WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 39-63. Research Article ISSN 2277– 7105

# BOTANICAL AND GENETIC CHARACTERISTICS OF *DALBERGIA PANICULATA* ROXB. CULTIVATED IN EGYPT

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Article Received on
18 March 2015,

Revised on 09 April 2015,
Accepted on 03 May 2015

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## ABSTRACT

Genus *Dalbergia* (Fabaceae) comprises about 159 species, native to the tropical regions of Central and South America, Africa, Madagascar and Southern Asia. *Dalbergia paniculata* Roxb. is a perennial non climbing tree with yellowish white flowers. Its heartwood is used in making musical instruments and construction of buildings. Reports had shown its antimicrobial, antioxidant, anti-inflammatory, antiulcerogenic and antidiarrheal activities. The macro- and micro-morphological characters of the different organs (young and old stem, leaf and seed) of *Dalbergia paniculata* Roxb., grown in Egypt, were presented and illustrated for their identification in both entire and powdered form. Furthermore, the DNA of the plant was extracted from leaf samples and analyzed using 15 decamer random primers. A total of 179 random amplified polymorphic DNA (RAPD) markers were identified and compared with that of *Dalbergia sisso* Roxb. Both the botanical study and the DNA fingerprint play a role in the identification of the plant.

**KEYWORDS:** *Dalbergia paniculata*, botanical study, DNA fingerprint.

## INTRODUCTION

Family Fabaceae (Leguminosae) is one of the largest families of flowering plants, consisting of 730 genera and over 19,400 species.[1] The genus *Dalbergia* belongs to subfamily Faboideae (previously known as papilinoidae) which includes 274 species distributed all over

the world, especially in the tropical and subtropical regions. In Egypt, there are two species of *Dalbergia (viz. Dalbergia paniculata* Roxb. *and Dalbergia sisso* Roxb.*)*. Most *Dalbergia* species are widely used as timber trees and are valuable because of their decorative and fragrant wood.[2]

Members of the genus enjoy a number of traditional uses all over the world. Recent reports had showed their activity in the treatment of different ailments like aphthae, bleeding piles, cough, diarrhea, dysentery, dyspepsia, epigastria, epistaxis, gonorrhea, haemorrhages, leprosy, malaria, rheumatism, scabies, scalding urine, stomach ache, syphilis, traumatic injuries, and ulcers, etc. Species are also used for their analgesic, anthelmintic, antiinflammatory, antipyretic, anti-spermicidal, anti-ulcerogenic, aphrodisiac, astringent, expectorant, and larvicidal activities in traditional medicine.[3]

*Dalbergia paniculata* Roxb. is a perennial non climbing tree with yellowish white flowers. Its heartwood is used in making musical instruments and construction of buildings. Reports had shown its antimicrobial, antioxidant, anti-inflammatory and hepatoprotective activities.[4-6]

The aim of our work is to identify the macro- and microscopical characters of *Dalbergia paniculata* Roxb. *(*seed, leaf and stem).

Also to construct DNA profile of *Dalbergia paniculata* Roxb in comparison with the most common present species in Egypt (*Dalbergia sisso* Roxb.*)*. The concept of DNA fingerprinting has been increasingly applied to establish the ancestry of plants. It is reported as a promising tool for the authentication of medicinal plant species and especially useful in species or varieties that are morphologically and/or phytochemically indistinguishable.[7]

## MATERIALS AND METHODS

### Plant material

Samples of *Dalbergia paniculata* Roxb. were collected in 2010 from Al-Giza Zoo, Egypt and were kindly identified by Dr. Mohamed El-Gebaly, Botany Specialist.

### I- Botanical Profiling

Specimens for morphological studies were dried according to standard herbarium techniques and voucher samples (23-4-2015) were kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. Photographs were taken using a Casio Digital camera.

Anatomical investigations were performed on cross-sections of the old and young stems, leaves and seeds which were preserved in 70% alcohol and on air-dried finely powdered samples. The photographs were taken using a Leica DFC500 digital camera.

**II- Genetic Profiling**

**A- DNA fingerprinting**

Entire fresh leaves of *Dalbergia paniculata* Roxb. and *Dalbergia sisso* Roxb. were separately freeze-dried and ground to fine powder under liquid nitrogen prior to DNA isolation.

**DNA Extraction:** DNA was extracted from 10 g of leaf tissue in 1.5 ml microfuge tubes using the DNA extraction method described by.[8]

**Oligonucleotide Primers:** A total of 15 random decamer oligonucleotide primers from A, B, D, E, and G kits (Operon Technologies Inc.) were used to amplify *Dalbergia* genomic DNA having the following sequences: OPA-20: GTTGCGATCC, OPB-01: GTTTCGCTCC, OPB-05: TGCGCCCTTC, OPB-06: TGCTCTGCCC, OPB-07: GGTGACGCAG, OPB-15: GGAGGGTGTT, OPB-17: AGGGAACGAG, OPB-18: CCACAGCAGT, OPD-06: ACCTGAACGG, OPD-07: TTGGCACGGG, OPD-16: AGGGCGTAAG, OPD-18: GAGAGCCAAC, OPE-02: GGTGCGGGAA, OPG-02: GGCACTGAGG, OPG-03: GAGCCCTCCA.

**Polymerase Chain Reaction (PCR):** PCR amplification was conducted with 25 µl of reaction mixture containing 1% Triton 10-X reaction buffer (100 mM Tris-HCl (pH=8.3), 500 mM KCl, 0.01% (w/v) gelatin), 2.0 µl $MgCl_2$ (25 mM), 2.5 µl of each dNTP (2 mM), 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 2 drops of mineral oil. The amplification reaction was carried out in a Thermocycler Perkin Elmer Cetus 480 (Warrington, UK). The thermocycler was programmed for 1 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 1 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 mM **T**ris-HC1, 1.0 mM EDTA, pH=8.0) with a constant

power of 100 volts 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

### I- Botanical Profiling

### 1-Macromorphology of *Dalbergia paniculata* (Roxb.)

***Dalbergia paniculata*** **(Roxb) (Fig. 1)** is a perennial, non climbing medium-sized to large tree cultivated in Egypt. It is an evergreen tree 12-17 m in height. The plant starts flowering in March. Fruits appear at the end of April and fully ripen in May.

The trunk is hard woody, with monopodial branches covered with dark, rough, scaly bark showing wrinkled cork with few scattered lenticels. The trunk is 3-6 m in height and 0.7-1 m in diameter.

#### a-The lateral stem branches (Fig. 1)

The old stem branches are cylindrical, solid about 2-5cm in diameter and having rough, longitudinally striated greyish brown colour surface. They are hard to break having fibrous splintery fracture, exhibiting a pale- yellow interior.

Young stem branches (**Fig. 2 B)** are thinner, about 0.2-2 cm in diameter, cylindrical, green in colour, showing fine longitudinal striations and having glabrous surface. The intenodes are short, 2-3 cm long. The stem has faint characteristic odour and slightly bitter astringent taste.

#### b-The leaf (Fig. 2 A-D)

The leaves are alternate, compound imparipinnate, shortly petiolate and exstipulate. The compound leaf is composed of 11-15 alternate leaflets arranged on a rachis.The lamina of the leaflet is elliptic to ovate in shape with entire margin and emarginate sometimes obtuse apex and symmetric base. Lamina measures from 2-4.5 cm in length and from 1.5-2.5 cm in breadth. The venation is pinnate reticulate, the veins leave the midrib at angle 50°C and running towards the margin where they anastomose. Texture is leathery. The upper surface is dark green, smooth and glabrous to the naked eye while the lower is light green, smooth and glabrous to the naked eye. The midrib is prominent on the lower surface. The petiole is short 1-2 cm long, green, cylindrical showing a swollen pulvinous at its base. The rachis is green in colour. 12-19 cm in length and cylindrical. The petiolule of the leaflet is very short 0.2-0.8 cm long, green in colour and cylindrical. The leaf has a faint characteristic odour and astringent taste.

**c-The fruit: (Fig. A-C)**

The fruits are short stalked legumes (pods). The legume is elliptical to lanceolate thin walled, containing one or two seeds. It is green in colour, changing to yellowish brown on ripening. The stalk is cylindrical, brown in colour, and glabrous. The fruit measures from 6 to 9 cm in length and 1-1.7cm in width.

**d-The seed: (Fig. 3D-G)**

The seeds are kidney shaped dark glossy brown in colour. The surface is smooth and finely pitted. The seed curved near the hilum which present near the micropyle in a depression near the proximal end. The raphe is lighter in colour and extends from the depression of the hilum and micropyle to chalaza end. Transverse (Fig. 3G) and longitudinal (Fig. 3F) cuts in the seed show brownish testa surrounding two large yellowish white cotyledons. The seed is albuminous derived from anatropus ovule. The seeds measure from 0.6-1.1cm length and 0.35-0.45 cm width.

**2- Micromorphology of *Dalbergia paniculata* (Roxb.)**

**The stem**

**a-The old stem branch (Fig.4 )**

A transverse section in the old stem branch is circular in outline. It is formed of a cork followed by a narrow cortex. The endodermis is indistinct. The pericycle is formed of patches of lignified fibres interrupted with parenchyma. The vascular tissue is comparatively wide representing about 3/4 of the diameter forming a ring traversed by medullary rays. The central pith is relatively narrow constituting about 1/5 of the diameter and formed of parenchyma containing calcium oxalate prisms.

**The cork (Fig. 4B and 6)**

It is formed of several rows (3-6) of brown radially arranged, tangentially elongated cells having thin suberized walls appearing polygonal nearly isodiametric in surface view. The **phellogen** consists of 1-2 rows of tangentially elongated cells having thin cellulosic walls.

**The cortex (Fig. 4B)**

The cortex is formed of 5-7 rows of polygonal more or less isodiametric or elongated thick walled parenchyma cells containing calcium oxalate prisms.

**The pericycle (Fig. 4 B and 6)**

It is formed of patches of lignified fibres interrupted with parenchyma. The fibres are fusiform long, having straight walls, moderately wide to narrow lumina, acute apices and mostly are surrounded by parenchyma cells containing prisms of calcium oxalate forming a crystal sheath.

**The vascular tissue**

**The phloem** consists of soft tissue formed mainly of sieve tubes, companion cells and thin walled parenchyma cells.

The **xylem** is lignified formed of radially arranged elements. The **vessels** which are either simple or in small radial groups, are with pitted and spiral thickenings **. Wood fibres** having moderately wide to narrow lumina, straight thin lignified walls and acute apices. **Wood parenchyma** consists of rectangular cells with lignified pitted walls. **Tracheids** are elongated with thin lignified walls and blunt ends. Medullary rays are uniseriate **,** formed of rectangular radially elongated cells with lignified walls**. The pith** is formed of rounded parenchymatous cells containing prisms of calcium oxalate.

**b- The young stem branch (Fig.4Cand D)**

The structure of the young branch is almost similar to that of the old branch with the following differences:

**1- The epidermis (Fig. 4C-D and 5)** consists of polygonal isodiametric or slightly axially elongated cells with straight anticlinal walls and covered with thin smooth cuticle. The stomata are mostly of paracytic (rubiaceous) type.

**2- The trichomes** being of covering (non- glandular) type. They are non-glandular multicellular uniseriate trichomes which are usually curved, occasionally straight with short basal cells (2-4) accompanied by an elongated terminal cell having acute apices and covered with warty cuticle.

**3-** The cortex is collencymateous

**4- The vascular tissue** is narrower (1/3 of the diameter) and the pith is wider (more than 1/3 of the diameter).

**5- The vessels** show pitted and spiral thickenings.

**6- The Pith** is formed of rounded parenchyma cells having thin cellulosic walls and containing prisms of calcium oxalate.

**Powdered stem (Fig. 5)**

The powdered stem is yellowish brown in colour having faint characteristic odour and slightly bitter astringent taste. It is characterized microscopically by the presence of the following.

1- Fragments of yellow, polygonal nearly isodiametric **cork cells** with thin suberized walls.

2- Fragments of polygonal isodiametric or slightly axially elongated **epidermal cells** having straight anticlinal walls, covered with smooth cuticle and accompanied with paracytic stomata.

3- Fragments showing non-glandular multicellular uniseriate **trichomes** which are usually curved, occasionally straight with short basal cells (2-4) accompanied by an elongated terminal cell having acute apices and covered with warty cuticle.

4- Fragments of thin walled parenchymatous cells containing **prisms of calcium oxalate.**

5- Numerous fragments of **pericyclic fibres** which are long, having acute apices with moderately wide or narrow lumina and mostly surrounded by parenchyma cells containing prisms of calcium oxalate forming a crystal sheath.

6- Fragments of lignified **wood fibres** having moderately wide to narrow lumina, straight thin lignified walls and acute apices.

7- Fragments of lignified **xylem vessels** having pitted and spiral thickenings.

8- Fragments of **wood parenchyma,** consists of rectangular cells with lignified walls and blunt ends.

9- Fragments of the **medullary rays,** formed of rectangular radially elongated cells with lignified walls.

10- Fragments of lignified **tracheids** with blunt ends.

The microscopical measurements of different elements present in the stem are recorded in table (1)

**2-The Leaf**

**The leaflet**

A transverse section in the leaflet **(Fig. 6)** shows upper and lower epidermises enclosing a dorsiventral mesophyll. The palisade consists of one row of columnar cells and is interrupted in the midrib region with collenchyma cells.

The midrib is prominent on the lower side and shows a crescent shaped collateral vascular

bundle which is surrounded by upper patch and lower arch of lignified pericyclic fibres.

**The upper epidermis (Fig. 6 B-D and 9 A)**

The upper epidermis is formed of polygonal isodiametric to slightly axially elongated cells having wavy anticlinal walls, covered with smooth cuticle, devoid of stomata and containing mucilage (stained red with ruthenium red T.S.).

**The lower epidermis (Fig. 6 B-D, 9 B-D)**

The lower epidermis is formed of polygonal isodiametric or slightly axially elongated cells having more wavy anticlinal walls, covered with smooth cuticle, showing stomata of paracytic type rarely anomocytic and containing mucilage.

**The trichomes (Fig. 9 H)**

They are present on the upper and lower epidermis, of non-glandular type, resembling those present in the stem together with unicellular hair usually curved occasionally straight and covered with smooth cuticle, trichomes arising from cicatrices.

**The neural epidermis (Fig. 9 E and F)**

The cells of the upper and lower neural surfaces (over the midrib and big veins) are polygonal isodiametric to axially elongated with straight anticlinal walls, covered with smooth cuticle and devoid of stomata. Trichomes are of non-glandular type similar to those of the lower and upper surfaces.

**The mesophyll (Fig. 6 B , D and 9 H)**

The mesophyll is heterogenous dorsiventral, the palisade is discontinuous over the midrib region and is formed of one row of columnar, closely packed cells, having straight thin walls with no intercellular spaces containing green chloroplasts. The spongy tissue consists of 2-5 rows of thin wall irregular shaped parenchyma cells with wide intercellular spaces containing scattered prisms of calcium oxalate. Small vascular bundles of the lateral veins are embedded within the spongy tissue.

**The midrib (Fig. 6 B-C)**

The cortical tissue of the midrib consists of parenchyma and subepidermal collenchyma formed of 2-3 rows on both sides. The parenchyma cells are thin-walled, more or less rounded, in about 2-3 rows on the upper side and 8-12 rows on the lower side of the vascular stele. Some parenchyma cells contain scattered prisms of calcium oxalate. The endodermis is indistinct.

**The pericycle (Fig. 6 B and C)**

IT IS formed of an almost upper patch and lower arch of lignified fibres**.** The fibres **(Fig. 6 C and 9 H)** are long having wide lumina. straight walls, acute apices and they are mostly surrounded by thin called parenchyma cells containing prisms of calcium oxalate forming crystal sheath.

**The vascular tissue (Fig. 6B-C and 9H)**

It consists of crescent shaped, collateral vascular bundle formed of xylem towards the upper side and phloem towards the lower one. The **xylem** consists of radial rows of vessels separated by wood parenchyma and wood fibres. The **vessels** are lignified with spiral and annular thickenings. **Wood parenchyma** consists of rectangular cells having slightly lignified walls. **Wood fibres** with straight or slightly undulating walls, showing wide lumina and having pointed apices. The **phloem** is formed of sieve elements and phloem parenchyma. Uni- to bi-seriate **medullary rays** traverses the xylem and phloem in the form of radiating lines.

**The petiole and rachis (Fig 7 A and B)**

The petiole and the rachis showing the same shape and structure as the transverse section of both is more or less rounded in outline. It consists of an epidermis surrounding a narrow cortex (about 1/7 of the diameter). The innermost layer of the cortex is indistinct. The vascular system consists of a large collateral vascular bundle surrounding central non-lignified pith. The vascular bundle is surrounded by discontinuous ring of lignified, wide lumen pericyclic fibres. The pericyclic fibres are surrounded by parenchyma cells containing prisms of calcium oxalate forming crystal sheaths.

**The epidermis (Fig. 7 B and 9 G)**

The epidermis of the petiole and of the rachis is similar to that covering the midrib and big veins of the leaflet, being polygonal slightly axially elongated with straight anticlinal walls and covered with smooth cuticle. Stomata are very rare and of paracytic type, trichomes are of non-glandular type similar to those of the lamina **(Fig. 9 H**).

**The cortical tissue (Fig. 7 B)**

The cortical tissue is formed of parenchyma cells (9-11rows) separated from the epidermis by 1-2 rows of collenchyma cells. Prisms of calcium oxalate are scattered in the cortex. The endodermis is indestinct.

**The pericycle (Fig. 7 B and 9 H)**

The pericycle consists of 5-7 rows of discontinuous ring of fibres surrounding the vascular bundle. The fibres have lignified walls, wide lumina. pointed apices and are surrounded by parenchyma cells containing prismatic crystals of calcium oxalate forming crystal sheaths.

**The vascular strand (Fig. 7 B)**

There is one large vascular bundle of collateral type.

**The phloem** consists of sieve tubes; companion cell and phloem parenchyma. **The xylem** is lignified formed of xylem vessels, wood fibres and wood parenchyma. **The xylem vessels** are radially arranged showing pitted, spiral and annular thickenings. **Wood fibres** with straight or slightly undulating walls, showing wide lumina and having pointed apices. **Wood parenchyma** consists of rectangular cells having slightly lignified walls. The pith is formed of thin-walled rounded parenchymatous cells.

**The petiolule (Fig. 8 A and B)**

The microscopical structure of the petiolule closely resembles that of the petiole and the rachis but is characterized by the following.

1. **A transverse section** in the petiolule of the leaflet is irregular in outline.
2. **The cortex** is wider in the petiolule (more than 1/2 of diameter).
3. **The pericycle** is formed of a complete ring of non-lignified fibres surrounded by parenchyma cells containing prismatic crystals of calcium oxalate forming crystal sheath.
4. **The vascular system** consists of only one crescent shaped collateral vascular bundle.

The microscopical measurements of the different elements present in the leaf are recorded in table (1).

**Powdered leaf (Fig. 9)**

The powdered leaf is grayish green in colour having a faint characteristic odour and astringent mucilaginous taste.

It is characterized microscopically by the presence of the following elements:

**1-** Fragments of the **upper and lower epidermises** which are polygonal Isodiametric to slightly axially elongated cells having wavy anticlinal walls, covered with smooth cuticle. The upper epidermis is devoid of stomata while the lower epidermis is more wavy and showing paracytic stomata rarely anomocytic. Both epidermises contain mucilage.

**2-** Fragments showing **non-glandular trichomes,** which are mainly unicellular, straight or

curved and covered with smooth cuticle. Occasionally, multicellular uniseriate with short basal cells (2-4) accompanied by an elongated terminal cell having acute apices and covered with smooth cuticle. Fallen trichomes leave rounded cicatrices.

3- Fragments of **neural epidermal** cells of the leaflets which are polygonal axially elongated having straight walls and covered with smooth cuticle. They are devoid of stomata.

4- Fragments of **epidermis of the petiole** with polygonal axially elongated cells, having straight walls and covered with smooth cuticle.

5- Fragments of **palisade cells,** which are columnar, thin walled containing green chloroplasts.

6- Fragments of thin walled parenchymatous cells containing **prisms of calcium oxalate.**

7- Numerous fragments of lignified and some non-lignified pericyclic fibres having straight walls, pointed apices with wide lumina and mostly surrounded by parenchymatous cells containing prisms of calcium oxalate forming **crystal sheaths.**

8- Fragments of lignified **xylem vessels** with pitted, spiral and annular thickenings.

9- Fragments of lignified **wood fibres** with straight or slightly undulating walls, wide lumina and having pointed apices.

10- Fragments of **wood parenchyma,** rectangular in shape with lignified walls.

11- Scattered **prisms of calcium oxalate.**

The microscopical measurements of different elements present in the leaf are recorded in table (1).

**The seed (Fig. 10)**

The transverse section of the seed **(Fig. 10 A)** begins with the testa which is narrow and differentiated into palisade like epidermal cells and bearer hypodermis. The testa is followed by the endosperm and the embryo.

**The testa**

**The epidermis**

The epidermis **(Fig. 10 A-C)** is formed of closely packed palisade like epidermal cells which are radially elongated without intercellular spaces. The lumen is narrow and the walls are thick, cellulosic and lamellate. The epidermis is covered with thin cuticle.

**The hypodermis (bearer layer)**

The hypodermis **(Fig. 10 A and D)** consists of one row of radially elongated cells with considerably narrow intercellular spaces resembling the tibia bone (refered as bone-shaped cells or hourglass cells), The cell lumen is narrow in the middle parts and then wider at both the extremities due to the uneven cellulosic thickening as shown in **(Fig. 10 D).**

The hypodermis is followed by a layer consisting of 5-7 rows of parenchymatous cells with thin cellulosic walls. **(Fig 10 A)**.

**The endosperm**

The endosperm **(Fig. 10 A and 10 E)** is represented first by the aleurone layer **(Fig. 10A and E)** consisting of one row of polygonal nearly isodiametric to slightly tangentially elongated cells with no intercellular spaces filled with aleuron grains. (stained yellow with picric acid T.S.). The reminder of the endosperm is formed of 3-5 rows of thin walled cellulosic slightly elongated cells.

**The embryo**

A transverse section in the cotyledon **(Fig 10 A)** is bounded by a typical epidermis of cubical shaped thin walled cells that are filled with aleurone grains. The reminder of the cotyledon (**Fig. 10 A and F**) is composed of thin walled parenchyma cells which are rounded in shape and filled with oil droplets (stained red with sudan III), protein masses (stained yellow with picric acid T.S.) and micro-rosettes of calcium oxalate.

**The powdered seed (Fig. 10 B-F)**

The powdered seed is creamy yellow with brown fragments, having a faint characteristic odour and a bitter oily taste. It is characterized microscopically by the presence of the following elements.

1. Fragments of epidermal cells that are radially elongated (polygonal, nearly rounded from surface view) without intercellular spaces. The lumen is narrow and the walls are thick, cellulosic and lamellate.
2. Fragments of hypodermis with hourglass or bone shaped.
3. Fragments of aleurone layer of isodiametric cells with no intercellular spaces filled with aleuron grains
4. Fragments of cotyledon in which the cells are thin walled parenchyma, rounded in shape and filled with oil droplets, protein masses and micro-rosettes of calcium oxalate.

The microscopical measurements of different elements present in the seed are recorded in table (1).

**II- Genetic Profiling**

In this study the extracted DNA of each of the two *Dalbergia* species was amplified using fifteen decamer primers to detect genetic variability between them. Each of the fifteen primers successfully directed the amplification of a genome-specific fingerprint of DNA fragments. The obtained banding profiles produced by the primers used in the RAPD analysis are represented in **figures 11-12**. The fifteen primers generated a total of 179 fragments in *Dalbergia paniculata* Roxb. and 156 fragments for *Dalbergia sisso* Roxb. Results are recorded in Table 2. RAPD markers obtained through primer screening of *Dalbergia paniculata* Roxb. and *Dalbergia sisso* Roxb.DNA, were reproducible and differentiated both *Dalbergia* species.

The fifteen primers produced multiple band profiles with the number of amplified DNA fragments ranging from 15 with OPD-18 in *Dalbergia paniculata* Roxb. and 14 with **OPB-01** and **OPG-02** in *Dalbergia sisso* Roxb. While the least number of fragments was 7, produced by **OPE-02** in *Dalbergia paniculata* Roxb. and 6 produced by **OPD-07** in *Dalbergia sisso* Roxb.

**Table (1): Microscopical measurement of different organs of *Dalbergia paniculata* Roxb. in microns.**

| Item | L | W | H | D |
|---|---|---|---|---|
| **The stem** | | | | |
| **Cork cells** | 20-33-40 | 13-26-30 | 15-22-37 | |
| **Epidermal cells** | 18-23-30 | 8-13-23 | 5-6-9 | |
| **Non-glandular trichomes** | 173-220-242 | 13-16-18 | | |
| **Pericyclic fibres** | 355-466-500 | | | 7-15-22 |
| **Wood fibres** | 116-230-315 | | | 5-7-9 |
| **Xylem vessels** | | | | 22-52-70 |
| **Wood parenchyma** | 76-87-95 | 16-20-23 | | |
| **Medullary rays** | 20-23-30 | 10-23-25 | | |
| **Calcium oxalate prisms** | 12-13-19 | 6-7-10 | | |
| **Tracheids** | 73-85-106 | 13-20-24 | | |
| **The leaf** | | | | |
| **Upper epidermal cells of leaflet** | 32-49-76 | 21-44-55 | 12-18-24 | |
| **Lower epidermal cells of leaflet** | 25-42-45 | 17-20-25 | 6-12-18 | |
| **epidermal cells of petiole** | 10-12-15 | 3-4-6 | 5-7-8 | |
| **Upper neural epidermis** | 69-72-80 | 12-17-19 | 12-18-27 | |
| **Lower neural epidermis** | 14-17-19 | 6-8-11 | 7-10-12 | |

| | | | | |
|---|---|---|---|---|
| **Trachieds** | 68-74-89 | 9-12-17 | | |
| **Xylem vessels** | | | | 11-20-28 |
| **Wood parenchyma** | 44-55-63 | 11-13-18 | | |
| **Prism of calcium oxalate** | 9-12-15 | 3-5-7 | | |
| **Non glandular trichome** | 120-174-400 | 10-12-13 | | |
| **Pallisade** | 48-52-61 | 6-8-11 | | |
| **Wood fibre** | 570-715-816 | | | 3-5-7 |
| **Pericyclic fibre** | | | | 10-12-15 |
| **Seed** | | | | |
| **Epidermal cells** | | 11-17-22 | | 59-65-85 |
| **Hypodermis** | | 11-18-25 | | 17-27-37 |
| **Aleurone layer** | 20-22-25 | 11-17-19 | | |
| **Embryo** | | | | 25-42-60 |
| **Micro rosette of calcium oxalate** | | | | 3-5-9 |

**Table (6): The total number of RAPD-PCR fragments, distribution of monomorphic (common) and polymorphic bands and similarity coefficients generated by 15 decamer arbitary primers in the two *Dalbergia* species.**

| **Primers** | **Number of RAPD fragments** | | **Monomorphic fragments** | **Polymorphic fragments** | **% of polymorphism** | **Similarity coefficient*** |
|---|---|---|---|---|---|---|
| | **DP** | **DS** | | | | |
| **G-3** | 14 | 12 | 4 | 18 | 69.23 | 30.76 |
| **G-2** | 12 | 14 | 7 | 12 | 46.15 | 53.84 |
| **D-6** | 12 | 9 | 5 | 11 | 52.38 | 47.61 |
| **D-7** | 13 | 6 | 2 | 15 | 78.94 | 21.05 |
| **D-16** | 9 | 7 | 1 | 14 | 87.5 | 12.50 |
| **D-18** | 15 | 7 | 4 | 14 | 63.63 | 36.36 |
| **A-20** | 10 | 8 | 4 | 10 | 55.55 | 44.44 |
| **B-1** | 14 | 14 | 6 | 16 | 57.14 | 42.85 |
| **B-5** | 12 | 10 | 3 | 16 | 72.72 | 27.27 |
| **B-6** | 13 | 13 | 4 | 18 | 69.23 | 30.76 |
| **B-7** | 14 | 10 | 3 | 18 | 75 | 25.00 |
| **B-15** | 12 | 13 | 6 | 13 | 52 | 48.00 |
| **B-17** | 13 | 11 | 7 | 10 | 41.66 | 58.33 |
| **B-18** | 9 | 12 | 4 | 13 | 61.90 | 38.09 |
| **E-2** | 7 | 10 | 5 | 7 | 41.17 | 58.82 |
| **Total** | 179 | 156 | 65 | 205 | **Mean** 61.61 | **Mean** 38.80 |

**Fig. (1): A photograph of the tree of *Dalbergia paniculata* Roxb. (X:0.01)**

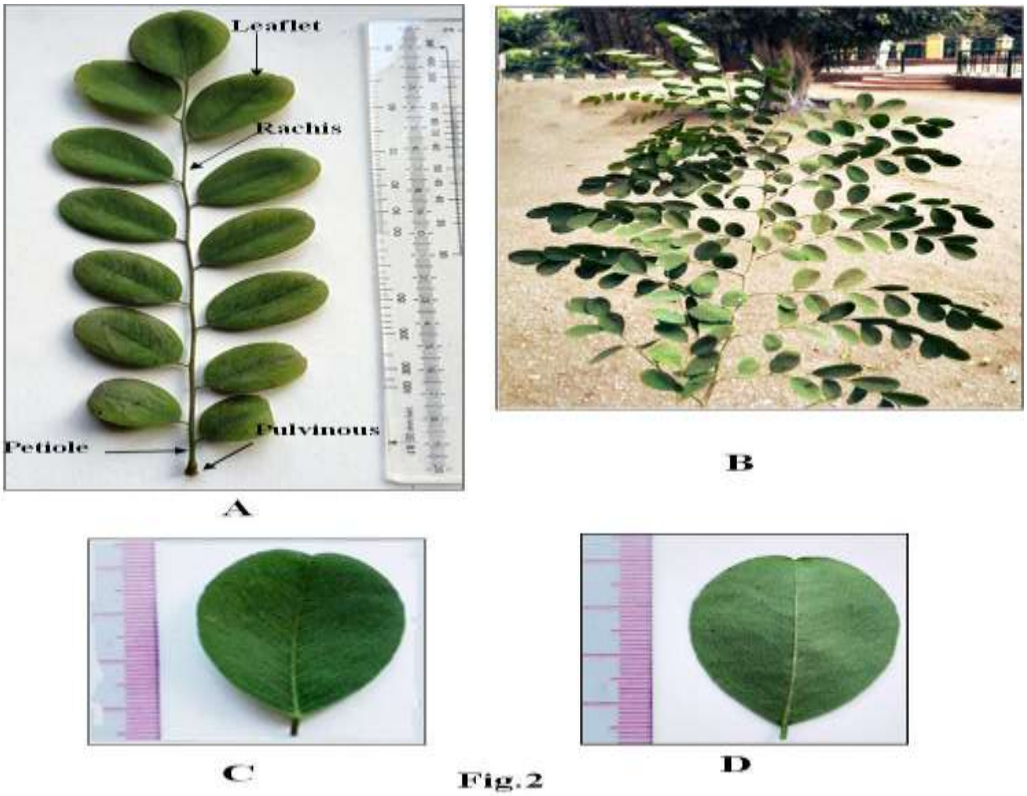


**Fig. (2): A photograph of the leaf of *Dalbergia paniculata* (Roxb.)**

**A. Compound leaf showing pulvinous.(X:0.5)**
**B. Lateral branch carrying leaves. (X:0.09)**
**C. The upper surface of the leaflet. (X:1.2)**
**D. The lower surface of the leaflet. (X:1.2)**

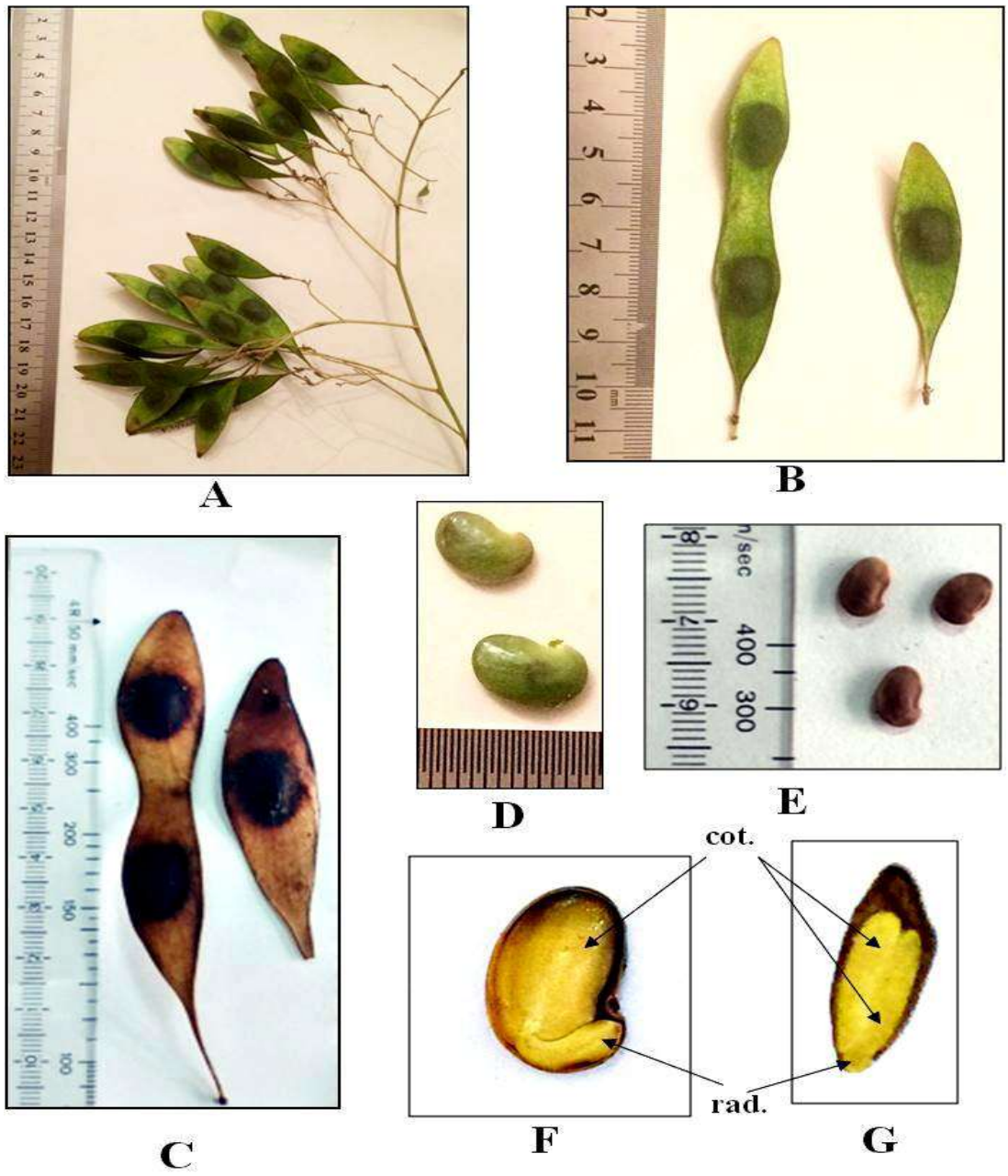


Fig.3

**Fig. (3): A photograph showing the fruit and the seed of *Dalbergia paniculata* (Roxb)**

**A. Fruiting branch. (X :0.3)**
**B. The unripe fruit. (X :0.7)**
**C. The ripe fruit. (X:0.7)**
**D. The unripe seed. (X:1.5)**
**E. The ripe seed (X :1.3)**
**F. L-cut in the seed. (X:5)**
**G. T-cut in the seed. (X:10)**
**cot., cotyledon; rad., radical.**

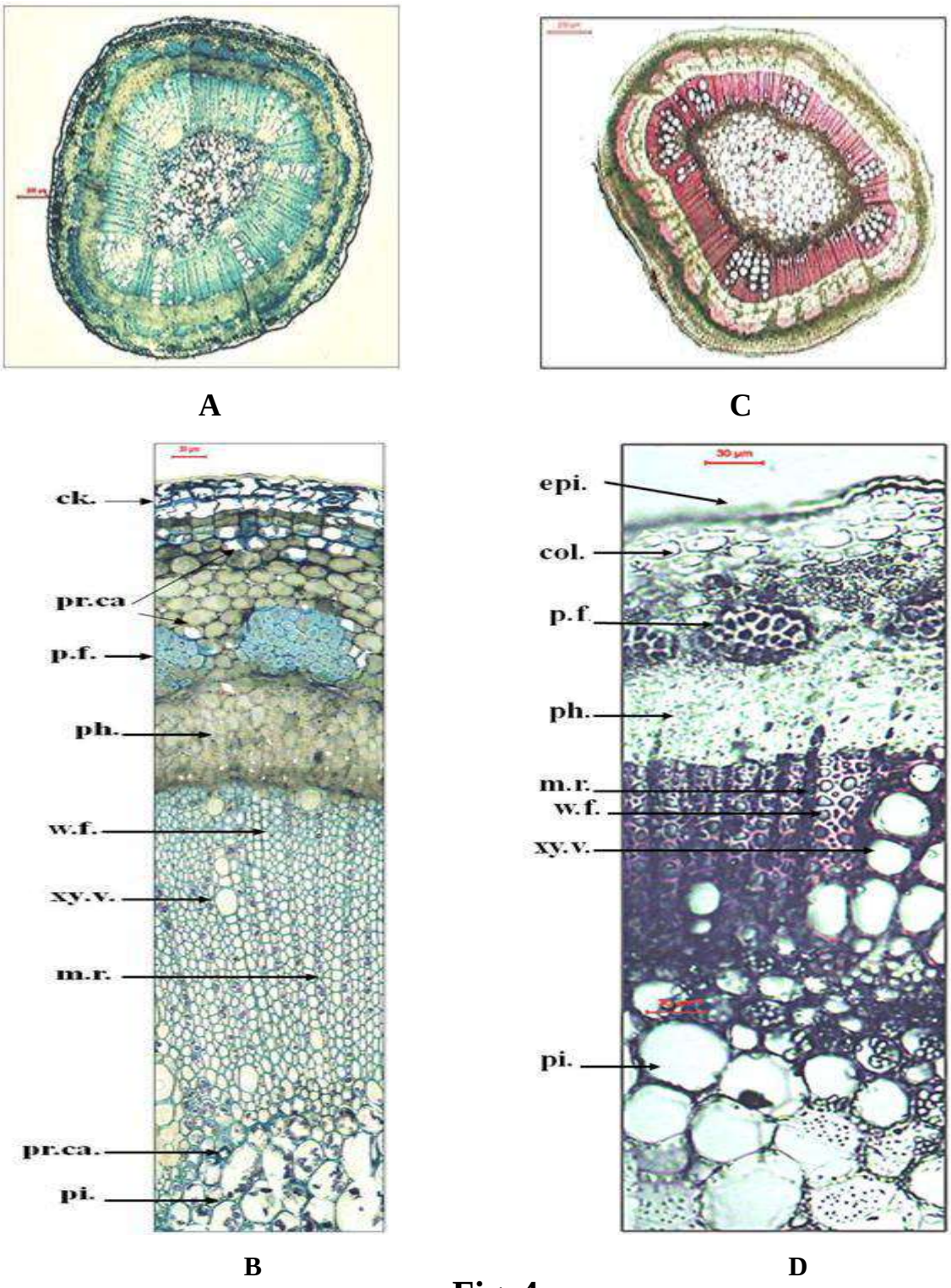


**Fig. 4**

**Fig. (4): Micromorphology of the old and young stem branch of *Dalbergia paniculata* (Roxb.)**

A. Low power view of T.S. in the old stem branch.(X:40) B. Detailed sector in the old stem branch.(X:133) C. Low power view of T.S. in young stem (X:35) D. Detailed sector in

young stem (X:300). Ck.,cork cells; m.r., medullary rays; p.f., pericyclic fibres; ph., phloem; pi., pith; pr.ca., prism of calcium oxalate; xy.v., xylem vessel; w.f.; wood fibres.

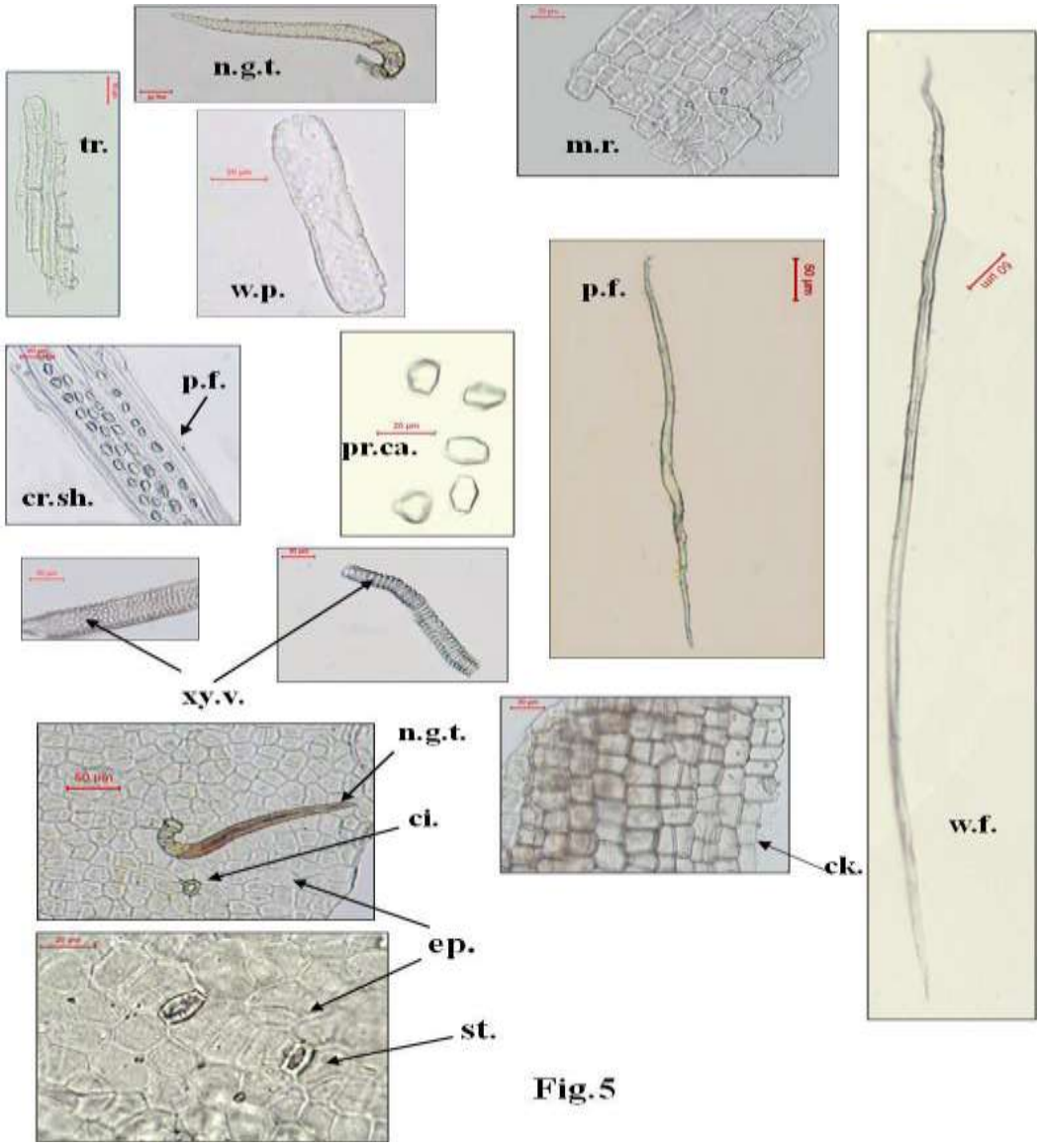


**Fig. (5): Micromorphology of the powdered stem of *Dalbergia paniculata* (Roxb.)**

**The powder**

Ci, cicatrix; ck, cork (X:150); cr.sh., crystal sheath (X:150); ep., epidermis; m.r., medullary rays (X:150); n.g.t., non glandular trichome; (X:150); pr.ca., prism of calcium oxalate (X:400) ; p.f., pericyclic fibres (X:150); st, stomata; tr., tracheids (X:150); xy.v., xylem vessel (X:150); w.f., wood fibres (X:150); w.p., wood parenchyma (X:400).

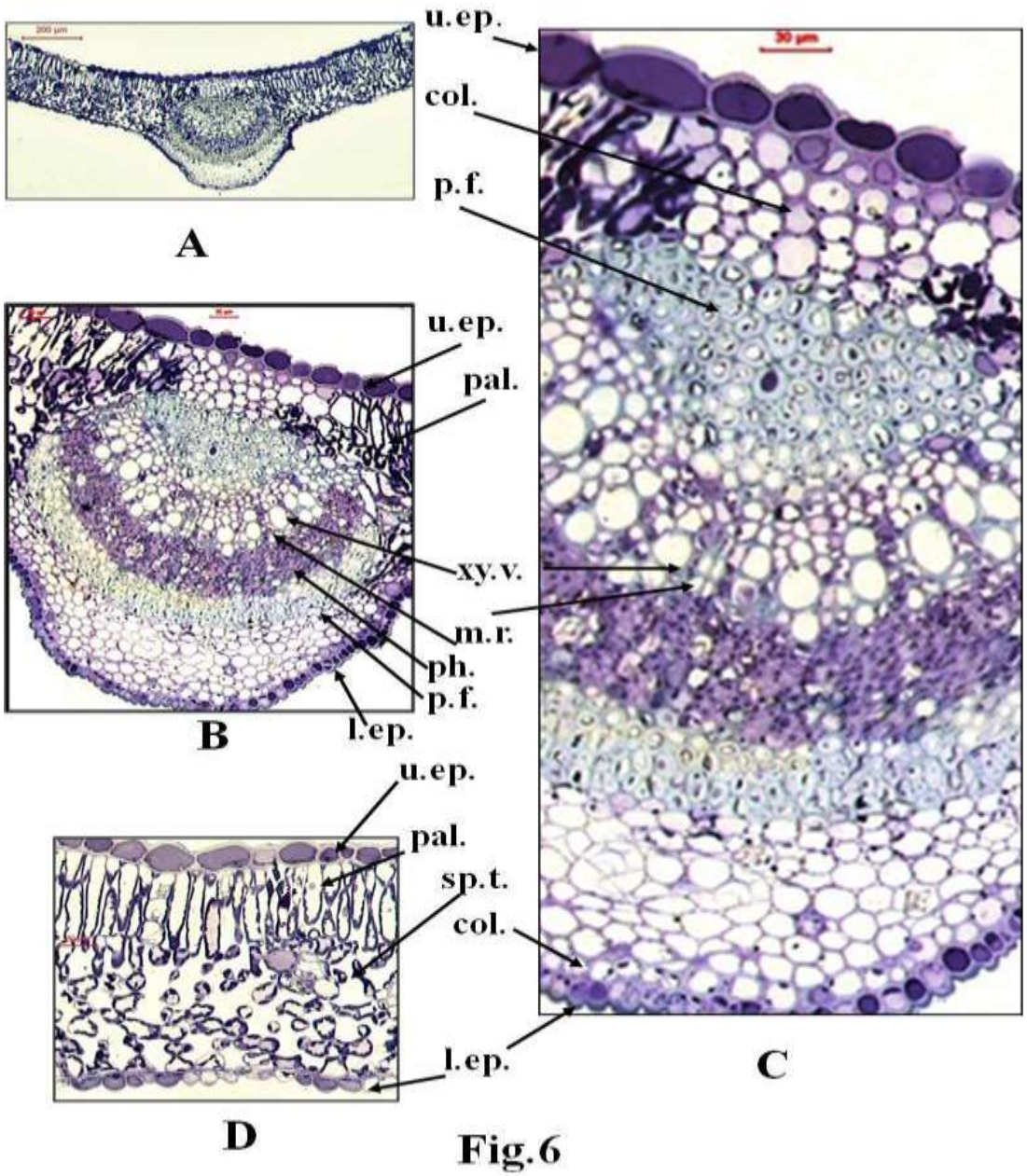


**Fig. (6): Micromorphology of the leaflet of *Dalbergia paniculata* (Roxb.)**

**A. Low power view of T.S. in the leaflet.(X:40)**

**B. High power view of T.S. in the leaflet.(X:100 )**

**C. Detailed sector in the midrib region.(X: 333)**

**D. Detailed sector in the lamina region. (X:166)**

**col., collenchyma; l.ep., lower epidermis; m.r., medullary rays; p.f., pericyclic fibres; pal., palisade; ph., phloem; sp.t., spongy tissue; u.ep., upper epidermis; xy.v., xylem vessels.**

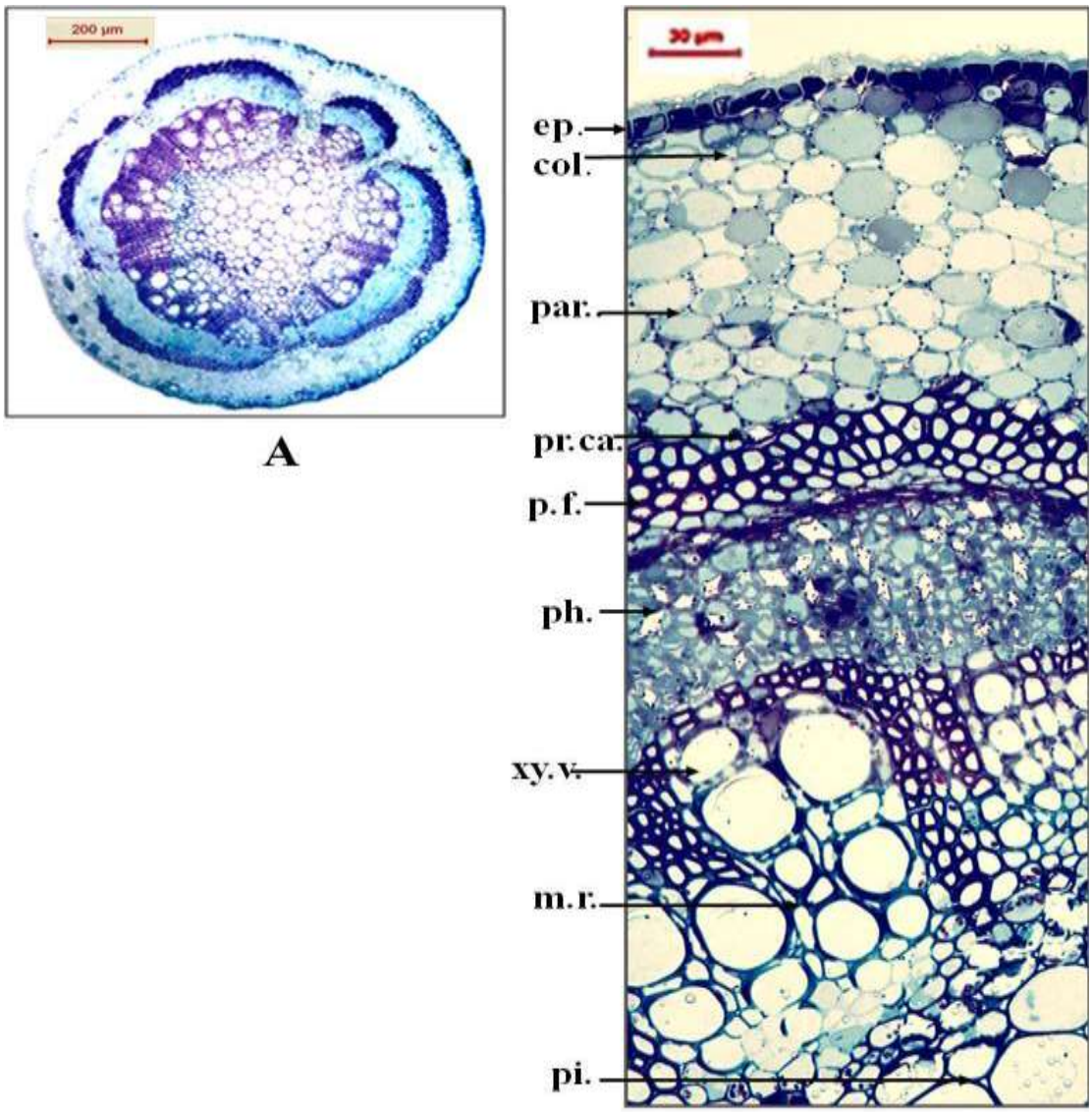


Fig 7

**Fig. (7): Micromorphology of the leaf petiole and rachis of *Dalbergia paniculata* (Roxb.)**

A. Low power view of T.S. in the leaf petiol and rachis.(X:62)

B. Detailed sector in the leaf petiole and rachis.(X:383)

col., collenchvma; ep., epidermis;

m.r., medullary rays;par., parenchyma;

p.f., pericyclic fibres; ph., phloem;

pi., pith; pr.ca.; prism of calcium oxalate;

xy.v., xylem vessel.

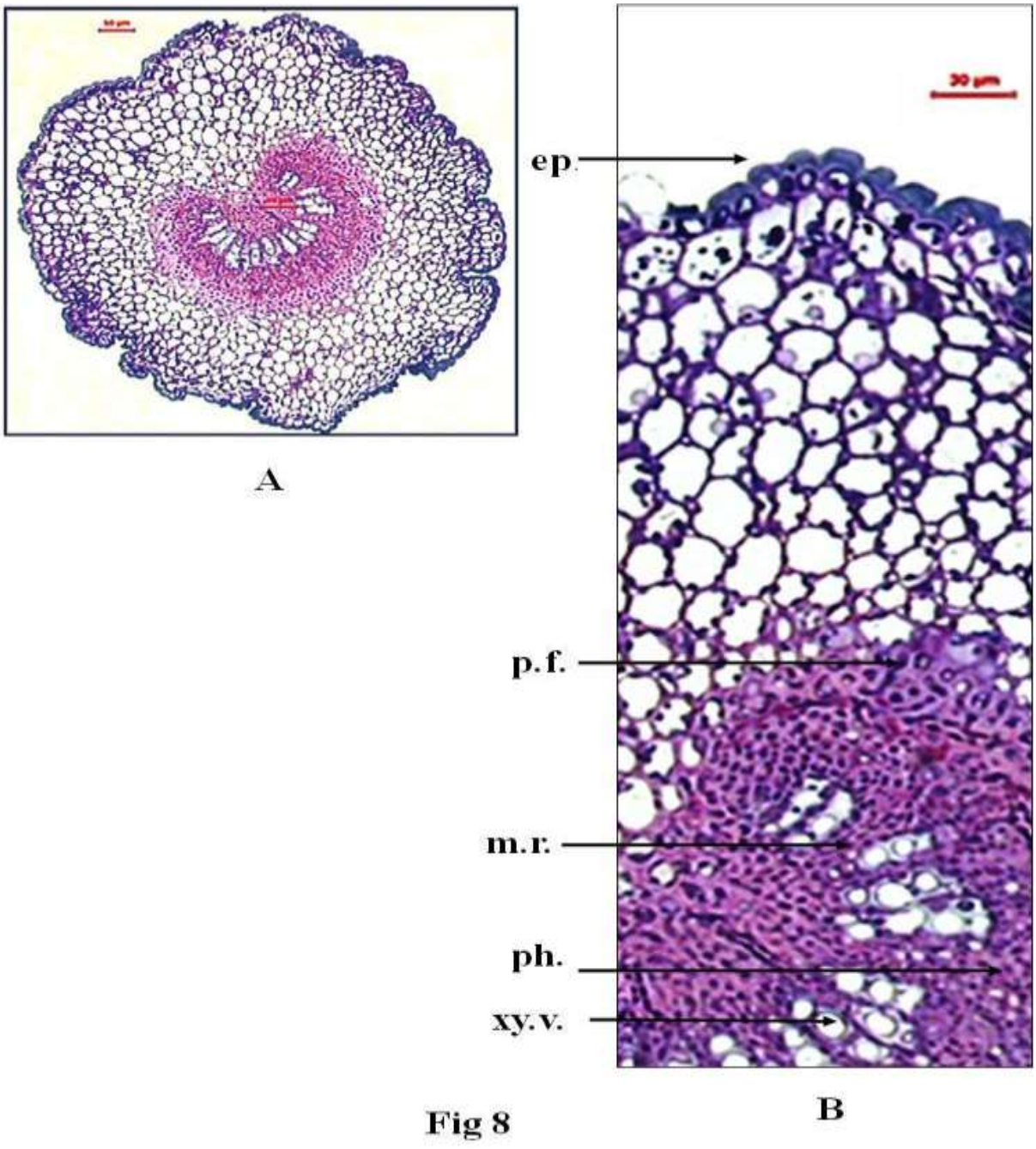


**Fig (8): Micromorphology of the leaflet petiolule of *Dalbergia paniculata* (Roxb.)**

A. Low power view of T.S. in the leaflet petiolule. (X:90)

B. Detailed sector in the leaflet petiolule.(X:333)

ep., epidermis; m.r., medullary rays;

p.f., pericyclic fibres; ph., phloem;

xy.v., xylem vessel.

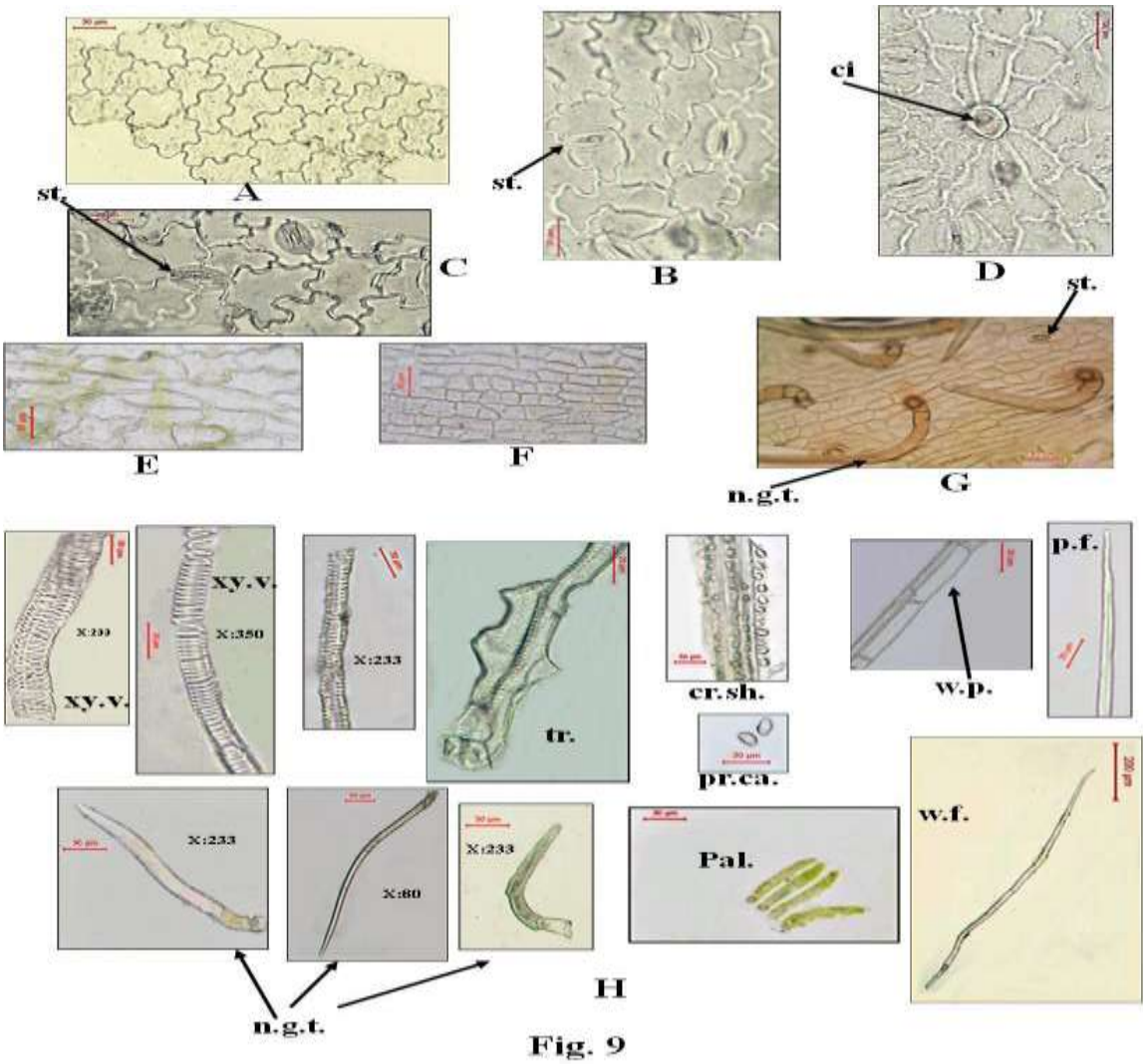


Fig. 9

**Fig. (9): Micromorphology of the powdered leaf of *Dalbergia paniculata* (Roxb.)**

**A. Surface preparation of the upper epidermis. (X: 233)**

**B. Surface preparation of the lower epidermis. (X:350)**

**C. Surface preparation of the lower epidermis showing anomocytic stomata (X:350)**

**D. Surface preparation of the lower epidermis showing cicatrix. (X:350)**

**E. Surface preparation of upper neural epidermis. (X: 233)**

**F. Surface preparation of lower neural epidermis. (X: 233)**

**G. Surface preparation of petiole epidermis. (X: 233)**

**H. Other elements of the leaf.**

**ci., cicatrix; cr.sh., crystal sheath (X:80); n.g.t., non-glandular trichomes; pal., palisade (X: 233); p.f., pericyclic fibre (x:233); pr.ca., prism of calcium oxalate (X:233); st., stomata; tr, tracheids (X:350); w.f., wood fibre (X:60); w.p., wood parenchyma (X:233); xy. v., xylem vessel.**

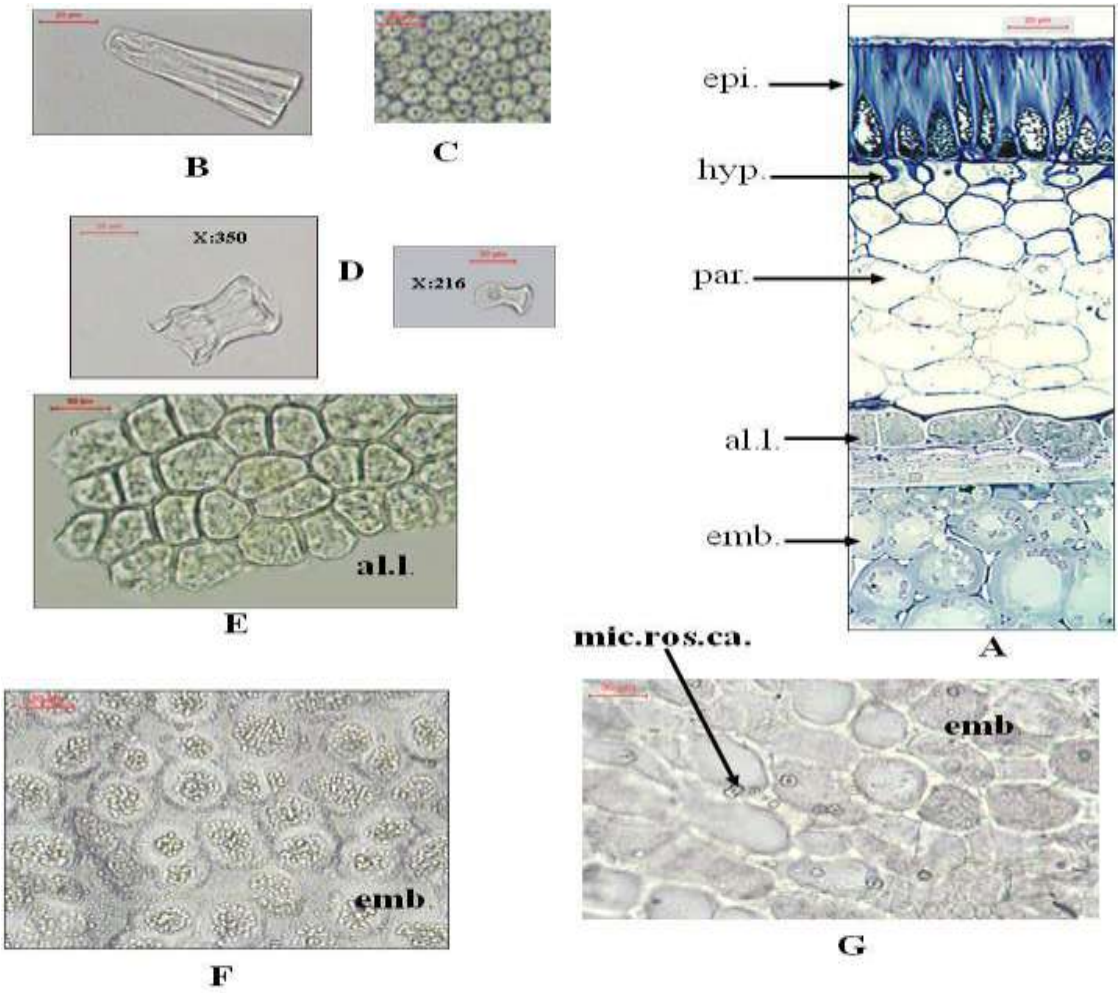


Fig. 10

**Fig. (10): Micromorphology of the seed of *Dalbergia paniculata* (Roxb.)**

A. Detailed sector in the seed.(X:350)

**Powdered seed**

B. Epidermis in side view.(X:350)

C. Epidermis in surface view.(X:133)

D. Hypodermis in side view.

E. Aleurone layer.(X:350)

F. Cotyledon. (X:216)

G. Cotyledon showing micro-rosette crystal of calcium oxalate. (X:216)

al.l.,aleuron layer ;emb.,embryo; epi.,epidermis; hyp.,hypodermis; mic.ros.ca**.,** microrossete crystal of calcium oxalate; par.,parenchyma.

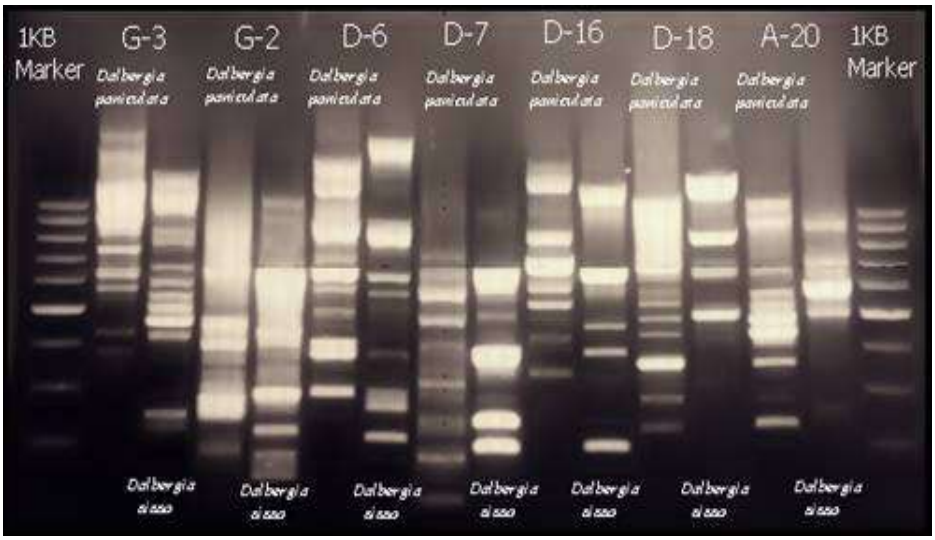


**Fig. ( 11 ): The RAPD electrophoretic profile of *Dalbergia paniculata* Roxb. and *Dalbergia sisso* Roxb. generated by primers (G-3, G-2, D-6, D-7, D-17, D-18 and A-20)**

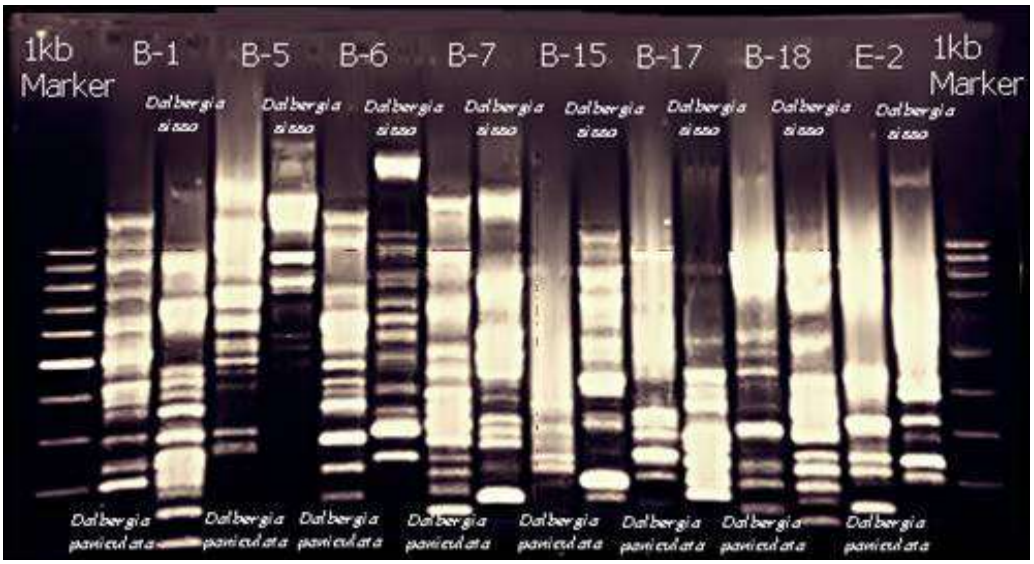


**Fig. ( 12 ): The RAPD electrophoretic profile *Dalbergia paniculata* Roxb. and *Dalbergia sisso* Roxb.generated by primers (B-1, B-5, B-6,B-7, B-15, B-17, B-18 and E-2)**

## CONCLUSION

On the total number of fragments was 335 bands, 205 were polymorphic representing a level of polymorphism of 61.61%. The highest degree of similarity (58.82%) was observed using primers **OPE-02**, while the least degree of similarity (12.50%) was observed using primers **OPD-16** in Table 2.

The genetic characterization of both *Dalbergia* species using random amplified polymorphic DNA (RAPD) showed that primers **OPD-18** and **OPE-02** could be used as an indicator for obtaining genetic markers. In addition, the primers **OPD-16**, **OPD-07** and **OPB-05** were found to be the most effective in generating polymorphic bands on application of the RAPD technique to both plants, and therefore can act as markers for species authentication beside the morphological characteristics.

**REFERENCES**


1. Stevens PF. Angiosperm Phylogeny Website Version., 2008; 9.
2. Chopra RN, Nayar SL and Chopra IC Glossary of Indian medicinal plants. Council of scientific & industrial research, New Delhi., 1980; 191-258.
3. Saha, S, Shilpi, J A, Mondial, H, Hossain, F, Anisuzzman, M, Hasan, MM, and Cordell, GA. Ethnomedicinal, phytochemical, and pharmacological profile of the genus *Dalbergia L. (*Fabaceae*).*, 2013; 4(2): 291-346.
4. Ramachandraiah, P Antimicrobial activity of *Dalbergia paniculata* seed oil. *Fitoterapia*, 1991; 62: 281.
5. Ganga, RB, Madhu, KP and Raju, ADV Investigation of antioxidant and anti-inflammatory activity of leaves of *Dalbergia paniculata* Roxb. *Asian Pac J Trop Med*, 2012; 5(6): 455-458.
6. Raju, ADV. Pharmacological and preliminary phytochemical studies of Indian traditional plants, *Dalbergia paniculata, cyathea gigantean, colebrookea* and *entada pursaetha.* PHD.Thesis, Acharya Nagarjuna University, Nagarjuna Najar, Gunter, India., 2013.
7. Herbal Drug Standardization, Posted July 1999. Retrieved at: www.swatijaininst.com/etechno/arunjain.doc.
8. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Research, 1990; 18: 6531-6535.