



Morphoanatomical study of the leaves of *Ocotea duckei* Vattimo (Lauraceae-Lauroideae)

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RESUMO: “Estudo morfoanatômico das folhas de *Ocotea duckei* Vattimo (Lauraceae-Lauroideae)”. *Ocotea duckei* Vattimo é uma espécie nativa do nordeste brasileiro, conhecida popularmente como “Louro-de-cheiro”. É rica em alcalóides, lignanas e óleos essenciais. Um dos constituintes mais importantes obtidos de *Ocotea duckei* é iangambina, uma lignana furofuranica, que têm demonstrado várias atividades farmacológicas como: antagonista do receptor do fator de agregação plaquetária (PAF), efeito protetor contra colapso cardiovascular e choque anafilático, antialérgica, analgésica, anticonvulsivante e depressora do sistema nervoso central. O estudo morfo-anatômico das folhas de *O. duckei* foi realizado com o objetivo de contribuir com a sua identificação. O estudo morfológico foi realizado com auxílio de estereomicroscópio e através de observações de campo. O estudo anatômico foi feito a partir de cortes paradérmicos e transversais da folha (lâmina e pecíolo), descorados e corados com azul de astra e fucsina básica. As folhas desta espécie são alternas, elípticas a oblongas, glabras, ápice agudo a acuminado e base aguda a ligeiramente atenuada. As características anatômicas principais são: epiderme uniestratificada, folha hipostomática, com estômatos do tipo paracítico, mesófilo dorsiventral, presença de células secretoras no parênquima e feixes vasculares colaterais.

Unitermos: *Ocotea duckei*, Lauraceae, morfo-antomia, planta brasileira.

ABSTRACT: *Ocotea duckei* Vattimo is a plant native of Northeastern Brazil and popularly known as “Louro-de-cheiro”. It is rich in alkaloids, lignans and essential oils. One of the most important constituents found in *Ocotea duckei* is yangambin, a furofuran lignan having significant pharmacological activities: as platelet activating factor (PAF) receptor antagonist, protective against cardiovascular collapse and anaphylactic shock, anti-allergic, analgesic, anti-convulsant and as depressant of central nervous system. A morphoanatomical study of the leaves of *O. duckei* was carried out in order to contribute to separate it from the other species of the genus *Ocotea*, which would be helpful in its identification. The morphological study was done with stereomicroscope and by field observations. The anatomical studies were made by paradermic and transversal sections of leaves (blade and petiole), cleared and stained with Astrablue and basic Fuchsin. The leaves are alternate, leathery, elliptic to oblong, glabrous, acute to acuminate at the apex, and acute and lightly attenuate at the base. The main anatomical features are the epidermis unistratified, the hypostomatic leaves with stomata of the paracytic type, mesophyll is dorsiventral with secretory cells in the parenchyma and collateral vascular bundles.

Keywords: *Ocotea duckei*, Lauraceae, morphoanatomy, Brazilian plant.

INTRODUCTION

Lauraceae is a large and predominant tropical family of trees and shrubs with 50 genera and about 3.000 species (Van der Werff, 1991). It is an important group of Angiosperm which is notoriously difficult to recognize to genus level even fertile plant (Van der Werff, 2002).

Ocotea Aublet is one of the largest genera of the Lauraceae and is comprised of about 350 species with wide distribution in the neotropical region but having a few species in Africa and Madagascar (Rohwer, 1993). In Brazil, the genus is well represented having about

150 species (Baitello, 2001). According to Van der Werff (2002), the features of *Ocotea* are quite variable and in not easy to separate from, being other genera of the family such as *Cinnamomum* and *Nectandra*. The difficult to make its delimitation, as a store, have been considered by Rohwer (1986) as well as the less defined genus of the family.

Ocotea duckei Vattimo, popularly known as “louro-de-cheiro” and “louro-canela”, is a big tree growing to about 10 m and it is native of the Atlantic Forest areas of Northeastern Brazil. Chemical studies of the plant showed the presence of various groups of constituents

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such as, alkaloids (Silva et al., 2002; Dias et al., 2003, Morais et al. 1998a), lignans and essential oils (Morais et al., 1996; Morais et al., 1998b; Morais et al., 1999; Barbosa-Filho et al., 1999). However, its most important constituent is yangambin, a lignan isolated from the leaves that has several pharmacological properties such as selective platelet activation (PAF) receptor antagonist (Castro-Faria-Neto et al., 1995a,b; Herbert et al., 1997); anti-allergic (Serra et al., 1997); analgesic (Almeida et al., 1995), and protective against cardiovascular collapse and anaphylactic shock (Tibiriçá et al., 1996; Ribeiro et al., 1996; Araújo et al., 2001). In addition, yangambin aids in increasing the sleeping time induced by pentobarbital and the blockage of convulsion induced by pentyllenetrazole (Pachú et al, 1993; Souza et al., 2005). Marques et al. (2003) demonstrated that this substance is not mutagenic when it was subjected to the Ames test.

Reticuline and coclaurine, the benzyltetrahydroisoquinoline alkaloids, are the other substances isolated from *Ocotea duckei* by Morais et al., 1998a, Silva et al., 2002 and Dias et al., 2003, respectively. Reticuline is known to produce alteration of pattern of behavior, prolongation of pentobarbital induced sleep, reduction in motor coordination, D-amphetamine-induced hyper motility and suppression of conditioned avoidance response, suggesting that it has potent depressant activity of central nervous system (Morais et al., 1998a).

Although *O. duckei* have demonstrated to be an important source of substances with pharmacological properties and there is no study done on the leaves of this plant species. The present work on the analysis of the morphoanatomical features of the leaves was carried out to aid in its identification and to distinguish it from others species of *Ocotea*.

MATERIAL AND METHODS

The plant material was collected in rural area of the municipality of Cruz do Espírito Santo, State of Paraíba, Brazil. Morphological studies were carried out for the botanical identification and macroscopic morphodiagnosis with fresh and fixed (formaldehyde, ethanol, acetic acid, water, 2:10:1:3,5) leaves, complemented by field observations. The voucher specimen (Agra & Coutinho 6482) is deposited at the Herbarium Professor Lauro Pires Xavier (JPB), Universidade Federal da Paraíba.

The epidermis was obtained by scraping of both fresh and fixed material and also by dissociation using nitric acid (10%), and was stained with astrablue and basic fuchsin. The transversal sections of the blade leaves and petiole were made by hand microtome, cleared by conventional methods, and were stained with safranin and astrablue. The sections were mounted in glycerin and observed at optical microscope. The terminology of Hickey (1979) was used for the architecture. The anatomical descriptions of the leaf were based on Metcalfe and Chalk (1972)

and Fahn (1974). Original drawings were made with a drawing device. The photomicrographs were performed using the Axiolab Zeiss Automatic System.

RESULTS

Ocotea duckei Vattimo

Tree to 4-15 m tall; stem and branches cylindrical, green when young becoming brown in adult plant. *Leaves* entire, simples, alternates and glabrous; blade elliptic to oval-elliptic, leathery and shining; the blade size has the notophyll type, 10-14 cm long, 3-5 cm wide; the shape is elliptic to oblong and symmetrical, acute to attenuate at the base, acute to acuminate at the apex, margin entire, slightly wavy, glabrous on both surfaces, discolor, being dark green and shining in upper surface, pale green and opaque in the lower, the midrib is prominent beneath, with 7 to 8 pairs lateral veins; the petiole is marginal, glabrous, 4-7 mm long and 1-2 mm wide (Figs. 1A and 4A). *Inflorescence* paniculate, many-flowered, 2-6 cm long. Pedicels teretes, 1-2.5 mm long, pubescent, with simple and non glandular hairs. *Flowers* unisexual, 5-7 cm long; tepals 6, equal, pale yellow, pubescent, lanceolate to elliptic, apex acute, 3-4 mm long. *Stamens* 9, 2.5-3 cm long, filaments narrower than the anthers, which are glabrous, fertile anthers with 4 valves for liberation of pollen grains; the outer 6 stamens have one round gland (staminoid) about 1 mm long, at the base of each filament, which are joined to the base of the tepals; the other inner 3 stamens surround the pistil. *Pistil* is glabrous, 4 mm long, pale green; ovary globose and shorter than style; stigma-1, 3-lobed. *Fruit* is oblong, 0.5-3 cm long and 0.8-1 cm width with evident cupola.

Common names: “louro-de-cheiro”, “louro-canela” and “louro-pimenta”.

Morphological characteristics of the leaf

Architecture: Leaf with the primary vein pinnate and the secondary vein weak brochidodromous with irregular spacing and the angle is smoothly decreasing toward base. Intersecondary veins are absent. The tertiary veins are alternate percurrents and have the angle inconsistent. Quaternary veins are regular polygonal reticulate with the areolation well developed, 5 or more sided, 1-2-branched and has absent type. The marginal veinlets are classified as looped and no toothed (Fig. 4B).

Anatomical characteristics of the leaf

Blade

Epidermis: In frontal section, the epidermal cells of the blade showed wavy anticlinal walls recovered by a thick and smooth cuticle in both surfaces (Fig. 1B-C), which are smaller in the lower surface (Fig. 1C). The

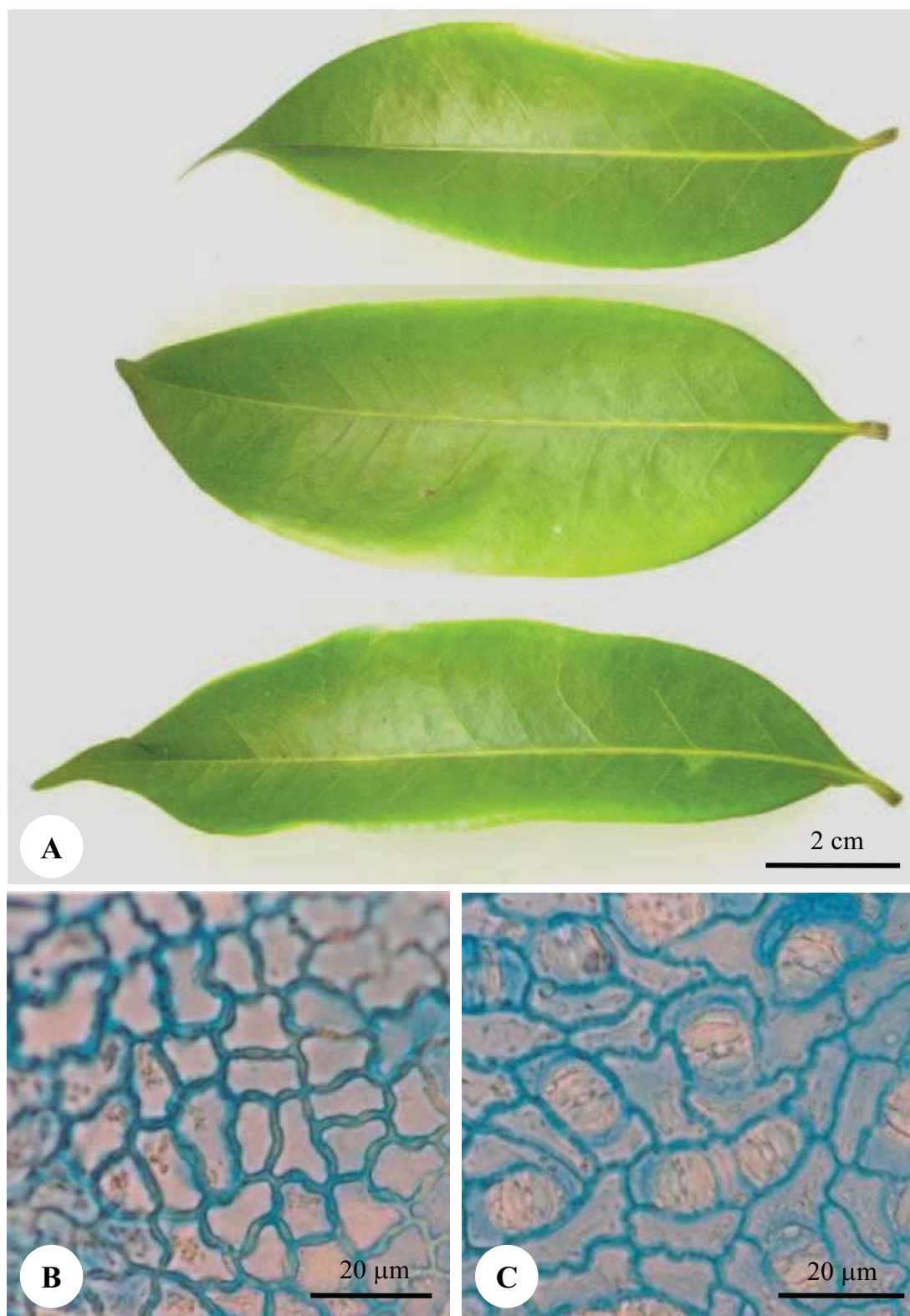


Figure 1. A. Variation of the leaf blade of *Ocotea duckei* Vattimo; B. Frontal view of the epidermis in adaxial surface; C. Surface view of epidermis in abaxial side showing paracytic stomata.

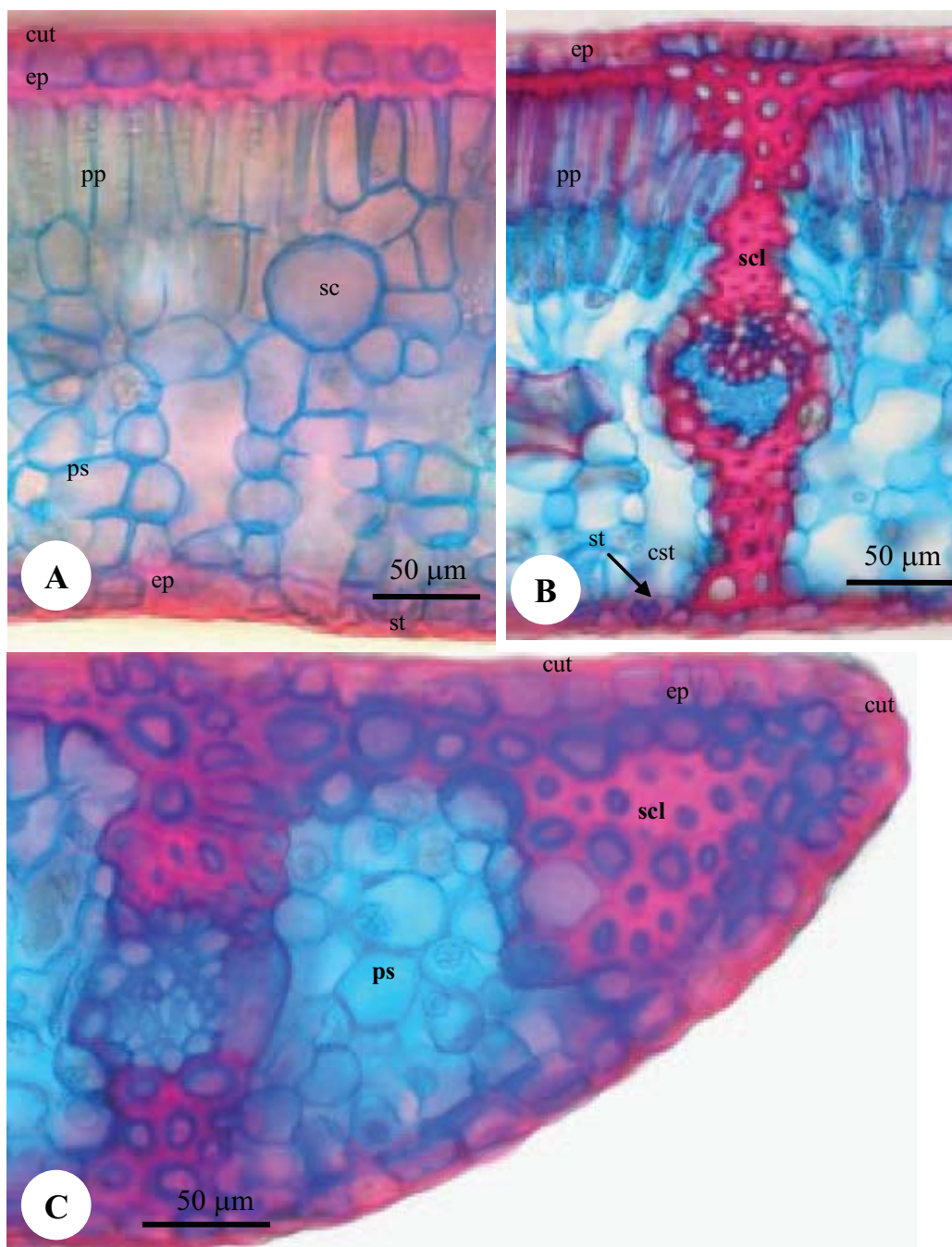


Figure 2. A. Portion of cross-section of the blade leaf of *Ocotea duckei* Vattimo showing the cuticular membrane on the epidermis and the mesophyll with the palisade parenchyma on the adaxial side and the spongy parenchyma on the abaxial side; B. Detail of the secondary collateral vascular bundle with sclerenchymatic sheath; C. Detail of the edge in medium portion of the leaf blade. **Legends:** secretories cells (sc), cuticle (cut), epidermal cells (ep), parenchyma palisade (pp), parenchyma spongy (ps), sclerenchyma (scl), sub-stomatal chamber (cst).

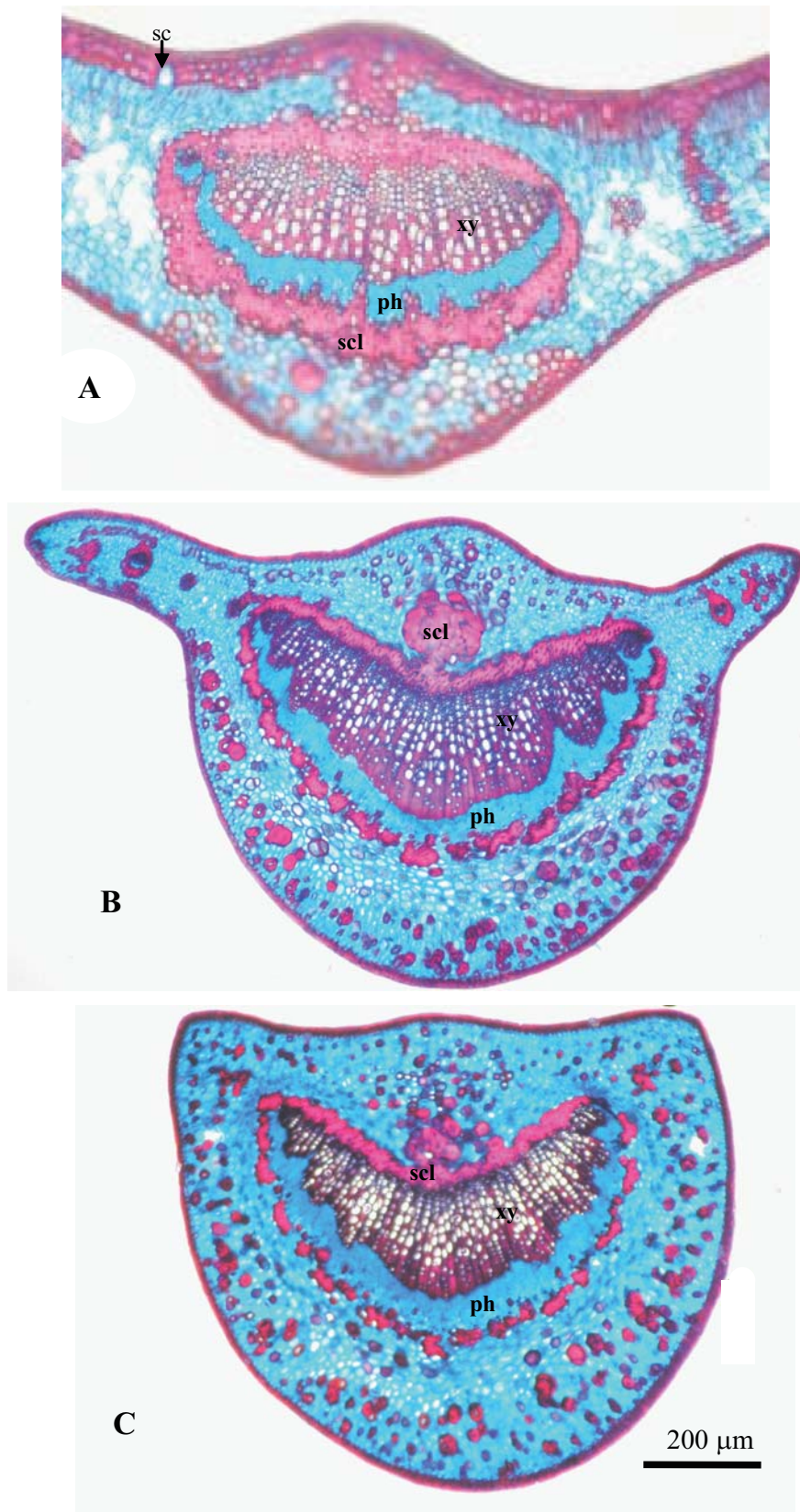


Figure 3. A. Cross-section of the blade leaf midrib in which the portion around midrib is enlarged; B-C. Cross-sections of the petiole in which can be seen the apical portion with wings lateral differing from the basal portion. **Legends:** xylem (xy), phloem (ph), sclerenchyma (scl), secretories cells (sc).

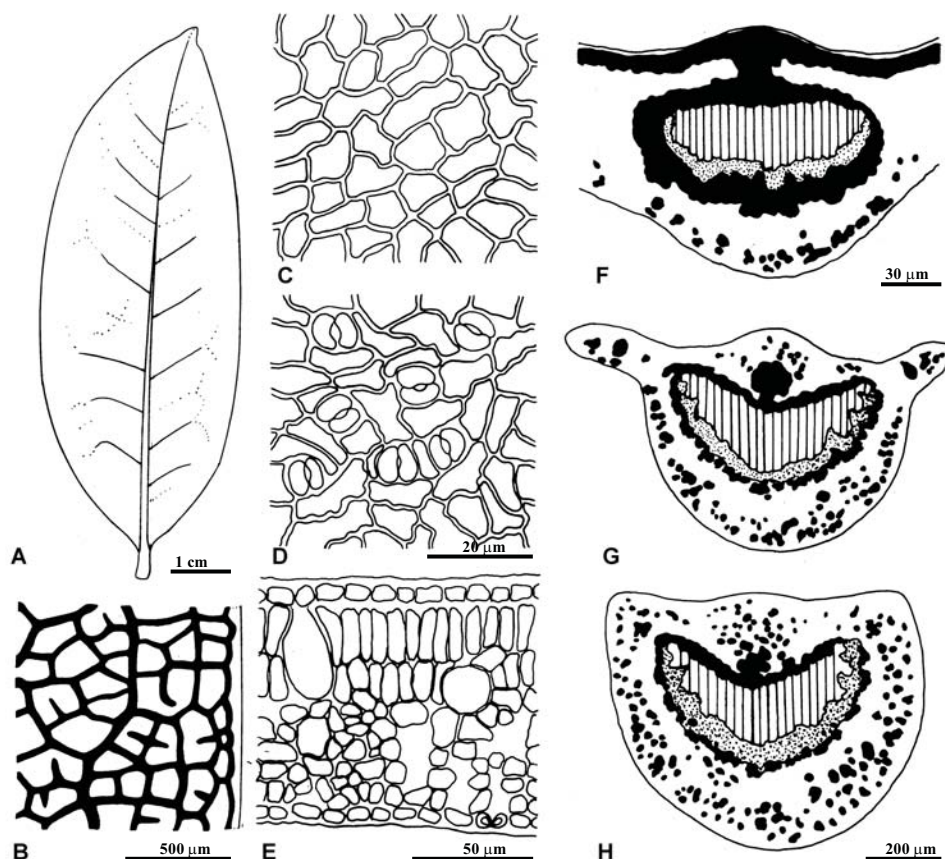


Figure 4. Diagrammatic representation of the leaf of *Ocotea duckei* Vattimo: A. Variations of the leaf; B. Surface view in which the marginal veins and areolation can be seen; C-D: Surface view in which the epidermis in adaxial and abaxial sides can be distinguished; E. Cross-section of the blade leaf in which can be observed the mesophyll; F. Cross-section of the midrib of the blade leaf; G-H. Cross-sections of the petiole in which the bundle is arranged in semi arch and the apical portion with lateral wings differs from the basal portion. White areas-collenchyma; stippled areas-phloem; hatched areas-xylem; solid black areas-sclerenchyma.

blade leaf is hypostomatic with stomata of the paracytic type. In transversal section, the epidermis is uniseriate with rectangular cells of periclinal walls that are thick and straight in the upper surface and a slowly wavy in the lower surface with sunken stomata (Fig. 2A-B).

Mesophyll: In transverse section, the mesophyll is dorsiventral with one or two layers of palisade and seven to eight layers of the spongy parenchyma (Fig. 2A-B and 4E). In both, secretory cells ovoid to round are present with spaced distribution. The collateral vascular bundles are involved by the sclerenchymatic sheath (Fig. 2B). The edge is acute and smooth having a thick and slowly wavy cuticle and a dense sclerenchymatic sheath (Fig. 2C).

Midrib: It is biconvex and very protuberant in the lower surface (Fig. 3A and 4F). In transversal section, the midrib is composed by only one central vascular

bundle semicircular to arch with a belt of continuous sclerenchymatic sheath. The annular collenchyma is followed by the cells of parenchyma, which has many sclerenchymatic fibers and round secretory cells between of these tissues. The epidermis is uniseriate with a thick cuticle. The position of xylem is turned up to the upper surface and the phloem is turned up to the lower (Fig. 3A).

Petiole

In transverse section, the petiole is plane-convex on basal and median portions, and biconvex with lateral prominences at the apical portion, winged, from the attenuated blade (Fig. 3B-C and 4G-H). The epidermis is uniseriate with rectangular and smaller cells of different

sizes and recovered by a thick and smooth cuticle. The cortex is constituted by the fundamental parenchyma formed by many layers of sclerenchymatic cells, discontinuous surround of the vascular bundle near of the epidermis. The vascular bundle in opened V-shaped has the collateral type and is involved by sclerenchymatic sheath having only one bundle at the apex and median portions and four bundles at the base. At the apex the lateral wings are bigger than in intermediary and basal regions. The wings have a small vascular bundles in the both sides (Fig. 3B-C and 4G-H).

DISCUSSION

The leaf morphoanatomy of *O. duckei* corresponds to the foliar pattern reported for the family Lauraceae especially for the subfamily Lauroidae and for the genus *Ocotea* by Metcalfe and Chalk (1972). The leaf morphology and phyllotaxy of *O. duckei* follows the description by Cronquist (1981) and Judd et al. (1999) for the species of the Lauraceae family. The leaves features observed are closely similar to others species of *Ocotea* described by Vattimo (1961) and mislead it correct species identification among the population mainly in the young plants.

The observation of a conspicuous thick and smooth cuticle coating the adaxial epidermis is similar to those found in *O. puberula* by Farago et al. (2005) and contradicts partially Metcalfe and Chalk (1972), who have mentioned the punctate cuticle for the *Ocotea* genus. According to the latter authors, the cuticle helps to prevent the water loss efficiently, as well as the leaf from collapsing when the cells dehydrate. Moreover, the cuticle is ornamented and the occurrence of cuticular flanges have been considered of taxonomic value and used for diagnostic purposes (Metcalfe, 1979).

The occurrence of paracytic and sunken stomata confined to the lower surface in *O. duckei* are common in Lauraceae and differs from that found in *O. puberula* (Farago et al., 2005). The mesophyll is dorsiventral and the midrib is surrounded by a mixed sclerenchymatous sheath similar to that in the region of pericycle, which has been recorded in other species of the Lauraceae family by Metcalfe and Chalk (1972). The only citation of isobifacial mesophyll for Lauraceae was in leaves of *Ocotea gardneri* by Coutinho et al (2006).

The presence of secretory cells containing oil or mucilage in *Ocotea duckei* constitutes one of the most important characteristic feature of the Lauraceae family. According to Metcalf and Chalk (1972), it was recorded in the leaf of all investigated species belonging to different genera of the family.

CONCLUSION

Despite the lack of outstanding morphoanatomical characters for *O. duckei* the leaf showing a set of

characters such as a conspicuous and sclerenchymatic ring continuous on the midrib and discontinuous in the petiole, the epidermal cells coated by a very thick cuticle, the dorsiventral mesophyll with 1-2 layers of palisade having secretory cells and ducts containing oil and mucilage that are relevant for the diagnosis of the species.

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