

Leaf and stem microscopic identification of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae)

Márcia do Rocio Duarte*, Cláudia Bonissoni Empinotti

Laboratory of Pharmacognosy, Departamento f Pharmacy, Federal University of Paraná

Tithonia diversifolia (Hemsl.) A. Gray is an Asteraceae shrub, popularly known as Mexican sunflower and cultivated for ornamental and therapeutic uses in different countries. In folk medicine, it is of value for treating diabetes, malaria and infectious diseases. These indications have been corroborated by various pharmacological assays. Given the lack of data on anatomical aspects of *T. diversifolia*, this work aimed to investigate the leaf and stem microscopic characters of this medicinal plant and potential vegetal drug. Samples of mature leaves and young stems were sectioned and stained. Histochemical tests and scanning electron microscopy were also performed. The leaf has anomocytic stomata on both sides, dorsiventral mesophyll and several collateral vascular bundles arranged as a ring in the midrib. The stem shows angular-tangential collenchyma, an evident endodermis and sclerenchymatic caps adjoining the phloem. The main characters for anatomical identification are the different types of trichome (non-glandular, capitate and non-capitate glandular), the midrib features and the localization of the secretory ducts near the vascular system.

Uniterms: *Tithonia diversifolia*/pharmacognosy. *Tithonia diversifolia*/anatomical characters. Medicinal plants. Mexican sunflower.

Tithonia diversifolia (Hemsl.) A. Gray é um arbusto da família Asteraceae, popularmente conhecido como girassol-mexicano e cultivado como ornamental e medicinal em vários países. Tradicionalmente, é usado no tratamento de diabetes, malária e doenças infecciosas. Essas indicações têm sido corroboradas por diversos ensaios farmacológicos. Em razão das escassas informações sobre aspectos anatômicos de *T. diversifolia*, este trabalho objetivou investigar os caracteres microscópicos foliares e caulinares dessa planta medicinal e potencial droga vegetal. Amostras de folhas adultas e caules jovens foram fixadas, seccionadas e coradas. Testes histoquímicos e microscopia eletrônica de varredura foram também realizados. A folha apresenta estômatos anomocíticos em ambas as superfícies, mesofilo dorsiventral e vários feixes vasculares colaterais dispostos em círculo na nervura central. O caule exhibe colênquima angular-tangencial, endoderme nítida e calotas esclerenquimáticas apostas ao floema. Os principais caracteres para a identificação anatômica são os diferentes tipos de tricoma (tector, glandular capitado e não capitado), os aspectos da nervura central e a localização dos dutos secretores nas proximidades do sistema vascular.

Unitermos: *Tithonia diversifolia*/farmacognosia. *Tithonia diversifolia*/caracteres anatômicos. Plantas medicinais. Girassol-mexicano.

INTRODUCTION

Tithonia diversifolia (Hemsl.) A. Gray (Asteraceae, tribe Heliantheae) (Figures 1A, 1B) is a prolific shrub, perennial and erect, native to Mexico and Central

America, and introduced in Africa, Australia, Asia and South America (Duke, 1982). It is widely cultivated as an ornamental shrub and for its medicinal value in different regions where it is commonly known as Mexican sunflower or tree marigold, as well as “nitobegiku” (Hui, Tang, Go, 2009).

In folk medicine, the aerial parts of *T. diversifolia* are of value for the treatment of diabetes (Hui, Tang, Go, 2009), malaria (Njoroge, Busmann, 2006a) and

*Correspondence: M. R. Duarte. Departamento de Farmácia, Setor de Ciências da Saúde, Universidade Federal do Paraná. Av. Pref. Lothário Meissner, 632 - Jardim Botânico - 80210-170 - Curitiba - PR, Brazil. E-mail: marciard@ufpr.br



FIGURE 1 - A, B - *Tithonia diversifolia* (Hemsl.) A. Gray: A. Reproductive apical branches; B. Leaves. Bar = 2 cm.

infectious diseases (Heinrich *et al.*, 1998; Njoroge, Bussmann, 2006b; Maregesi *et al.*, 2007). The species is of particular interest for phytomedicine and health care research since it has shown diverse pharmacological activities, such as antiplasmodial (Goffin *et al.*, 2002; Madureira *et al.*, 2002; Elufioye, Agbedahunsi, 2004; Maregesi *et al.*, 2009; Muganga *et al.*, 2010), antiamebic (Tona *et al.*, 1998; 2000), antiviral (Cos *et al.*, 2002; Chiang *et al.*, 2004), anti-inflammatory (Rüngeler *et al.*, 1998; Owoyele *et al.*, 2004) and antidiabetic (Miura *et al.*, 2002; 2005). Therefore, it has potential to become a reference vegetal drug.

Concerning the phytochemical analysis, the non-volatile fractions of *T. diversifolia* are a rich source of flavonoids and sesquiterpene lactones (Pereira *et al.*, 1997; Gu *et al.*, 2002; Kuroda *et al.*, 2007; Ambrósio *et al.*, 2008), while the essential oil comprises predominantly monoterpene hydrocarbons, such as β -ocimene, α -pinene and limonene (Gbolade, Biondi, Ruberto, 2008).

Although many studies on *T. diversifolia* have been carried out in different research fields, there are few reports on its botanical investigation. The studies available focused on trichome aspects (Sasikala, Narayanan, 1998) and the secondary thickening pattern of the stem (Liasu, Ogunkunle, 2007). Thus, the aim of the present work was to provide data for microscopic identification of this medicinal species.

MATERIAL AND METHODS

Samples of *Tithonia diversifolia* (Hemsl.) A. Gray, Asteraceae were collected from at least five specimens grown in a sunny area in Palotina, a city in western Paraná, Brazil, at coordinates 24°17'S and 53°40'W, and an altitude of 290m in height, in December 2004. A voucher was

lodged as MBM 293886 at the Herbarium of the Museu Botânico Municipal de Curitiba, Paraná, Brazil.

The microscopic analysis (anatomy) was carried out using mature leaves and young stems, obtained 5-20 cm from the shoot apex, fixed in FAA70 (Johansen, 1940) and kept in 70% (v/v) ethanol (Berlyn, Miksche, 1976). The examined leaf region was the lower third of the blade and petiole. Semi-permanent slides were prepared with the fixed material sectioned transversely and longitudinally freehand and stained with astra blue and basic fuchsin (Roeser, 1972). Permanent slides were prepared with the fixed material embedded in glycol-methacrylate, sectioned using a rotary microtome, stained with toluidine blue (O'Brien, Feder, McCully, 1964) and mounted in synthetic resin (Kraus, Arduin, 1997).

Histochemical tests were performed using the following reagents: aqueous solution of ferric chloride for detecting phenolic compounds (Johansen, 1940), lugol for starch grains (Berlyn, Miksche, 1976), hydrochloric phloroglucin for lignin (Foster, 1949), Sudan III for lipophilic substances (Sass, 1951) and diluted solution of sulphuric acid for oxalate and carbonate calcium crystals (Oliveira, Akisue, 1997).

For scanning electron analysis, leaf fragments were fixed, dehydrated in a graded ethanol series and, using the CO₂ critical point procedure, gold coated and examined in a high vacuum (Souza, 1998).

RESULTS

In surface view, the leaf blade has epidermal cells with thin anticlinal walls, which are sinuous in the intervein region (Figures 2A, 2B) and polygonal on the veins. A slightly striated cuticle covers the epidermis (Figure 2C), and anomocytic stomata (Figure 2B) are present

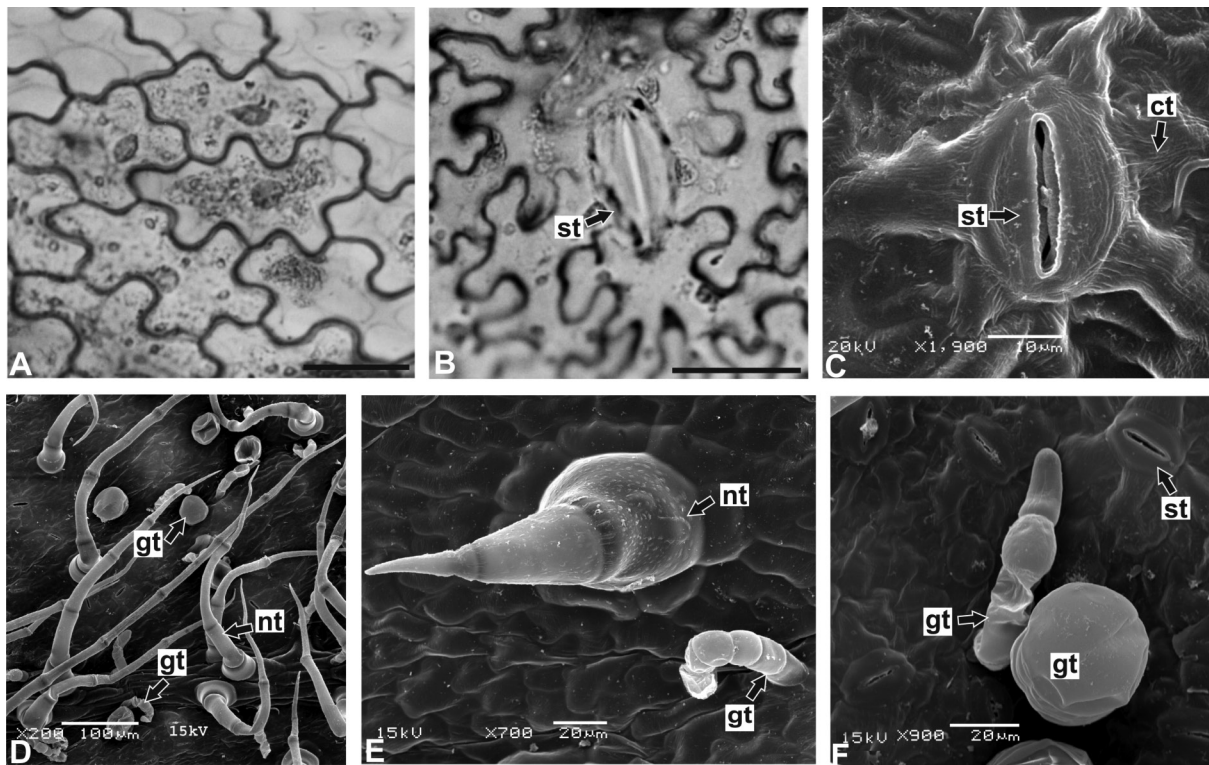


FIGURE 2 - A-F: *Tithonia diversifolia* (Hemsl.) A. Gray – leaf blade in surface view: **A.** Adaxial side; **B.** Abaxial surface, showing a stoma; **C.** Detail of a stoma and the slightly striated cuticle; **D-F.** Glandular and non-glandular trichomes. Abbreviations: **ct** – cuticle, **gt** – glandular trichome, **nt** – non-glandular trichome, **st** – stoma. Bar = 20 μm (A,B).

on both surfaces. There are glandular and non-glandular trichomes (Figures 2D-2F) predominantly on the abaxial side. The latter (Figures 2D, 2E) are multicellular, consisting of three to eight cells in a single rank, with acute apex, larger base and relatively thick cell walls, and coated with a granular cuticle. The glandular trichomes secrete lipophilic substances and are of two types. The capitate type (Figures 2D, 2F) have a short and multicellular stalk and ovate and biseriate head. The non-capitate trichomes (Figures 2E, 2F) are bent, multicellular (three to six cells), and uniseriate with round apex.

In transverse section, the epidermis is uniseriate, with comparatively larger cells on the adaxial side (Figures 3A, 3D), due to slightly higher anticlinal walls. Stomata are located on the same level as the surrounding cells and the guard-cells show evident outer cuticular ledges. The mesophyll is dorsiventral (Figures 3A, 3D), encompassing two or three layers of palisade parenchyma and four to six rows of spongy parenchyma, which represents around 50% of the chlorenchyma height. Minor collateral vascular bundles are found encircled by a parenchymatic sheath (endodermis with no evident Casparian strips). Some of these bundles can present parenchymatic bundle-sheath extensions (Figure 3D).

The midrib has a biconvex shape in transverse section, showing a prominent curvature on the abaxial side (Figure 3A). Angular collenchyma are found adjacent to the epidermis (Figure 3B), consisting of eight to ten strata on the adaxial side and two or three layers on the abaxial side. Several vascular bundles, collateral with fascicular cambium, ovate and arranged as a ring traverse the ground parenchyma. There are one major, around six medium-size and two small bundles (Figures 3A-3C). Various secretory ducts (Figures 3B, 3C) presenting uniseriate epithelium, often aligned by four cells, with dense cytoplasm, evident nucleus and secreting lipophilic substances, are found proximal to the vascular bundles.

The petiole has a plano-convex contour, with small lateral wings in the distal region. It exhibits uniseriate epidermis, a continuous angular collenchyma (seven to ten layers), and secretory ducts near vascular bundles (Figure 3E). These are collateral with fascicular cambium and arranged in an open arc in the proximal region and as a ring in the distal region.

The stem, in transverse section, has a circular shape and exhibits incipient secondary growth. The epidermis is uniseriate (Figures 4B, 4C) and may be detaching as the superficial development of the phellogen, which has

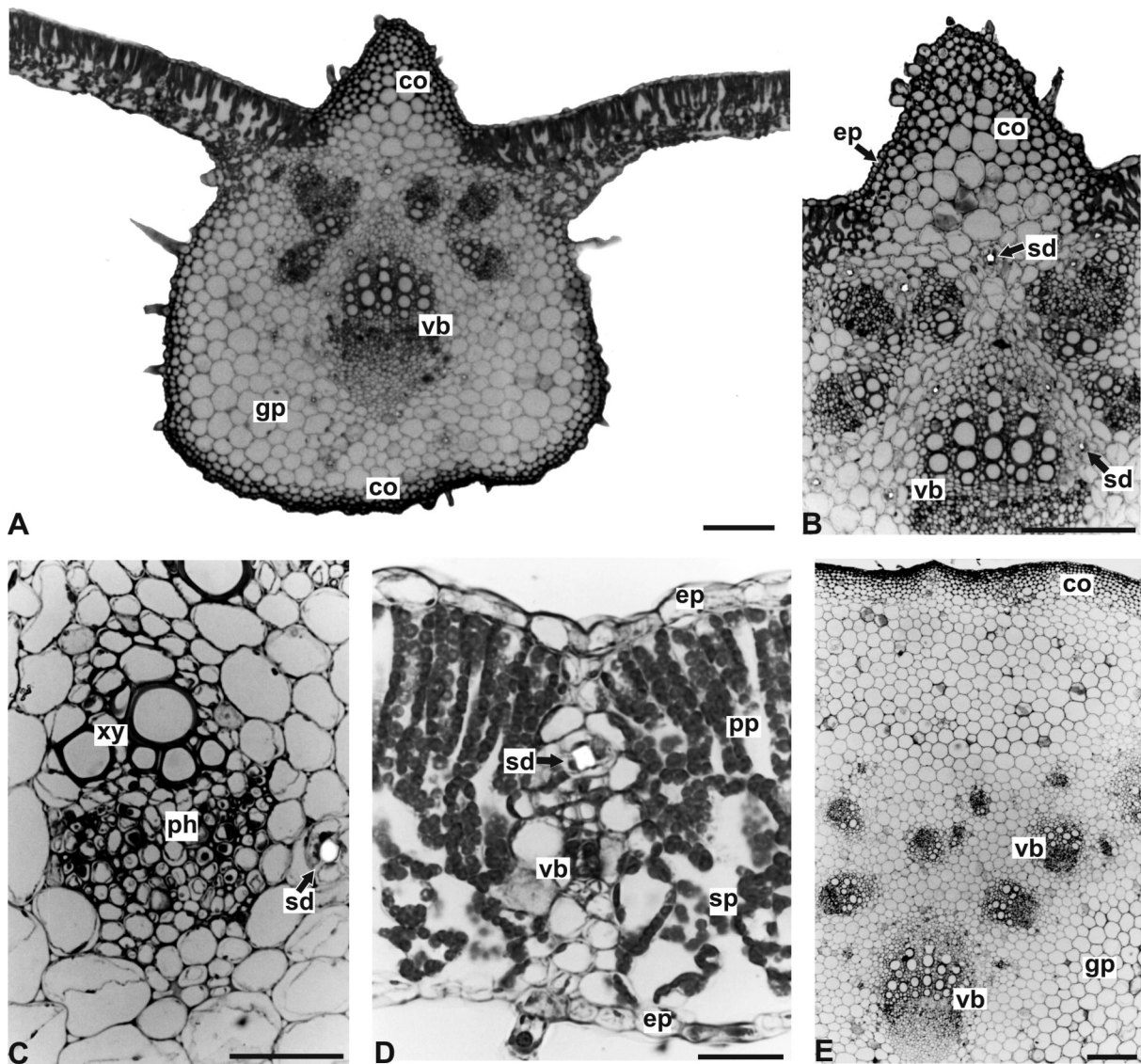


FIGURE 3 - A-E: *Tithonia diversifolia* (Hemsl.) A. Gray – leaf in transverse section: A. Intervein region and midrib with biconvex contour; B. Epidermis, collenchyma and vascular bundles in the midrib; C. Detail of a collateral vascular bundle and a secretory duct; D. Intervein region, displaying dorsiventral mesophyll and minor vascular bundle; E. Petiole. Abbreviations: **co** – collenchyma, **ep** – epidermis, **gp** – ground parenchyma, **ph** – phloem, **pp** – palisade parenchyma, **sd** – secretory duct, **sp** – spongy parenchyma, **vb** – vascular bundle, **xy** – xylem. Bar = 200 μ m (A,B,E), 20 μ m (C,D).

subepidermal origin (Figure 4C). It follows a continuous strand of angular-tangential collenchyma (about six strata), multiseriate cortical parenchyma and an evident endodermis (Figure 4B) presenting Casparian strips. In the vascular cylinder, collateral vascular bundles with a cambial zone can be distinguished (Figure 4B). Sclerenchymatic caps adjoin the phloem (Figures 4A, 4B) and secretory ducts (Figure 4C), similar to those of the leaf, are found adjacent to the phloem and in the pith, typically near the xylem. A few small druses of calcium oxalate are seen in the pith as well as the ground parenchyma of the midrib.

DISCUSSION

The microscopic characteristics of the leaf and stem of *T. diversifolia* are in concordance with the general aspects of Asteraceae. Therefore, amphistomatous leaf with predominantly anomocytic stomata, uniseriate epidermis whose cells are sinuous in surface view, dorsiventral mesophyll and collateral vascular bundles were also reported in *Vernonia condensata* Baker (Barreto, Alves, Neves, 1994), *Bidens pilosa* L. (Duarte, Estelita, 1999), *Mikania conferta* Gardner (Oliveira, Rodrigues, Kato, 1999) and *Taraxacum officinale* F.H. Wigg. (Alquini, Takemori, 2000).

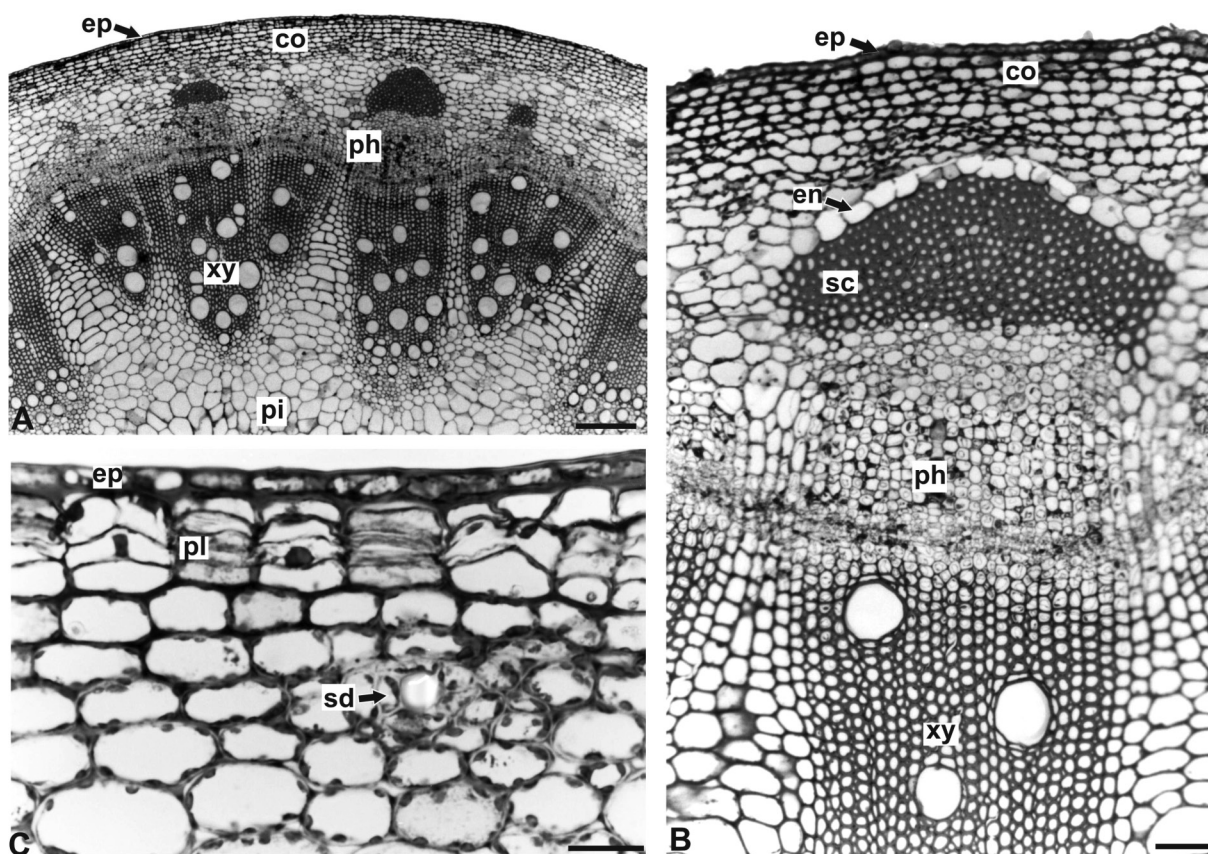


FIGURE 4 - A-C: *Tithonia diversifolia* (Hemsl.) A. Gray – stem in transverse section: **A.** Overall organization; **B.** Epidermis, cortex and vascular cylinder aspect; **C.** Detail of the superficial phellogen onset. Abbreviations: **co** - collenchyma, **en** - endodermis, **ep** - epidermis, **ph** - phloem, **pl** - phellogen, **sc** - sclerenchymatic cap, **sd** - secretory duct, **xy** - xylem. Bar = 200 μm (A), 50 μm (B), 20 μm (C).

The leaf cuticle ornamentation, a relevant character for diagnosis (Metcalf, Chalk, 1988), is described herein as slightly striated, akin to other species studied by different authors, for example, *Calea longifolia* Gardner (Farago *et al.*, 2006), *Mikania laevigata* Sch. Bip. ex Baker (Budel *et al.*, 2009) and *Baccharis singularis* (Vell.) G.M. Barroso (Souza *et al.*, 2011).

Regarding trichomes, these are of three types in *T. diversifolia*. The non-glandular trichomes are multicellular and coated with a cuticle which exhibits granular micro-ornamentation. These were also observed in *B. pilosa* (Duarte, Estelita, 1999), *Ageratum conyzoides* L. (Ferreira *et al.*, 2002) and different species of *Mikania* (Rodrigues, Oliveira, Kato, 1996; Oliveira, Rodrigues, Kato, 1999; Oliveira *et al.*, 2000). Among the glandular trichomes of *T. diversifolia*, there are both capitate and non-capitate types. The former are biseriate and were also reported in *Santolina leucantha* Bertol. (Pagni, Masini, 1999) and *Tanacetum parthenium* (L.) Sch. Bip. (Simmons, Krishna-Raj, Saxena, 2002). The non-capitate trichomes are uniseriate and bent, and were also mentioned in *Bidens*, *Calea*, *Eupatorium*, *Verbesina*

(Castro, Leitão-Filho, Monteiro, 1997) and *Mikania* (Rodrigues, Oliveira, Kato, 1996; Oliveira, Rodrigues, Kato, 1999; Oliveira *et al.*, 2000). Although these trichomes are not exclusive in *T. diversifolia*, the simultaneous presence of the three types contributes to its identification. The same trichomes were described for the same species by Sasikala and Narayanan (1998) and play an important role in ecological interactions. Ambrósio *et al.* (2008) stated that glandular trichomes of *T. diversifolia* store sesquiterpene lactones as main constituents and act as an antimicrobial defense and antifeedant agent for caterpillars.

In general, the midrib plays an important role in distinguishing species. *Tithonia diversifolia* showed a bi-convex midrib, in transverse section, and various vascular bundles in a circular arrangement. In comparison with other medicinal plants of the Heliantheae tribe, since scant information is available on the genus *Tithonia*, the midrib of *C. longifolia* has a concave-convex contour (Farago *et al.*, 2006). Although having a biconvex shape, *Calea uniflora* Less. has one single vascular bundle (Budel *et al.*, 2006).

Concerning the stem features, the results of this

work were similar to the description published for the same species by Liasu and Ogunkunle (2007). These authors reported the occurrence of persistent epidermis, angular collenchyma and collateral vascular bundles with a strip of cambial cells, however without remarking on the phellogen initiation or endodermis description. According to Van Fleet (1961) and Mauseth (1988), the typical endodermis with evident Casparian strips, as seen in this study, is an important diagnostic character, being less frequent in stem than in root. The sclerenchymatic caps adjoining the phloem, observed in *T. diversifolia* in this work, were also seen in the investigation of Liasu and Ogunkunle (2007) and may be related to the pericyclic fibre strands mentioned by Melo-de-Pinna and Menezes (2002) in Asteraceae stems.

The secretory ducts of Asteraceae, according to Castro, Leitão-Filho and Monteiro (1997), present a single-layered epithelium. Pagni and Masini (1999) suggested that they are schizogenous. For the leaf, in some representatives of the Heliantheae tribe such as *Aspilia*, *Bidens* and *Calea* (Castro, Leitão-Filho, Monteiro, 1997), ducts were found next to the phloem and xylem, in the same manner as *T. diversifolia*. In this investigation of the stem, these secretory structures were found near the vascular system, in the cortex and pith.

For identification purposes, the characteristics of *T. diversifolia* described herein should be taken together, with emphasis on the different types of trichomes, the midrib features and localization of the secretory ducts, since isolated characters can also be present in other species of the group.

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