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Morphological, Anatomical, Genetical and High Performance Thin Layer Chromatography Profiling of *Buddleia indica* (Scrophulariaceae)

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ABSTRACT

Buddleia indica Lam is an evergreen African shrub, native to Madagascar. Botanical investigations were done on the entire fresh plant leaves, old and young roots and stems, their histological sections as well as air-dried finely powdered samples. Genetic profiling and DNA fingerprinting were accomplished using RAPD-PCR technique (Random Amplified Polymorphic DNR- Poymerase Chain Reaction). Preliminary metabolic screening was performed using high performance thin layer chromatography (HPTLC) analysis. Botanical study of *Buddleia indica* revealed the presence of non –glandular 4-armed stellate trichomes, cruciferous stomata in the leaves, and the existence of U- shaped median vascular strand accompanied by small accessory bundles in the wings of its petiole all showed its close similarity to the Buddleioideae. Additionally, the cork in the stem that arouses from the pericycle (pericylic cork) and the pericycle which forms a loose ring of fibers surrounding the collateral vascular bundles are common characteristic among the members of Buddleioideae On the genetic level, it can be concluded that the most discriminative and relevant fragment resulting from the successful combination of template and primer was that produced by two primers OPA-09 and OPA-06 followed by OPA-07 RAPD primers attributing to the generation of 14, 13 and 12 amplified DNA fragments. Examination of HPTLC chromatograms of the leaves methanol extract showed ten major spots existing at different percentages that give bluish green color upon spraying with ferric chloride solution indicating its richness by phenolic compounds. *Buddleia indica* is rich in phenolic compounds and it belongs to Buddleioideae owing to the botanical resemblance.

1. Introduction

Genus *Buddleia (Buddleja)* has recently been classified as a member in the family Scrophulariaceae. It comprises about 100 species that are native to Africa, Asia, North and South America (Cortes et al., 2006). *Buddleia* species showed a little contribution in the traditional medicine in many areas around the globe, where they are indigenous, and highly reputed as a topical antiseptic and a potent diuretic in folk medicine (Houghton, 1984). It is worthy to mention that genus *Buddleja* has been placed in several families including Scrophulariaceae Juss. and Loganiaceae (Van Laere et al., 2009). That is due to the morphological similarities with both families that can be seen in the flowers which are cymes of various kinds, usually in globose heads, panicles, or verticils; tetramerous, actinomorphic, except for the often unequal calyx lobes. Calyx is gamosepalous, campanulate, cup-shaped, or tubular, persistent often unequal but not bilabiate, Corolla is gamopetalous, imbricate or valvate, tubular, funnelform, salverform, or campanulate. Additionally stamens are four, often inserted on the upper half of the corolla tube; all are equal, free and alternating with the corolla lobes. Anthers are bilocular, tetrasporangiate, dehiscing with longitudinal slits and introrse. Pollen grains are 3–4(5)-colporate and 2-celled. Ovary is superior, bilocular, rarely 4-celled, with anatropous ovules, with a single style and a bilobed stigma which are common characheters of all the genera and common with schrophulariaceae (Oxelman et al., 2004).Besides, *Buddleja* has strong affinities within the Scrophulariaceae molecular systematics (Thompson and Abbott, 2013).

Many biological and pharmacological activities have recently been reported regarding its species, including antioxidant, anti-inflammatory, analgesic, antipyretic and immunosuppressive activities (Martinez-Vazquez et al., 1996; Houghton et al., 2003; Piao et al., 2003; Zhang et al., 2010). Moreover, they exhibited remarkable antihypertensive, antidiabetic, hepatoprotective as well as antiviral, antimicrobial and anticancer properties (Pardo et al., 1993; Duschatzky et al., 2005; Oliveira et al., 2007; El-Domiaty et al., 2009; Gilani et al., 2009; Lee et al., 2010). These pronounced activities may partially reside in the prevalence of medicinally valuable secondary metabolites in

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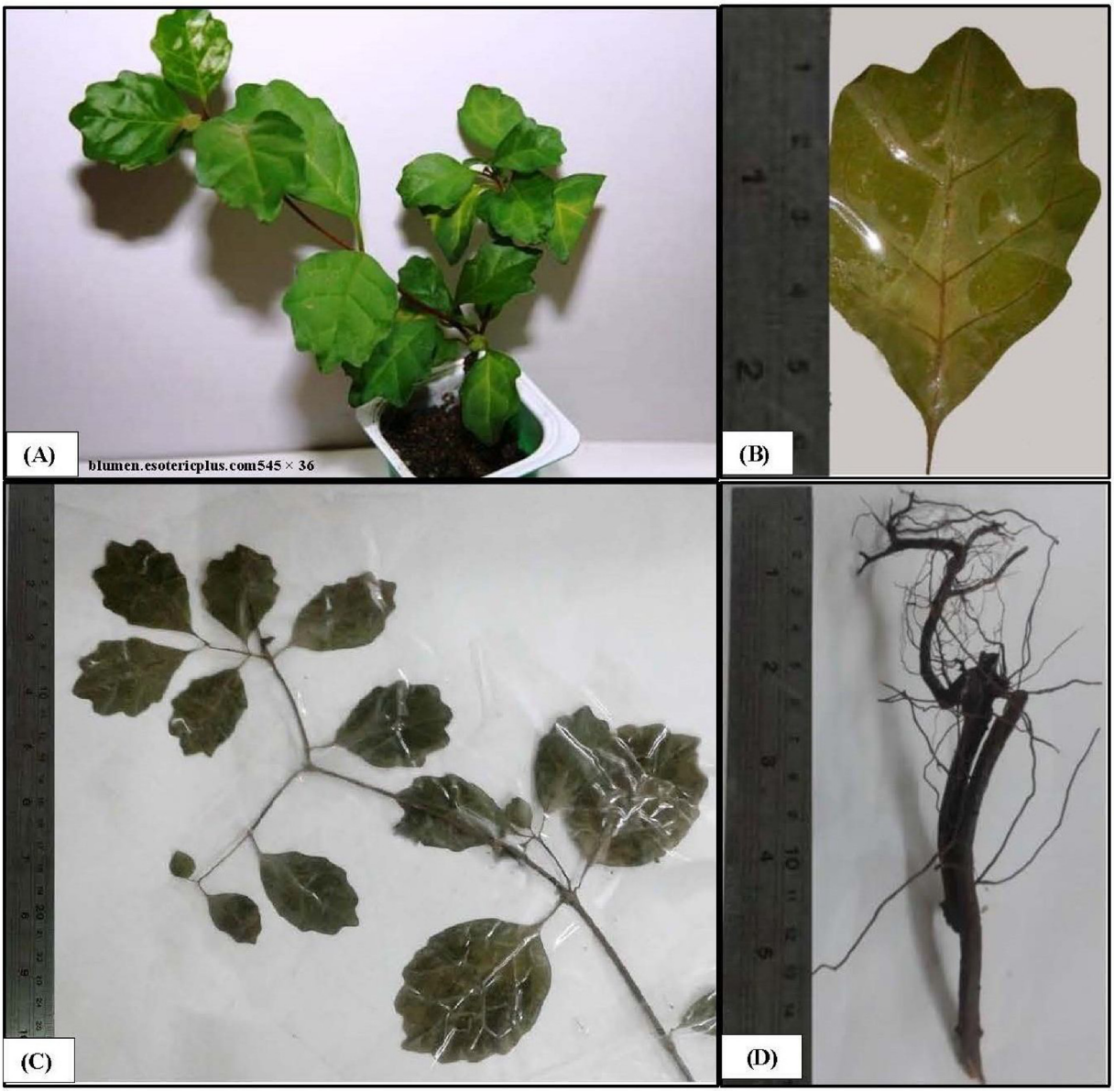

Fig. 1. Morphological characterization of *Buddleia indica* showing (A) entire plant, (B) leaf (X 0.86), (C) leafy branch (X 0.35) and (D) a part of the main root (X 0.6).

most of *Buddleia* species. An arising picture about the nature of these compounds revealed the predominance of considerable amounts of flavonoid and iridoid glycosides, which are common throughout the genus up to date (Houghton, 1984).

Buddleia indica Lam (Scrophulariaceae) is an evergreen African shrub native to Madagascar. It grows from the coast of Southeast Africa to the mountains up to 2000 m high. It was identified by Lamarck in 1785 whereas *Nicodemia diversifolia* Ten. is among its synonyms. It is grown almost exclusively as a houseplant and its leaf shape, is extremely diverse from orbicular to oak-like, hence it is named as an indoor oak (Leeuwenberg, 1979). However, through an elaborative tracing of the current literature, it seems that the botanical profiling, the phytochemical investigation as well as the biological assessment of this species has been somewhat neglected.

Herein, in depth morphological and anatomical investigations were done on the entire fresh plant leaves, old and young roots and stems, their histological sections as well as air-dried finely powdered samples. Genetic profiling and DNA fingerprinting were accomplished using RAPD-PCR technique (Random Amplified Polymorphic DNR-Poymerase Chain Reaction). Furthermore, preliminary metabolic screening was also performed using high performance thin layer chromatography (HPTLC).

2. Materials and Methods

2.1. Plant material

The different organs (leaves, stems and roots) of *Buddleia indica* Lam. were purchased in January 2013 from El-Orman Botanical Garden, Giza, Egypt. The plant was kindly identified and authenticated morphologically by Mrs Therease Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and Director of Orman Botanical Garden, Giza, Egypt. Voucher specimens of the plant material are kept at Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University (voucher number PHG-P-BI-163)

2.2. Preparation of B. indica extract

Air-dried leaves of *B. indica* (50 g) were crushed into coarse powder, exposed to exhaustive percolation in distilled MeOH (500 mL × 3) and