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Research Article

Pharmacognostical and Genetic Characterization of *Tecoma smithii* Will. Wats.

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ABSTRACT
Objective: very limited information were traced on the macromorphology of *Tecoma Smithii* Will. Wats. and nothing was found regarding the micromorphology. Furthermore, there were no reports regarding the genetic profiling of the plant although it is claimed to be a hybrid of two other species. Therefore, the study aims to of find out the diagnostic characters for identification and differentiation of this species. Method: the macro- and micromorphology of the leaves, stems and flowers of the plant cultivated in Egypt were carried out. DNA samples of three species of *Tecoma*: *Tecoma Smithii* Will. Wats.; as the hybrid, and the parents: *Tecoma mollis* Humb. & Bonpl., and *Tecomaria capensis* (Thunb.) Lindl. were extracted from fresh leaves and Random Amplified Polymorphic DNA (RAPD) analysis was conducted using ten primers of arbitrary sequences. Results: botanical characters of different organs of the plant were identified. On the other hand, the ten primers of arbitrary sequences generated a total of 224 fragments in all of the three species; distributed as 69, 73, and 82 for *Tecoma mollis* Humb. & Bonpl., *Tecoma Smithii* Will. Wats., and *Tecomaria capensis* (Thunb.) Lindl. respectively. A 100% genetic similarity percentage was observed between the three species using the primers OPC-01 and OPE-03, supporting the claim of the hybridization and strong relation between the three species. The highest level of polymorphism was recorded using the primer OPAX-16 thus could be a good tool for differentiation between the three species or each two of them. Conclusion: in the present study, macro and micromorphological characters, as well as DNA fingerprint can be considered as discriminating features to authenticate and differentiate *Tecoma Smithii* Will. Wats.

Keywords: *Tecoma Smithii*, DNA fingerprinting, botanical profiling

INTRODUCTION

Characterization of plants using DNA markers is an ideal approach for identification of medicinal plant species and varieties of the same species because it is based on the unique nucleotide sequences which are not affected by age, environmental factors, and physiological conditions and not tissue specific and thus can be detected at any stage of plant development[1].

The plant used for the present investigation is belonging to the family Bignoniaceae which belongs to Lamiales and includes 82 genera and 827 species[2], plants of the Bignoniaceae usually produce large flowers, and many species are widely cultivated as ornamentals. These species are widely spread at famous Egyptian botanical gardens for their showy view[3]. *Tecoma Smithii* Will. Wats. is a hybrid between *Tecoma mollis* Humb. & Bonpl. [Synonym of *Tecoma stans* (L.) Juss. Ex Kunth var. *velutina* DC.] and *Tecomaria capensis* (Thunb.) Lindl. as stated by Bailey[4]. The name of the genus *Tecoma* (Trumpet bush) is derived from the Mexican name Tecomaxochitl**,** which was applied by the indigenous peoples of Mexico to plants with tubular flowers[5].

Polymerase chain reaction (PCR), invented by Kary Mullis, 1983, is a method used to generate billions of copies of genomic DNA within a very short time. Random amplified polymorphic DNA (RAPD) is one of the most commonly used primary assays for screening the differences in DNA sequences between plant species[6], where plant genomic DNA is cut and amplified using short synthetic oligonucleotide primers at low annealing temperatures, primers are designed for specific amplification of a particular locus in targeted species[7].

It is known that the same plant growing in different areas may have different chemical components and different biological activities. Consequently, the establishment of a pharmacognostic profile of the plant will assist in standardization for quality, purity and sample identification[8].

Tecoma has been described as a taxonomically difficult group with overlapping vegetative characters, therefore, other methods are essential in identifying hybrids[9].

Reviewing the available literature, nothing was found concerning neither the DNA fingerprinting nor the botanical characteristics of *Tecoma Smithii* Will. Wats. Data on the macromorphology[5] and confirmation of hybridity in genus *Tecoma* were traced[9]. In addition, macro-and micro-morphological characters of *Tecoma stans* Linn. were reported[8,10,11].

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The macro- and micromorphology of the stem, leaf, and flower of *Tecoma Smithii* Will. Wats. cultivated in Egypt are carried out with the aim of finding out the diagnostic characters for identification and differentiation of this species as well as confirmation of hybridity of the plant through DNA fingerprinting. The objective of this work was targeted towards an establishment of different botanical and genetic criteria for profiling of this species.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Tecoma Smithii* Will. Wats. used in this study were collected during November (autumn) 2012 from plants cultivated in a field belonging to the Applied Research Center for Medicinal Plants (ARCMP), National Organization of Drug Control and Research (NODCAR). The plant was kindly authenticated by Dr. Wafaa M. Amer, Professor of Flora, Botany Department, Faculty of Science, Cairo University. A voucher specimen (no. 4122012) is kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Botanical profiling

The stems, leaves and flowers were separated and examined either fresh or after keeping in ethanol (70 %) containing 5 % glycerol, as well as after being dried and reduced to a fine powder for microscopical examination. Samsung digital camera was used for macromorphological photography and Leica DFC500 digital camera was used for anatomical investigation.

Genetic profiling

DNA fingerprinting

The fresh leaves of three *Tecoma* species *viz*. *Tecoma Smithii* Will. Wats., *Tecoma mollis* Humb. & Bonpl. and *Tecomaria capensis* (Thunb.) Lindl. were separately freeze-dried, and ground to fine powder under liquid nitrogen.

DNA extraction

DNA extraction was performed using DNeasy® plant Mini Kit, (QIAGEN, Hilden, Germany), for micro-preparation of nucleic acid samples QIAshredder spin column (QIAGEN, Hilden, Germany) disposable lysate homogenizing vials in a microcentrifuge was used.

Oligonucleotide primers

OPAX-16	5´ GTC TGT GCG G 3`
OPAX-19	5´ CCC TGT CGC A 3´
OPC-01	5´ TTC GAG CCA G 3´
OPC-14	5´ TGC GTG CTT G 3`
OPC-16	5´ CAC ACT CCA G 3`
OPD-05	5´ TGA GCG GAC A 3`
OPD-07	5´ TTG GCA CGG G 3`
OPE-03	5´ CCA GAT GCA C 3`
OPE-19	5´ ACG GCG TAT G 3`
OPG-05	5´ CTG AGA CGG A 3`

Ten primers, purchased from Operon® Technologies Inc. (Almeda, California, USA), were used for Random Amplified Polymorphic DNA (RAPD) analysis, with the following sequences:

Polymerase Chain Reaction PCR

PCR was performed in 30-µl volume tubes according to Williams *et al*.[12] that contained the following: dNTPs (2.5 mM) 3 µl, MgCl2 (25 mM) 3 µl, Buffer (10 x) 3 µl, Primer (10 pmol) 2 µl, Taq DNA polymerase (5U/µl) 0.2 µl, Template DNA (25 ng) 2 µl, H2O(d.w.) 16.8 µl. The amplification reaction was carried out in a Perkin-Elmer Cetus 480 DNA Thermal Cycler (Perkin-Elmer, Warrington, UK), The thermocycler 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

The PCR products were separated on A mini-sub® cell GT agarose gel electrophoresis system (Bio-Rad, USA) in Tris-borate (TBE) buffer, pH 8.0, (5X stock solution; Tris-base 5.40 g, boric acid 2.75 g, 500 mM EDTA, PH 8.0 2 ml, distilled water up to 100 ml). The products were stained using Ethidium bromide (1 % solution in distilled water) and then visualized and photographed using a UV Polaroid camera type 57 (ASA 3000).

Sample loading dye (5X; Na-EDTA, pH 8.0 (500 mM) 2 ml, glycerol 5 ml, bromophenol blue 2% 0.75 ml, distilled water 1.5 ml).

Data analysis software

Gel works ID advanced software UVP-England.

RESULTS AND DISCUSSION

Botanical profiling

Macromorphology of Tecoma Smithii Will. Wats. (Figs. 1 & 2)

Tecoma Smithii Will. Wats. cultivated in Egypt is an evergreen small tree, attains a height of 1.5 - 2.5 m and diameter up to 5 cm (Fig. 1-A).

The stem

The main stem (trunk) and old branches (Fig. 1-B) are hard, solid, cylindrical, with rough surface showing fine longitudinal fissures and scars. The old branches measure 0.5-1.5 cm in diameter. The young branches (Fig. 1-C) are herbaceous, glabrous to the naked eye, green, flexible and measure 2-5 mm in diameter. Branching is monopodial. Stem bark is thin papery, dotted and light brown in color.

The leaf

The leaf (Fig. 1-D) is compound imparipinnate, measuring 6-15 cm in length and 3-6 cm in width, carries 7-13 leaflets, bright green color, exstipulate and show opposite decussate arrangement. The rachis of the compound leaf is cylindrical with light green color and measures 2-7.5 cm in length and 0.5-0.7 mm in diameter. The petiole is cylindrical, light green color, glabrous and measures 1.5 - 4.5 cm in length and 1-2 mm in diameter. The leaf blade shows a narrow thin extension along the length of the rachis until the end of the petiole.

The leaflets (Fig. 1-E) are opposite, ovate to oblong in shape with an acute apex, serrate margin, and asymmetric base. The leaflet measures 1.5 - 3 cm in length and 0.7 - 1.2 cm in width. The venation is pinnate reticulate and the leaflets are nearly sessile or have short petiolules. The midrib is more prominent on the lower surface. The upper

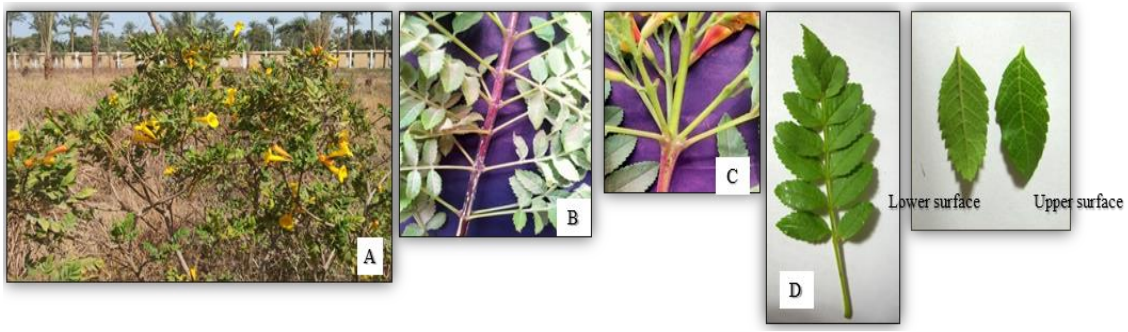

Figure 1: Photographs of *Tecoma Smithii* Will. Wats. (A) The whole plant (X=0.06), (B) The old stem (X=0.2), (C) The young stem (X=0.5), (D) The leaf (X=0.24), The leaflet (X=0.5)

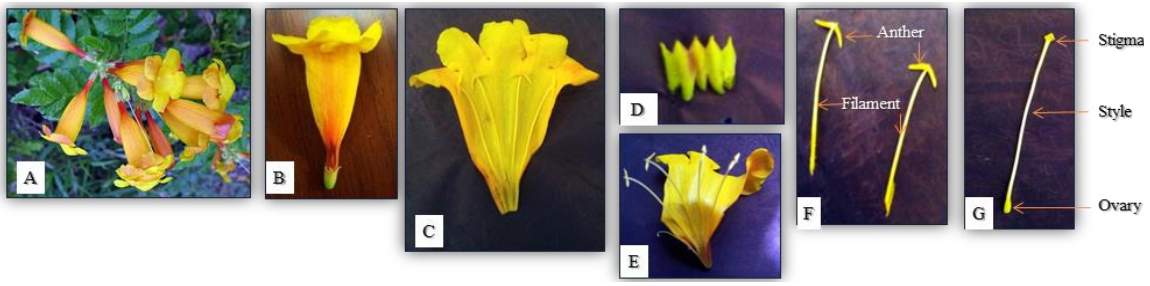

Figure 2: Photographs of the inflorescence of *Tecoma Smithii* Will. Wats. (A) The inflorescence (X=0.2), (B) The flower (X=0.4), (C) The fused corolla (opened longitudinally) (X=0.45), (D) The fused calyx (opened longitudinally) (X=0.8), (E) The flower exposing the epipetalous stamens (X=0.4), (F) The stamens (X=0.6), (G) The gynoecium (X=0.45)

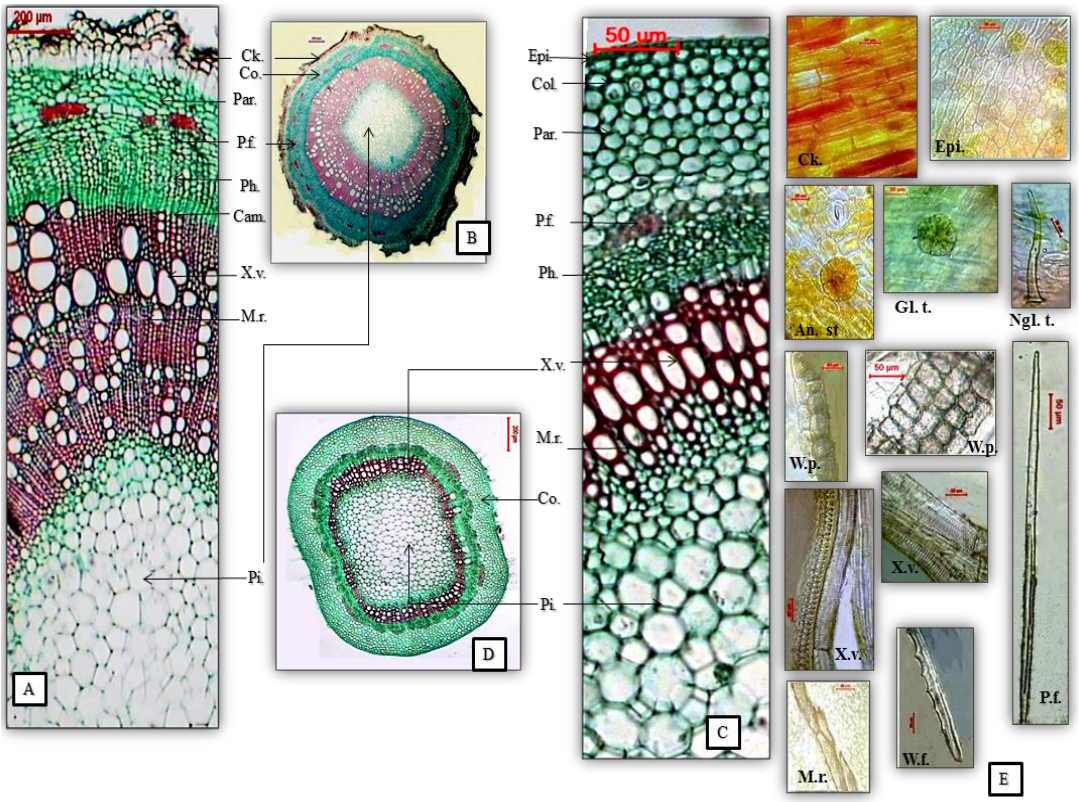

Figure 3: Transverse section of the stem of *Tecoma Smithii* Will. Wats. (A) High power view of the old stem (X=47), (B) Low power view of the old stem (X=8.8), (C) High power view of the young stem (X=210), (D) Low power view of the young stem (X=20), (E) Powdered stem (X=85)

An.st., anomocytic stomata; Cam., cambium; Ck., cork; Col., collenchymas; Epi., epidermis; Gl.t., glandular trichomes; M.r., medullary ray; Ngl.t., non-glandular trichomes; Par., parenchyma; Ph., phloem; Pi., pith; P.f., pericyclic fibers; W.f., wood fibers; W.p., wood parenchyma; X.v., xylem vessel

surface is vivid green while the lower surface is pale green, but both sides are finely pubescent and the texture is papery. It has a mild characteristic odor and an astringent taste.

The flower (Fig. 2)

The flower is zygomorphic, hermaphrodite and has no characteristic odor (however attractive to insects owing to its nectar disk). It measures 2.5 - 5.5 cm in length and 1.3-1.6 cm in width, tubular or funnel-shaped (Fig. 2-A) and rises in panicle inflorescence. The panicles are crowded at the apex of the main stem and lateral branches like hanged bells (Fig. 2-B).

The calyx (Fig. 2-D) is formed of five sepals, greenish yellow in color, gamosepalous; where the fused sepals appear as a five-toothed short tube. The fused sepals measure 4 - 8 mm in length and 7-9 mm in width. The corolla (Fig. 2-C and E) is formed of five petals, showy bright yellow tinged with orange, pubescent, gamopetalous; where the fused petals resemble a funnel form with rounded reflexed ends like lips. The fused corolla measures 2.5 - 5.5 cm in length and 13-16 mm in diameter at the widest area. The androecium (Fig. 2-E, F) consists of four epipetalous stamens. The filament is yellowish white and measures 2-3 cm in length and 0.4-0.5 mm in diameter. The anther is yellow in color, two celled and dorsifixed. It measures 5-7 mm in length and 0.6-0.8 mm in width. The gynoecium (Fig. 2-G) consists of an ovary which is superior, syncarpous, bicarbillary forming two locules separated by a septum, each locule contains numerous ovules showing axile placentation. The ovary measures 3-4 mm in length and 0.4-0.5 mm in diameter. The style is cylindrical, yellowish white color and measures 3.5- 4.2 mm in length 0.3-0.4 mm in diameter. The stigma is bifid and measures 0.7- 1 mm in length.

Micromorphology of Tecoma Smithii Will. Wats.:

The stem (Figs. 3)

A transverse section of the old stem (Fig. 3-A and B) is almost circular in outline, formed of somewhat narrow cork, formed of 4 - 6 rows of brown, polygonal, tangentially elongated cells, having thick suberized walls (Fig. 3-E). followed by a narrow secondary cortex, formed of 5 - 7 rows of thin-walled cellulosic parenchyma cells with narrow intercellular spaces. The endodermis is indistinct. The well developed vascular system consists of a circumference of pericycle which is formed of patches of fibers interrupted by parenchyma cells. The fibers appear in the powder (Fig. 3-E) to have lignified walls, narrow lumina, and acute apices. The vascular bundles are collateral and arranged in a continuous ring traversed by uni- and biseriate medullary rays. Phloem region (outer tissue) consists of thin-walled elements formed of sieve tubes, companion cells and phloem parenchyma with no phloem fibers, followed by 2-3 rows of thin-walled tangentially elongated, radially arranged cells of cambium. Xylem region (inner tissue) consists of lignified radially arranged elements, formed of thick walled spiral and annular vessels (Fig. 3-E), wood fibers have undulated sides, acute to rounded apices and narrow lumina (Fig. 3-E), wood parenchyma are rectangular with moderately thin lignified pitted walls (Fig. 3-E), and pitted lignified radially elongated cells of the medullary rays (Fig. 3-E). The pith is relatively narrow and formed of large rounded

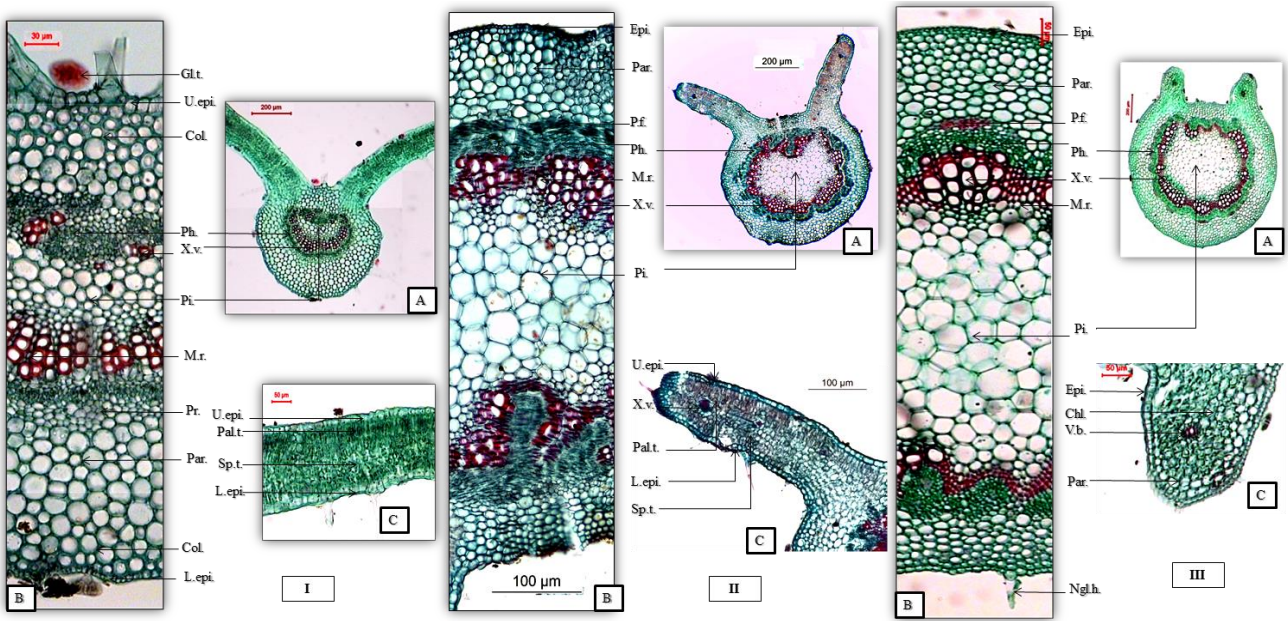

Figure 4: Transverse section of the compound leaf of *Tecoma Smithii* Will. Wats.

(I-A) Low power view of the leaflet (X=20), (I-B) High power view of the midrib region of the leaflet (X=110), (I-C) High power view of the lamina region of the leaflet (X=40), (II-A) Low power view of the rachis (X=22), (II-B) High power view of the rachis (X=88), (II-C) High power view of the region of the extension of the rachis (X=55), (III-A) Low power view of the petiole (X=15), (III-B) High power view of the petiole (X=55), (III-C) High power view of the region of the extension of the petiole (X=60).

Chl., chlorenchyma; Epi., epidermis; L.epi., lower epidermis; M.r., medullary rays; Ngl.h., non-glandular hair; Par., parenchyma; P.f., pericyclic fibers; Ph., phloem; Pi., pith; Sp.t., sponge tissue; Pal.t., palisade tissue; U.epi., upper epidermis; V.b., vascular bundle; X.v., xylem vessel

thin walled parenchymatous cells with narrow intercellular spaces.

The young stem

The structure of the young stem (Figs. 3-C and D) is almost similar to that of the old stem with the following differences:

- The transverse section is more or less rectangular in outline.
- The absence of cork cells; instead there are epidermal cells that are tubular, axially elongated cells, with thin straight anticlinal walls, and covered with thin, smooth cuticle. Stomata are rare and of anomocytic type (Fig. 3-E).
- Two types of trichomes are present: They are of glandular and non-glandular types:
  - Glandular trichomes: typical peltate hair: with a very short unicellular stalk and a broad, round multicellular secretory head consisting of four to twelve radiating cells, all in a single shield (Fig. 3-E).
  - Non-glandular trichomes: They are multicellular (2-4 cells, mostly 2), uniseriate, unbranched, conical, curved or straight at the apex and covered with thick smooth cuticle (Fig. 3-E).
- The cortex region is formed of 3-4 rows collenchyma followed by 5-6 rows of parenchyma.
- The pericyclic fibers are characterized by their relatively wider lumen.
- The cambium is indistinct.
- The vascular tissue is narrow and the pith is wider.

The powdered stem

Powdered stem (Fig. 3-E) is yellowish brown in color with no characteristic odor or taste. The diagnostic microscopical features are: fragments of axially elongated cork cells with thick suberized, fragments of polygonal, axially elongated epidermal cells with straight anticlinal walls, covered with smooth cuticle and showing few anomocytic stomata, numerous glandular and non-glandular trichomes, fragments of lignified spiral and annular xylem vessels, fragments of lignified pitted wood parenchyma, fragments of medullary rays showing radially elongated cells with thin lignified walls. fragments of wood fibers, they have moderately thin–lignified walls, with undulated sides, wide lumina, and acute to rounded apices, fragments of fusiform pericyclic fibers, with moderately lignified walls, narrow lumina, and acute apices. The absence of calcium oxalate crystals and starch granules was observed.

The leaf

A transverse section of the leaflet through the lamina and midrib (Fig. 4- I) shows a biconvex midrib and the lamina with dorsiventral mesophyll. The palisade is formed of two rows being interrupted in the midrib region by collenchymatous tissue. The midrib appeared more prominent on the lower surface. The vascular tissue of the midrib consists of an arc of crescent shaped collateral vascular bundle and a smaller inverted arc towards the upper side, enclosing in-between a narrow pith.

The upper epidermal cells (Fig. 5) are polygonal, nearly isodiametric with straight anticlinal walls, showing anomocytic stomata and covered with thin smooth cuticle (sometimes striated around stomata). Besides, glandular and non-glandular trichomes are present.

The lower epidermal cells (Fig. 5) are similar to those of the upper epidermis, but they are slightly axially elongated and showing more frequent stomata and trichomes.

Trichomes (Fig. 5) are very numerous on both surfaces. They are of glandular and non-glandular types. The glandular trichomes are more frequent on the lower epidermis. They are similar to those in the stem but of smaller size.

The lamina region (Fig. 4- IC)

The mesophyll in lamina region is dorsiventral and differentiated into palisade tissue and spongy parenchyma. The palisade tissue is formed of a closely packed layer of two rows of radially elongated columnar cells that show straight anticlinal walls and contain chloroplasts. They are discontinued in the midrib region by collenchymatous tissue. The spongy tissue is formed of irregular round shaped parenchyma cells with wide intercellular spaces. Small vascular bundles of the lateral veins are embedded within the spongy tissue.

The midrib region (Fig. 4- IB)

The upper cortical tissue consists of 4-5 rows of collenchyma cells followed by 3-4 rows of large, thin-walled rounded parenchyma cells, while the lower cortical tissue consists of 2-3 rows of collenchyma cells followed by 4-6 rows of parenchyma cells. The endodermis is indistinct. The vascular tissue is arranged in two crescent shaped groups forming an almost continuous ring, showing xylem towards the pith and the phloem outwards and surrounded by parenchyma cells of the pericycle. The phloem is comparatively narrow and consists of thin-walled cellulosic phloem elements; sieve tubes, companion cells and phloem parenchyma with no fibers. The xylem is composed of lignified vessels (Fig. 5) with annular and spiral thickening and wood rectangular parenchymatous cells (Fig. 5) having pitted lignified walls. The cambium is almost indistinguishable. Uni- and biseriate, radially elongated parenchyma cells of medullary rays traverse the vascular bundle. The pith is formed of large thin-walled parenchymatous cells.

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Table 1: Microscopical measurements of the different elements of *Tecoma Smithii* Will. Wats.

Element	L*	W*	H*	D*
The stem				
Epidermal cells	20-38-46	12-17-20	3-4-5	-
Stomata	15-17-19	4-5-7	-	-
Glandular trichomes				
Peltate type				
➢ The stalk	3-4-9	10-18-20	-	-

Table 1: Microscopical measurements of the different elements of *Tecoma Smithii* Will. Wats.

Element	L*	W*	H*	D*
➢ The head	-	-	-	42-50-65
Non-glandular trichomes	70-104-138	6-17-20	-	-
Cork	75-84-127	21-24-25	15-18-23	
Medullary rays	25-29-35	17-21-33	-	-
Pericyclic fibers	305-610-703	7-15-18	-	-
Wood fibers	500-552-600	10-12.5-22	-	-
Wood parenchyma	50-60-105	10-15-18	-	-
Xylem vessels	-	-	-	12-22-44
Sclereids	31-47-104	17-22-30	-	-
The leaflet				
Upper epidermal cells	23-30-44	15-20-25	18-20-22	-
Lower epidermal cells	18-26-30	10-18-24	7-14-15	-
Stomata	5-6-7	3-5-7	-	-
Glandular trichomes				
Peltate type				
➢ The stalk	9-10-13	6-9-14	-	-
➢ The head	-	-	-	25-30-40
Non-glandular trichomes	100-116-138	6-10-16	-	-
Palisade cells	25-30-35	7-11-13	-	-
Medullary rays	50-56-66	12-16-26	-	-
Wood parenchyma	52-90-123	7-10-14	-	-
Xylem vessels	-	-	-	4-9-12
Volatile oil droplets	-	-	-	2-3-4
The rachis				
Epidermal cells	28-33-50	11-15-20	8-9-11	-
Glandular trichomes				
Peltate type				
➢ The stalk	9-10-13	6-9-10	-	-
➢ The head	-	-	-	40-46-52
Non-glandular trichomes	37-45-63	4-5-7	-	-
Palisade cells	14-22-23	7-8-11	-	-
Wood fibers	200-226-250	10-13-16	-	-
Wood parenchyma	52-90-123	7-10-14	-	-
Xylem vessels	-	-	-	5-14-16
Volatile oil droplets	-	-	-	2-3-4
The petiole				
Epidermal cells	30-34-50	10-12-16	21-23-25	-
Glandular trichomes				
Peltate type				
➢ The stalk	9-10-13	6-9-14	-	-
➢ The head	-	-	-	31-41-43
Non-glandular trichomes	60-77-80	8-10-13	-	-
Wood fibers	200-226-250	10-13-16	-	-
Wood parenchyma	52-90-123	7-10-14	-	-
Xylem vessels	-	-	-	15-25-35
Volatile oil droplets	-	-	-	2-3-4
The flower				
a. The calyx				
Epidermal cells	16-23-28	11-12-15	13-14-16	-
Stomata	11-12-14	4-5-6	-	-
Xylem vessels	-	-	-	4-5-6
b. The corolla				
Inner epidermis at the tip	32-40-59	22-36-40	-	-
Outer epidermis at the tip	32-36-45	20-22-26	-	-
Glandular trichomes				
➢ The stalk	307-333-384	50-55-69	-	-
➢ The head	-	-	-	40-44-46
Xylem vessels	-	-	-	5-8-10

Table 1: Microscopical measurements of the different elements of *Tecoma Smithii* Will. Wats.

Element	L*	W*	H*	D*
c. The androecium				
Epidermis of filament	111-129-137	14-16-27	14-18-20	-
Epidermis of anther	33-48-57	12-15-21	9-15-21	-
Fibrous layer of anther	18-24-27	9-12-15	-	-
Pollen grains	-	-	-	34-37-40
d. The gynoecium				
Epidermis of ovary	11-16-21	12-15-20	9-10-12	-
Epidermis of style	81-115-136	10-16-24	12-15-20	-
Epidermis of stigma	-	-	9-10-14	
Glandular trichomes				
➢ The stalk	10-12-14	7-9-10	-	-
➢ The head	-	-	-	28-40-42

*L, W, H, D are length, width, height and diameter scaled in micrometer

Table 2: The total number of RAPD-PCR fragments, the distribution of monomorphic and polymorphic bands, the percentage of polymorphic fragments, and genetic similarity percentage generated by ten primers with *Tecoma mollis* Humb. & Bonpl. (Sp1) and *Tecoma Smithii* Will. Wats. (Sp2)

Primer code	RAPD fragments		Monomorphic fragments	Polymorphic fragments	Percentage of polymorphism	Genetic similarity
	Sp1	Sp2				
OPAX-16	7	7	4	6	42.85	57.14
OPAX-19	3	5	3	2	25	75
OPC-01	8	8	8	-	0	100
OPC-14	6	7	5	3	23.07	76.93
OPC-16	6	3	3	3	33.33	66.67
OPD-05	7	9	7	2	12.50	87.5
OPD-07	10	10	8	4	20	80
OPE-03	8	8	8	-	0	100
OPE-19	5	6	4	3	27.27	72.73
OPG-05	9	10	9	1	5.26	94.74
Total	69	73	59	24	16.9	83.09

A transverse section in the rachis (Fig. 4- II) is more or less circular in outline with two long projections representing the leaf blade extension. It shows an outer epidermis (Fig. 5) consists of polygonal axially elongated cells with straight anticlinal walls and covered with thin cuticle and trichomes which are similar to those of the leaflet but less in number, stomata are rare. The cortex is formed of 7-10 rows of parenchymatous cells. The vascular tissue consists of patches of collateral vascular bundles, connected to each Other forming a wavy ring and surrounded by discontinued patches of nonlignified fibers of pericycle. The phloem consists of thin-walled cellulosic elements and the xylem is formed of annular and spiral lignified vessels, with thin lignified wide lumina, rounded apices wood fibers and irregular shaped pitted lignified wood parenchyma (Fig. 5). The cambium is indistinct. Uni- and biseriate, radially elongated parenchyma cells of medullary rays traverse the vascular bundle. The pith region is wide and formed of parenchyma cells. At the region of the two projections (Fig. 4- IIC), the cortical tissue represents a typical structure of the mesophyll region of the leaflet, showing two rows of palisade columnar cells, followed by chlorenchymatous cells surrounding small vascular bundle.

The microscopical structure of the petiole (Fig. 4- III) closely resembles that of the rachis, but the outline of the section (Fig. 4- IIIA) shows much shorter projections, trichomes are scarce, The cortical region of the two projections consists of one or two rows of parenchyma, followed by several rows of chlorenchyma (Fig. 5), all surrounding a narrow vascular bundle. The patches of the pericycle are more condensed and lignified (Fig. 5).

Powdered Leaf (Fig. 5)

Powdered leaf is pale green in color with characteristic odor and slightly bitter taste. The diagnostic microscopical features of the powder are: fragments of polygonal isodiametric epidermal cells of the leaflet having straight anticlinal walls, covered with thin smooth cuticle and showing anomocytic stomata, fragments of axially elongated epidermal cells of the rachis and petiole with straight anticlinal walls and covered with smooth cuticle, glandular trichomes of peltate type, uniseriate non-glandular trichomes, spiral and annular lignified xylem vessels, rectangular lignified pitted wood parenchyma, radially elongated lignified cells of medullary rays, long, fusiform, lignified pericyclic fibers of the petiole, short lignified wood fibers of the petiole with blunt apices, columnar thin walled palisade cells, oily droplets dispersed in the field.

The flower

Sections were made for the whole flower and its individual parts (Fig. 6). A transverse section of the sepal (Fig. 6- A

Table 3: The total number of RAPD-PCR fragments, the distribution of monomorphic and polymorphic bands, the percentage of polymorphic fragments, and genetic similarity percentage generated by ten primers with *Tecoma Smithii* Will. Wats. (Sp2) and *Tecomaria capensis* (Thunb.) Lindl. (Sp3).

Primer code	RAPD fragments		Monomorphic fragments	Polymorphic fragments	Percentage of polymorphism	Genetic similarity
	Sp2	Sp3				
OPAX-16	7	8	4	7	46.66	53.34
OPAX-19	5	8	5	3	23.07	76.93
OPC-01	8	8	8	-	0	100
OPC-14	7	7	4	6	42.85	57.15
OPC-16	3	6	3	3	33.33	66.67
OPD-05	9	5	4	6	42.85	57.15
OPD-07	10	14	9	6	25	75
OPE-03	8	8	8	-	0	100
OPE-19	6	8	6	2	14.28	85.72
OPG-05	10	10	10	-	0	100
Total	73	82	61	33	21.29	78.71

and D) shows polygonal cells of the inner and outer epidermises covered with a thin cuticle. Anomocytic stomata and trichomes are present only on the outer epidermis (Fig 7 a). The trichomes are glandular peltate, similar to those of the stem and leaf. The mesophyll is homogenous and consists of several rows of large rounded parenchymatous cells, traversed by small vascular bundles, which is formed of xylem and phloem.

A transverse section of the petal (Fig. 6- C and E) shows outer and inner epidermises, enclosing a homogenous mesophyll consists of several rows of loosely attached parenchymatous cells and traversed by vascular bundles. The inner epidermis at the upper part (Fig. 7 b) is composed of polygonal, isodiametric cells with wavy anticlinal walls, covered with smooth cuticle and show glandular protrusions of a short unicellular stalk and unicellular head. At the lower part (Fig. 7 c) the cells are polygonal axially elongated showing glandular trichomes with giant unicellular stalk and unicellular head. The outer epidermis (Fig 7 d) at the upper part is composed of polygonal, isodiametric cells with wavy anticlinal walls, covered with smooth cuticle and show protrusions of finger-like papillae. At the lower part, the cells are similar to those of the inner epidermis, but devoid of any trichomes.

A transverse section in the anther (Fig. 6- F) shows two lobes attached by the connective tissue and has a central vascular bundle that consists of phloem and a few xylem vessels. The epidermis of the anther (Fig. 7 e) consists of elongated cells, with wavy anticlinal walls and covered with a thin faintly striated cuticle. Anomocytic stomata are present but rare. Trichomes are absent. Each anther lobe is formed of pollen sac containing numerous translucent pollen grains. The pollen grains (Fig. 7 f) are more or less triangular in shape with smooth surfaces and three germination pores, one at each angle. The fibrous layer of the anther (Fig. 7 g and h) is formed of one row of radially elongated cells, nearly polygonal in surface view showing lignified bar-like thickening.

a transverse section in the filament (Fig. 6- G) is more or less triangular in outline. The epidermis (Fig. 7 i) consists of flattened axially elongated cells with straight anticlinal walls, covered with thin cuticle and devoid of stomata and trichomes. The epidermis encloses a ground tissue formed of parenchymatous cells, within which small vascular bundles are embedded at the center.

A transverse section in the ovary (Fig. 6- H and I) is rounded in outline and shows 2 distinctive cuts at the two extremities. The line drawn between the two cuts is perpendicular to the septum. These two cuts represent the point of dehiscence of the fruit pericarp after ripening of the ovary. It is bilocular with axial placentation. The ovary wall consists of an outer and inner epidermises enclosing in-between a condensed parenchymatous mesophyll traversed by small vascular bundles scattered all around the circumference of the mesophyll and two special vascular bundles supporting the cut extremities. The outer and inner epidermises of the ovary are formed of polygonal isodiametric cells. The outer epidermal cells appear indistinguishable (Fig. 6- I) may be as it is heavily covered with numerous glandular trichomes of peltate type (Fig. 7 j).

A transverse section of the style is almost rounded in outline (Fig. 6- J). The epidermis of the style is formed of polygonal axially elongated cells (Fig. 7 k). Some cells of the outer epidermis contain certain pigments and appear dark. Inner epidermis appears more tangentially elongated, not pigmented, slightly papillosed and loosely attached to the underneath layers. Both enclosing loose parenchymatous cells turn smaller towards the center. The ground tissue is traversed by two vascular bundles appear as two poles. the transverse section of the stigma appears as two separate lips (Fig. 6- K and L). The stigmatic surface shows a distinctly papillosed epidermis. Papillae are cylindrical and protrude sometimes as finger-like shape (Figs. 6- L and 7 h).

Powdered Flower (Fig. 7)

The powdered flower is pale yellow in color with no characteristic odor and with mucilaginous taste. The diagnostic microscopical features of the powder are: several fragments of the epidermises of the different parts: the sepal (Fig. 7 a) showing polygonal isodiametric cells having straight anticlinal walls, covered with thin smooth cuticle and showing few anomocytic stomata and peltate

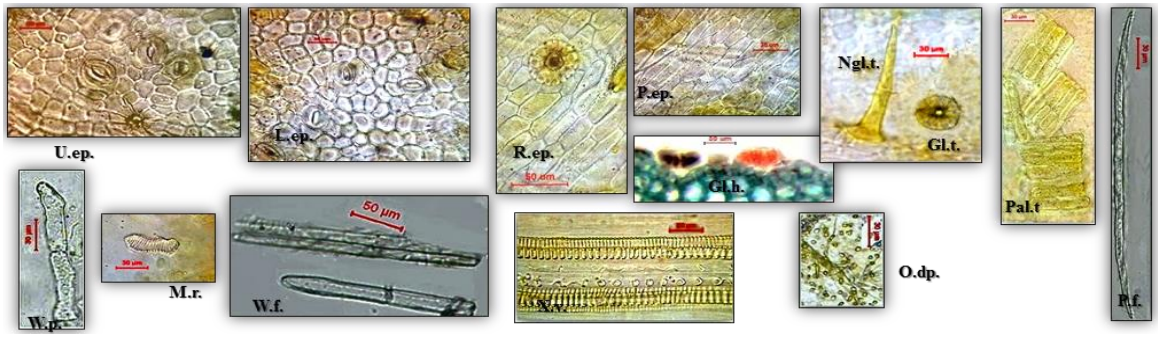

Figure 5: Powdered leaf of *Tecoma Smithii* Will. Wats. (X=120) Gl.t., glandular trichomes; L.ep., lower epidermis; M.r., medullary rays; Ngl.t., non-glandular trichomes; O.dp., oily droplets; Pal., palisade; P.ep., petiole epidermis; P.f., pericyclic fibers of petiole; R. epi., rachis epidermis; U.ep., upper epidermis; W.f., wood fibers; W.p., wood parenchyma; X.v., xylem vessels

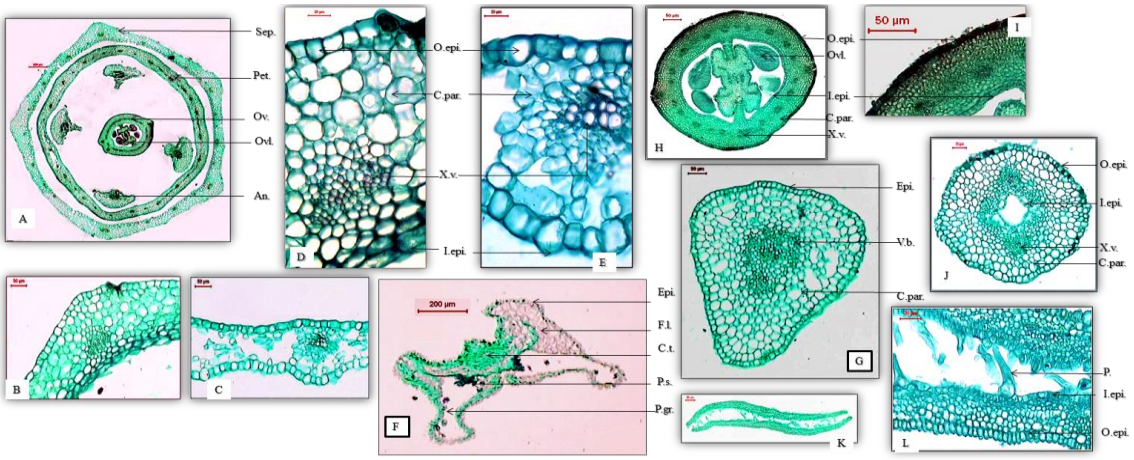

Figure 6: Transverse sections of the flower parts of *Tecoma Smithii* Will. Wats.
(A) Transverse section of the whole flower At the upper part of the calyx (X= 10), (B) low power view of the sepal (X=35), (C) low power view of the petal (X=35), (D) High power view of the sepal (X=125), (E) High power view of the petal (X=125) (F) Transverse section of anther (X=28), (G) Transverse section of filament (X=42) (H) Transverse section of ovary (X=40), (I) High power view of the surface of ovary (X=110), (J) Transverse section of style (X=55), (K) Transverse section of stigma (X=22), (L) High power view of the surface of stigma (X=80)
Ant., anther; C.par., cortical parenchyma; C.t., connective tissue; Epi., epidermis; F.l., fibrous layer of anther; I.epi., inner epidermis; O.epi., outer epidermis; Ov, ovary; Ovl., ovule; p., papillae; Pet., petals; P.gr., pollen grains; P.s., pollen sac; Sep., sepals; V.b., vascular bundle

glandular trichomes, the petal (Fig. 7 b and c) consists of polygonal isodiametric cells with wavy anticlinal walls at the upper region and axially elongated cells with straight anticlinal walls at the lower region, and covered with smooth cuticle. The inner epidermis of the petal is covered with glandular trichomes (Fig. 7 d), the anther (Fig. 7 e) with polygonal axially elongated cells with wavy anticlinal walls and showing few anomocytic stomata, the epidermis of the filament (Fig. 7 i) consists of flattened, thin-walled, axially elongated cells. The ovary outer epidermal cells (Fig. 7 j) are indistinctive and covered with numerous glandular trichomes of peltate type, the style epidermal cells (Fig. 7 k) are axially elongated with thin walls, the stigma (Fig. 7 l) showed numerous crowded papillae of various sizes. Beside the epidermal fragments, there are fragments of the fibrous layer of the anther (Fig. 7 g and h) formed of radially elongated cells, nearly polygonal in surface view showing lignified bar-like thickenings. Fragments of small lignified spiral vessels (Fig. 7 m). Numerous pollen grains more or less triangular in shape with three germination pores, are dispersed in the field (Fig. 7 f).

The microscopical measurements of the different elements of *Tecoma Smithii* Will. Wats. are illustrated in table (1).

Genetic profiling

DNA fingerprinting

The extracted DNA of each of the three species, *Tecoma mollis* Humb. & Bonpl., *Tecoma Smithii* Will. Wats., and *Tecomaria capensis* (Thunb.) Lindl. was amplified using ten decamer primers to detect their genetic relationship. Each of the primers had successfully directed the amplification of a genome-specific fingerprint of DNA fragments and consequently serves to evaluate interspecific diversity between these species. The obtained

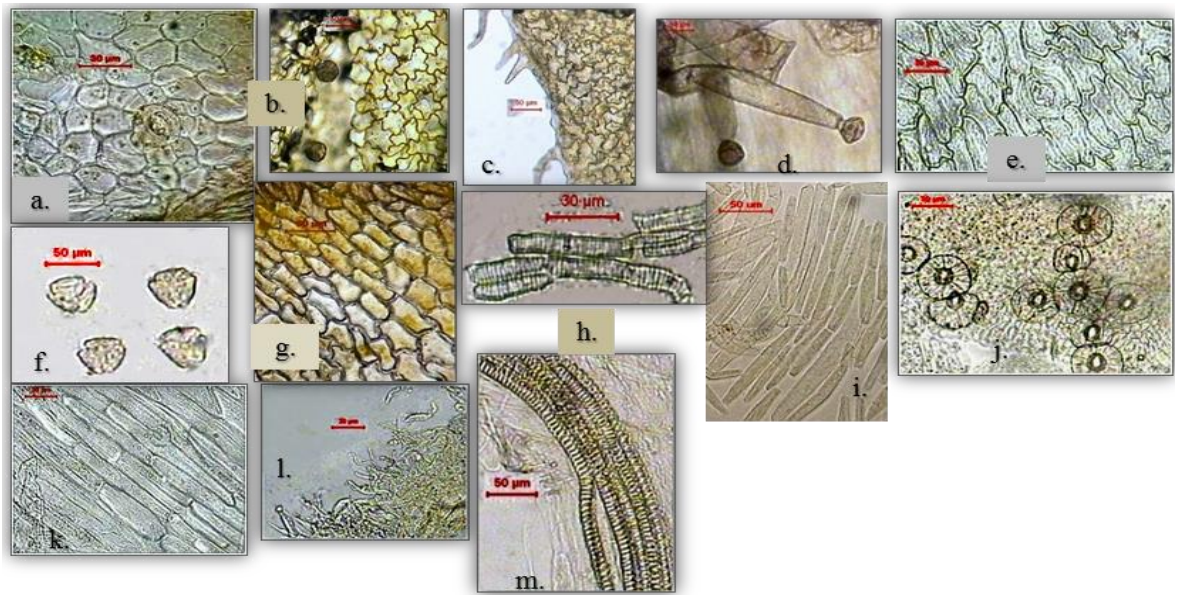

Figure 7: Powdered flower of *Tecoma Smithii* Will. Wats.
a) Epidermis of the sepal (X=180), b) Inner epidermis of the petal (X=60), c) Outer epidermis of the petal (X=65), d) Glandular trichomes of the petal epidermis (X=100), e) Epidermis of the anther (X=145), f) Pollen grains (X=120), g) Fibrous layer of anther (top view) (X=175), h) Fibrous layer of anther (side view) (X=250), i) Epidermis of the filament (X=110), j) Outer epidermis of the ovary (X=150), k) Epidermis of the style (X=115), l) Epidermis of the stigma (X=105), m) Xylem vessels (X=110),

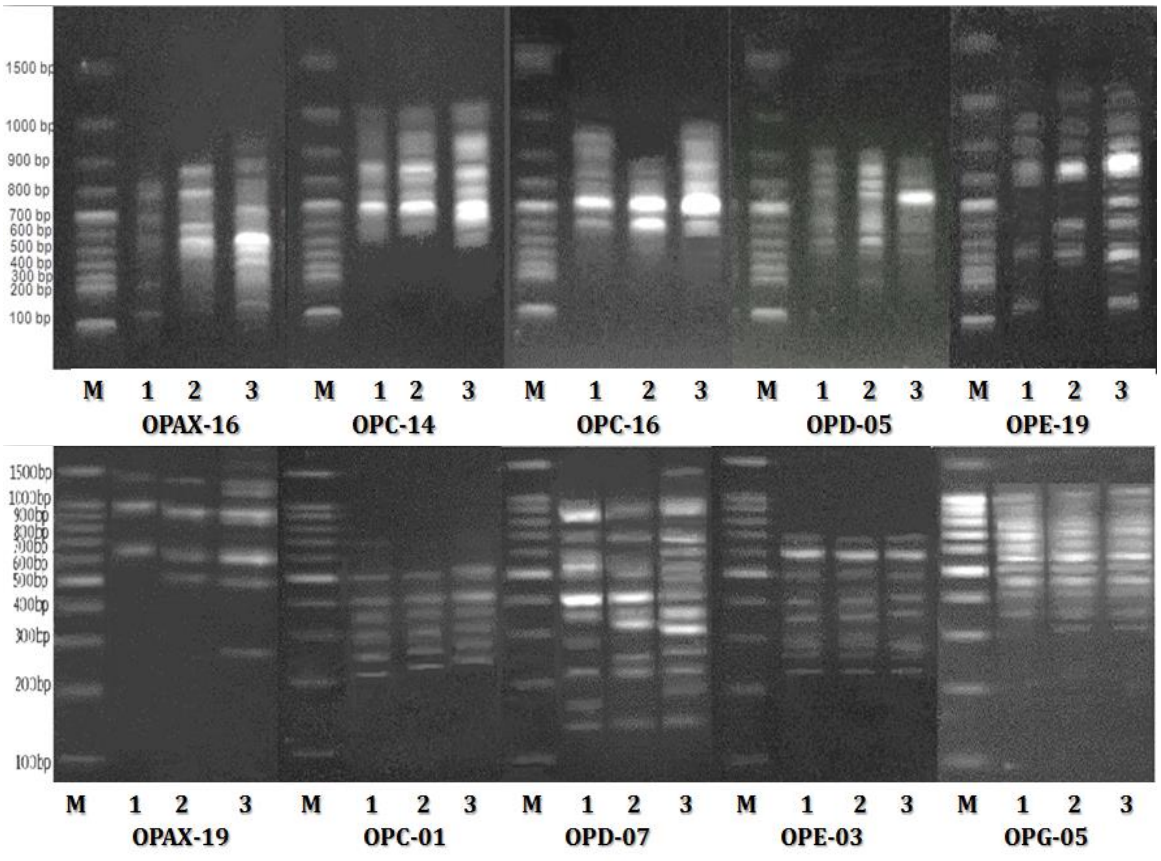

Figure 8: The RAPD electrophoretic profile of the three species under investigation generated by the ten decamer primers; Lane1: *Tecoma mollis* Humb. & Bonpl., Lane2: *Tecoma Smithii* Will. Wats., Lane3: *Tecomaria capensis* (Thunb.) Lindl., M: Marker

photographs of RAPD-PCR using the ten decamer primers, as detected by gel electrophoresis, for the three *Tecoma* species are represented in Fig. (8).

The ten primers had produced multiple band profiles with a number of amplified DNA fragments ranging from 3 when OPAX-19 is used with *Tecoma mollis* Humb. & Bonpl. and OPC-16 with *Tecoma Smithii* Will. Wats. and the maximum number of bands was 14 at OPD-07 with *Tecomaria capensis* (Thunb.) Lindl. The total number of fragments were 224 in all of the three species, distributed as 69, 73, and 82 for *Tecoma mollis* Humb. & Bonpl., *Tecoma Smithii* Will. Wats., and *Tecomaria capensis* (Thunb.) Lindl. respectively.

For estimating genetic distance among the tested samples, the RAPD electrophoretic profile of *Tecoma Smithii* Will. Wats. was compared independently with each one of the other two species (i.e.: the parent species). Table (2) represents *Tecoma mollis* Humb. & Bonpl. (Sp1) versus *Tecoma Smithii* Will. Wats. (Sp2), and table (3) represents *Tecoma Smithii* Will. Wats. (Sp2) versus *Tecomaria capensis* (Thunb.) Lindl. (Sp3).

When relating *Tecoma mollis* Humb. & Bonpl. (Sp1) to *Tecoma Smithii* Will. Wats. (Sp2), the highest degree of similarity (GS %= 100%) was corresponding to the primers OPC-01 and OPE-03, this can strongly support the claim of close genetic relation. The total genetic similarity percentage (calculated by averaging all the resulted values of genetic similarity for every combination) is 83.09%. while relating *Tecoma Smithii* Will. Wats. (Sp2) to *Tecomaria capensis* (Thunb.) Lindl. (Sp3), the highest degree of similarity (GS %= 100%) was corresponding to the primers OPC-01, OPE-03, and OPG-05, which again supports the claim of close genetic relation The total genetic similarity percentage equals 78.71%. The lowest degree of similarity in both cases was corresponding to the primer OPAX-16, means that this primer was found to be the most effectively generating polymorphic bands on applying the RAPD technique for the three species and so can be the one of choice for differentiation between the three species or each two of them.

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 *Tecoma Smithii* Will. Wats., besides, the primer OPAX-16 could be used to discriminate between *Tecoma Smithii* Will. Wats. and the parent species *Tecoma mollis* Humb. & Bonpl., *Tecoma Smithii* Will. Wats., and *Tecomaria capensis* (Thunb.) Lindl. depending on the low values of similarity coefficients and high level of polymorphism. While, the primers OPC-01, OPE-03 could be used in the authentication of the three *Tecoma* species as highest similarity coefficients were indicated.

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