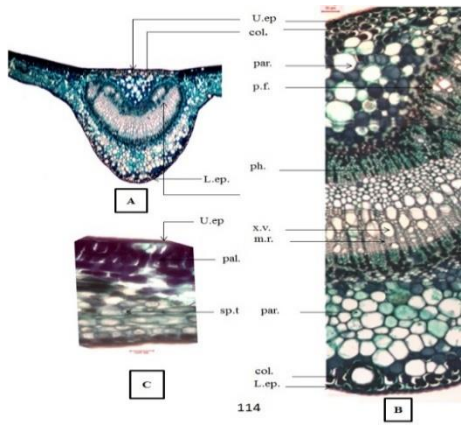

Figure 7: The transverse section of the leaf of *E. jambolana* A:Low power view (X=25), B: High power view at the midrib region (X=150), C: High power view at the lamina region (X=120); U.ep., upper epidermis; col., collenchyma; L.ep., lower epidermis; m.r., medullary rays; par., parenchyma; ph., phloem; p.f., pericyclic fibers; sp.t., sponge tissue; pal., palisade tissue; x.v., xylem vessels.

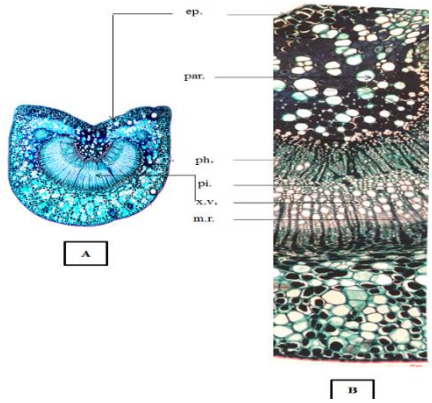

Figure 8: The transverse section of the petiole of *E. jambolana* A: Low power view (X=25), B: High power view (X=125); ep., epidermis; m.r., medullary rays; par., parenchyma; ph., phloem; pi., pith; x.v., xylem vessels.

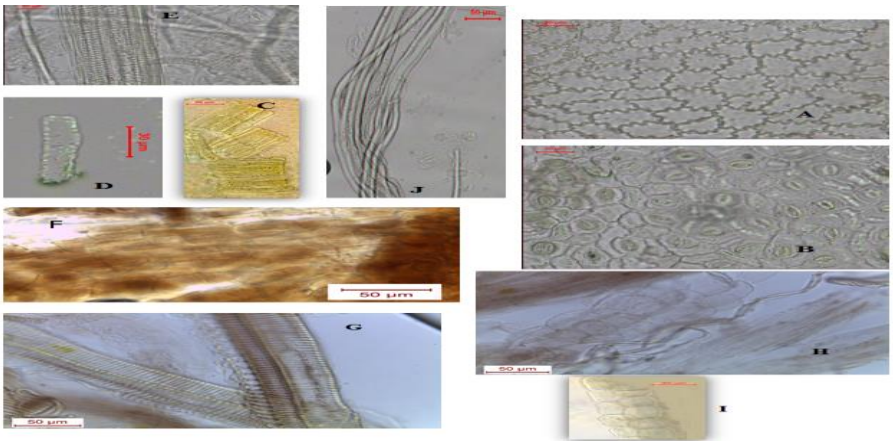

Figure 9: The isolated elements of the leaf and petiole of *E. jambolana* A: upper epidermis (X = 200), B: lower epidermis (X = 200), C: palisade tissue (X= 120), D: wood parenchyma (X = 65), E: spiral xylem vessels of leaf (X = 125), F: petiole epidermis (X = 200), G: spiral and annular xylem vessels of petiole (X = 250), H: petiole parenchyma (X = 280), I: medullary ray (X = 150), J: pericyclic fibers (X = 75)

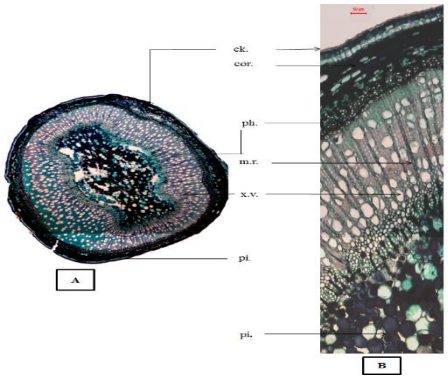

Figure 10: The transverse section of the stem of *E. jambolana* A: Low power view (X=25), B: High power view (X=150); ck., cork; cor., cortex; m.r., medullary rays; ph., phloem; pi., pith; x.v., xylem vessels

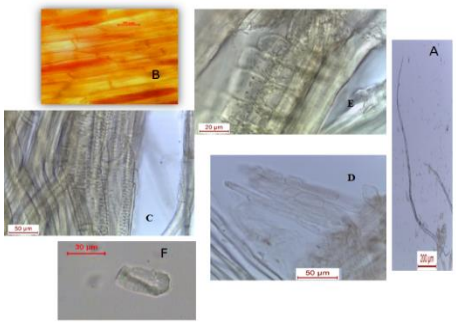

Figure 11: The isolated elements of the stem of *E. jambolana* A: pericyclic fibres (X= 70), B: cork (X= 200), C: spiral xylem vessels (X= 185), D: parenchyma (X = 200), E: medullary ray (X = 175), F: wood parenchyma (X = 75).

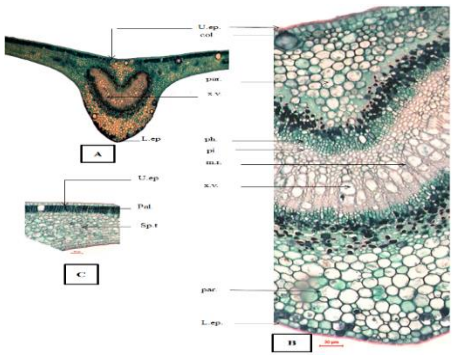

Figure 12: The transverse section of the leaf of *E. jambos* A:Low power view (X=35), B: High power view at the midrib region (X=150), C: High power view at the lamina region (X=110); U.ep., upper epidermis; col., collenchymas; L.ep., lower epidermis; m.r., medullary rays; par., parenchyma; Ph., phloem; Pi., pith; Sp.t., sponge tissue; Pal.t., palisade tissue; x.v., xylem vessel

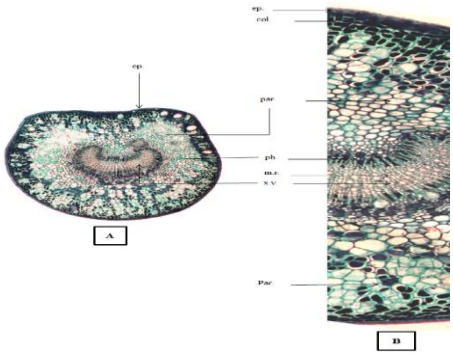

Figure 13: The transverse section of the petiole of *E. jambos* A: Low power view (X=40), B: High power view (X=165), ep., epidermis; m.r., medullary rays; Par., parenchyma; col., collenchyma; Ph., phloem; pi., pith; x.v., xylem vessels

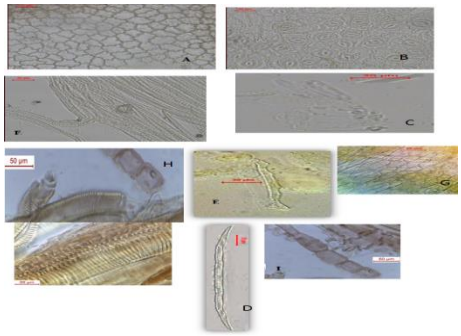

Figure 14: The isolated elements of the leaf and petiole of *E. jambos* A: upper epidermis (X = 200), B: lower epidermis (X = 200), C: palisade tissue (X= 120), D: pericyclic fibers (X = 150), E: wood parenchyma (X=75), F: spiral and annular xylem vessels of leaf (X = 125), G: petiole epidermis (X = 200), H: spiral xylem vessels of petiole (X = 250), I: medullary ray of petiole (X = 150)

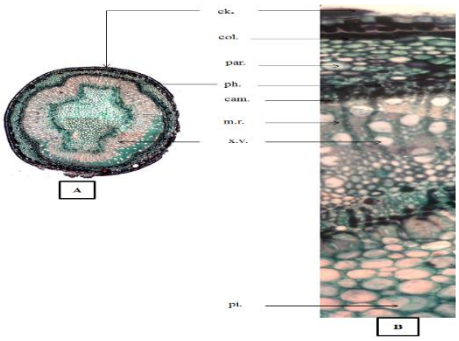

Figure 15: The transverse section of the stem of *E. jambos* A: Low power view (X=42), B: High power view (X=180); ck., cork; col., collenchyma; Par., parenchyma; m.r., medullary rays; ph., phloem; pi., pith; x.v., xylem vessel; cam., cambium.

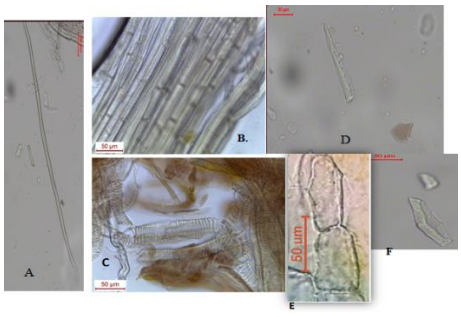

Figure 16: The isolated elements of the stem of *E. jambos* A: pericyclic fibres (X= 70), B: cork (X= 200), C: spiral xylem vessels (X= 185), D: non glandular hair (X = 100), E: medullary ray (X = 100), F: wood parenchyma (X = 70)

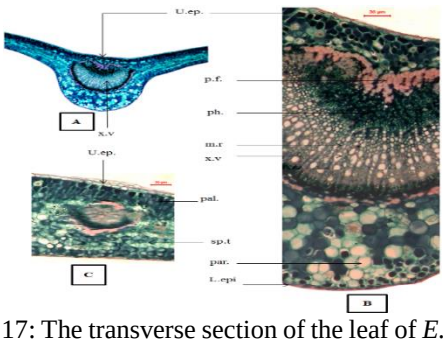

Figure 17: The transverse section of the leaf of *E. supra-axillaris* A:Low power view (X=25), B: High power view at the midrib region (X=135), C:High power view at the lamina region (X=85); U.ep., upper epidermis; L.ep., lower epidermis; m.r., medullary rays; Par., parenchyma; Ph., phloem; p.f., pericyclic fiber; Sp.t., sponge tissue; Pal.t., palisade tissue; x.v., xylem vessel

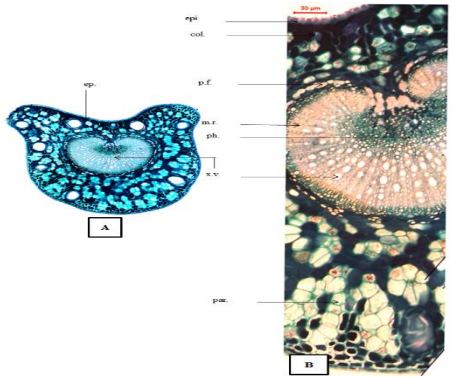

Figure 18: The transverse section of the petiole of *E. supra-axillaris* A: Low power view (X=30), B: High power view (X=150); ep., epidermis; m.r., medullary rays; Par., parenchyma; col., collenchyma; Ph., phloem; x.v., xylem vessel; P.f., pericyclic fibers

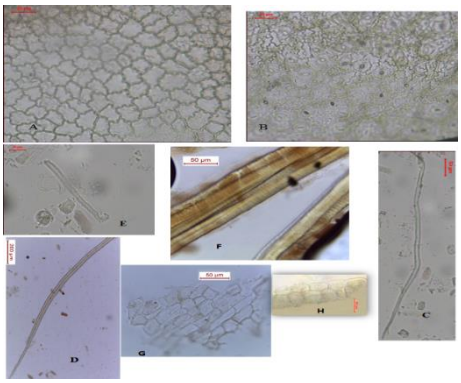

Figure 19: The isolated elements of the leaf and petiole of *E. supra-axillaris* A: upper epidermis (X = 200), B: lower epidermis (X= 200), C: pericyclic fibers of leaf (X = 150), D: pericyclic fibers of petiole (X = 150), E: wood parenchyma (X = 65), F: xylem vessel (X= 125), G : petiole epidermis (X = 200), H: medullary ray (X=150)

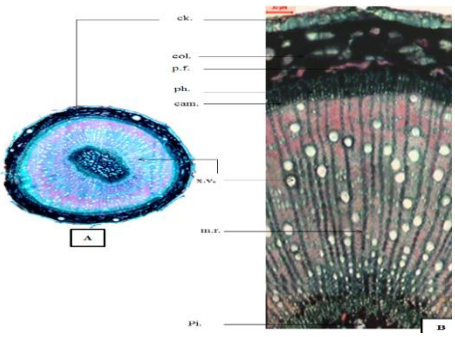

Figure 20: The transverse section of the stem of *E. supra-axillaris* A: Low power view (X=30), B: High power view (X=125); ck., cork; col., collenchyma; P.f., pericyclic fibers; m.r., medullary rays; ph., phloem; pi., pith; x.v., xylem vessel; cam., cambium.

Figure 21: The isolated elements of the stem of *E. supra-axillaris* A: spiral xylem vessel (X= 185), B: pericyclic fibres X= (70), C: cork (X= 125), D: non glandular hair (X = 100), E: medullary ray (X = 100), F: wood parenchyma (X = 70)

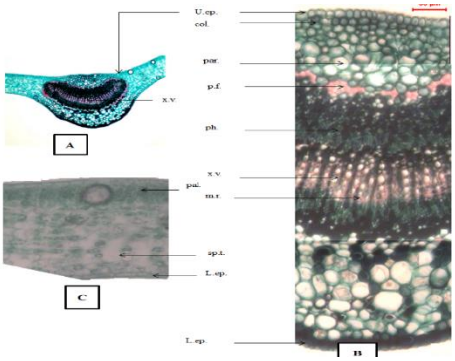

Figure 22: The transverse section of the leaf of *E. uniflora* A:Low power view (X=35), B: High power view at the midrib region (X=150), C: High power view at the lamina region (X=115)*;* U.ep., upper epidermis; col., collenchyma; par., parenchyma; L.ep., lower epidermis; m.r., medullary rays; Ph., phloem; Sp.t., sponge tissue; Pal.t., palisade tissue; x.v., xylem vessel; p.f. pericyclic fibers.

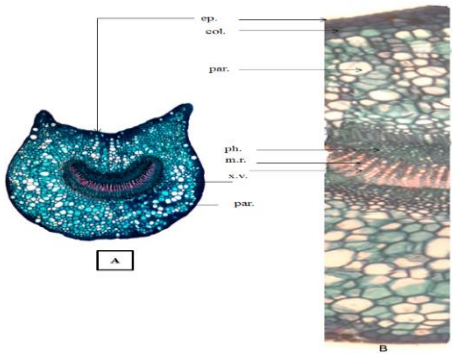

Figure 23: The transverse section of the petiole of *E. uniflora* A: Low power view (X=37), B: High power view (X=175); ep., epidermis; m.r., medullary rays; col., collenchyma; Par., parenchyma; Ph., phloem; x.v., xylem vessel

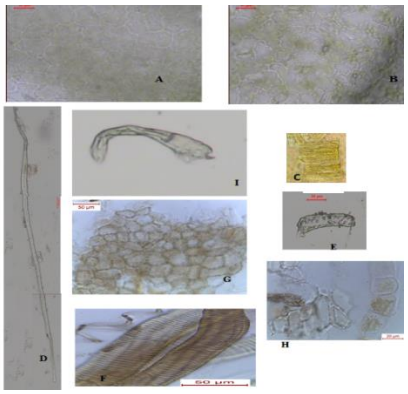

Figure 24: The isolated elements of the leaf and petiole of *E. uniflora* A: upper epidermis (X = 200), B: lower epidermis (X = 200), C: palisade tissue X = (100), D: pericyclic fibers (X = 150), E: wood parenchyma (X = 65), F: xylem vessel (X = 150), G : petiole epidermis (X = 200), H: medullary ray (X = 150), I: non glandular hair (X = 150)

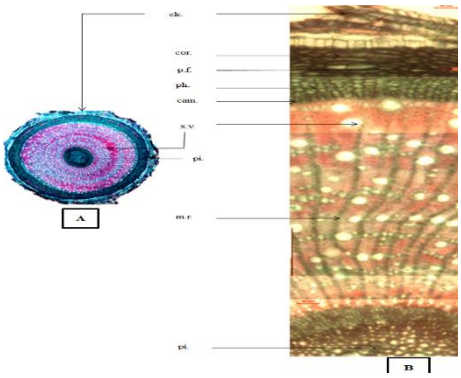

Figure 25: The transverse section of the stem of *E. uniflora* A: Low power view (X=44), B: High power view (X=165) , ck., cork; cor., cortex; m.r., medullary rays; ph., phloem; pi., pith; x.v., xylem vessel; cam., cambium; p.f., pericyclic fibers.

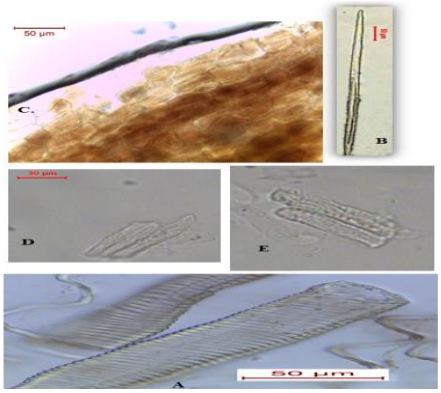

Figure 26: The isolated elements of the stem of *E. uniflora* A: spiral xylem vessels (X= 200), B: pericyclic fibres (X= 70), C: cork (X=150), D: medullary ray (X = 100), E: wood parenchyma (X = 70)

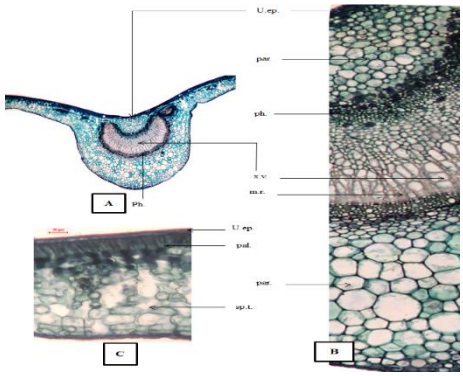

Figure 27: The transverse section of the leaf of *E. aquea* A: Low power view (X=30), B: High power view at the midrib region (X=150), C: High power view at the lamina region (X=115) U.ep., upper epidermis; par., parenchyma; L.ep., lower epidermis; m.r., medullary rays; Ph., phloem; Sp.t., sponge tissue; Pal.t., palisade tissue; x.v., xylem vessel.

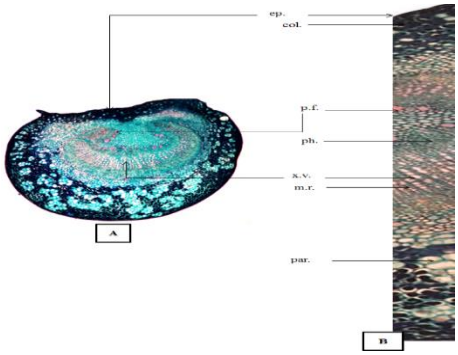

Figure 28: The transverse section of the petiole of *E. aquea* A:Low power view (X=59), B: High power view (X=155); ep., epidermis; m.r., medullary rays; col., collenchyma; Par., parenchyma; Ph., phloem; x.v., xylem vessel; p.f. pericyclic fibers.

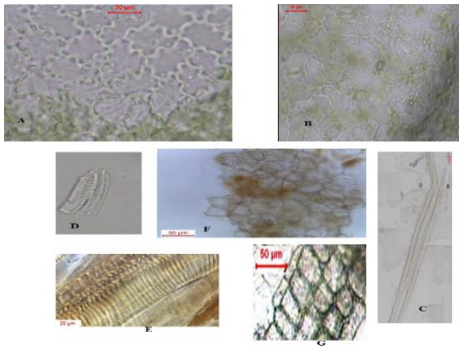

Figure 29: The isolated elements of the leaf and petiole of *E. aquea* A: upper epidermis (X = 200), B: lower epidermis (X = 200), C: pericyclic fibers (X = 100), D: wood parenchyma (X = 75), E: xylem vessels (X = 180), F: petiole epidermis (X = 200), G: medullary ray X = (150)

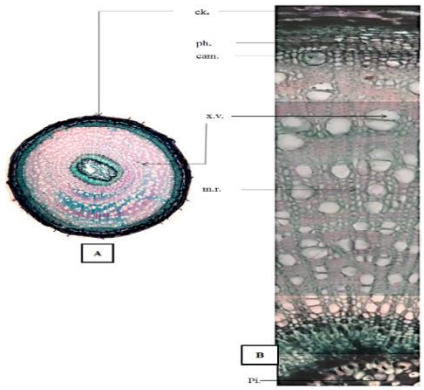

Figure 30: The transverse section of the stem of *E. aquea* A: Low power view (X=35), B: High power view (X=185), ck., cork; m.r., medullary rays; ph., phloem; pi., pith; x.v., xylem vessel; cam., cambium

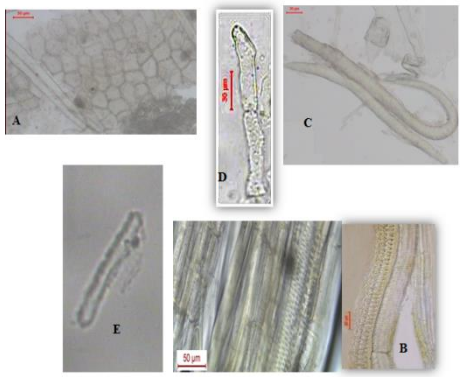

Figure 31: The isolated elements of the stem of *E. aquea* A: cork (X= 150), B: spiral and annular xylem vessels (X= 200), C: pericyclic fibres (X= 100), D: medullary ray (X = 100), E: wood parenchyma (X = 70)

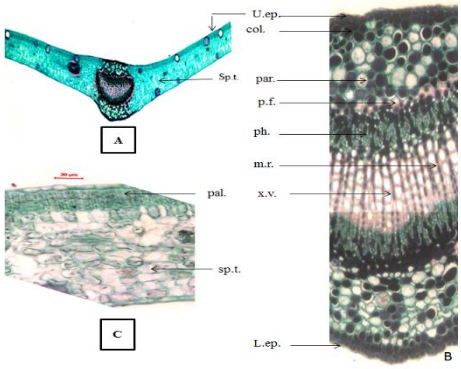

Figure 32: The transverse section of the leaf of *E. javanica* A: Low power view (X=27.5), B: High power view at the midrib region (X=166), C: High power view at the lamina region (X=110)*;* U.ep., upper epidermis; L.ep., lower epidermis;col., collenchyma; m.r., medullary rays; Par., parenchyma; Ph., phloem; Sp.t., sponge tissue; Pal.t., palisade tissue; x.v., xylem vessel; p.f. pericyclic fibers.

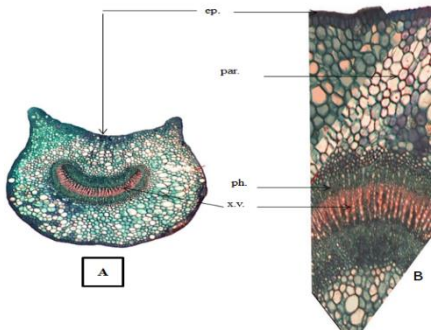

Figure 33: The transverse section of the petiole of *E. javanica* A: Low power view (X=35), B: High power view (X=120); ep., epidermis; Par., parenchyma; Ph., phloem; x.v., xylem vessel.

Figure 34: The isolated elements of the leaf and petiole of *E. javanica* A: upper epidermis (X = 200), B: lower epidermis (X = 200), C: pericyclic fibers (X = 100), D: wood parenchyma (X = 75), E.: xylem vessels (X = 180), F: petiole epidermis (X=200), G: medullary ray (X = 150)

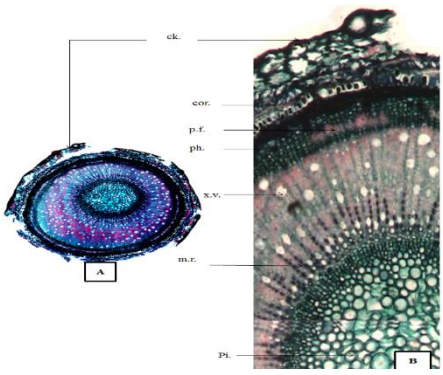

Figure 35: The transverse section of the stem of *E. javanica* A: Low power view (X=34), B: High power view (X=110), ck., cork; cor., cortex; m.r., medullary rays; ph., phloem; pi., pith; x.v., xylem vessel; p.f., pericyclic fibers.

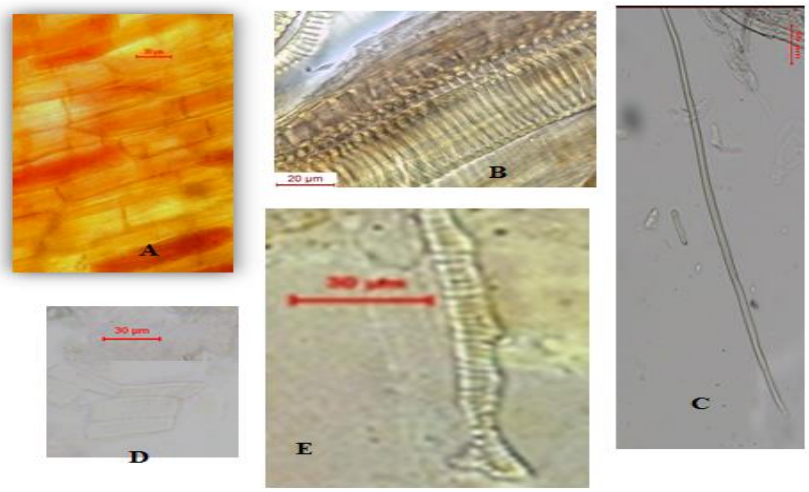

Figure 36: The isolated elements of the stem of *E. javanica* A: cork (X= 150), B: spiral and annular xylem vessels (X= 200), C: pericyclic fibres (X= 100), D: medullary ray (X = 100), E: wood parenchyma (X=70)

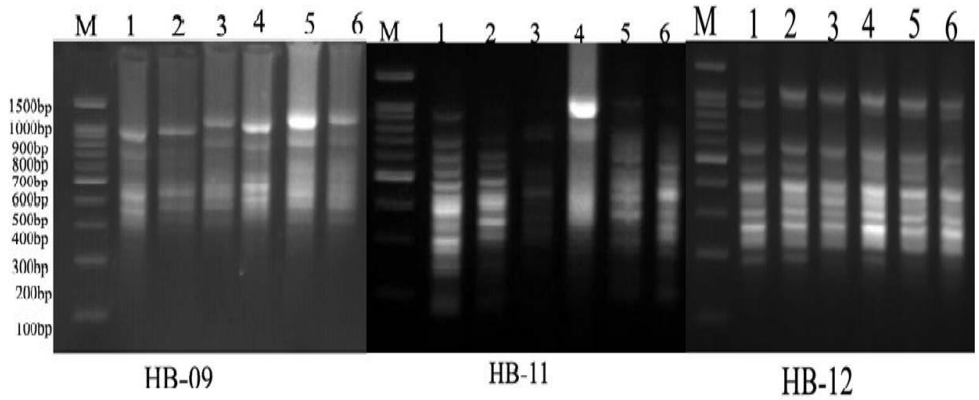

Figure 37: Photograph of electrophoresis of DNA shows ISSR bands of HB-09, HB-11, HB-12. M; marker, Lane 1: *E. jambolana*, Lane 2: *E. jambos*, Lane 3: *E. uniflora*, Lane 4: *E. supra-axillaris*, Lane 5: *E. aquea*, Lane 6: *E. javanica*

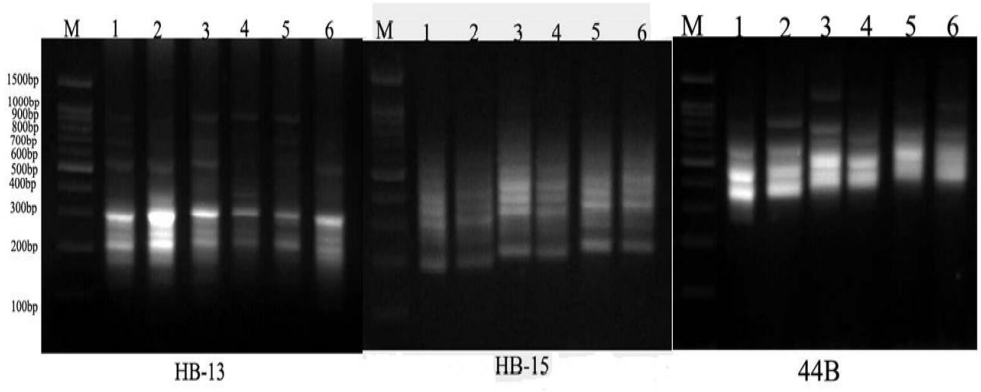

Figure 38: Photograph of electrophoresis of DNA shows ISSR bands of HB-13, HB-15, HB-44. M; marker, Lane 1: *E. jambolana*, Lane 2: *E. jambos*, Lane 3: *E. uniflora*, Lane 4: *E. supra-axillaris*, Lane 5: *E. aquea*, Lane 6: *E. javanica*

Table 1: Macromorphological characteristics of different *Eugenia* species:

Organ	Item	*E. jambolan* Fig.(1)	*E. jambos* Fig.(2)	*E. supra-axillaris* Fig.(3)	*E. uniflora* Fig.(4)	*E. aquea* Fig.(5)	*E. javanica* Fig.(6)
Leaf	Shape	Elliptical, broadly oblong	Lanceolate or narrow-elliptical	Ovate to ovate-lanceolate	Ovate	Elliptical or oblong	Broadly ovate
	Apex	Acute, acuminate	Acute	Acute	Acuminate	Acute	Acute
	Margin	Entire					
	Texture and surface	Leathery texture with smooth glossy surface					
	Length	14 -19 cm	10 – 20 cm	4 - 7 cm	3 - 4 cm	10 -15 cm	3 - 5 cm
	Width	4.5 -5 cm	2 - 4 cm	3 - 3.5 cm	2 - 3 cm	4 – 6 cm	2 – 2.5 cm
Petiole	Shape	Cylindrical, thick, green color	Cylindrical, pale green	Cylindrical, Brown	Cylindrical, brown, short	Cylindrical, green color	Cylindrical, brown, short
	Length	10 -12 mm	7 – 8 mm	7 mm	3 mm	9 – 10 mm	3 mm
	Diameter	3 – 4 mm	2 mm	1 - 1.5 mm	1 – 2 mm	2 – 3 mm	1 -2 mm
Stem	Shape	Cylindrical, slightly rough	Cylindrical, woody, smooth	Cylindrical, woody, smooth	Cylindrical, woody, rough	Cylindrical, rough	Cylindrical, woody, rough
	Color	Pale brown	Brown	Greyish brown	Brown	Brownish green	Brown
	Diameter	4 mm	6 mm	5 mm	5 mm	4 mm	4 – 5 mm
Fruit	Shape	Ellipsoid or oblong	Rounded or oval	Rounded with 4 fleshy calyx lobes at the apex	Rounded with 4 fleshy calyx lobes at the Apex	Pear-shaped	Rounded with 4 fleshy calyx lobes at the apex
	Color of ripe fruit	Purple	Pale-yellow	Red to purple	Scarlet-red	Light-red or red	Red to crimson red
	Length	2.5 cm	4.5 - 6.5 cm	1.5 - 2.5 cm	1.5 - 2.5 cm	2 - 4 cm	1.5 - 2.5 cm
	Width	1.5 cm	3 – 4 cm	1.5 - 2 cm	2 – 2.5 cm	2.5 - 3.5 cm	1.5 - 2 cm
Seed	No. & color and shape	One large oval seed, purple in color.	1 or 2 large brown rounded seeds	1 or 2 rounded, small, brown seeds	1, 2 or 3 brown, rounded small seeds	seedless	1 or 2 rounded, brown seeds

Table 2: Microscopical characteristics of different *Eugenia* species:

Organ	Item	*E. jambolana*	*E. jambos*	*E. supra-axillaris*	*E. uniflora*	*E. aquea*	*E. javanica*
Leaf	Stomata Lower epidermis	It is formed of polygonal cells with thick cellulosic, beaded, wavy anticlinal walls; they are covered by thin striated cuticle.					
	Stomata Upper epidermis	The cells have thick beaded anticlinal walls, less wavy than upper epidermis. The stomata are absent in the upper epidermis, stomata of paracytic type rarely of anisocytic type are present in lower epidermis.					
	Spongy tissue	7-10 rows of elongated or round shaped parenchyma cells having intercellular spaces.	8 to 12 rows of polygonal or round shaped parenchyma cells.	9 to 13 rows of round shaped parenchyma cells traversed by bundle	9 to 13 rows of irregular shaped loosely packed parenchyma cells.	9 to 11 rows of irregular or rounded shape loosely packed parenchyma cells.	9 to 10 rows of elongated or irregular shaped loosely packed parenchyma cells.

			sheath extension.				
Petiole	Epidermis	The epidermal cells are polygonal, elongated with straight thin anticlinal walls, covered with thin cuticle and stomata are absent.			The epidermal cells are polygonal with straight thick beaded anticlinal walls, covered with thick cuticle and stomata are absent.		
	Cortex	2-4 rows of collenchyma followed by 5-9 rows of parenchyma cells.	4-6 rows of collenchym a followed by 9-12 rows of parenchyma cells.	2-3 rows of collenchyma cells followed by 10-12 rows of polygonal or rounded parenchyma.		2-3 rows of collenchyma cells followed by 9-13 rows of polygonal or rounded, small parenchyma.	
	Phloem	Relatively narrow	Narrower	Narrower	Narrower	Narrower	Narrower
	Xylem	Relatively narrow	Narrower	Wider	Narrower	Narrower	Wider
	Pith	Small arcuate parenchyma cells.	Absent				
	Cork	3-7 rows Polygonal, elongated cells, having thick suberized walls.	6-9 rows tangentially	3-4 rows of Polygonal, thick walled cells.	7-8 rows of Polygonal, thick walled cells.	3-4 rows of Polygonal, beaded thick walled cells.	6-8 rows of Polygonal, tangentially elongated cells with thick walled.
Stem	Cortex	3-4 rows of collenchyma followed by 4-5 rows of parenchyma .	4-5 rows of collenchyma followed by 5-7 rows of parenchyma cells.		7-9 rows of collenchym a followed by 6-8 rows of parenchyma cells.	3-4 rows of collenchym a followed by 4-5 rows of parenchyma .	2-3 rows of collenchyma followed by 4-5 rows of parenchyma.
	phloem	Relatively narrow	Narrower	Wider	Wider	Wider	Wider
	Xylem	Relatively narrow	Narrower	Wider	Wider	Wider	As *E. jambolana*
Stem	Pith	Relatively wide	Narrower	Narrower	Narrower	Narrower	Narrower

The cortical tissue of midrib

The upper cortical tissue of the midrib consists of 4-5 rows of collenchyma cells. Followed by 4-6 rows of parenchyma cells, while the lower cortical tissue consists of 2-3 rows of collenchyma cells followed by 4-6 rows of thin walled rounded parenchyma cells. The endodermis is indistinct. Volatile oils and tannins were detected in the parenchyma of midrib and lamina (positive with sudan III and ferric chloride).

The vascular bundle

The pericycle is formed of an almost continuous ring of pericyclic fibers above and below the vascular bundle. These fibers appear in the transverse section usually polygonal in outline having narrow lumina. In surface view they are long usually tapering towards both ends having pointed or rounded tips, thick, straight lignified walls. The vascular bundle in the midrib region is arranged in two crescent shaped groups forming an almost continuous ring. It is collateral showing xylem and phloem. The phloem consists of phloem vessels and phloem parenchyma. The cambium is undifferentiated. The xylem is wholly lignified consisting of vessels, fibers and wood parenchyma. The vessels are mostly spiral thickened. The fibers have thin or moderately thickened walls and narrow or wide lumina. They are tapering towards both ends with rounded or pointed tips. The medullary rays form radiating lines traversing the xylem, uni- to biseriate consisting of radially elongated non lignified parenchymatous cells.

The Petiole - Fig. (8)

A transverse section in the petiole is more or less circular in outline with two projections representing the leaf blade extension. It shows an outer epidermis surrounding 2-4

Table 3: Microscopical measurements of the leaf of the six Eugenia species in (µm).

Organ	Element	Dimensions	Plant name					
			E. jambolana	*E. jambos*	*E.supra-axillaris*	*E. uniflora*	*E. aquea*	*E. javanica*
Leaf	Upper Epidermis	L	30-43-56	40-48-55	30-35-39	47-53-58	43-52-60	30-35-39
		W	26-24-22	20-15-30	17-20-22	26-33-39	12-21-30	13-17-21
		H	13-15-17	9-11-13	8-12-15	6-8-10	14-16-18	7-9-11
	Lower Epidermis	L	30-39-47	21-28-34	22-26-30	43-60-77	39-44-48	22-30-13
		W	18-24-30	17-22-26	9-13-17	17-24-30	17-24-30	13-18-22
		H	10-12-13	5-7-9	5-7-8	5-6-7	10-11-12	5-6-7
	Sto-mata	D	17-20-22	25-28-30	9-15-21	10-18-25	9-16-22	9-11-13
	Palisade	L	36-39-42	58-61-64	47-52-57	48-52-55	38-40-42	44-47-51
		W	12-15-18	6-11-15	12-15-18	8-10-12	15-17-19	12-14-16
		Th	58-60-62	50-60-70	50-55-60	41-51-61	53-58-63	44-48-52
	Sponge tissue	Th	120-124-128	190-205-220	115-135-155	143-154-165	212-221-230	120-143-165
	Pericyclic fibers	L	420-460-500	420-470-520	550-575-600	660-730-800	300-363-425	350-390-430
		W	8-13-17	13-16-18	8-13-17	4-9-14	17-20-23	8-10-12
	Xylem vessels	D	13-15-17	9-12-15	10-13-15	12-13-14	16-18-19	7-9-11
	Wood parenchyma	L	68-72-75	78-81-84	108-117-126	60-66-72	---	---
		W	15-19-23	4-6-8	12-14-16	15-23-30	---	---
	Non-glandular hairs	L	---	---	---	128-150-170	---	---
		D	---	---	---	9-18-26	---	---

L: Length, W: Width, H: Height, D: Diameter, Th: Thickness

rows of collenchyma followed by 5-9 rows of parenchyma cells. Tannins were detected in the cortex (positive with ferric chloride). The cortex is followed by the pericycle which encloses vascular tissue. There are many rounded cavities scattered irregularly near the epidermis. The epidermal cells Fig. (9 F) are polygonal, elongated with straight thin anticlinal walls, covered with thin cuticle and stomata are absent. The cortex consists of 2-4 rows of collenchyma followed by 5-9 rows of parenchyma cells. The two projections are supported by several rows of collenchymatous cells surrounding a group of small vessels. The pericycle is formed of discontinuous patches of lignified fibers separated by parenchyma and surrounding the vascular tissue. The vascular tissue consists of wide collateral vascular bundle, forming a ring. The vascular tissue is formed of the phloem which is comparatively narrow and consists of thin-walled cellulosic parenchyma cells surrounding the xylem, the medullary rays which are uni- to biseriate, being cellulosic, radially elongated and the xylem which formed of xylem vessels, wood fibers and wood parenchyma. All elements are lignified. The fibers are short or moderate length, have

Table 4: Microscopical measurements of the petiole and stem of the six *Eugenia* species in (µm).

Organ	Element	Dimensions	Plant name					
			E. jambolana	*E. jambos*	*E.supra-axillaris*	*E.uniflora*	*E. aquea*	*E. javanica*
Petiole	Epidermis	L	27-41-55	20-33-65	41-50-58	41-51-61	40-49-58	19-23-27
		W	11-14-17	5-10-15	16-23-30	17-25-33	17-23-29	10-13-16
		H	15-22-29	21-24-27	13-15-17	12-16-20	13-18-22	12-15-18
	Cork	L	48-52-56	50-65-80	31-41-51	50-63-75	36-44-52	47-51-55
		W	8-10-12	16-21-25	12-16-20	16-23-30	17-22-27	9-13-17
		H	20-23-26	23-29-35	18-24-30	40-50-60	42-45-48	45-51-57
	Cortex	H	50-52-54	52-55-58	55-60-65	50-58-65	48-53-58	55-58-60
	Pericyclic fibers	L	275-283-291	360-380-420	625-825-1025	450-550-650	490-510-530	700-800-900
		W	10-12-14	10-13-16	17-20-23	20-25-30	30-35-40	17-21-24
stem	Wood parenchyma	L	38-44-50	50-57-64	67-76-85	72-79-85	60-65-70	71-77-83
		W	21-25-29	22-28-34	31-35-39	22-26-29	21-24-27	18-22-26
	Xylem vessels	D	25-28-31	28-31-34	50-54-58	33-38-43	36-41-46	27-31-34
	Non-glandular hairs	L	---	55-60-65	59-65-71	---	---	---
		W	---	19-22-24	18-23-28	---	---	---

L: Length, W: Width, H: Height, D: Diameter, Th: Thickness

straight, thin lignified walls, wide lumina and rounded apices. The vessels are lignified arranged in radial rows, with spiral thickenings. Wood parenchyma is rectangular in shape, pitted lignified walls. The pith is small arcuate, consists of thin-walled parenchymatous cells.

The stem - Fig. (10)

A transverse section in the old stem is almostly circular. It consists of; cork which is formed of 3-7 rows of polygonal, tangentially elongated cells, having thick suberized walls Fig. (11), cortex which is formed of 3-4 rows of collenchyma followed by 4-5 rows of parenchyma, with indistinct endodermis. The pericycle is formed of patches of fibers interrupted by parenchyma cells; fibers have lignified pitted walls with narrow lumina and acute apex. The phloem consists of sieve tubes, companion cells and phloem parenchyma. The xylem Fig. (11) is wide, consists of lignified radially arranged items, formed of thick walled vessels, fibers, wood parenchyma and pitted lignified polygonal cells of medullary rays. The vessels are with spiral thickenings. Fibers have straight sides and tapered apices. Wood parenchyma is rectangular lignified cells with pitted walls. They are traversed with uni to biseriate medullary rays. Pith is relatively wide and formed of large rounded thin walled parenchymatous cells. Tannins were detected in the pith (positive with ferric chloride). The comparison between different species and *E. jambolana* was tabulated in Table (2). The Figures of other species were illustrated from Fig.(12) to Fig. (36).

DNA fingerprinting

The extracted DNA of each of the six *Eugenia* species was amplified using six decamer primers to detect their genetic variability. The obtained ISSR-PCR products using the six decamer primers, as detected by gel electrophoresis, for the six *Eugenia* species are represented in Fig. (37, 38). The total number of amplified product was 68 fragments, which were generated by the six used primers with average of 11 fragments per primer. The total number of polymorphic bands was 23 ranging from 17 as maximum amplified fragments and 9 as minimum amplified fragments. The primer HB-09 had the highest percentage of polymorphism recording 55.5% polymorphism. Followed by, the primer HB-44 produced 11 bands, 6 of them were polymorphic bands recording 54.5% polymorphism. The primer HB- 15 recorded the highest

Table 5: The total number of ISSR-PCR fragments, distribution of unique and polymorphic bands, species specific percentage for primer and percentage of polymorphism generated by six decamer arbitrary primers in *Eugenia* species.

Primer	Size of amplification products (bp)	Number of bands	Mono-morphic bands	Unique bands	Poly-morphic bands	% of Poly-morphism	Genetic similarity %
HB-09	995-297	9	4	1	5	55.5	44.5
HB-11	1127-91	17	10	4	7	41.2	58.8
HB-12	976-178	11	9	1	2	18.2	81.8
HB-13	867-172	10	8	0	2	20	80
HB-15	907-199	10	9	0	1	10	90
HB-44	1126-253	11	5	3	6	54.5	45.5
Total		68	45	9	23		
Mean		11.33	7.5	1.5	3.83		
Percentage					33.8	33.2	66.8

degree of similarity (90%), followed by primer HB-12 which recorded (81.2%), The primers HB-09 and HB-44 could be used to discriminate between the six *Eugenia* species depending on their low values of genetic similarity and high level of polymorphism. While, HB-15 primer could be used in the authentication of different *Eugenia* species as highest value of genetic similarity were indicated.

CONCLUSION

From the previous findings, the macro and micro-morphological characters, as well as, DNA fingerprinting can be considered as the identifying parameters to authenticate and differentiate between the six plants under study. Where, HB-09and HB-44 ISSR primers could be used to discriminate between the six *Eugenia* species depending on their high level of polymorphism, while, HB-15 primer could be used in the authentication of different *Eugenia* species as highest value of genetic similarity were indicated.

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