INTRODUCTION
Macadamia nut, known as Queensland nut, Australian nut, and bauple nut,[1] is the kernel obtained from two species of the genus Macadamia: Macadamia integrifolia (Maiden and Betche) and Macadamia tetraphylla (L.A.S Johnson). M. integrifolia is a dark green spreading semi-hard tree that can grow up to 20 meters in height[2] and belongs to family Proteaceae. The genus Macadamia consists of nine species, but only the smooth-shelled M. integrifolia (Maiden and Betche) and the rough-shelled M. tetraphylla (L.A.S. Johnson) are cultivated for their edible nuts.[3,4] M. integrifolia (Maiden and Betche) occurs naturally only in Southeast Queensland while M. tetraphylla is native to the northernmost part of New South Wales and the southernmost part of Queensland.[5] The edible part of M. sintegrifolia and M. tetraphylla which is the kernel[6,7] can be eaten raw, fried or roasted, and salted. Furthermore, oil extracted from the kernel can be used for salads or manufacturing of cosmetics, and it is similar in composition to olive oil with high content of monounsaturated fatty acids reaches to 58.2%.[8,9] M. integrifolia is considered among the highly economically valuable nuts world widely where its largest production comes from Australia with approximately 42,000 tons followed by Hawaii 22,000 tons a year. In addition to its wide usage as a snack and in different confectionaries, M. integrifolia nuts reported as healthy food for heart due to their good cholesterol-lowering potential[9] and also moderate antioxidant activity.[10] This encouraged the authors to design an authentication process for this highly valuable plant through its botanical characters and DNA fingerprinting by random amplified polymorphic DNA (RAPD) analysis which is a modified polymerase chain reaction (PCR) technique involving the amplification of whole-plant DNA extracted from leaves or other plant organs and considered commonly used by plant biologists to perform a number of tasks including the genetic fingerprinting of plant varieties.[11]
this study were collected since March 2015 from Horticulture Research Institute, Agriculture Research Center, Giza, Egypt, and its identity was authenticated by Dr. Reem Sameer Hamdi assistant professor of plant taxonomy at the Department of Botany, Faculty of Sciences, Cairo University and a voucher specimen number (19.9.16) was kept at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

**Method for Botanical Study**

Fresh samples of leaf, stem, and petiole were fixed for at least 48 h in formalin-acetic acid-70% alcohol in ratio of 5:5:90 v/v and dehydrated, paraffin infiltrated and embedded in wax using customary techniques. Transverse sections were cut on a rotary microtome to a thickness of 15 microns and then stained with erythrosin and crystal violet. A light microscope was used to view the slides. Microphotographs were obtained using image analyzer at Cairo University Research Park at Faculty of Agriculture, Cairo University.

**Materials for DNA Fingerprinting**

**Plant material**

Samples of freeze-dried whole leaves of *M. integrifolia* were ground to a fine powder grinder before DNA isolation.

**Kits**

The DNA isolation kit: Qiagen DNeasy kit including Cell lysis buffer (AP1), RNAse Protein-depleting buffer (AP2), AP3/E Buffer, AW Buffer, AE Buffer, TBE Buffer (Qiagen Santa Clara, CA).

**Amplification reagents**

The amplification reaction was carried out in 25 μl reaction volume containing 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs (Pharmacia, Sweden), 1 μM primer, 1 U Taq DNA polymerase (PerkinElmer/Cetus, USA; Advanced Biotechnologies, UK), and 25 ng template DNA.

**Molecular weight marker**

New England Biolabs Co., UK.

**Primers**

Five primers were used for randomly amplified polymorphic DNA (RAPD) analysis obtained from (Operon Technologies Inc., Alameda, California, USA) with the following sequence:

- OPA-09: 5’-GGGTAACGCC -3’
- OPA-16: 5’-AGCCAGCGAA -3’
- OPB-01: 5’-GTTTCGCTCC -3’
- OPB-18: 5’-CCACAGCAGT -3’
- OPC-05: 5’-GATGACCGCC -3’.

**Method for DNA fingerprinting**

**DNA extraction and quantification**

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

**Amplification of RAPD markers**

The amplification reactions were carried out in 25 μl reaction volume containing 19X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1 μM primer F, 1 μM primer R, 1U Taq DNA polymerase, and 25 ng template DNA following a thermal cyclic program. Amplified products were analyzed by electrophoresis on 1.5% agarose gel and finally stained with ethidium bromide (0.5 μg/ml). A molecular size marker of 1 Kbp was used as standard marker. PCR products were visualized on UV light and photographed using Polaroid camera.

**Table 1: DNA fragments produced by randomly amplified polymorphic DNA analysis of Macadamia integrifolia Maiden and Betche**

<table>
<thead>
<tr>
<th>Molecular size (bp)</th>
<th>OPA-09</th>
<th>OPA-16</th>
<th>OPB-01</th>
<th>OPB-18</th>
<th>OPC-05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1118</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>996</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>866</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>803</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>795</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>736</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>718</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>693</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>610</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>588</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>566</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>492</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>436</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>389</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>214</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Number of fragments</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
Mohammed Abubaker, et al.

Analysis of RAPD Data

RAPD bands obtained for *M. integrifolia* genetic DNA were all two state characters only and coded as presence (+) and absence (-).

RESULTS AND DISCUSSION

DNA Fingerprinting

Results are explained in Figure 1 and Table 1, where a total of 16 different RAPD fragments were recorded showing four bands by each of OPA-09 and OPB-01 ranging from 0.803 to 0.214 Kbp and 0.996 to 0.492 Kbp, respectively.
Mohammed Abubaker, et al.

three bands by each of OPA-16 and OPB-18 ranging from 1.118 to 0.736 Kbp and 0.693 to 0.389 Kbp, respectively, and two bands by OPC-05 at 0.795 and 0.566 Kbp.

Botanical Study

Macromorphology

*M. integrifolia* is an evergreen tree native to Australia with a spread of 13 m, rough trunk with a diameter of 30 cm and reaching heights of 12–20 m and a roundish crown [Figure 2].

Leaves

They are 10–15 cm long, dark green in color. They are tough, in whorls of three, simple, narrow-elliptical to narrow-oblong in shape. Mature leaves have a serrated margin but juvenile leaves have smooth margins or broadly and sparingly serrated with <14 serrations. Petiole is pubescent, cylindrical 4–18 mm in length and is grooved on the upper surface [Figure 3a-d].

Fruit

Fruit is a globular follicle with an apical horn, 20–30 mm in diameter, consisting of a fleshy green pericarp (husk) 3 mm thick that hardens when ripe. The fruit encloses a globose to broadly ovoid smooth testa seed (nut). Seed is 15–25 mm across. Testa is hard, brown, smooth, or nearly so enclosing an edible cream-colored kernel [Figure 3e].

Micromorphology

The leaf lamina

A transverse section in the leaf lamina shows upper and lower epidermis enclosing a dorsiventral mesophyll [Figure 4]. The palisade tissue consists of two rows of columnar cells [Figure 4a] interrupted in the midrib region by collenchymatous cells. The midrib is more prominent to the lower surface showing ring-shaped vascular bundle, which lower part formed of patches of fibers interrupted with parenchymatous cells.

The epidermis

The upper epidermis of the lamina consists of a single raw of polygonal isodiametric cells with straight somewhat thick anticlinal walls, covered with thin, smooth cuticle, and devoid of stomata [Figure 5a].

The lower epidermis consists of elongated less regularly polygonal cells, showing sinuous somewhat thick anticlinal walls, covered with thin, smooth cuticle [Figure 5b]. Stomata are frequent and of paracytic type being surrounded by two cells. Trichomes are unicellular [Figure 5d] non-glandular or bicellular with a single basal cell in the epidermis.

The neural epidermis

The cells of the neural epidermis are rectangular having straight anticlinal walls, devoid of stomata.
Neural trichomes are unicellular or bicellular with a single short basal cell and a short stalk cell. The mesophyll

Mesophyll is dorsiventral and the palisade appears in two rows of upright columnar closely packed cells. It is interrupted in the midrib region with collenchymatous cells. The spongy tissue is formed of 4–6 rows of more or less rounded to irregular parenchyma cells with wide intercellular spaces which are more spongy near the surface. Small vascular bundles may be embedded within the spongy tissue [Figure 4b].

The midrib

Cortex is formed of 3–5 rows of collenchyma in the upper side and 4–7 rows in the lower side.

The pericycle is formed of an almost continuous ring of lignified fibers interrupted by parenchymatous cells [Figure 4c].

The vascular tissue

It consists of 7–10 arc-shaped collateral vascular bundles of various sizes, each with a fiber cap on both sides showing xylem toward the upper side and phloem toward the lower side. Bundles are separated by lignified parenchyma cells.

The xylem consists of lignified spiral, annular vessels as shown in Figure 5f and wood parenchyma. The phloem consists of sieve elements and phloem parenchyma.

The leaf petiole

A transverse section in the petiole showed it nearly rounded on the lower side and bigrooved on the upper [Figure 6a]. The epidermis is followed by cortical tissue enclosing seven arches of collateral vascular bundles which are accompanied by three inverted bundles near the base of the petiole.

The epidermis

It consists of polygonal, isodiametric, or slightly elongated cells with straight lightly thick anticlinal walls and covered with smooth cuticle. Trichomes are non-glandular, unicellular.

The cortex and ground tissue

It is formed of 1–3 rows of collenchymatous cells followed by 2–3 rows of parenchyma at the lower epidermis [Figure 6b]. Beneath the upper epidermis, three rows of collenchyma followed by 2–4 rows of parenchyma with occasional appearance of sclereids which are either isodiametric or elongated with pitted walls [Figure 7e].
The pericycle
The pericycle is formed of separated bands of fiber caps above the vascular bundles interrupted with parenchymatous cells [Figure 6b]. The fibers are long with either straight or undulating walls showing tapering ends and narrow lumen with dark content [Figure 7a].

The vascular tissue
Each vascular bundle has an oval shape with fiber cap on each side. Sclerenchymatous tissue consists of fiber with undulating walls and brown content and sclereids with pitted walls and dark content [Figure 6b]. The phloem is formed of sieve elements and phloem parenchyma traversed by broad multiseriate medullary rays consisting of thin-walled cells. The xylem elements are radially arranged and consist of lignified spiral and annular vessels traversed by broad multiseriate medullary rays [Figure 7b] with occasional wood fibers showing blunt apices and wide lumen [Figure 7d].

The powdered leaf
The powdered leaf is dark green in color, odorless with astringent taste. The powder of the leaf is characterized microscopically by the following elements: [Figures 5 and 7]
1. Fragments of the upper epidermis of lamina consist of polygonal isodiametric cells with straight somewhat thick anticlinal walls, covered with thin, smooth cuticle, and devoid of stomata.
2. Fragments of the lower epidermis of lamina showing less regularly polygonal cells, with simuous thick anticlinal walls, covered with thin, smooth cuticle and showing frequent paracytic stomata.
3. Fragments of the upper and lower neural epidermis with rectangular cells having straight anticlinal walls, devoid of stomata.
4. Fragments of lignified pericyclic fibers of both leaf lamina and petiole having narrow lumina and acute apices surrounded with parenchyma.
5. Fragments of lignified spiral, annular xylem vessels with those from petiole showing wide diameters.
7. Numerous lignified pericyclic fibers, having acute apices with undulating walls and narrow lumen with brown content.

The stem
A transverse section in the stem showed that it is almost circular in outline [Figure 8a]. It is formed of an outer cork followed by cortex, which is formed of collenchyma containing frequent sclerenchymatous cells. The endodermis is undifferentiated. The pericycle is formed of groups of fibers interrupted by parenchyma cells and/or sclereids showing dark content. The vascular tissue is associated with abundant parenchyma in curved tangential bands and is traversed
by broad medullary rays. The central pith is wide and formed of parenchyma containing starch granules.

The cork
It consists of 5–6 rows of brown flattened radially arranged cells with polygonal suberized walls [Figure 8b and c]. The phellogen cells consist of one row of tangentially elongated cells having thin cellulosic walls.

The cortex
It is formed of 4–5 rows of nearly oval collenchymatous cells [Figure 8b] with frequent lignified isodiametric sclereids with wide lumen showing dark content [Figure 9d].

The pericycle
It is formed of patches of lignified fibers interrupted by parenchymatous cells some showing dark content. Some sclereids are present. The fibers are elongated with a narrow lumen, straight or undulating lignified walls with acute apices and some of the fibers are accompanied with sclereids [Figure 9b].

The vascular tissue
The phloem consists of soft tissue, devoid of fibers, formed mainly of thin-walled parenchymatous cells, sieve tubes, and companion cells. The xylem: It is formed of lignified radially arranged elements. The vessels are mainly pitted or showing annular thickenings [Figure 9c]. Wood fibers are present in groups, fusiform with wide lumen and blunt apices. The wood parenchyma consists of oval cells with pitted lignified walls. The medullary rays are formed of rectangular cells with pitted walls.

The pith: It consists of more or less rounded parenchymatous cells similar to wood parenchyma.

The powdered stem
The powder is yellowish brown in color and odorless. Microscopically, it is characterized by the following elements: [Figure 9]

1. Fragments of polygonal isodiametric cells of cork with suberized wall.
2. Fragments of lignified pericyclic fibers having narrow lumen, acute apices. Some are accompanied with sclerenchymatous cells showing dark content.
3. Fragments of lignified annular xylem vessels.
4. Fragments of elongated sclereids arranged in groups.
5. Non-glandular, unicellular trichomes in young stem.
6. Fragments of the epidermis from young stem with rectangular cells having straight anticlinal walls, devoid of stomata.

CONCLUSION
The DNA fingerprinting as well as the botanical study discussed in this study can be considered as an identifying parameters for authentication of the plant.

ACKNOWLEDGMENTS
Our special thanks to horticultural research institute and assistant professor Reem Hamdi for providing and identifying the plant.

REFERENCES