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ANATOMY OF MONOCOTYLEDONS: STEMS AND RHIZOMES OF LAND HERBS USED AS MEDICINAL IN THE RIO DE LA PLATA AREA (ARGENTINA)

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Resumen: Novoa, M. C., M. N. Colares & A. M. Arambarri. 2012. Anatomía de Monocotiledóneas: tallos y rizomas de hierbas terrestres medicinales rioplatenses (Argentina). Bonplandia 21(2): 149-157.

Se estudió la anatomía de los tallos aéreos de *Cenchrus spinifex* Cav., *Commelina erecta* L., *Sisyrinchium chilense* Hook. subsp. *chilense* y *Tripogandra diuretica* (Mart.) Handlos, y los rizomas de *Arundo donax* L., *Cynodon dactylon* (L.) Pers. y *Kyllinga odorata* Vahl. Estos órganos son usados en la medicina popular de la región rioplatense. El objetivo fue obtener caracteres diagnósticos para elaborar una clave que permita la identificación de los taxones cuando se encuentren los órganos caulinares enteros o desmenuzados. Para ello, se aplicaron técnicas histológicas y métodos microquímicos convencionales. Las principales diferencias se encontraron en la forma de las células epidérmicas, posición y tipo de estomas, tipos de tricomas, distribución de las fibras, características de los haces vasculares y cristales. Todas las especies dieron reacción positiva para almidón, mientras que el resultado fue positivo para mucílagos solo en las especies de Commelinaceae. La mayoría de las especies dio resultado negativo para sustancias lipofílicas y fenólicas, sin embargo, el rizoma de *Kyllinga odorata* dio una reacción altamente positiva para aceites en forma de pequeñas gotas.

Palabras clave: Anatomía, Monocotiledóneas, plantas medicinales, rizoma, tallo aéreo.

Summary: Novoa, M. C., M. N. Colares & A. M. Arambarri. 2012. Anatomy of Monocotyledons: stems and rhizomes of land herbs used as medicinal in the Río de La Plata area (Argentina). Bonplandia 21(2): 149-157.

Aerial stems of *Cenchrus spinifex* Cav., *Commelina erecta* L., *Sisyrinchium chilense* Hook. subsp. *chilense*, and *Tripogandra diuretica* (Mart.) Handlos, and rhizomes of *Arundo donax* L., *Cynodon dactylon* (L.) Pers., and *Kyllinga odorata* Vahl were studied. These organs are used in folk medicine in the Río de La Plata area. The objective was to get diagnostic characters to elaborate a key to distinguish the taxa from whole or fragmented caulinar samples. Conventional histological and microchemical methods were used. The main differential traits were found in epidermal cell shapes, position and stomata type, trichome types, distribution of fibers, characteristics of vascular bundles, and crystals. Every species gave positive reaction for starch, and for mucilages the reaction was positive in Commelinaceae species. Most of the species gave negative reaction for lipophilic and phenolic substances, however *Kyllinga odorata* rhizome showed interesting content of secretory substances and gave positive reaction for oils droplets.

Key words: Anatomy, medicinal plants, Monocotyledons, rhizome, aerial stem.

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Introduction

The Río de La Plata region is situated in central-eastern of Argentina, it starts approximately in the city of Diamante in Entre Ríos province, reaching San Fernando in Buenos Aires province, and ending in the Río de La Plata. It comprises the delta of the Paraná (upper, middle and lower delta), Martín García Island, and the Río de La Plata shore (from NE Buenos Aires to Punta Lara, near La Plata city). The taxa studied in this paper are living in the lower delta, Martín García Island and Río de La Plata shore (Fig. 1). This area has a temperate climate, warm and humid. Annual rainfall ranges between 850 and 1000 mm, with the intermediate seasons (spring and fall) more rainy. The land is low and flooded sandy clay loam (Lahitte & al., 1997). The native and exotic species have value to the people living in this area, who use as ornamental, in the construction, and medicinal. The area has been widely studied from the systematic and floristic point of view (Hauman, 1925; Cabrera & Dawson, 1944; Cabrera, 1949; Burkart, 1957; Cabrera & Zardini, 1978; Hurrell & al., 2005; Bacigalupo & Hurrell, 2008; Roitman & Hurrell, 2009). Studies of popular uses of these plants have also been developed (Lahitte & Hurrell, 1996; Lahitte & al., 1997, 1998; Pochettino & al., 1997; Hernández & al., 2009, 2010, 2011; Hurrell & al., 2011). Several authors reported their medicinal properties (Hieronymus, 1882; Domínguez, 1928; Martínez Crovetto, 1964, 1981; Toursarkissian, 1980; Lahitte & Hurrell, 1996; Lahitte & al., 1998; Chifa & Ricciardi, 2001; Carrizo & al., 2002, 2005; Alonso & Desmarchelier, 2005; Barboza & al., 2006; Sawchuk Kobalchuk, 2006). Some morphological and anatomical characters of the studied taxa in this paper have been examined by other authors (Freire & al., 1997; Vizcaíno & al., 1998; Arambarri & Bayón, 1998; Monti & Mandrile, 1998; Cristóbal & al., 2008; González & al., 2009). Due to, there is not a study of comparative anatomy of the organs used as medicinal drugs, the deepening of the knowledge in this sense is of a great value in the identification process. This paper is one part of our project of a comparative anatomy study of vegetative organs of 75 species of land herbs (Spermatophytes) cited as used in folk medicine in the Martín García Island and Río de La Plata shore (Lahitte & Hurrell, 1995, 1996, 1997; Pochettino & al., 1997; Hernández & al., 2009, 2010). The aim of this paper is to describe aerial stems and rhizomes anatomy traits of Monocotyledons land (terrestrial) herbs, not aquatic or marsh, which are used in folk medicine in the region mentioned. The structural features of these stems could be used for diagnostic value to distinguish them in the herbal products.

Materials and Methods

Plant materials studied

Complete and fresh plants were collected in the area under study. One part of material was deposited in the LPAG herbarium, and another part was used to perform the study. For each species, specimen of herbaria LP and LPAG (abbreviations according to Thiers, 2011), were also surveyed. The botanical nomenclature follows Zuloaga & al. (2008), and the sites www.darwin.edu.ar, and ITIS (Integrated Taxonomic Information System), United States Department of Agriculture (USDA) www.itis.gov, were consulted on July 2012. Synonyms are not listed, except when they are still frequently used in papers.

Specimen vouchers

Commelinaceae: *Commelina erecta* L. "flor de Santa Lucía". **Buenos Aires**: Pdo. La Plata, La Plata, 6-XII-2011, *Arambarri 370* (LPAG); Pdo. La Plata, Jardín Botánico y Arboretum (JBA) "C. Spegazzini", Facultad de Ciencias Agrarias y Forestales (FCAyF), UNLP, 15-XII-2011, *Arambarri s.n.* (LPAG). *Tripogandra diuretica* (Mart.) Handlos, [*Tripogandra elongata* (G.F.W. Mey) Woodson]. "flor de Santa Lucía". **Buenos Aires**: Pdo. San Fernando, Delta del Río Paraná Miní, 18-V-1950, *Cabrera 10635* (LP); Pdo. Ensenada, Río Santiago, 18-IV-1964, *Cabrera 15914* (LP).

Cyperaceae: *Kyllinga odorata* Vahl [*Cyperus sesquiflorus* (Torr.) Mattf. & Kük. ex Kük.] "canelilla". **Buenos Aires**, Pdo. La Plata, Isla Martín García, 13-X-1997, *Hurrell & al., 3692* (LP).

Iridaceae: *Sisyrinchium chilense* Hook. subsp. *chilense* [*Sisyrinchium iridifolium* Kunth subsp. *valdivianum* (Phil.) Ravena]. "canchalagua". **Buenos Aires**: Pdo. La Plata, La Plata, JBA "C. Spegazzini", FCAyF, UNLP, 8-IX-1995, *Colares 46* (LPAG); La Plata, 11-X-2011, *Arambarri 357* (LPAG).

Poaceae: *Arundo donax* L. "caña de Castilla". **Buenos Aires**: Pdo. La Plata, La Plata, Facultad de Agronomía, UNLP, s. f., *Fabris 375, 376* (LPAG). *Cenchrus spinifex* Cav. (*Cenchrus pauciflorus* Benth.). "cadillo". **Buenos Aires**: Pdo. La Plata, La Plata, FCAyF, UNLP, 21-II-1995, *Bayón 119, 121* (LPAG). *Cynodon dactylon* (L.) Pers. "gramilla". **Buenos Aires**: Pdo. La Plata, La Plata, 15-XII-2000, *Bayón 577* (LPAG); La Plata, JBA "C. Spegazzini", FCAyF, UNLP, 28-XII-2011, *Arambarri 373* (LPAG).

The seven studied taxa alphabetically ordered by family, and ethnobotany use are shown in Appendix 1.

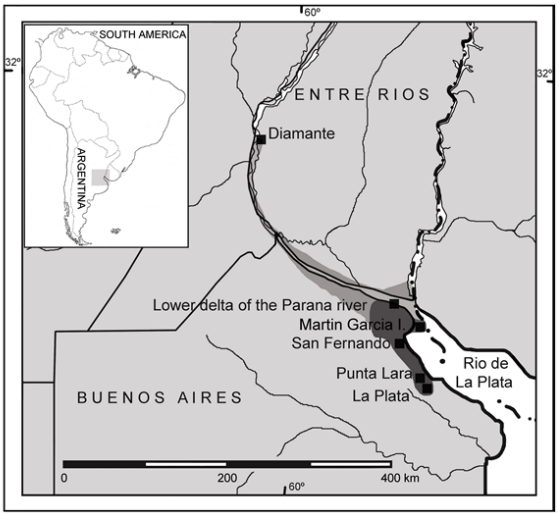

Fig. 1. Map of the Río de La Plata region (the shaded area), and the area under study: lower delta of the Paraná river, Martín García Island, and Río de La Plata shore (dark shaded area).

Anatomy, microchemical methods and microscopy

Portions of the middle part of aerial stems length were used. To avoid alterations on fresh stem and rhizome samples, they were immediately fixed in formalin-aceto-alcohol (FAA 70%) (Johansen, 1940). Portions of dried stems were reconstituted in water with a drop of detergent and placed in an oven at 30ºC for 24-72 h, then fixed in FAA. To analyze the stomata and trichomes, the peeling technique was used (D'Ambrogio de Argüeso, 1986). The fixed material was sectioned transversally by freehand. To distinguish different structures the sections were bleached in 50% sodium hypochlorite (NaOCl), washed three times with distilled water, and stained with 80% alcoholic solution of safranin, 1% aqueous solution of cresyl violet or 0.5% toluidine blue, then they were mounted on glycerin jelly (Johansen, 1940). Microchemical tests were performed for starch with iodine-potassium iodide (IKI) (Ruzin, 1999), for lipophilic substances with a saturated alcoholic solution of Sudan IV (Johansen, 1940), for phenolic compounds with ferric chloride (Domínguez, 1973), and for mucilage was used color reaction (D'Ambrogio de Argüeso, 1986), and on fresh material was also performed a phytochemical assay (alcohol precipitate test) using alcohol 100º (Korwar & al., 2010). Microscopic studies were performed by means of a Leitz SM lux light microscope. The images were captured with a color PAL CCD camera attached to a microscope, and they were digitalized by means of Hyper Media Center software. Terminology used in species descriptions is according to Metcalfe & Chalk (1979). On the basis of diagnostic characters a dichotomic key was elaborated and is offered.

Results

Commelinaceae: aerial vegetative stems of *Commelina erecta* and *Tripogandra diuretica* were studied. They have in common the epidermis in surface view formed by large polygonal cells, tetracytic stomata (Fig. 2A), glandular trichomes which are multicellular with cutinized basal cells and a thin-walled apical cell, oblong and obtuse at the tip (Fig. 2B). The epidermis in transection has one single layer of thick-walled quadrangular cells, and striated cuticle. Stomata are disposed at level with respect to the remaining epidermal cells. Cortex presents parenchyma and a fibers ring. The fibers ring has joined collateral vascular

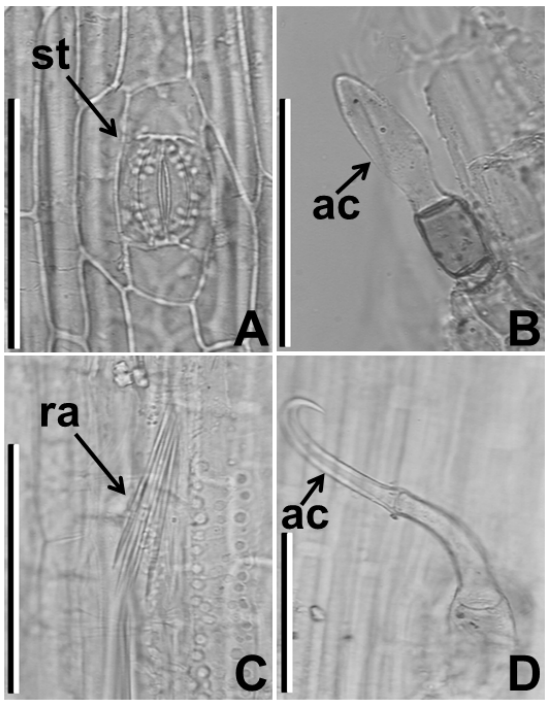

Fig. 2. Commelinaceae characteristics. A: Epidermis in surface view, showing (st) tetracytic stomata. B: Glandular trichome (ac: apical cell, tenuous, oblong and obtuse at the tip). C: Raphides (ra). D: Eglandular trichome (ac: apical cell curved at the tip). Scales: 100 µm.

bundles located toward the pith. These bundles together with the remaining vascular bundles randomly distributed in the pith form the Monocotyledons stele. The medullar vascular bundles are characterized by the formation of a large xylem lacuna, and frequently they are reduced to phloem and the lacuna. Inside cortical parenchyma are found solitary microcrystals, macles and raphides (Fig. 2C). Both species have secretory structures. The chemical test revealed the presence of starch in the cortical and medullar parenchyma, and the color test and phytochemical test were positive for mucilages.

Observation: *Commelina erecta* is characterized by having glandular and eglandular trichomes. The last, are simples, multicellular, with basal cell swollen, and the apical cell with curved tip (uncinate) (Fig. 2D).

Cyperaceae: the rhizome of *Kyllinga odorata* was studied. The epidermis in transection has one single layer of thin-walled and rectangular cells. Cortex presents attached to the epidermis 4-6(-8) very thick-walled fibers clustered. In the cortical parenchyma there are randomly distributed vascular bundles characterized by a prominent fiber cap on the xylem side, and the most inner cortical layer is an endodermis with thick-walled cells. Inside of the pith (mainly in the peripheral area) and randomly distributed, there are numerous concentric amphivasal vascular bundles with a fiber cap (Fig. 3A), and without it. The chemical test revealed the presence of starch in cortical and medullar parenchyma. The reagent Sudan IV revealed large amount of oils in spherical droplets in the parenchyma tissue (Fig. 3B).

Iridaceae: aerial floriferous stems of *Sisyrinchium chilense* subsp. *chilense* were studied. The epidermis in surface view comprises large polygonal cells and anomocytic stomata (Fig. 4A). The epidermis in transection has one single layer of thick-walled quadrangular to rectangular cells, elongated perpendicularly to the surface (Fig. 4B), sometimes papillose and with a wide and smooth cuticle. Stomata are disposed sunken respect to the remaining epidermal cells (Fig. 4B). Cortex presents 6-8 layers of parenchyma in the flat portion of the stem, and more than those into the wings. Inside of the cortical parenchyma there are collateral vascular bundles encircled by a parenchymatous sheath. The inner most layer of the cortical parenchyma forms a sheath surrounding a fibers ring 5-8 layered. The fibers ring has joined collateral vascular bundles located toward the pith. The pith is formed by parenchyma, with thin-walled cells enlarged to the center, and without vascular bundles. The chemical test revealed the presence of starch only in stomata guard cells.

Poaceae: rhizomes of *Arundo donax* and *Cynodon dactylon*, and aerial vegetative stem of *Cenchrus spinifex* were studied. They have in common epidermal characteristics; inside of the cortex there is a fiber ring, and in the pith randomly distributed collateral vascular bundles encircled by fibers. In *Arundo donax* rhizome, the epidermis is formed by thin-walled quadrangular cells. Cortex presents 5-8(-12) layers of thin-walled cells of parenchyma (Fig. 5A), where there are randomly distributed concentric amphivasal vascular bundles. The

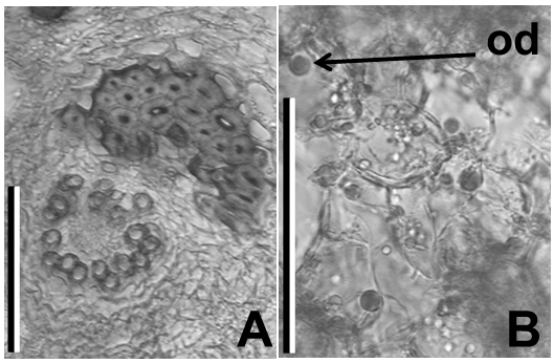

Fig. 3. *Kyllinga odorata*. A: Concentric vascular bundle with a conspicuous fiber cap. B: Parenchyma with abundant essential oil droplets (od). Scales: 100 µm.

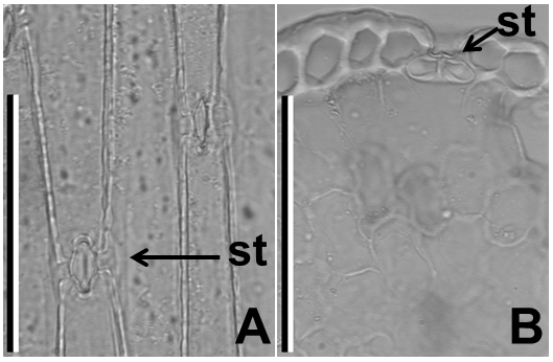

Fig. 4. *Sisyrinchium chilense* subsp. *chilense*. A: Epidermis in surface view showing (st) anomocytic stomata. B: Epidermis in transection showing (st) sunken stomata. Scales: 100 µm.

most inner layer of cortical parenchyma has abundant starch. It surround the thick fibers ring 8-10 layered. The chemical test revealed the presence of starch in cortical and medullar parenchyma. In *Cynodon dactylon* rhizome the epidermis is formed by thick-walled quadrangular cells. Cortex presents attached to the epidermis 1-2 layers of thick-walled cells, followed by 3-4(-12) layers of parenchyma (Fig. 5B), where there are randomly distributed a few concentric amphivasal vascular bundles. The most inner cortical layer presents thick-walled cells. It surround the fibers ring 6-8 layered. The fibers ring has joined collateral vascular bundles located toward the cortex and others located toward the pith (Fig. 5C). The chemical test revealed the presence of starch predominantly in the medullar parenchyma. Reaction with Sudan IV was negative for oils, however, exhibited the existence of suberin impregnating the thick-walled cells of epidermis, one-two peripheral cortical layers, and the inner most cortical layer. Phenolic substances were also present in cortical parenchyma. *Cenchrus* aerial stem transection presents epidermal and cortex characteristics similar to *Cynodon,* however some differences were found: the cortical parenchyma is distributed alternately with fibers forming columns perpendicular to the epidermis, which connect the epidermis with the inner fibers ring (Fig. 5 C, D), and in the cortex there are not concentric vascular bundles. The chemical test revealed the presence of starch predominantly in the cortical parenchyma.

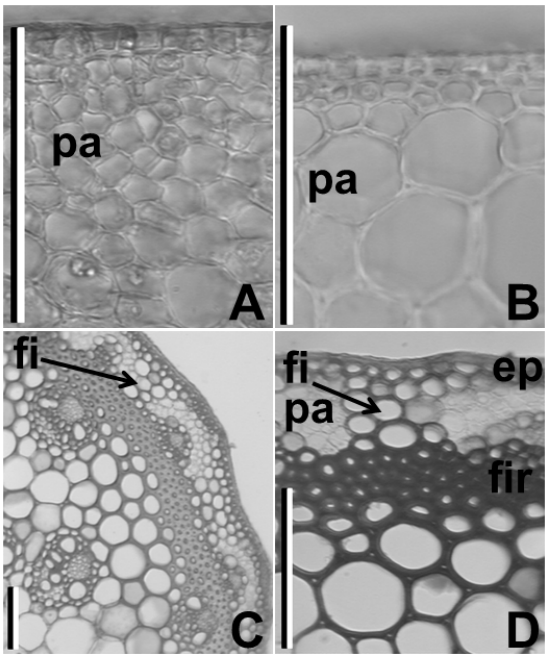

Fig. 5. Poaceae characteristics. A: *Arundo donax*, transection of epidermis and cortical parenchyma (pa). B: *Cynodon dactylon*, transection of epidermis and cortex, showing 1-2 layers attached to the epidermis with suberized thick-walled cells, and parenchyma (pa). C: *Cenchrus spinifex*, stem transection, showing the fibers (fi) that connect the epidermis with the fibers ring. It may be see the fibers ring which has joined collateral vascular bundles located toward the cortex and others located toward the pith, and in the pith randomly distributed collateral vascular bundles encircled by fibers. D: Enlarged view of the epidermis (ep), cortical parenchyma (pa), and the fibers (fi) connecting the epidermis with the fibers ring (fir). Scales: 100 µm.

A key to distinguish the studied medicinal stems and rhizomes, on the basis of their anatomy traits

1. Raphides present.
   2. Eglandular and glandular trichomes.

Commelina erecta

   2'. Only glandular trichomes.

Tripogandra diuretica

1'. Raphides absent.
   3. Essential oil droplets present. Vascular bundles with or without prominent fibers cap.

Killinga odorata

   3'. Essential oil droplets absent. Vascular bundles without prominent fibers cap.
      4. Epidermal cells elongated perpendicularly to the surface. Stomata sunken respect to the remaining epidermal cells. Pith without vascular bundles.

Sisyrinchium chilense subsp. *chilense*

      4'. Epidermal cells not elongated perpendicularly to the surface. Stomata at level respect to the remaining epidermal cells. Pith with vascular bundles.
         5. Cortex totality formed by parenchyma.

Arundo donax

         5'. Cortex formed by parenchyma and suberized cell walls and/or fibers.
            6. Cortex presents attached to the epidermis 1-2 layers of suberized thick-walled cells, followed by 3-4(-12) layers of parenchyma, and the most inner cortical layer presents suberized thick-walled cells surrounding the fibers ring.

Cynodon dactylon

            6'. Cortex presents parenchyma distributed alternately with fibers forming columns which connect the epidermis with the inner fibers ring.

Cenchrus spinifex

Discussion

The characters found in the studied species are unique to Monocotyledons, such as the elongated shape of epidermal cells, the distribution of stomata in the epidermis and the stele of the stem (Esau, 1982; Strasburger & al., 1994). The types of stomata were found tetracytic in the two studied species of Commelinaceae, which coincides with Cronquist (1988), who reported that this family have members with tetracytic type of stomata, although generally have paracytic. In *Sisyrinchium chilense* subsp. *chilense* belongs to Iridaceae family, we found anomocytic stomata, and according to the same author (Cronquist, 1988), the species contained in this family often have anomocytic type of stomata. Stem structural characters found in *Commelina erecta* (Commelinaceae) are in agreement with Vizcaíno et al. (1998) they showed the secretory structures in stems and leaves. The essential oils droplets found in *Kyllinga* coincide with Monti & Mandrile (1998) who indicated the presence of essences, resins, flavones, tannins, phenols and organic acids, however in our material we did not find phenolic substance (tannins). Noteworthy that Tucker & al. (2006) examined the chemistry of essential oils of *Kyllinga odorata* and they identified twenty three different constituents. Structural characters of *Kyllinga* are in agreement with Gonzalez et al. (2009). Species of Poaceae showed characters own family. Freire et al. (1997) and Arambarri & Bayón (1998) reported *Cynodon dactylon* stems features, and they do not mentioned vascular bundles in cortical

parenchyma. We agree with them for aerial stems characteristics, and we added that *C. dactylon* rhizome has a few concentric vascular bundles inside of cortical parenchyma.

Conclusions

Results showed that the species belonging to the same family have similar anatomical characters. It becomes difficult to distinguish among species, however it is possible on the basis of anatomy characters. The species *Cenchrus spinifex, Commelina erecta, Sisyrinchium chilense* subsp. *chilense*, and *Tripogandra diuretica*, whose aerial stems were studied can be differentiated by epidermal cells shape, stomata type, position of stomata respect to the remaining epidermal cells, trichomes, crystals, fiber and vascular bundles characteristics. The species of *Arundo donax, Cynodon dactylon*, and *Kyllinga odorata*, whose rhizomes are used as medicinal drug, can be distinguished by fibers distribution, vascular bundles characteristics, and presence of essential oils droplets. Results demonstrated that anatomical traits are an important tool in pharmacognostic control quality. On the basis of diagnostic characters a key for all species is offered.

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Appendix 1. List of studied species ordered by family with ethnobotany use.

Commelinaceae. *Commelina erecta*: the juice with mucilage accumulated in flower bracts is used as ophthalmic (Lahitte & Hurrell, 1996; Lahitte & al., 1997, 1998; Toursarkissian, 1980; Martínez Crovetto, 1981; Chifa & Ricciardi, 2001; Carrizo & al., 2002; Alonso & Desmarchelier, 2005). Besides, this species possesses properties to control hemorrhage and herpes (Toursarkissian, 1980). The juice of the plant in external use, calm itching (Lahitte & al., 1998; Hurrell & al., 2005). The plant is consumed in cooking against leucorrhoea and gonorrhea and the decoction of stems and leaves would increase fertility properties (Alonso & Desmarchelier, 2005; Bacigalupo & Hurrell, 2008). *Tripogandra diuretica*: is used in ophthalmology as *Commelina* (Martínez Crovetto, 1981; Lahitte & al., 1998). The decoction of the plant is drunk as a diuretic remedy, also consumed as sudorific, to treat rheumatism and digestive and in external application, to treat hemorrhoids (Lahitte & Hurrell, 1996; Lahitte & al., 1997, 1998; Hurrell & al., 2005). **Cyperaceae**. *Kyllinga odorata*: the rhizome is used as antidiarrheal, antispasmodic, diaphoretic, digestive, diuretic, tonic, and in treating leucorrhea (Toursarkissian, 1980; Lahitte & Hurrell, 1996; Lahitte & al., 1998; Hurrell & al., 2005; Barboza & al., 2006; Sawchuk Kobalchuk, 2006; González & al., 2009). **Iridaceae.** *Sisyrinchium chilense* subsp. *chilense*: the plant in folk medicine is used as a tonic (Toursarkissian, 1980), digestive, carminative, and to treat rheumatism and in external application to combat thrushes (Toursarkissian, 1980; Lahitte & al., 1998; Hurrell & al., 2005; Barboza & al., 2006; Roitman & Hurrell, 2009). **Poaceae**. *Arundo donax*: the decoction of the rhizomes is used as diuretic, depurative, sudorific, and to reduce the milk of nursing mothers (Lahitte & al., 1997, 1998; Barboza & al., 2006; Sawchuk Kobalchuk, 2006). *Cenchrus spinifex*: is used in infusion and decoction as hypotensive and cardiac tonic (Lahitte & Hurrell, 1996; Lahitte & al., 1998; Martínez Crovetto, 1981; Barboza & al., 2006). *Cynodon dactylon*: the rhizomes decoction is used as diuretic, refreshing, purifying, hepatic, laxative, and to treat rheumatism (Hieronymus, 1882; Toursarkissian, 1980; Martínez Crovetto, 1981; Lahitte & Hurrell, 1996; Lahitte & al., 1998; Carrizo & al., 2005; Hurrell & al., 2011). The rhizome of this species possesses properties to treat diseases hepatic, nephritis, diuretic, laxative (Barboza & al., 2006).