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Artigo

Anatomical characters of the phyllode and stem of *Acacia podalyriifolia* A. Cunn. ex G. Don (Fabaceae)

M.R. Duarte*, S. Wolf

Laboratório de Farmacognosia, Departamento de Farmácia, Universidade Federal do Paraná, Rua Prefeito Lothário Meissner 3400, Jardim Botânico, 80210-170, Curitiba, PR, Brasil

ABSTRACT: The *Acacia* genus has presented various secondary metabolites, such as tannins, flavonoids, alkaloids and gums. Preparations from different species have been applied for diabetes, gastrointestinal disorders and inflammatory diseases in the traditional medicine and have demonstrated cytotoxic, antimicrobial and antiparasitic activities. *Acacia podalyriifolia* A. Cunn. ex G. Don (Fabaceae) is a small wood, indigenous to Australia and cultivated worldwide for its ornamental feature. This work aimed to characterize the anatomy of the phyllode and stem, in order to contribute to the species identification. The botanical material was fixed, sectioned and prepared according to usual light and scanning microtechniques. The epidermal cells, in surface view, are polygonal and coated with striate and thick cuticle, and filaments of epicuticular wax. Paracytic stomata and unicellular non-glandular trichomes are seen. Palisade and ground parenchymas, and minor collateral bundles with xylem directed alternately to upper and lower sides occur in the blade. The midrib shows two collateral bundles facing each other. The stem, in incipient secondary growth, exhibits epidermis, annular collenchyma, sclerenchymatic sheath and collateral vascular organization. Cells containing phenolic compounds and prisms of calcium oxalate are observed.

Keywords: *Acacia podalyriifolia*, phyllode, stem, wattle.

INTRODUCTION

The genus *Acacia* comprises about 1200 species (Guinet; Vassal, 1978; Barroso, 1991), indigenous to tropical and subtropical savannas, mainly in Australia and Africa (Cronquist, 1981). Several secondary metabolites have been reported in the taxon, such as hidrolyzable and condensed tannins, flavonoids, terpenes, alkaloids, cyanogenic glycosides and gums (Malan; Roux, 1975; Secor et al., 1976; Flath et al., 1983; Bennie et al., 2001; Seigler, 2003).

Ethnobotanical data revealed that preparations from different parts of *Acacia* spp. have been applied for diabetes, gastrointestinal disorders and inflammatory diseases in the traditional medicine (Gupta; Mishra, 2002; Wickens; Pennacchio, 2002; Bhatt et al., 2003; Li et al., 2003). Pharmacological researches have demonstrated cytotoxic, antimutagenic, antimicrobial and antiparasitic activities (Fournet et al., 1994; Ghosh et al., 1996; Omer et al., 1998; Popoca et al., 1998; Arora et al., 2003). Besides, *Acacia* members are popularly called as wattle and have also been employed in the development of adhesives, food additives, demulcents and emollients (Karnik et al., 1973; Saayman, 1978; Muhammad et al., 1998).

Acacia podalyriifolia A. Cunn. ex G. Don is a small wood, perennial, indigenous to Australia and distributed worldwide. It has been cultivated for its ornamental feature, due to the showy yellow inflorescences contrasting with the gray phyllodes. These are modified leaves consisting of reduced blade and broadened petiole, which performs the photosynthetic role. Similarly to *Acacia* species employed in folk medicine, *A. podalyriifolia* has drawn attention, although only few studies have been carried out, mostly devoted to ecological aspects (Hawkeswood, 1988; 1990; 1991; Andrade et al., 2003) and chemical analysis, such as the investigation of free proline (Li et al., 1999) and alkaloids contents (White, 1943), and the identification of a naphthoquinone and naringenin (Andrade, 2003). The lack of available data on the morphology of *A. podalyriifolia* has led to this investigation, which dealt with the anatomy of the phyllode and stem, aiming to contribute to the species identification for pharmacognostic purposes.

MATERIAL AND METHODS

The botanical material was collected from cultivated specimens in the garden at the Sede Botânico, Setor de Ciências da Saúde, Universidade Federal do Paraná (approximately 25º 26' 40"S and 49º 14' 20"W, 930m height), in January 2004. The species was identified by the voucher MBM285767 at the Museu Botânico Municipal de Curitiba.

Fully developed phyllodes and stem fragments, obtained 5-30cm from the caulinar apex, were fixed in FAA 70 (Johansen, 1940) and kept in 70% ethanol solution (Berlyn; Miksche, 1976). The examined region of the phyllodes was the lower half. Transverse and longitudinal freehand sections were stained either with toluidine blue (O'Brien et al., 1965) or astra blue

* *E-mail: marciard@ufpr.br, Tel. + 55-41-3604064*

and basic fuchsine (Roeser, 1962). Microchemical tests were applied with iodine-iodide to reveal starch (Berlyn; Miksche, 1976), Sudan IV to lipophilic substances (Foster, 1949), hydrochloric phloroglucin to lignin (Sass, 1951), ferric chloride to phenolic compounds (Johansen, 1940) and sulphuric acid to calcium oxalate crystals (Oliveira; Akisue, 1989). The results were registered by botanical illustration and photos taken by a light microscope Olympus BX40 attached to the control unit PM20.

For the ultrastructure study (Souza, 1998), phyllodes fixed in FAA 70 were dehydrated in a graded ethanolic series and by the critical point procedure (Bal-Tec CPD-030), coated with gold (Balzers SCD-030) and analysed by a scanning electron microscope Jeol JSM-6360LV.

Figure 1. *Acacia podalyriifolia* A. Cunn. ex G. Don (Fabaceae) – reproductive apical branches.

RESULTS

Phyllode anatomy

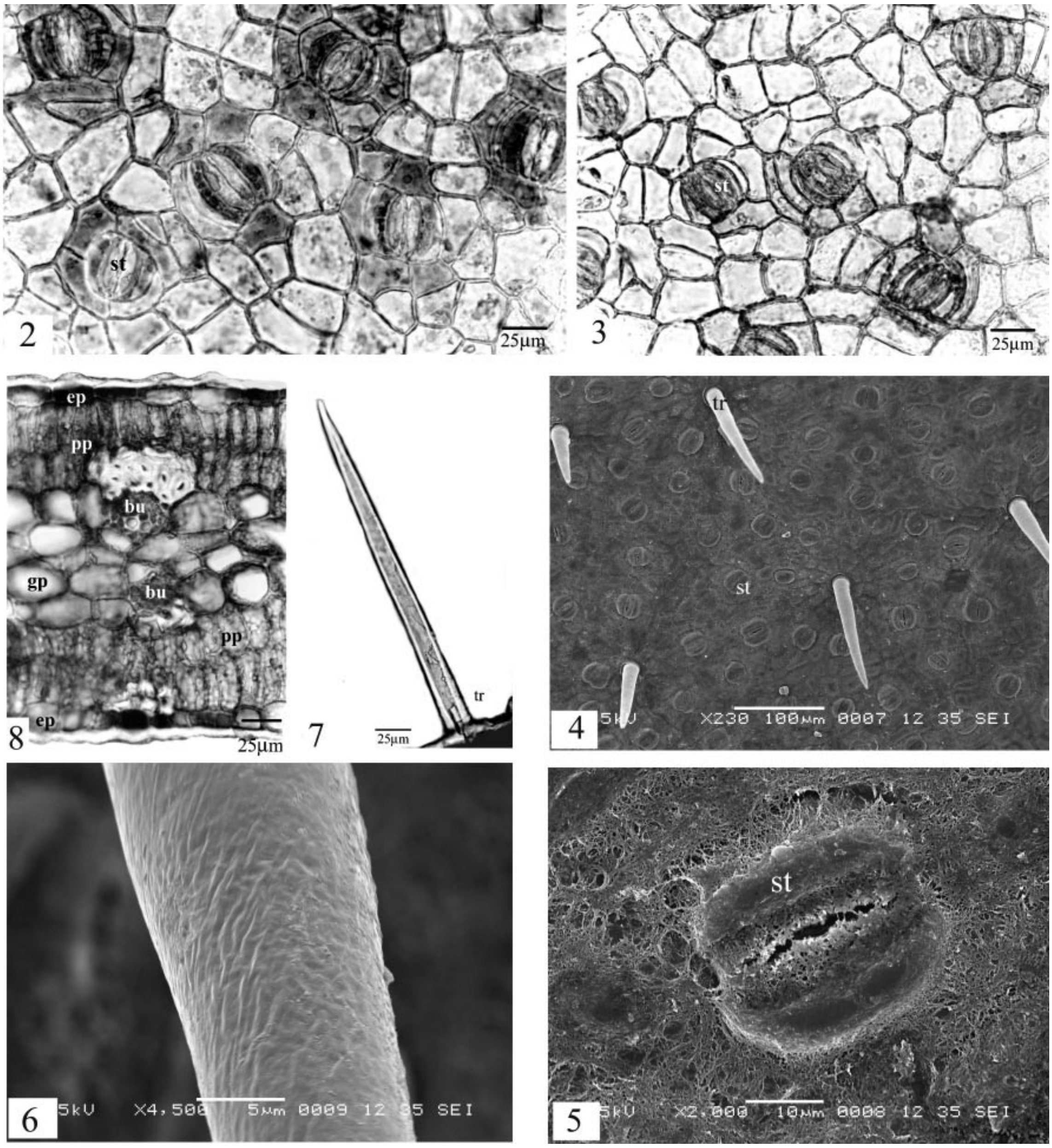
The phyllodes (Figure 1) are alternate, oblong-elliptic, uninerved, with asymmetric blade, mucronate acute apex, acute base, entire margin, waxy texture and gray colour, measuring about 5cm long and 2cm wide. The blade, in surface view of both surfaces, has epidermal cells exhibiting a polygonal shape (Figures 2, 3), coated with a striate and thick cuticle and presenting filaments of epicuticular wax (Figure 5). The epidermis is uniseriate (Figure 8) and has paracytic stomata on both surfaces (Figures 2, 3) and on the same level as the other surrounding cells. Unicellular non-glandular trichomes, erect (Figures 4, 7), having thick cell walls (Figure 7) and coated with a striate cuticle (Figure 6) are seen. They show an acute apex and a base encircled by epidermal cells arranged as a rosette, which originate distinct detachment areas. Adjacent to the upper and lower epidermal sides, a two-layered palisade parenchyma occurs. In the central region, it is encountered three to five strata of ground parenchyma, harbouring few chloroplasts (Figure 8). Many minor collateral bundles with a cap of perivascular fibres next to the phloem are embedded in the mesophyll. Each bundle directs the xylem to the upper and lower surfaces alternately (Figure 8). The midrib in transection is biconvex (Figure 9) and the epidermis is similar to the blade. In the region below, an annular collenchyma is seen, consisting of about four rows, and two major collateral bundles facing each other. The xylem is directed to the centre, and adjoining the phloem a perivascular fibre cap is encountered. The parenchymatic cells may contain amiloplasts and calcium oxalate prisms. Phenolic compounds are detected in the epidermis, ground parenchyma and phloem.

Stem anatomy

The stem transection is approximately circular (Figure 10) and, in incipient secondary growth, the epidermis is present, showing trichomes similar to the phyllode ones. The cortex is composed of a strand of annular collenchyma, few strata of parenchymatic cells and an evident sclerenchymatic sheath, practically complete, that comprises stone cells and many fibres (Figure 11). A cylinder of phloem is formed outward and xylem, inward. Parenchymatic rays having one to three rows traverse the xylem and phloem (Figures 12, 13). In the latter, they broaden owing to the increasing size of the cells (Figure 12). Tracheary elements are encountered isolated or in small groups (Figure 13). The pith (Figure 14) is formed by cells with thick walls and prisms of calcium oxalate are found in the cortex and pith. The epidermis, sub-epidermal layers, parenchymatic cells in the phloem and parenchymatic rays traversing the phloem and xylem harbour phenolic compounds.

DISCUSSION

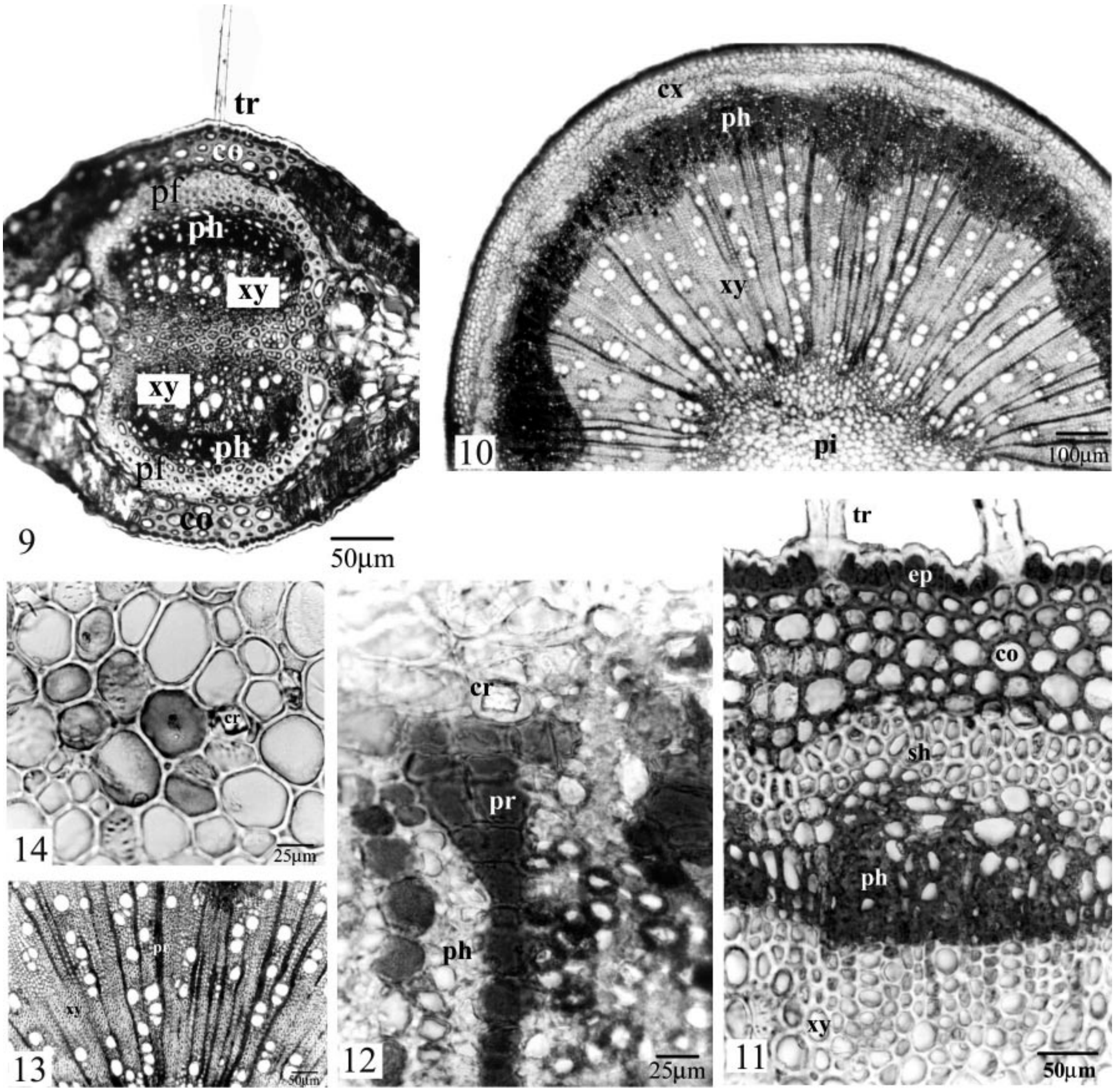
Concerning the phyllodes, which are leaf-like photosynthetic organs commonly found in many species of *Acacia* from Australia (Fahn, 1990), Boke (1940) has mentioned that they are homologous with the petiole/rachis of a pinnate foliage leaf, and the apical pointlet is an abortive terminal leaflet. This point of view is shared with Kaplan (Fahn, 1990), who has reported that this organ is homologous with the rachis of the compound leaf plus a small sector of the petiole. However, the metamorphosis of the phyllodes does not involve distal laminar suppression and compensatory petiolar elaboration but rather a single adaxial meristem responsible for the development of the phyllode. On the other hand, Metcalfe and Chalk (1988) have stated that these aerial organs in *Acacia* may be



Figures 2-8. *A. podalyriifolia* – phyllode: 2, 3. epidermal cells in surface view, upper and lower sides respectively; 4. lower epidermal side; 5. stomatum and filaments of epicuticular wax; 6. detail of a non-glandular trichome coated with striate cuticle; 7. thick cell-walled trichome; 8. transection of the blade. bu – vascular bundle, ep – epidermis, gp – ground parenchyma, pp – palisade parenchyma, st – stomatum, tr – non-glandular trichome

without leaflets, due to the failure to develop leaflets in maturation, or the abscission of leaflets at various stages of development. Phyllodes have been described in many species of *Acacia*, such as *Acacia auriculiformis* A. Cunn. (Yu; Cen, 1990; Sinha et al., 1996), *Acacia koa* A. Gray (Hansen, 1996) and *Acacia suaveolens* (Smith) Willd. (Morrison; Rupp, 1995).

In general, the epidermis is the outer most layer of leaves, consisting of tabular and sinuous cells in Magnoliopsida (Mauseth, 1988; Fahn, 1990). Differing from this pattern, *Acacia podalyriifolia* A. Cunn ex G. Don has epidermal cells with polygonal anticlinal cell walls, in surface view. Besides, it has not shown hemispherical papillae in the lower epidermal cells, often registered in the *Acacia* genus (Metcalfe; Chalk, 1950). The occurrence of thick cuticle is usually typical in plants growing in dry habitats (Fahn, 1990) and wax is a universal adjunct to the outer wall of the epidermis (Mauseth, 1988), and both features are consistent with the xeric origin of this species. Similarly, white deposit of a wax-like substance was reported for other species of *Acacia* (Metcalfe; Chalk, 1950) and epicuticular wax



Figures 9-14. *A. podalyriifolia* – phyllode and stem: 9. midrib of the phyllode, in transection, exhibiting two vascular bundles facing each other; 10. structural organization of the stem; 11. detail of the previous figure, showing the uniseriate epidermis, annular collenchyma, sclerenchymatic sheath and vascular system; 12, 13. phloem and xylem, respectively, traversed by parenchymatic rays; 14. pith cells and crystal. co – collenchyma, cr – crystal, cx – cortex, ep – epidermis, pf – perivascular fibres, ph – phloem, pi – pith, pr – parenchymatic ray, sh – sclerenchymatic sheath, tr – non-glandular trichome, xy – xylem.

was described for *Acacia greggii* A. Gray var. *arizonica* (Bleckmann et al., 1980).

Paracytic stomata and non-glandular trichomes have been found in Fabaceae members (Metcalfe; Chalk, 1950), as seen in the studied species. Similarly, in *A. auriculiformis*, non-glandular trichomes are characterized as unicellular, straight or curve (Banerjee et al., 2002). In addition, in Metcalfe and Chalk's opinion (1950), the structural leaf organization observed is due to the phyllode arranged in a compressed ring, corresponding with the flattening of the petiole and the xylems of the opposed pairs of bundles being directed towards one another. Consequently, the mesophyll is classified as centric and its central portion contains relatively little chlorophyll. In this investigation, the minor bundles embedded in the mesophyll and the major ones of the midrib are accompanied by perivascular fibres, which are equivalent to sclerenchymatic cells of pericyclic origin adjoined to the phloem.

According to Fahmy (1997), the reduction of the leaf area, the presence of trichomes, amphistomacy, isobilateral leaf construction and two to four-layered palisade parenchyma represent xeromorphy and are ecophysiological adjustments of the desert plants to their habit, compatible to this species origin.

On the caulinar level analysed, the epidermis persists in *A. podalyriifolia*, as can be seen even in barks of considerable thickness in *Acacia*. The cork arises superficially, in the second or third layer of cells below the epidermis and the cortical region is internally bounded by a sclerenchymatic ring, composed of stone cells (Metcalfe; Chalk, 1950). In this study, the lignified sheath consists of stone cells and fibres. In contrast, the latter is only mentioned in the phloem of the Australian wattles, according to the previous authors. Comparatively, non-continuous layers of sclereids and fibres have also been registered in *Acacia montana* Benth. (Chen et al., 2003), while a sclerenchymatous fibre sheath located between the cortex and phloem is encountered in the stem of *Acacia baileyana* F. Muell. (Schwarz et al., 1999) and, correspondently, a sclerenchymatous pericycle is observed in *Acacia moniliformis* Griseb. (Bose; Mondal, 1975). Metcalfe and Chalk (1950) have described solitary or small clusters of tracheary elements, as well as narrow rays traversing the vascular system in the genus, as found in this investigation.

Regarding the crystal presence, *A. podalyriifolia* has prisms of calcium oxalate in the leaf and stem, following the family pattern. They are solitary and often rhombohedral (Metcalfe; Chalk, 1950), and various functions have been attributed to them, such as avoidance of toxic accumulation of oxalate, calcium storage and mechanical support against foraging animals (Franceschi; Horner Jr., 1980). Wattendorff (1978) has studied the development of the calcium oxalate crystal cells in the bark and secondary xylem of *Acacia senegal* Willd. and stated that crystals could lie in vacuoles, in which they are covered by a thin cytoplasmic layer that is connected with the bulk of the cytoplasm. He has suggested that the crystal compartment contained the oxalate ion while the calcium can be transported within the cytoplasmic compartment, which is in close contact with the crystal, through its membrane. In this study, crystal cells are not found in the xylem as they are not found in the wood of *Acacia gaumeri* S. F. Blake (Rebollar; Quintanar, 1998). Several African *Acacia* species show growth rings as narrow bands of marginal parenchyma filled with long calcium oxalate crystal chains (Gourlay; Grime, 1994; Gourlay, 1995a,b).

The outlined morpho-anatomical characters of *Acacia podalyriifolia* A. Cunn. ex G. Don correspond to the structures described for the family and can be applied for this species identification, although it lacks information about correlated species of the genus to evaluate their usefulness for taxonomic purposes.

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REFERENCES

Andrade CA 2003. *Flores de Acacia podalyriaefolia A. Cunn. (Leguminosae – Mimosoideae): avaliação fitoquímica e das atividades antibacteriana e alelopática*. Curitiba, 91 p. Dissertação de Mestrado – Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Paraná.

Andrade CA, Miguel MD, Miguel OG, Ferronato ML, Peitz C, Cunico M, Dias JFG, Balestrin L, Kerber VA 2003. Efeitos alelopáticos das flores da *Acacia podalyriaefolia* A. Cunn. *Visão Acadêmica 4*: 93-98.

Arora S, Kaur K, Kaur S 2003. Indian medicinal plants as a reservoir of protective phytochemicals. *Teratogen Carcin Mut Supp.1*: 295-300.

Banerjee A, Rahaman CH, Kar RK, Mandal S 2002. Micromorphology of foliar epidermis of some tropical tree legumes. *Phytomorfology 52*: 223-230.

Barroso GM 1991. *Sistemática de angiospermas do Brasil*. Viçosa: Universidade Federal de Viçosa. vol. 2, p. 71-77.

Bennie L, Coetzee J, Malan E, Woolfrey JR, Ferreira D 2001. Oligomeric flavanoids. Part 34: Doubly-linked proteracacinidin analogues from *Acacia caffra* and *Acacia galpinii*. *Tetrahedron 57*: 661-667.

Berlyn GP, Miksche JP 1976. *Botanical microtechnique and cytochemistry*. Ames: Iowa State University, p. 121, 276.

Bhatt DC, Patel NK, Mitaliya KD, Ant HM 2003. Herbal magic by contact therapy among tribals and rurals of Gujarat. *J Econ Taxon Bot 27*: 205-207.

Bleckmann CA, Hall HM, Hoshaw RW 1980. Cuticular ultrastructure of *Prosopis velutina* and *Acacia greggii* leaflets. *Botanical Gazette 141*: 1-8.

Boke NH 1940. Histogenesi and morphology of the phyllode in certain species of *Acacia*. *Am J Bot 27*: 73-90.

Bose TK, Mondal DP 1975. Anatomy of stem in relation to adventitious rooting. *Plant Sci 7*: 23-27.

Chen YQ, Xu T, Chen XM, Li K 2003. Study on comparative anatomy of bark of seven host tree species of *Kerria lacca* (Kerr.) and its parasitic characteristics. *Forest Res 16*: 411-417.

Cronquist A 1981. *An integrated system of classification of flowering plants*. New York: Columbia University, p. 592-595.

Fahmy GM 1997. Leaf anatomy and its relation to the ecophysiology of some non-succulent desert plants from Egypt. *J Arid Environments 36*: 499-525.

Fahn A 1990. *Plant anatomy*. 4th ed. Oxford: Pergamon Press, 152-184, 223, 262.

Flath RA, Mon TR, Lorenz G, Whitten CJ, Mackley JW 1983. Volatile components of *Acacia* spp. blossoms. *J Agr Food Chem 31*: 1167-1170.

Franceschi VR, Horner Jr. HT 1980. Calcium oxalate crystals in plants. *Bot Rev 46*: 361-427.

Foster AS 1949. *Practical plant anatomy*. 2.ed. Princeton: D. Van Nostrand, p. 218.

Fournet A, Barrios AA, Munhoz V 1994. Leishmanicidal and trypanocidal activities of Bolivian medicinal plants. *J Ethnopharmacol 41*: 19-37.

Ghosh NK, Sinha BSP, Sukul NC, Ito A 1996. Cestocidal activity of *Acacia auriculiformis*. *J Helminthol 70*: 171-172.

Gourlay ID 1995a. Growth ring characteristics of some African *Acacia* species. *J Trop Ecol 11*: 121-140.

Gourlay ID 1995b. The definition of seasonal growth zones in some African *Acacia* species – a review. *IAWA Journal 16*: 353-159.

Gourlay ID, Grime GW 1994. Calcium oxalate crystals in African *Acacia* species and their analysis by scanning proton microprobe (SPM). *IAWA Journal 15*: 137-148.

Guinet P, Vassal J 1978. Hypotheses on the differentiation of the major groups in the genus *Acacia* (Leguminosae). *Kew Bulletin 32*: 509-527.

Gupta AK, Mishra SK 2002. Indigenous phytotherapy for diabetes from Chhattisgarh. *Adv Plant Sci 15:* 407-409.

Hansen DH 1996. Establishment and persistence characteristics in juvenile leaves and phyllodes of *Acacia koa* (Leguminosae) in Hawaii. *Int J Plant Sci 157*: 123-128.

Hawkeswood TJ 1988. The food plants of the Australian butterfly *Prosotas felderi* (Murray) (Lepidoptera: Lycaenidae). *Giornale Italiano di Entomologia 4*: 89-92.

Hawkeswood TJ 1990. A new larval host plant for *Jalmenus daemeli* Semper (Lepidoptera: Lycaenidae) from Queensland. *Victorian Entomology 20*: 118.

Hawkeswood TJ 1991. Review of the history, biology and host plants of the Australian weevil *Chrysolopus spectabilis* (Fabricius). *Spixiana 14*: 17-25.

Johansen DA 1940. *Plant microtechnique*. New York: McGraw Hill, p. 41, 193.

Karnik MG, Sharma OP, Dobhal NP 1973. Note on catechin (from heartwood) of *Acacia catechu* (Khair wood) and effect on some food additives on its coloured solutions. *Indian J Forestry 99*: 149-151.

Li K, Zeng JM, Zhao H 1999. Drought resistance in relation to free proline in the major tree species in the dry and hot river valley of Jinsha. *Forest Res 12*: 103-107.

Li RW, Myers SP, Leach DN, Lin GD, Leach G 2003. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. *J Ethnopharmacol 85*: 25-32.

Malan E, Roux DG 1975. Flavonoids and tannins of *Acacia* species. *Phytochemistry 14*: 1835-1841.

Mauseth JD 1988. *Plant anatomy*. Menlo Park: Benjamin Cummings, p. 167-198.

Metcalfe CR, Chalk L 1988. *Anatomy of dicotyledons*. 2nd ed. Oxford: Clarendon Press, vol. 1, 276 p.

Metcalfe CR, Chalk L 1950. *Anatomy of the dicotyledons: leaves, stem, and wood in relation to taxonomy*. Oxford: Clarendon Press, vol. 1, p. 476-487.

Morrison DA, Rupp AJ 1995. Patterns of morphological variation within *Acacia suaveolens* (Mimosaceae). *Aust Syst Bot 8*: 1013-1027.

Muhammad S, Mushtaq A, Ashfaq A 1998. Chemistry of the medicinal plants of genus *Acacia*. *Hamdard Medicine 41*: 63-67.

O'Brien TP, Feder N, Mccully ME 1965. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma 59*: 368-373.

Oliveira F, Akisue G 1989. *Fundamentos de farmacobotânica*. Rio de Janeiro: Atheneu, p. 45-48.

Omer MEA, Al-Magboul AZ, El-Egami AA 1998. Sudanese plants used in folkloric medicine: screening for antibacterial activity. Part IX. *Fitoterapia 69*: 542-545.

Popoca J, Aguilar A, Alonso D, Villarreal ML 1998. Cytotoxic activity of selected plants used as antitumorals in Mexican traditional medicine. *J Ethnopharmacol 59*: 173-177.

Rebollar S, Quintanar A 1998. Wood anatomy of several tropical species from Quintana Roo, Mexico. *Rev Biol Trop 46*: 1047-1057.

Roeser KR 1962. Die nadel der schwarzkiefer-massenprodukt und kunstwerk der natur. *Mikrokosmos 61*: 33-36.

Saayaman HM 1978. The chemistry and development of wood adhesives from wattle bark extract. *Wood Southern Africa 3*: 14-34.

Sass JE 1951. *Botanical microtechnique*. 2.ed. Ames: Iowa State College, p. 97.

Schwarz JL, Glocke PL, Sedgley M 1999. Adventitious root formation in *Acacia baileyana* F. Muell. *J Hortic Sci Biotech 74*: 561-565.

Secor JB, Conn EE, Dunn JE, Seigler DS 1976. Detection and identification of cyanogenic glucosides in six species of *Acacia*. *Phytochemistry 15*:1703-1706.

Seigler DS 2003. Phytochemistry of *Acacia – sensu lato*. *Biochem Syst Ecol 31*: 845-873.

Sinha AK, Shirke PA, Pathre UV, Behl HM 1996. Spectral properties of *Acacia auriculiformis*: Effect of leaf position. *Indian Forester 122*: 496-500.Souza W 1998. *Técnicas básicas de microscopia eletrônica aplicadas às ciências biológicas*. Rio de Janeiro: Sociedade Brasileira de Microscopia Eletrônica, p. 1-44.

Wattendorff J 1978. Ultrastructure and development of the calcium oxalate crystal cells with suberin-like crystal sheaths in the bark and secondary xylem of *Acacia senegal* Willd. *Protoplasma 95*: 193-206.

White EP 1943. Alkaloids of the Leguminosae. *New Zeal J Sci Technol* 93-162.

Wickens K, Pennacchio M 2002. A search for novel biologically active compounds in the phyllodes of *Acacia* species. *Conserv Sci, Western Australia 4*: 139-144.

Yu QZ, Cen BZ 1990. A study on the pathological anatomy of the phyllode of *Acacia auriculiformis* and its resistance to the powdery mildew pathogen, *Oidium* sp. *J South China Agric Univ 11*: 72-78.