ELSEVIER

Fitoterapia 74 (2003) 207–225

FITOTERAPIA

www.elsevier.com/locate/fitote

Morpho-histological studies in the aromatic species of *Chenopodium* from Argentina

N.E. Bonzani*, G.E. Barboza, M.A. Bugatti, L. Ariza Espinar

Botánica, Departamento de Farmacia, Facultad de Ciencias Químicas,
Universidad Nacional de Córdoba e Instituto Multidisciplinario de Biología Vegetal
(IMBIV-CONICET), Casilla de Correo 495, Córdoba 5000, Argentina

Received 3 December 2001; accepted 27 November 2002

Abstract

A morpho-histological study of the vegetative organs (stem and leaf) of the aromatic species of *Chenopodium* L. from Argentina [*C. ambrosioides* L., *C. burkartii* (Aellen) Vorosch., *C. carinatum* R. Br., *C. chilense* Schrad., *C. graveolens* Willd. var. *bangii* (Murr) Aellen, *C. haumanii* Ulbr., *C. multifidum* L., *C. oblanceolatum* (Speg.) Giusti, *C. pumilio* R. Br., *C. retusum* (Moq.) Moq., and *C. venturii* (Aellen) Cabrera] was carried out. Classifications for the glandular and non-glandular trichomes are established and their presence among species is presented. A variant in both the dorsiventral and isobilateral mesophyll is reported; some data are valuable for systematic purposes and for the identification of dried and smashed material used as vegetal drug.

Keywords: *Chenopodium*; Aromatic species; Argentina; Morphoanatomic features

1. Introduction

In different opportunities, some dried stem and leaf fragments known as 'paico' were sent to us for identification by the authorities at the Hospital de Niños de Córdoba (Argentina). The reason for the request was the result of children suffering from severe intoxication and in one case, death. The patients have been taking concentrate infusions of 'paico' for its digestive properties. The 'paicos' belong to

---

*Corresponding author. Tel./fax: +54-351-4332104.
E-mail address: gbarboza@imbiv.unc.edu.ar (N.E. Bonzani).

0367-326X/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0367-326X(03)00024-8

the genus *Chenopodium* L. (*Chenopodiaceae*). This cosmopolitan genus comprises approximately 250 species, 33 of which occur in Argentina [1].

Some aromatic species of *Chenopodium,* known as 'paicos', are used in infusions mainly for their digestive disorders, and anthelmintic properties [2–5].

When we analyzed the samples sent by Hospital de Córdoba authorities and others bought at different places (pharmacies, herbal shops, markets, etc.) we could identify more than one species. *Chenopodium ambrosioides* is the traditional species used in folk medicine, and is easily recognized when the samples contain its fragments with reproductive organs. On the contrary, the sterile fragments of any *Chenopodium* species are difficult to identify.

Few published data regarding the vegetative anatomy of the genus are reported [6–13]. For this reason, we decided to investigate this aspect in all the aromatic species of *Chenopodium* from Argentina.

From a phytochemical point of view, the information is more abundant. In fact, secondary metabolites of several species of *Chenopodium* have been isolated, especially those of *C. ambrosioides* [14,15]. The 'chenopodium essence', with no less than 65% of ascaridole, is registered in some official publications such as the Codex Medicamentarius Argentino [16] as in many other Pharmacopoeias [4,17]. In addition, many authors [18–20] have studied the chemical composition of the essential oil of *C. ambrosioides* and *C. multifidum* due to their medicinal properties.

Vegetative anatomy of the 11 aromatic Argentinean species is studied in this paper. The aims are to contribute to the knowledge of the aromatic and medicinal plants of Argentina, with the purpose that will allow to make a proper quality control of samples whenever it is required, and to clarify the delimitation of some closely related species.

2. Experimental

2.1. Plant material

The materials, from Argentina, are deposited at the Herbarium of Museo Botánico de Córdoba (CORD), Museo de La Plata (LP) and Instituto Darwinion, San Isidro (SI). The species studied are: *C. ambrosioides* L. Prov. Córdoba Departamento Capital, Ariza Espinar 3176, 3177, 3178, 3179 and 3191 (CORD), Bonzani 25 (CORD); Departamento Colón, Hunziker 10906, 10935, (CORD), Ariza Espinar 3323, 3326, 3154 and 3156 (CORD), Bonzani 49 (CORD). *Chenopodium burkartii* (Aellen) Vorosch. Prov. Córdoba Departamento Capital, Hunziker 8393, 17455 and 15380 (CORD). *Chenopodium carinatum* R. Br. Prov. Córdoba Departamento Río Primero, Hunziker 11923 (CORD); Departamento Santa María, Hunziker 10605 (CORD); Prov. La Rioja. Departamento Chamical, Biurrum 20342 (CORD); Prov. Santiago del Estero. Departamento Choya, Hunziker 24330 (CORD). *Chenopodium chilense* Schrad. Prov. Córdoba. Departamento Cruz del Eje, Hunziker 8825 (CORD); Departamento Ischilín, Hunziker 9730 (CORD); Departamento Minas, Hunziker 9833 (CORD); Departamento Pocho, Hunziker 14004 (CORD). *Chenopodium graveolens* Willd. var. *bangii* (Murr) Aellen. Prov. Tucumán. Departamento

Tafí, Legname y Cuezzo 4794 (LIL). *Chenopodium haumanii* Ulbrich. Prov. Córdoba Departamento Colón, Burkart 20544 (CORD); Prov. Entre Ríos. Departamento Concordia, Burkart and Crespo 22960 (SI). *Chenopodium multifidum* L. Prov. Córdoba Departamento Capital, Hunziker 8114 (CORD); Departamento Río Seco, Hunziker 10333 (CORD); Departamento Colón, Ariza Espinar 3320 (CORD). *Chenopodium oblanceolatum* (Speg.) Giusti. Prov. Chubut. Departamento Cushamen, Soriano 2326 (LIL). *Chenopodium pumilio* R. Br. Prov. Córdoba Departamento Tercero Arriba, Hunziker 16517, 16519 and 16520 (CORD); Departamento Capital, Hunziker 17379 (CORD); Departamento Roque Saénz Peña, Hunziker 18661 (CORD). *Chenopodium retusum* Juss. ex Moquin. Prov. Entre Ríos. Departamento Gualeguaychú, Burkart 23321 (SI). *Chenopodium venturii* (Aellen) Cabrera. Prov. Santiago del Estero. Departamento Robles, Luna 1296 (LIL); Prov. Tucumán. Departamento Burruyacú, Cuesta 3391 (LIL).

2.2. Analysis

Herbarium specimens, fresh and preserved material in formalina–acetic acid–ethanol (5:5:90) were used. Two types of preparations of both leaves and stem were made.

a. Temporary: by making very thin cross-sections and peeling off the epidermis after clearing the leaf with sodium hypochlorite (30%). In both cases, several differential staining procedures (neutral red, Sudan III and IV, safranine, alcian blue and iodo–iodure–potassium) were used.
b. Permanent: by dehydrating the material in an ethanol–xylene gradients and then embedding it in paraffin; finally, some microtomic cuttings of 9–14 μm stained with triple combination were made [21].

Different sectors of at least five mature leaves per specimen were analyzed. As it is known that the lignification degree of the mechanical elements varies in relation to the developmental stage and growing conditions, young and mature stem sectors were selected from several plants of each species, and growing in different habitats. In this way, it was possible to draw some valid taxonomic conclusions only by comparing stems that were at an equivalent stage of development. The environment conditions demonstrated to have low influence in the lignification of the tissues.

The illustrations were drawn with a camera lucida. The photomicrographs were taken with an Axiophot optical microscope.

3. Results

3.1. General characters

The aromatic species of *Chenopodium* are annual or perennial herbs, sometimes ligneous at the base; from pilose to glabrous, with glandular and non-glandular trichomes. Stems aristate. Leaves from narrowly elliptical to elliptical, pinnatifid to pinnatisect. Sepals three–five free or partly or totally united; stamens five–three

(1), free or rarely with adnate filaments. Ovary spherical compressed; style short. Fruit completely or partially enclosed by the calyx.

3.2. Leaf anatomy

In cross-section, the leaf outline shows straight to slightly revolute margins in *C. ambrosioides, C. carinatum, C. graveolens* var. *bangii* and *C. pumilio* (Fig. 1b), revolute in *C. haumanii, C. multifidum, C. oblanceolatum* and *C. retusum* (Fig. 1c) and very revolute in *C. burkartii, C. chilense* and *C. venturii* (Fig. 1d).

3.2.1. Epidermis

Uniseriate, rectangular epidermal cells with slightly convex outer and inner tangential walls occur in both epidermis surfaces; abaxial epidermis have smaller cells, except on the nerves; smooth cuticle (Fig. 1a).

According to the epidermal cell walls, at surface view, two different epidermal models are recognized:

a. Sinuate model: with slightly to very sinuous cellular walls (Fig. 1g).
b. No-sinuate model: with straight or scarcely convex/concave walls (Fig. 1e,h).

In *C. ambrosioides, C. burkartii, C. carinatum, C. chilense, C. graveolens* var. *bangii, C. multifidum* and *C. pumilio*, the adaxial surface is organized as in the non-sinuate model and the hypophyllous surface as in the sinuate one. The remaining species (*C. haumanii, C. oblanceolatum, C. venturii* and *C. retusum*) have epiphyllous and hypophyllous surfaces according with the non-sinuate model.

3.2.2. Stomata apparatus

Three types of stomata are found in both leaf surfaces; the anomocytic stomata are the most frequent (Fig. 1g), the anisocytic (Fig. 1h) and tetracytic (Fig. 1e) ones appear in less proportion; guard cells are slightly raised on the epidermal surface.

3.2.3. Indumentum

In general, pubescence is more concentrated on primary veins of the hypophyllous surface and in the margin folds. Leaves and stems are glabrescent in *C. haumanii, C. graveolens* var. *bangii* and *C. oblanceolatum,* being pubescent in *C. carinatum, C. pumilio, C. multifidum* and *C. retusum.* In *C. ambrosioides, C. chilense, C. venturii, C. burkartii* and *C. haumanii,* the pubescence is variable, according to the age of the plant and the habitat ecology.

3.2.4. Morphology of the individual trichomes

In this section, leaf and stem trichomes are described.

3.2.4.1. Multicellular non-glandular trichomes. Some non-glandular trichomes have the proximal zone, above the basal cells (trichopodium, after Font Quer [22]), differentiated from the rest of the body by its size and cell shape or by the width

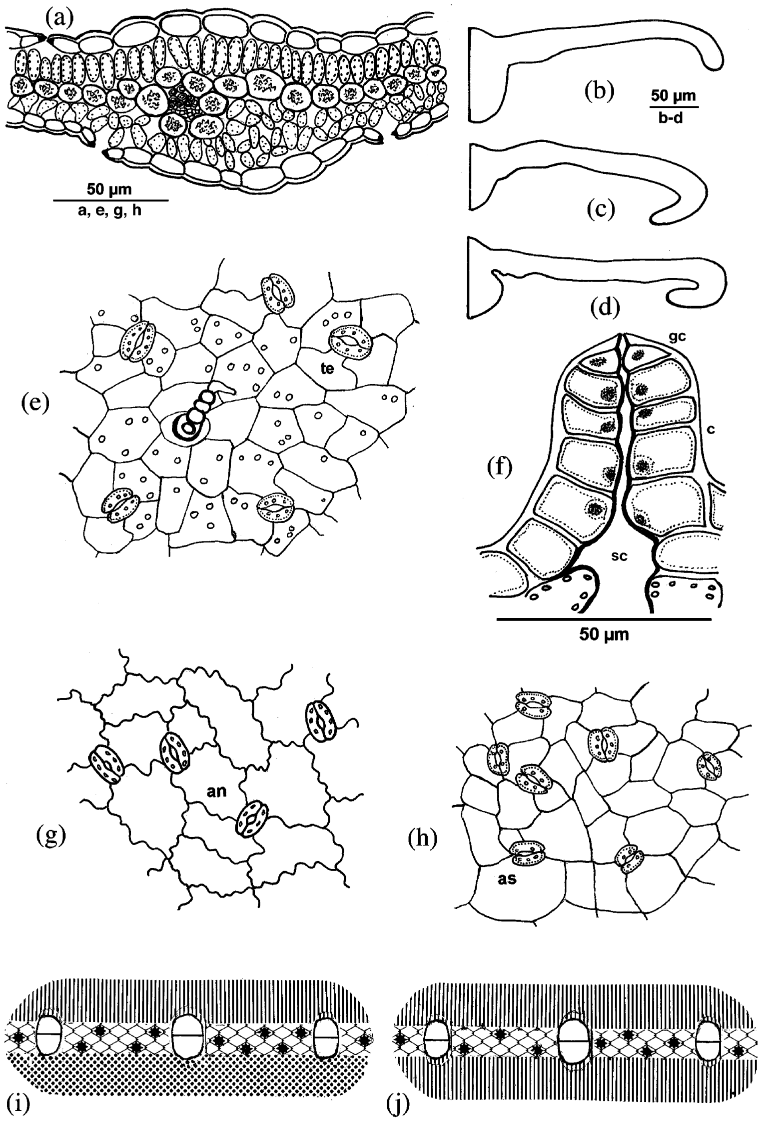

Fig. 1. Leaf structure of *Chenopodium* and stomatal complex of the stem. (a, b, f, g: *C. ambrosioides*; c, e: *C. multifidum*; d, h: *C. chilense*). (a) Detail of transverse section through leaf (notice the parenchyma with crystal sand in the mesophyll and surrounding a secondary nerve); (b–d) transverse sections through leaf showing different edges [(b) slightly revolute; (c) revolute; (d) very revolute]; (e, g, h) epidermal models in surface view [(e, h) no-sinuate model; (g) sinuate model]; (f) stoma raised on a column of the stem; (i, j) mesophyll structures [(i) dorsiventral structure, subtype 'ambrosioid'; (j) isobilateral structure, subtype 'retusoid']. *Abbreviations:* an: anomocytic; as: anisocytic; c: column; gc: guard cell; sc: substomatic chamber; te: tetracytic.

or composition of the cell wall or by the distribution of the cells (number of series or layers). This peculiar zone of the trichome is called 'epitrichopodium' in this paper. According to the absence or presence of the epitrichopodium as well as the general shape of the trichomes, we established the following classification:

Without epitrichopodium. Type I. Cylindrical-shaped trichomes (Fig. 2a,i). They consist of two–seven cells similar in width and variable in length. These trichomes are characteristic of *C. carinatum* and *C. graveolens* var. *bangii*.

Type II. Conical-shaped trichomes (Fig. 2e,g,l). The proximal cell of the body is broader than the three–four remaining cells; sometimes the proximal cell is globose. The terminal cell can be blunt or truncate (Fig. 2l).

This particular group can be conveniently subdivided into three subtypes:

Subtype 1. Conical appressed trichomes (Fig. 2e). Body with four–five cells; proximal cells with one of their radial wall longer; basal cells at the same level than the other adjacent epidermal cells. Observed in *C. carinatum, C. graveolens* var. *bangii, C. pumilio* and *C. retusum.*

Subtype 2. Conical erect trichomes (Fig. 2l). Body with two–four cells; basal cells surrounded by many-raised epidermal cells. Found in *C. graveolens* var. *bangii, C. carinatum* and *C. pumilio.*

Subtype 3. Conical incurvate trichomes (Fig. 2g). Body with five–six cells, sometimes very tortuous. The proximal cells are curved inwards from the leaf surface; the large basal cell is more elevated than the adjacent epidermal cells. This subtype is exclusive of *C. carinatum.*

With epitrichopodium. Type III. Flagelliform trichomes (Fig. 2d). With a short uniseriate epitrichopodium consisting of three–four small discoidal-depressed thick-walled cells. The distal portion of the trichome seems like an undulate band with one–two thin-walled cells that collapse at maturity; the basal cell is at the same level as the epidermis and is similar in shape and size to the remaining epidermal cells. Observed in *C. ambrosioides, C. burkartii, C. chilense, C. haumanii, C. multifidum* and *C. venturii.*

Type IV. Whip-shaped trichomes (Fig. 2c,h,k and m). With a long multiseriate or uniseriate epitrichopodium. The distal portion is band-shaped, one–three celled, attached to the epitrichopodium through the tangential cell wall.

This type is subdivided into the thin or basically uniseriate epitrichopodium and the more or less thickened or multiseriate ones, as follow:

Subtype 1 (Fig. 2c). Epitrichopodium wholly uniseriate of three–eight thin-walled cells, except the distal one with thick walls; band-shaped portion one–two-celled perpendicular to the epitrichopodium. They appear in *C. ambrosioides, C. chilense, C. multifidum, C. burkartii, C. haumanii* and *C. venturii*.

Subtype 2 (Fig. 2m). Epitrichopodium biseriate at the base and uniseriate at the apex, the four distal cells being smaller and with thicker walls; band-shaped portion one–two celled, oblique to the epitrichopodium. Seen only in *C. venturii* and *C. ambrosioides* stems.

Subtype 3. Epitrichopodium mostly multiseriate. It can be: (a) dome-shaped, then the band-shaped portion is one–three celled and completely curved (Fig. 2h); (b)

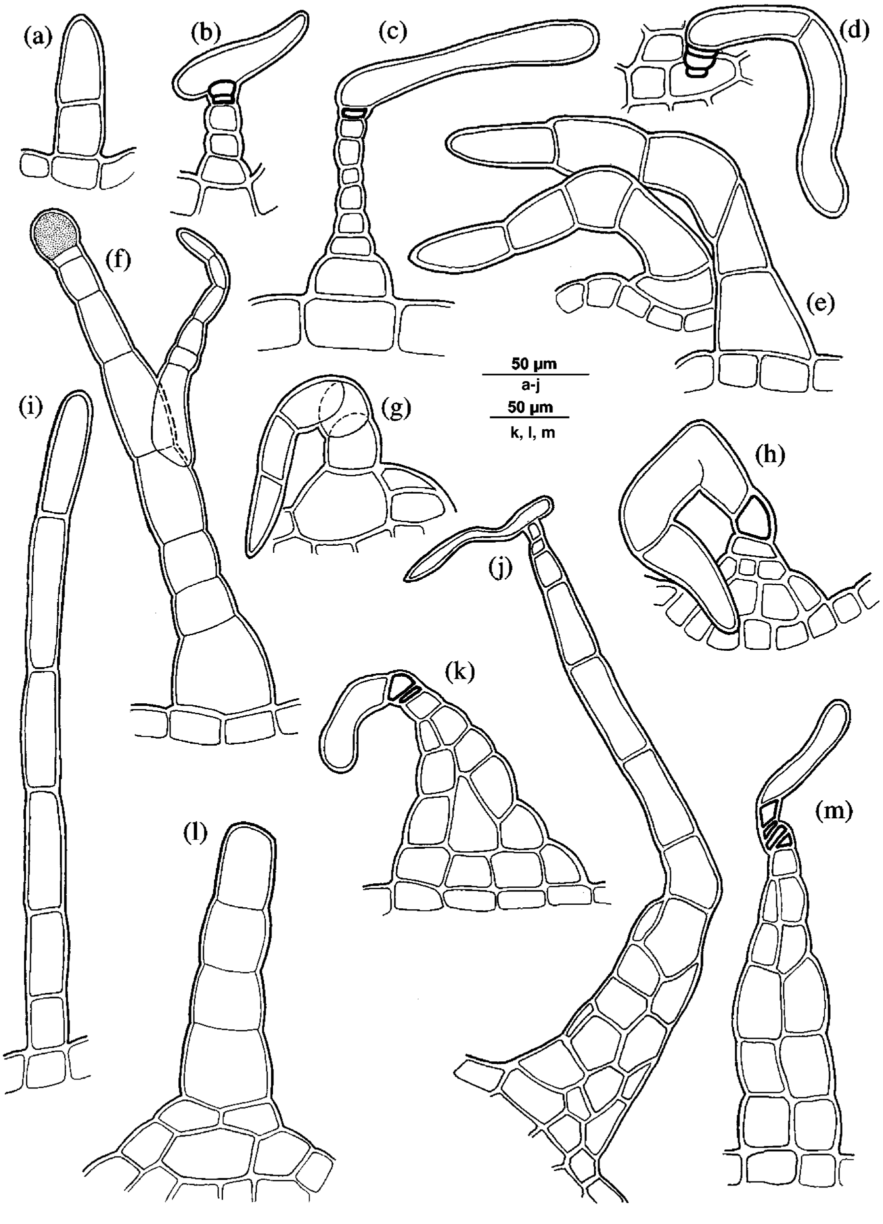

Fig. 2. Non-glandular trichomes of *Chenopodium.* (a, i) Cylindrical-shaped trichomes; (b) hammer-shaped, subtype 2; (c) whip-shaped, subtype 1; (d) flagelliform trichome; (e) conical appressed trichomes; (f) mixed trichome; (g) conical incurvate trichomes; (h) whip-shaped, subtype 3; (j): hammer-shaped, subtype 1; (k) whip-shaped, subtype 3; (l) conical erect trichome; (m) whip-shaped trichome, subtype 2.

pyramidal-shaped, then the band-shaped portion is short, one–two celled and slightly curved or parallel to the surface organ (Fig. 2k). In both cases, one or two distal cells of the epitrichopodium have thick walls. Observed in *C. venturii*.

Type V. Hammer-shaped trichomes (Fig. 2b,j). Multicellular epitrichopodium; unicellular band-shaped distal portion attached to the epitrichopodium through the basal third of one of the radial cell walls, and perpendicular to the rest of the body.

Subtype 1 (Fig. 2j). Three zones are distinguished in the trichome: the basal cells, the multicellular epitrichopodium multiseriate at its base and uniseriate at the apex and finally, the band-shaped portion, four–five times shorter than the epitrichopodium. Observed only in *C. ambrosioides.*

Subtype 2 (Fig. 2b). The epitrichopodium is uniseriate and subpyramidal in shape while the band-shaped portion is perpendicular to the epitrichopodium and of similar size. This subtype is present in *C. ambrosioides, C. chilense, C. multifidum* and *C. venturii.*

3.2.4.2. Glandular trichomes. The head can be well differentiated (type I and type II), or slightly differentiated (type III) from the rest of the cells.

Type I. Unicellular head. The cell has a green–yellowish secretor content and large vacuoles; the stalk is multicellular, cylindrical or pyramidal, with thick cellulosic cell walls.

According to the stalk and the head morphology, the following subtypes can be described:

Subtype 1 (Fig. 3f). The head is slightly asymmetrical, with a large vacuole; stalk short, two–five celled, with its distal cell slightly bowed. They are common in *C. ambrosioides, C. burkartii, C. chilense, C. haumanii, C. multifidum, C. retusum*, *C. venturii* and *C. oblanceolatum.*

Subtype 2 (Fig. 3a). The head is globose, symmetrical and erect; stalk short, cylindrical, one–three-celled. Found in *C. ambrosioides, C. burkartii, C. chilense, C. graveolens* var. *bangii, C. multifidum* and *C. oblanceolatum*.

Subtype 3 (Fig. 3c). The head is pear-shaped, appressed; stalk short, obpyramidal, with two–five thick-walled cells. Observed in *C. ambrosioides, C. burkartii, C. chilense, C. haumanii, C. multifidum, C. pumilio, C. retusum, C. venturii* and *C. oblanceolatum.*

Subtype 4 (Fig. 3g). The head is club-shaped, erect; stalk short four-celled. It is exclusive of *C. pumilio* and *C. carinatum*.

Subtype 5 (Fig. 3i). The head is small and transverse-elliptic; stalk short, three-celled, the distal cell with thick walls. Observed only in *C. carinatum* and *C. retusum.*

Subtype 6 (Fig. 3d). The head is subellipsoidal and parallel to the organ surface; stalk long, cylindrical, five–nine-celled and curved at the apex. Found in *C. ambrosioides, C. burkartii, C. retusum* and *C. carinatum*.

Subtype 7 (Fig. 3b). The head is ellipsoidal; stalk long, cylindrical, five–six-celled and scarcely curved. Observed in *C. pumilio.*

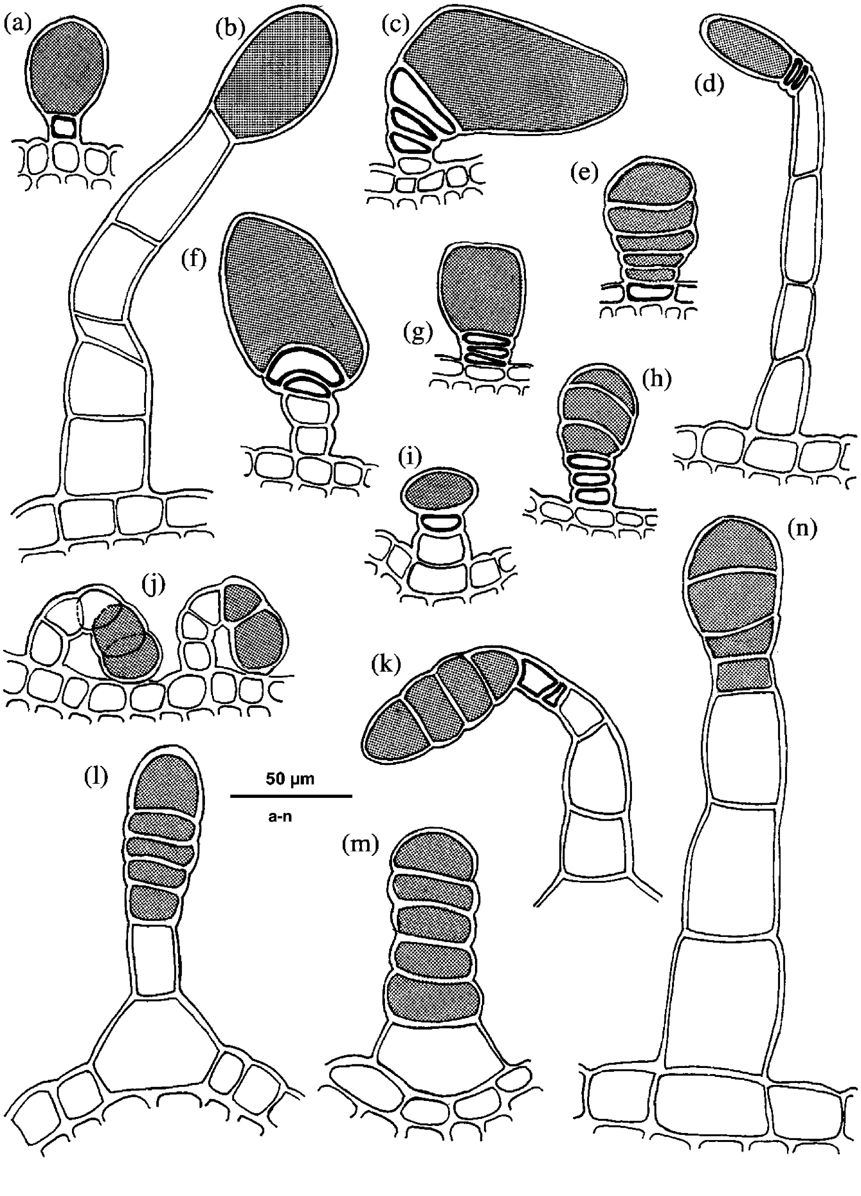

Fig. 3. Glandular trichomes of *Chenopodium.* (a) Symmetrical head (type I, subtype 2); (b) ellipsoidal head (type I, subtype 7); (c) pear-shaped head (type I, subtype 3); (d) subellipsoidal head (type I, subtype 6); (e, h) club-shaped head (type II, subtype 9); (f) asymmetrical head (type I, subtype 1); (g) club-shaped head (type I, subtype 4); (i) transverse-elliptic head (type I, subtype 5); (j) vermiform trichomes (Type III); (k–m) multicellular cylindrical head (type II, subtype 8); (n) ovoidal and stipitate head (type II, subtype 10).

Type II. Multicellular head. Cells originated by anticlinal divisions; stalk long or short.

Subtype 8 (Fig. 3l,m and k). The head is cylindrical, four–five-celled; stalk uni/bicellular, with the basal cell swiftly elevated from the epidermal surface (Fig. 3l,m), or stalk multicellular and curved (Fig. 3k); in this latter case, the two distal cells smaller with thicker cell walls. Observed in *C. carinatum, C. pumilio, C. retusum* and *C. oblanceolatum*
Subtype 9 (Fig. 3e,h). The head is club-shaped, three–five-celled; stalk unicellular (Fig. 3e) or two–four-celled (Fig. 3h). Observed in *C. pumilio, C. carinatum, C. ambrosioides* and *C. oblanceolatum*.
Subtype 10. The head is ovoid and stipitate; stalk more or less conical, with three–four long cells (Fig. 3n). Exclusive of *C. pumilio.*

Type III. Vermiform trichomes. Body with five spherical cells, completely bent one towards the epidermis. The secretory distal cells are spherical, and have no vacuoles (Fig. 3j). They appear in the majority of the species with the exception of *C. carinatum, C. graveolens* var. *bangii, C. haumanii* and *C. pumilio*.

3.2.4.3. Mixed bifurcate trichomes. Trichomes with body Y-shaped, equal-armed; both arms uniseriate and multicellular; one arm ends in a glandular cell and the other one in a non-glandular and cylindrical cell. They are present in *C. carinatum* and *C. pumilio* (Fig. 2f).

3.3. Mesophyll

In the majority of the species studied, the mesophyll is dorsiventral with the exception of *C. retusum* and *C. oblanceolatum* where it is isobilateral. In the species with dorsiventral structure, the palisade and spongy chlorenchyma are one–two-layered, the spongy mesophyll being more or less compact; in *C. carinatum*, the palisade cells have a very sinuate contour. In the species with isobilateral structure, the adaxial palisade tissue is denser than the abaxial one, which is discontinuous. A central well defined layer of large parenchymatic cells, without chloroplasts but with a lot of crystal sand, stands out in the middle of the mesophyll in both dorsiventral and isobilateral leaves (Fig. 1). The main vascular strand is surrounded by abundant parenchyma with crystal sand, and collenchyma protruding toward the abaxial and adaxial surfaces. The vascular bundles are collateral and arranged crosswise (in semicircle in the petiole); the presence and arrangement of the fibers surrounding the bundles are variable, they can be in-groups above the xylem or surrounding completely the central fascicle. The secondary bundles have a non-chlorenchymatous sheath with crystal sand (Fig. 1a).

3.4. Stem anatomy

All taxa investigated are very similar in their primary and secondary growth.

3.4.1. Primary growth

The stem has an angular contour with ridges and slightly depressed areas between ridges; most of the glandular trichomes are placed on the depressed areas.

The epidermis is uniseriate; the epidermal cells are isodiametric with convex tangential walls covered by a smooth and thick cuticle. Projecting stomata are common on stems. These stomata are raised on a column; the uniseriate column develops as epidermal evaginations bearing the stoma over the organ surface; the sub-stomatal chamber is partly included in the elevation and the remaining portion in the subepidermal tissue (Fig. 1f).

The cortex (Fig. 4b) consists of eight–nine layers of angular and laminar collenchyma in the ridges, and of two–three layers of chlorenchyma between the ridges. Below these tissues, two–three parenchyma layers, with abundant crystal sand, are developed. The vascular cylinder is enclosed by a sheath containing starch and with a conspicuous meristematic area inside. From this meristem a discontinuous procambium differentiates producing isolated open primary bundles; between the bundles interfascicular parenchyma arises (Fig. 4a). The primary bundles arrange in two or more concentric circles (Fig. 4b). The pith is abundant, its cells containing crystal sand.

3.4.2. Secondary growth

The epidermis is not replaced by a peridermis (a phellogen was never observed); in the ridges the collenchyma lignifies (*C. burkartii* and *C. retusum*) or remains cellulosic (the other species). An interrupted (*C. ambrosioides, C. multifidum, C. retusum* and *C. carinatum*) or a complete ring (*C. burkartii, C. pumilio, C. graveolens* var. *bangii* and *C. chilense*) of pericyclic fibers surrounds the meristematic area (Fig. 4c), but these fibers are absent in *C. haumanii* and *C. oblanceolatum*. Cambium produces an anomalous secondary thickening. The first cambium ring starts in peripheral cells of the central cylinder outside the vascular bundles giving rise to groups of phloem which lies embedded within the lignified tissue (Fig. 4c). The cambia of the later growth rings arise at the periphery of the last phloem; in this way, irregular bands of secondary thickening are produced consisting mainly of a fibrous conjunctive tissue (differently developed according to the species), xylem vessels, groups of phloem and finally, in the innermost area nearby the pith, groups of primary bundles (the latter partly or wholly or not included in the conjunctive tissue). Therefore, cortex and pith are reduced because of the great development of the vascular tissue (Fig. 4d).

4. Discussion

Chenopodiaceae trichomes have been studied by many authors [6,8–10,12,23]; however, descriptions known for *Chenopodium* trichomes are not complete enough since some of their numerous variants have not yet been registered. Thus, Solereder [23] and Metcalfe and Chalk [6] drew just some non-glandular (type IV, subtype 1 and type V, subtype 2) and glandular (type I, subtypes 1 and 3) trichomes. Carolin [8] described uniseriate trichomes in *C. ambrosioides* and *C. multifidum* similar to

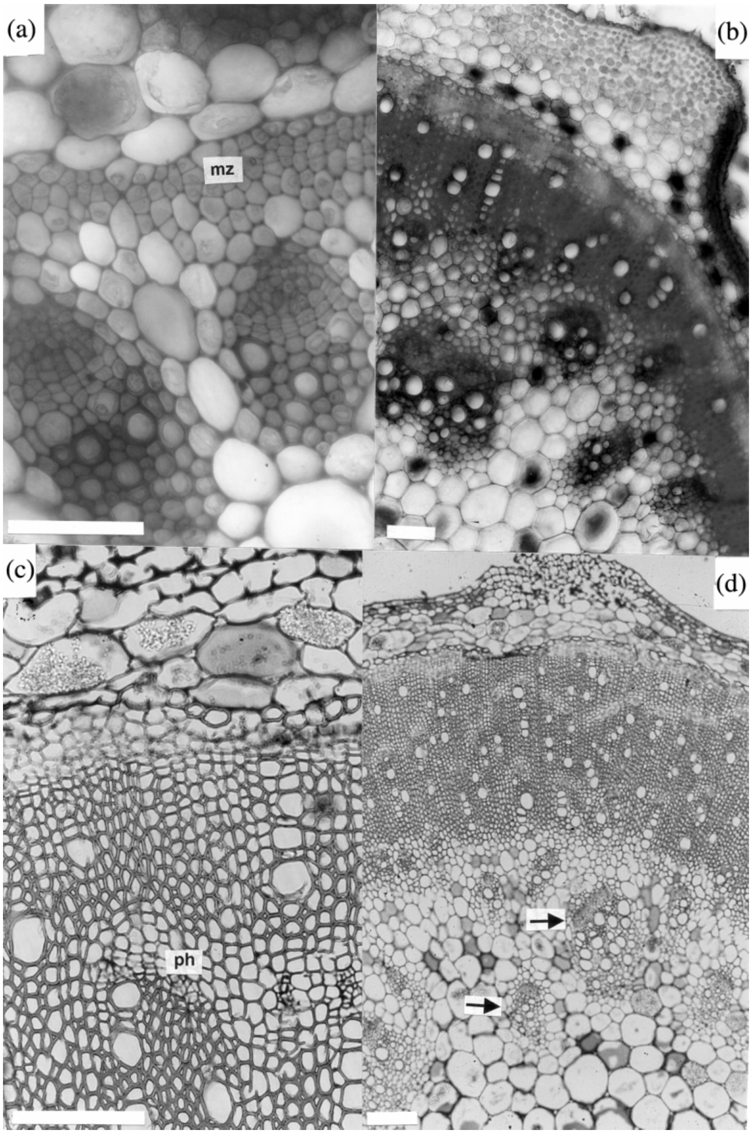

Fig. 4. Stem anatomy of *Chenopodium ambrosioides*. (a) Primary growth [observe the open vascular bundles and the meristematic zone (mz) above them]; (b) early secondary growth; (c) detail of a sector of the cortex showing crystal sand, groups of fibers and xylem with groups of phloem (ph); (d) late secondary growth (note the primary bundles, arrows, not included in the conjuctive tissue). Bar: 50 μm.

those which we call flagelliform trichomes but he did not mention the hammer-shaped and whip-shaped trichomes observed in these species and in another five species of *Chenopodium*. Gattuso and Gattuso [12] have studied the stomata and trichomes ontogeny in *C. ambrosioides*; they described only four types of trichomes: type I 'pelo secretor vesiculoso' (=type I, subtype 3: pear-shaped head, appressed trichome), type II 'pelo secretor no vesiculoso' (=type III: vermiform trichome), type III 'pelo pluricelular con una célula basal' (very similar to the flagelliform trichome) and type IV 'pelo pluricelular con 2 células basales' (=type IV: whip-shaped, subtype 1 trichome).

In *C. ambrosioides* and *C. venturii* the density of the indumentum is in relation to the habitat. It is usual that plants growing close to rivers are very pubescent instead of the glabrous specimens of the drier habitats. Furthermore, a variation in the types of trichomes is noticeable in the pubescent specimens. For example, in *C. ambrosioides*, the hammer-shaped trichomes, with multiseriate epitrichopodium, and in *C. venturii*, the whip-shaped trichomes with biseriate epitrichopodium, are only present in populations coming from wet areas. According to the literature, these two types of trichomes are exclusive of *Chenopodium* within the family.

Carolin [8] when referring to the glandular trichomes explained in detail their physiology and, judging by the figures included in his paper, these trichomes correspond to the multicellular head type (type II). This author observed the true glandular trichomes (the variant he called 8 b) in *C. ambrosioides* and *C. multifidum*; we did not find them in the latter species but they appear scarcely in *C. ambrosiodes* and more frequently in *C. carinatum*, *C. pumilio* and *C. oblanceolatum*.

The bladder trichomes (unicellular head containing ions) described by Thomson [24] and Carolin [8] are missing in the aromatic species of *Chenopodium* [1]; however, Simón [10] described salt secretory trichomes with tetracellular head for these species. Although Carolin [8] pointed out that *C. ambrosioides* and *C. multifidum* have modified bladder trichomes, in our opinion the unicellular head trichomes of these species are completely different in their morphology and physiology from the mentioned bladder trichomes. In fact, we observe many different types of glandular trichomes with unicellular head and multicellular stalk (two–five-celled), varying in shape and size. All these trichomes contain a characteristic yellowish aromatic resin in the head, and are present in the two species cited and in the remaining ones analyzed.

In *Chenopodiaceae* and other related families, the term 'bladder trichomes' is referred to those trichomes containing ions [8,24,25]; hence, we suggest avoiding the use of the word bladder when trichomes are described considering only their morphology.

As regards *C. ambrosioides*, glandular trichomes (type I with its variants), we agree with Holm [26] when he refers to their presence in both epidermises. This situation is different from what Simón [9] affirmed since she observed them 'always in abaxial position'. The indumentum density is much lesser in the adaxial epidermis. In all species studied, glandular trichomes are also present in both epidermis surfaces.

The different subtypes of glandular trichomes type I, have been observed at various densities in the 11 species analyzed; for this reason, we think it is very difficult to differentiate the 'paico' species, considering only the morphology of these glandular trichomes. These species can be recognized taking into account also the non-glandular trichomes, the epidermal model, the mesophyll and the primary structure of the stem.

Simón [9,10] pointed out the presence of 'pilose nests' when referring to *C. chilense* (sub nom. *C. sooianum* Aellen). According to her description of such structures, they do not correspond to the true concept of pilose nests. In fact, a pilose nest is a group of trichomes concentrated in a tiny and more or less profound cavity where they seem to originate from the same point [27]. In *C. chilense* we have only observed slightly depressed areas with a greater trichome concentration distributed all along its surface.

The vermiform trichomes (type III) are very common in all species studied and, as the rest of the glandular trichomes, produce a yellowish secretion. These trichomes are very similar to the ones observed in other related family, the *Amaranthaceae* [28].

As regards mesophyll, Carolin et al. [7] established different types within the non-Kranz and Kranz structures for the family. *Chenopodium* contains non-Kranz species, belonging the majority of the species studied (9) to the general *Dicotyledoneae* type, that is, with dorsiventral structure; the two remaining species (*C. retusum* and *C. oblanceolatum*) have isobilateral structure. These structures have already been cited in other genera of the family [6]. However, the mesophyll of the aromatic species of *Chenopodium* is very different to the one present in the other genera, since it is remarkable in these species the presence of a non-chlorophyllic parenchyma with abundant crystal sand in the middle of the mesophyll. For this reason, we propose 'the ambrosioid' subtype within the *Dicotyledoneae* type for the first case, and 'the retusoid' subtype within the isobilateral structure. It seems that there could be three morphological types within the mesophyll structure in *Chenopodium*: the typical dorsiventral type [6,23], the 'ambrosioid' subtype and the 'retusoid' subtype.

In relation to the stem, Balfour [29] has elucidated the origin of the anomalous secondary structure in *C. murale* L. The same ontogeny was found in other genera of the family by different authors [30–32] as well as in the 11 species investigated in this paper. Gattuso and Gattuso [13] differentiated five species of *Chenopodium* considering some features of the secondary growth, (e.g. lignification in the collenchyma cells; position of the collateral bundles in relation to the conjunctive tissue; distribution of pericyclic fibers, etc.). Our data reveal that the degree of lignification of the collenchyma and the presence and distribution of the pericyclic fibers are the most relevant characters for distinguishing the species. On the other hand, we observed that the collateral bundles can be embedded or not within the fibrous conjunctive tissue in different sectors of the stem within a species.

In relation to the taxonomic considerations when Hunziker [33] stated the presence of the adventitious species *C. carinatum* in Argentina, he himself distinguished it from two other allied ones, *C. pumilio* and *C. cristatum* (F. Muell.) F. Muell., considering mainly the fruiting sepals morphology. However, in the recent taxonomic synopsis of the Argentinean *Chenopodium* species, Giusti [1] asseverated the close resemblance between *C. carinatum* and *C. pumilio* and his doubt about the validity of *C. pumilio*.

Our analysis indicates that *C. carinatum* and *C. pumilio* can be distinguished from each other by their trichomes, the shape of the palisade cells (straight or sinuate walls) and the distribution of fibers in the secondary structure of the stem.

C. carinatum: leaves with cylindrical-shaped and conical incurvate non-glandular trichomes; glandular trichomes with transverse-elliptic unicellular head and short stalk and others with subellipsoidal head parallel to the epidermis and long stalk. Palisade cells with very sinuate walls. Pericyclic fibers in a discontinuous ring.

C. pumilio: leaves without cylindrical-shaped and conical incurvate non-glandular trichomes; glandular trichomes with pear-shaped and ellipsoidal head, both with short stalk; others with ovoid and stipitate head and long-stalked. Palisade cells with straight walls. Pericyclic fibers in a continuous ring.

Nevertheless, both taxa share the slightly revolute leaf margins, the same epidermal model and the presence of non-glandular trichomes type II (subtypes 1 and 2), glandular trichomes type I (subtype 4) and type II (subtypes 8 and 9) and the mixed trichomes.

Some species are easily characterized by the presence of an exclusive type of trichome among other types. Conical incurvate trichomes appear only in *C. carinatum*, whip-shaped trichomes subtype 3 (trichome uncinulate) only in *C. venturii*, hammer-shaped trichomes subtype 1 only in *C. ambrosioides* and finally, glandular trichomes with multicellular and stipitate head and long stalk only in *C. pumilio.*

The combination of some leaf features (indumentum, types of trichomes, epidermal model and leaf outline) and secondary structure of the stem (lignification of the collenchyma and distribution of the pericyclic fibers), allows to differentiate the aromatic species of *Chenopodium*, as we summarize in Table 1.

5. Conclusion

A classification and standardization of non-glandular and glandular trichomes of the 11 aromatic species of *Chenopodium* is established.

Stomata raised on a column, non-glandular trichomes: type IV (subtypes 2 and 3) and type V (subtype 2) and, glandular trichomes: type I (subtype 6 and 7), type II (subtype 8–10) are described for the first time in *Chenopodiaceae.*

In the mesophyll, a variant of the dorsiventral structure ('the ambrosioid' subtype) and a variant of the isobilateral structure ('the retusoid' subtype) are recognized for the aromatic species.

Differences between *C. carinatum* and *C. pumilio* are found taking into account leaf and stem features.

The main diagnostic characters to identify dried and smashed *Chenopodium* species material used as drug, are: type of trichomes; epidermal model; leaf margin; mesophyll; and the secondary growth of the stem.

Table 1
Leaf and stem characters of the aromatic species of *Chenopodium* in Argentina

	Indument	Leaf-blade outline	Epidermal model	Non-glandular trichomes										Mixed trichomes
				NI	NII_1	NII_2	NII_3	NIII	NIV_1	NIV_2	NIV_3	NV_1	NV_2	
C. ambrosioides	Glabrescent to pubescent	Straight to slightly revolute	E: NS H: S					* +	* +	* +		* +	* +	
C. burkartii	Mostly pubescent	Very revolute	E: NS H: S					+	+					
C. carinatum	Pubescent	Straight to slightly revolute	E: NS H: S	+	+	+	+							+
C. chilense	Mostly pubescent	Very revolute	E: NS H: S					+	*				+	
C. graveolens var. *bangii*	Glabrescent	Straight to slightly revolute	E: NS H: S	*	*	*								
C. haumanii	Glabrescent to pubescent	Revolute	E: NS H: S					+	*					
C. multifidum	Pubescent	Revolute	E: NS H: S					+	+				+	
C. oblanceolatum	Glabrescent	Revolute	E: NS H: S										+	
C. pumilio	Pubescent	Straight to slightly revolute	E: NS H: S		+	+								+
C. retusum	Pubescent	Revolute	E: NS H: S		+									
C. venturii	Glabrescent to pubescent	Revolute	E: NS H: S					*	+	*	+		+	

References: E: epiphyllous; H: hypophyllous; NS: no sinuate; S: sinuate; N: non-glandular trichomes; I–V: type of trichomes; II_{1-3} IV_{1-3} V_{1-2}: subtype of trichomes; +: common; *: rare.

Table 1 (*Continued*)

	Glandular trichomes											Collenchyma	Pericylic fibres
	GI_1	GI_2	GI_3	GI_4	GI_5	GI_6	GI_7	GII_8	GII_9	GII_{10}	GIII		
C. ambrosioides	*	*	* +						* +		* +	C	I
C. burkartii	+	+	+			+					+	L	Co
C. carinatum				+	+	+		+	+			C	I
C. chilense	+	+	+								+	C	Co
C. graveolens var. *bangii*		+										C	Co
C. haumanii	+		+									C	Absent
C. multifidum	*	+	+								+	C	I
C. oblanceolatum	*	*	*					*	*		*	C	Absent
C. pumilio			*	*			+	+	+	*		C	Co
C. retusum	+		+		*	+		*			+	L	I
C. venturii	+		+								+	C	Co

References: G: glandular trichomes: I–III: types of trichomes; I_{1-7}, II_{8-10} subtypes of trichomes; L: lignified; C: cellulosic; I: interrupted ring; Co: complete ring; +: common; *: rare.

Acknowledgments

The authors thank the late Prof. Ing. A.T. Hunziker for useful comments on the manuscript; Dr M.T. Cosa for her assistance in some anatomical features; Ing. L. Giusti (Fundación M. Lillo), and the authorities of Instituto Darwinion and Museo de la Plata for providing material of some species of *Chenopodium*; Biol. Marcelo Zak for linguistic correction to the text; the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR) and the Secretaría de Ciencia y Tecnología (Universidad Nacional de Córdoba, Argentina) for the economical support.

References

[1] Giusti L. Chenopodiaceae. In: Hunziker AT, editor. Flora fanerogámica Argentina, fasc 40. Argentina: Proflora-Conicet, 1997. p. 1.
[2] Sorarú B, Bandoni A. Plantas de la medicina popular Argentina. Buenos Aires: Albatros, 1978.
[3] Ratera EL, Ratera MO. Plantas de la flora Argentina empleada en medicina popular. Buenos Aires: Hemisferio Sur S.A, 1980.
[4] Gupta M. 270 Plantas medicinales iberoamericanas. Colombia: Cyted, 1995.
[5] Alonso JR. Tratado de Fitomedicina. Bases clínicas y farmacológicas. Argentina: Isis, 1998.
[6] Metcalfe CR, Chalk L. Anatomy of the Dicotyledons. Oxford: University Press, 1950.
[7] Carolin RC, Jacobs SW, Vesk M. Bot Jahrb Syst 1975;95:226.
[8] Carolin RC. Bot Jahrb Syst 1983;103:451.
[9] Simón LE. Notas Mus La Plata 21, 1987, Serie botánica no. 99: 100.
[10] Simón LE. Adansonia Sér 3 1997;19:293.
[11] Gattuso M, Gattuso S. Physis (Buenos Aires) Secc c 1985;43:53.
[12] Gattuso S, Gattuso M. Physis (Buenos Aires) Secc c 1987;45:85.
[13] Gattuso S, Gattuso M. Actas IV Congreso Argentino del Medicamento, Argentina, 1989. p. 169.
[14] Glasby JS. Dictionary of plants. Containing secondary metabolites. London: Taylor and Francis, 1991.
[15] Harborne JB, Baxter H, Moss G. Dictionary of plant toxins. England: J. Wiley & Sons Ltd, 1996.
[16] Codex Medicamentarius Argentino, 6th edition, Buenos Aires, 1978.
[17] Nájera M, Spegazzini E. Rev Farm 1984;126:7.
[18] Fester GA, Martinuzzi EA, Retamar JA, Ricciardi AI. Aceites esenciales de la República Argentina.
[19] Bandoni AL, Mendiondo ME, Rondina RV, Coussio JD. Econ Bot 1976;30:161.
[20] De Pascual TJ, Bellido IS, Torres C, Sastre BA, Grande M. Phytochemistry 1981;20(1):163.
[21] Conn HJ, Darrow MA, Emmel VM. Staining procedures. Baltimore: Williams & Wilkins Co, 1960.
[22] Font Quer P. Diccionario de botánica. Barcelona: Labor, 1979.
[23] Solereder H. Systematic anatomy of the Dicotyledons. Oxford: University Press, 1908.
[24] Thomson WW. The structure and function of salt glands. In: Poljakoff-Mayber A, Gale J, editors. Plants in saline environments, vol 15. Ecol Stud, 1975. p. 118.
[25] Pyykkö M. Ann Bot Fenn 1966;3:453.
[26] Holm T. Am J Sci 1923;6:157.
[27] Ariza Espinar L. Bol Acad Nac Ciencias Córdoba 1973;50:175.
[28] Filippa EM, Ariza Espinar L. Acta Farm Bonaerense 1993;12:79.

[29] Balfour E. Phytomorphology 1965;15:111.
[30] Yarrow GL, Popham RA. Am J Bot 1981;68:1042.
[31] Bhambie S, Goyal R, Gupta S. Acta Bot Indica 1990;18:252.
[32] Krumbiegel A. Nordic J Bot 1998;18:159.
[33] Hunziker AT. Rev Argent Agron 1955;22:184.