Pharmacognostic characterization of leaves and stem barks of *Eugenia brasiliensis* Lam. (Myrtaceae)

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RESUMO: Caracterização farmacognóstica de folhas e de cascas de caule de *Eugenia brasiliensis* Lam. (Myrtaceae). *Eugenia brasiliensis* Lam. (Myrtaceae) a "cereja brasileira" é usada na medicina popular brasileira em distúrbios gastrintestinais e em reumatismo. A estreita semelhança existente entre as mirtáceas, em particular, do gênero *Eugenia* torna necessário caracterizar a droga de forma a identificá-la. As análises macro e microscópicas das folhas e cascas de caule, que constituem as partes usadas, foram realizadas de acordo com a metodologia clássica. As características de valor diagnóstico foram indicadas e ilustradas com fotografias e fotomicrografias. A análise cromatográfica em camada delgada do extrato hidroetanólico (30:70) complementou o estudo morfológico. Flavonóides e terpenos foram detectados e a melhor separação deu-se empregando, respectivamente, os sistemas de solventes: (a) clorofórmio-ácido acético-metanol-água (60:32:12:8) e (b) clorofórmio. Tais sistemas permitiram, igualmente, diferenciar órgãos vegetais. A associação destas análises mostrou ser importante para a identificação da droga, além de ser de fácil execução.

Palavras-chave: *Eugenia brasiliensis* Lam., Myrtaceae, caracterização botânica, análise cromatográfica, plantas medicinais.

ABSTRACT: Pharmacognostic characterization of leaves and stem barks of *Eugenia brasiliensis* Lam. (Myrtaceae). *Eugenia brasiliensis* Lam. (Myrtaceae) the "brazilian cherry" is a folk brazilian medicine used in gastrointestinal disorders and in rheumatism. The close resemblance among the Myrtaceae, in particular, of the *Eugenia* genus render necessary to characterize the drug in order to identify it. Macro and microscopical analysis of the leaves and the stem barks, which are the used parts, followed the classical techniques. Some characters of diagnostical value are pointed out and illustrated by photographs and photomicrographs. The thin layer chromatographic analysis of the hydroethanolic extract (30:70) complemented the morphological characterization. Flavonoid and terpene components were detected and better separated, respectively, with the solvent systems (a) chloroform-acetic acid-methanol-water (60:32:12:8) and (b) chloroform. Those chromatographic systems allowed also to differentiate the plant organs. The combined analysis showed to be of value for identification of the drug and are of easy execution.

Key words: *Eugenia brasiliensis* Lam., Myrtaceae, botanical characterization, chromatographic analysis.

INTRODUCTION

Eugenia brasiliensis Lam. is one of the 3.5 thousand species of the family Myrtaceae (Cronquist, 1981; Barroso, 1986). This brazilian fruitbearing tree, known as *grumixama* or *brazilian cherry*, grows in the Atlantic rainforest (Correa, 1969; Legrand & Klein, 1969; Angely, 1970). Leaves and stem barks are traditional medicines claimed to be useful for gastrointestinal disorders, dysentery and vaginal infections due to their astringent properties (Correa, 1969; Coimbra, 1994). Antirheumatic and diuretic properties of the leaves are also reported (Cavalcante, 1988). The close morphological resemblance of the Myrtaceae species (Barroso, 1986), in particular, among those of the *Eugenia* (Haron & Moore, 1996) make it difficult to identify them. This paper aims at describing diagnostical macroscopical and microscopical features of the leaves and of the stem barks of *E. brasiliensis* as well as to perform their chromatographic analysis.

Recebido para publicação em 07/05/02
e aceito para publicação em 22/03/03.

MATERIAL AND METHOD

Plant Material

The leaves and the stem barks of *Eugenia brasiliensis* Lamarck (Myrtaceae) were collected, on October 1999, in the *campus* of the Universidade Estadual Paulista (UNESP), at Jaboticabal, in the São Paulo State, Brazil. The species was authenticated by Dr. Maria Lúcia Kawasaki, herbarium of the Instituto de Botânica de São Paulo, São Paulo, where *voucher* specimens are deposited (SP-331554).

https://doi.org/10.70151/xgykj872

Macro and microscopical analysis

Macroscopic characters were observed with a stereoscopic magnifying lens. Photographs were taken to illustrate descriptions. For the microscopic analysis of fresh and dried leaves and of the stem barks, transverse and longitudinal sections of the foliar lamina, midrib, petiole and stem bark were made. The dried material and the stem barks were rehydrated previously in boiling water. The sections were obtained by conventional methods (Johansen, 1940). The ocurrence of lignin, suberin, phenolic compounds and calcium oxalate were confirmed, respectively, with floroglucinol-hydrochloric acid, Sudam IV, 2% ferric chloride solution and sulfuric acid TS (Johansen, 1940; Sass, 1951; Jensen, 1962). The structures were observed with a light microscope (Olympus). Photomicrographs were taken with an FX-35WA Nikon Automatic System and Nikon UFX-IIA Microscope.

Chromatography

The thin layer chromatographic (TLC) analysis of the *E. brasiliensis* hydroethanolic extracts from leaves and from the stem bark (70 % v/v) was performed with different solvent systems of analytical grade (Merck). The two best ones were: (a) chloroform-acetic acid-methanol-water (60:32:12:8) and (b) chloroform. The chromatograms were visualized in UV light (254 and 365 nm) and they were sprayed with a 5:4 mixture of 1% diphenylboric acid-ethanolamine complex in methanol and 5% polyethyleneglycol 4000 in ethanol (for flavonoids) and anisaldehyde-sulfuric acid reagent (for terpenoids) (Wagner & Bladt, 1996). Methanolic solutions 0.1% of rutin, quercetin (Merck) and stigmasterol (Aldrich) were used as reference compounds.

RESULT AND DISCUSSION

Macro and micromorphological characters

LEAF

Morphological characteristics

The dried leaves of *E. brasiliensis* are glabrous and coriaceous. The rehydrated foliar lamina is 7-18 cm long and 2-7 cm wide, with an attenuated base. The apex is acute or obtuse. They are obovate-oblong, obovate or elliptic (Figure 1a). The leaf margins are entire, often curved towards the lower surface. The crude drug opposite margins can be completely overlapped, forming a tube. The upper surface is lustrous and, in general, dark green. It also can be entirely orange or shows orange spots. The lower side is light brown with a prominent midrib. The lateral veins (18-22 pairs) are convex on both surfaces, joined into a straight inframarginal vein at 3-5 mm from the margin. The lamina has translucent areas, which correspond to the secretory cavities scattered on the mesophyll. The petiole is 4 to 7 cm long, arched and slightly twisted. When rubbed, the odour is faintly aromatic, reminiscent of fruit scent. The taste is slightly astringent.

Anatomical characters

In transverse sections, the leaves show a dorsiventral mesophyll (Figure 1b) although an isobilateral structure may occur in *Eugenia* (Metcalfe & Chalk, 1950). A single layer of palisade parenchyma cells (Figure 1b - pp) is observed and comprises 1/4 to 1/8 of the mesophyll width. The spongy parenchyma (Figure 1b - sp) has round and periclinally elongated cells, the last ones occuring under the abaxial epidermis.

Secretory cavities of two different shapes (Figure 1b - sc) are scattered on the mesophyll bellow epidermis and in the median region. At the palisade parenchyma, the cavities are oval, and those at the spongy parenchyma are rounded. Metcalfe & Chalk (1950) previously described their subepidermal localization in Myrtaceae. The content of the secretory cells is dense and lipophyllic.

Numerous idioblasts with druses of calcium oxalate are scattered mainly on the spongy parenchyma. Epidermal cells (Figure 1b - e) on both surfaces are quadrangular or periclinally elongated. Cells on the abaxial epidermis are flattened with thickned outer walls and those on the adaxial one are entirely thick-walled. The cuticle is thick on both surfaces.

On surface view, the epidermal cells of the adaxial face are almost isodiametric, undulated and thick-walled (Figure 1c - ec). On the abaxial side, the epidermal cells are irregularly shaped and curved (Figure 1d - ec). Haron & Moore (1996) also observed two epidermal cell shapes in some *Eugenia* species and considered the pattern of the anticlinal walls a character of diagnostically importance in this genus. The cuticule is smooth on both surfaces.

The leaves are hypostomatic with anomocytic stomata (Figure 1d – gc; sb) as in the majority of the Myrtaceae (Metcalfe & Chalk, 1950) and in other *Eugenia* (Haron & Moore, 1996). However, anomostaurocytic stomata occur in some South African species (Van Wyk et al., 1982).

Trichomes are absent, although the occurence of non-glandular trichomes were reported in several *Eugenia* species (Metcalfe & Chalk, 1950; Landrum & Kawasaki, 1997), in general at a young stage (Haron & Moore, 1996) even remaining in some adult leaves.

The transverse section of the midrib (Figure 2d) is concave-convex. The epidermal cells are isodiametric, thick-walled and with convex outer walls on both faces. Secretory cavities are scattered under the epidermis as in other Myrtaceae (Metcalfe & Chalk, 1950). Cell walls of the ground parenchyma are thickened, not differentiated from those of the collenchyma cells. Large druses of calcium oxalate are found in that tissue.

The vascular system is arranged in a single arched bicollateral bundle with the ends directed towards the upper side. Xylem has series of single vessels alternating with the parenchymatic cells. Phloem contains numerous prismatic crystals of calcium oxalate. A sheat of sclerenchymatous fibres is related to phloem on both sides.

Phenolic compounds are detected all over the leaf, mainly in the palisade cells and at the phloematic region.

In transverse section, the median petiole is concave-convex (Figure 2a). Epidermal cells are quadrangular or elongated periclinally, with all thickned walls and the outer ones convex. The ground parenchyma cells are thick-walled, with a round shape or anticlinally elongated cells, in the peripheric region. In this region and in subepidermal positions, several anticlinally elongated secretory cavities are found (Figure 2b - sc). Druses of calcium oxalate are abundant in the ground parenchyma cells (Figure 2b - dr). Bellow the adaxial epidermis layers of cells with prismatic crystals of calcium oxalate are observed. Starch grains are numerous in cells surrounding the vascular system.

The vascular system is formed by a single strand in an incomplete cylinder with incurved ends. The midvein is bicollateral (Figure 2c – ph; x), a typical character in Myrtaceae (Metcalfe & Chalk, 1950). At the surrounding region it shows fibres (fig. 2d – f). Prismatic crystals of calcium oxalate are numerous in the phloem.

STEM BARK

Morphological characteristics

The bark is thin and 3-5 mm wide. The pieces are slightly curved with short fracture and inconspicuously fibrous at the inner part. The outer surface is weakly dipped-scaly, brown and shows transversal and longitudinal fissures (Figure 3a). When the cork is removed, the color of the exposed secondary phloem varies from pink to a redish shade. The inner surface is beige and contains longitudinal striae. The crude drug has no smell and its taste is weakly bitter and astringent.

Anatomical characters

The transverse section shows three different parts: the phellem, the pseudocortex and the phloem. The phellem is brown and the other parts are dark orange.

The stratified pattern of the phellem and the alternate arrangement of sclerosed and thin-walled cells are features commonly found in Myrtaceae (Metcalfe & Chalk, 1950; Van Wyk, 1985). In *E. brasiliensis* the phellem is also stratified, composed of one or two layers of sclerosed cells extended in tangential direction alternating with a layer of thin-walled cells. The inner walls, in contact with the phellogen remain thin and unsuberized. Some of these latter cells contain prismatic crystals of calcium oxalate. No conspicuous phelloderm was observed. The pseudocortex presents radially elongated and thin walled cells with starch and some druses of calcium oxalate.

The secondary phloem is composed of zones of tangentially elongated groups of phloem sclereids and fibres (Figure 3b e 3d – s)), enclosed in a sheath of parenchymatous cells containing prisms of calcium oxalate which alternate with sieve tubes and phloem parenchyma. The sclereids have thickened lignified walls, showing well-defined pit-canals. The lumen is clearly visible and sometimes contains starch. This arrangement, in regular tangential layers is characteristic and similar to the one described for *E. zulunesis* by Van Wyk (1985). Although, in this species, the author only reported fibres in those layers, in *E. brasiliensis* sclereids are observed, which is a character also described for other *Eugenia* (Chattaway, 1959).

The phloematic parenchyma has thin-walled cells. It contains starch (Figure 3c - st), prismatic crystals of calcium oxalate (Figure 3d - pc) and phenolic compounds. Deposits of them were abundant in the ray cells of *Eugenia* species from southern Africa (Van Wyk, 1985).

Parenchymatic rays are narrow (1-2 cells wide) in the inner phloem and enlarged in the outer region, with slightly radially elongated cells.

The macro and micromorphological analysis of *E. brasiliensis* showed that the majority of the characters are typical to the Myrtaceae (Metcalfe & Chalk, 1950; Landrum & Kawasaki, 1997). Also it confirmed the morphological resemblance with other *Eugenia* species (Haron & Moore, 1996). However, some of the following characteristics are of diagnostic value of the crude drug: **leaves** - glabrous, coriaceous, a part of them entirely orange or orange spotted, some of them have margins completely overlapped forming a tube, dorsiventral mesophyll, subepidermal secretory cavities, wavy-walled epidermal cells, anomocytic stomata, druses of calcium oxalate in

FIGURE 1 - Leaves of *Eugenia brasiliensis* Lam. (a) Macroscopical characters of leaves (b) Transverse section of the lamina. e= epidermis, pp= palisade parenchyma, sc= secretory cavity, sp= spongy parenchyma. (c) Adaxial surface of the leaf. ec=epidermal cell. (d) Abaxial surface with anomocytic stomata. ec=epidermal cell, sb=subsidiary cell, gc=guard cell.

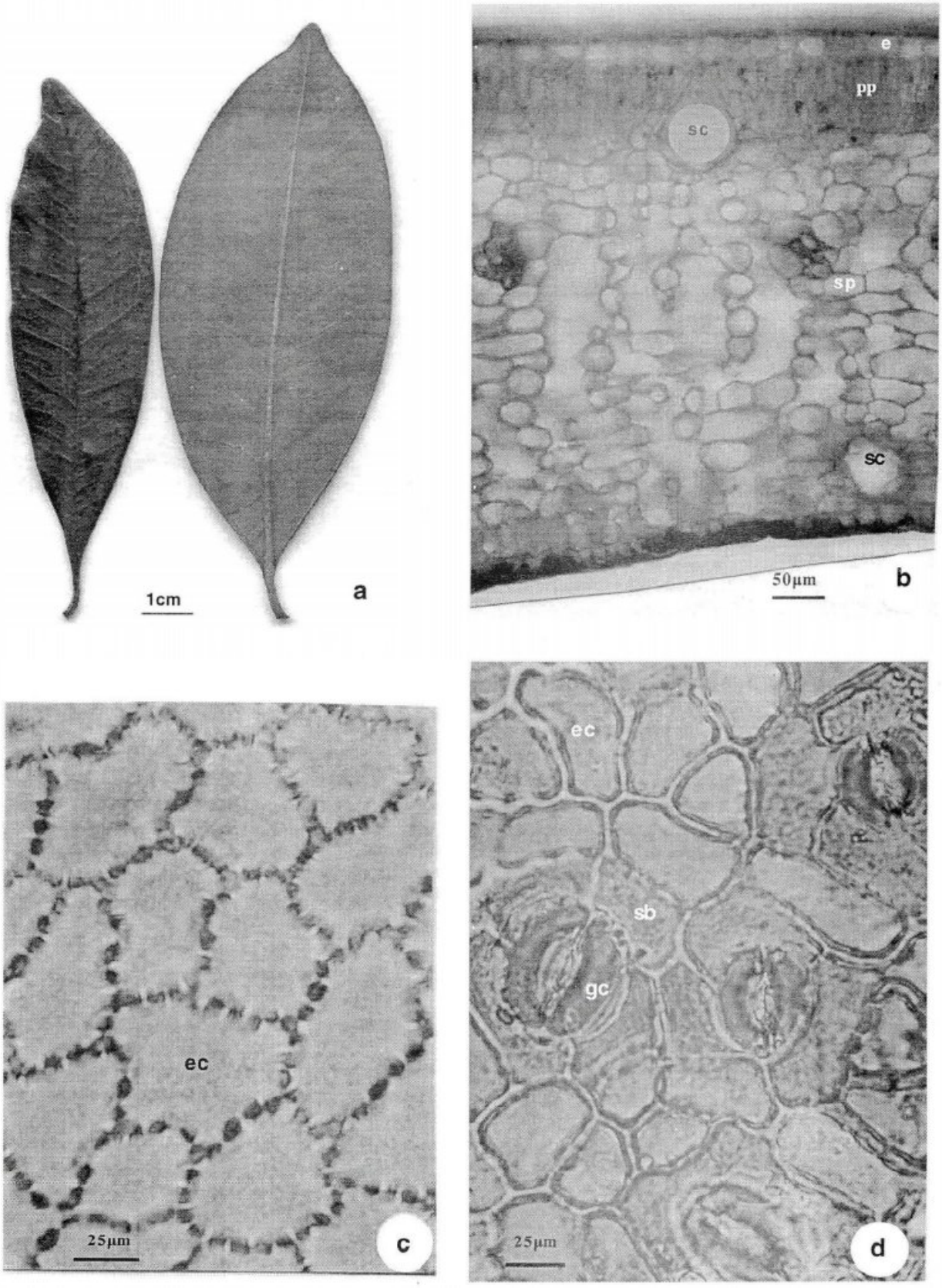

FIGURE 2 - Petiole of *Eugenia brasiliensis* Lam. (a) Vascular strand in arc with xylem ends curved inwards.sc= secretory cavity. (b) Adaxial region. sc=secretory cavity, dr= druse (c) Bicollateral vascular bundle. ph= phloem, x= xylem, f = fibers (d) Midrib with the arched bundle. sc= secretory cavity, ph= phloem, x= xylem, f = fibers

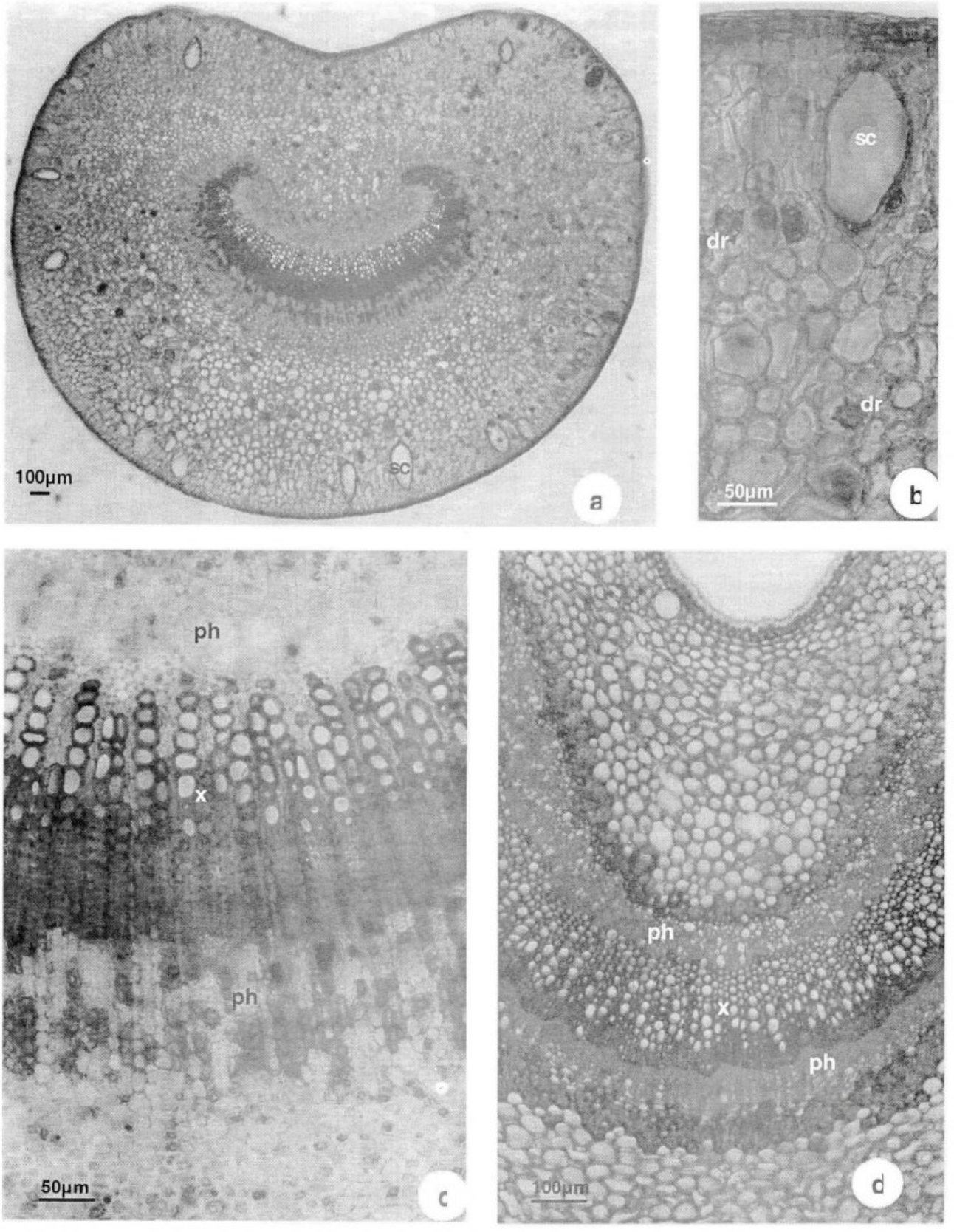

FIGURE 3 - Stem bark of *Eugenia brasiliensis* Lam. (a) Surface pattern. (b) Transverse section with detail of the tangentially elongated groups of sclerenchymatic cells. s= sclerenchyma. Secondary phloem: (c) Transverse section. st=starch grains, pc= prismatic crystal; (d) Radial section. s= sclerenchyma, pc= prismatic crystals.

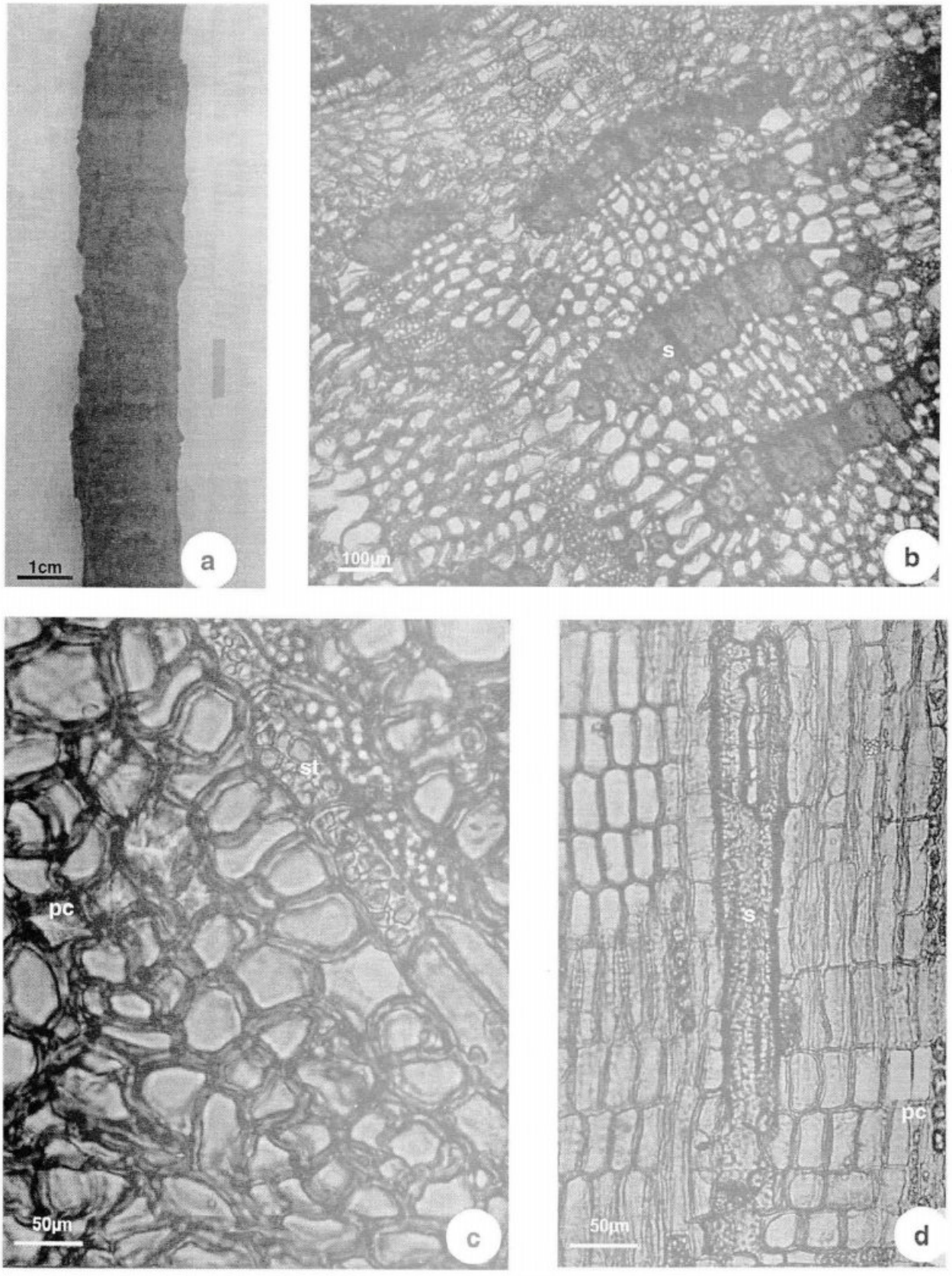

TABLE 1 - TLC of the hydroethanolic (30:70) extracts of the leaf and the stem bark of *Eugenia brasiliensis* Lam. in solvent-systems (a) and (b)

	Eugenia brasiliensis Lam.															
	System (a)								System (b)							
	NP/PEG				AS				NP/PEG				AS			
	Leaf		Stem bark		Leaf		Stem bark		Leaf		Stem bark		Leaf		Stem bark	
	R_f	colour	R_f	colour	R_f	colour	R_f	colour	R_f	colour	R_f	colour	R_f	colour	R_f	colour
	0.66	orange	0.75	orange	front	violet	front	violet	0.81	orange	-		0.44	purple	0.07	blue
	0.40	orange			0.62	violet			0.10	orange	-		0.36	purple		
	0.47	orange			0.34	purple							0.07	blue		
								Reference	compounds							
rutin	0.50	orange							0.04	orange						
quercetin	0.72	orange							0.91	orange						
stigmasterol					front	purple							0.54	purple		

(a) = chloroform-acetic acid-methanol-water (60:32:12:8)
(b) = chloroform
NP/PEG = 5:4 mixture of 1% diphenylboric acid-ethanolamine complex in methanol and 5% polyethyleneglycol 4000 in ethanol
AS = anisaldehyde-sulfuric acid reagent
(-) = no spot

the midrib ground tissue, numerous small prismatic crystals of calcium oxalate at the phloematic region, bicolaterall midvein arranged in arc. **Bark** – weakly dipped-scaly surface, transversal and longitudinal fissures, transverse section with three differentiated regions, the stratified phellem; the elongated and amiliferous cells of the pseudocortex, the tangential arrangement of sclerenchyma in secondary phloem, prismatic crystals mainly in the axial parenchymatic cells and phenolic compounds scattered all over the phloem.

Considering the narrow resemblance among *E. brasiliensis* and the other *Eugenia*, the chromatographic analysis had to be performed.

Chromatographic analysis

The ocurrence of terpenoid and phenolic compounds is common in Myrtaceae (Cronquist, 1981; Weyerstahl et al., 1988; Mendez et al., 1997; Lee et al. 1998) and was confirmed in *E. brasiliensis*.

The best TLC profile of the leaf hydroethanolic extract was achieved with the solvent system (a) chloroform-acetic acid-methanol-water (60:32:12:8), which better separated flavonoid components (Table 1). Rutin and quercetin were not detected in both extracts. The stem bark extract showed one separated component (R - 0.75) with this system, allowing to distinguish them from the leaves.

The standard tested flavonoids were also not detected in both of the plant parts using the chromatographyc solvent (b) chloroform, indicating the probable absence in *E. brasiliensis*, although they were identified in other *Eugenia* (Slowing et al., 1994; Schmeda-Hirshmann, 1988). Stem barks had no visualized spots with the NP/PEG reagent (Table 1).

The terpene components of *E. brasiliensis* were well separated with the solvent (b) chloroform, as seen in Table 1. Three major spots were observed in the leaves (R -0.07, 0.36 and 0.44), while only one was detected in the stem barks (R -0.07), which seems to be common to both the organs. None of the components corresponded to stigmasterol. The system (a) was too polar for separation of the *E. brasiliensis* terpenes.

In conclusion, it remains clear that for a correct identification of *E brasiliensis* the botanical characterization should be complemented by the chromatographic profile, that also allowed to differentiate the plant parts. Furthermore, both analysis are easy to perform in routine laboratories even in those supplied with few facilities.

ACKNOWLEDGMENT

The authors are grateful to FAPESP for the availability of the photomicroscope and to Roberto de Jesus Honório for his technical help.

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