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ORIGINAL ARTICLE

# Leaf and stem anatomy and essential oil composition of four Brazilian *Aldama* species (Asteraceae) and their taxonomic significance

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**Abstract** *Aldama* La Llave is one of several Asteraceae genera that pose phylogenetic problems. The close similarity between species, as well as the inconsistencies found in the most recent phylogenetic analysis, shows that new data are needed to help delimit group species. *Aldama anchusifolia* (DC) E.E.Schill. & Panero, *Aldama megapotamica* (Malme) Magenta & Pirani, *Aldama nudibasilaris* (S.F.Blake) E.E.Schill. & Panero and *Aldama pilosa* (Baker) E.E.Schill. & Panero are difficult to identify because they are very closely related. Therefore, the aim of this study was to detect anatomical and phytochemical characteristics to help elucidate phylogenetic issues raised by *Aldama*. Aerial vegetative organs were prepared using the standard histological techniques. Essential oils were obtained by hydrodistillation, and their components identified using a gas chromatograph coupled to a mass spectrometer and flame ionization detector. Each species presented a set of unique leaf and stem anatomical features. The front view of the epidermal cell walls in the leaves, the presence of secretory ducts in the phloem and medulla sclerification in the stems proved useful in delimiting these species. The essential oils were characterized by the predominance of sesquiterpenes such as *t*-caryophyllene, germacrene D and bicyclogermacrene. Some unique constituents in each species were also identified as potential chemical markers.

**Keywords** Compositae · Fructan · Secretory duct · Socket cells · Terpenes

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## Introduction

The South American species of *Viguiera* Kunth were transferred to *Aldama* La Llave due to results of molecular analyses (Schilling and Panero 2011) since these species were formed a cohesive group apart from the other species in this genus (Schilling and Jansen 1989; Schilling and Panero 1996, 2011). However, inconsistencies found in the phylogenetic analysis performed by Schilling and Panero (2011) highlighted the need for more data to circumscribe *Aldama*, whose species share many morphological similarities (Magenta and Pirani 2014).

Anatomical and chemical features in *Aldama* are important tools used to help identify the species (Bombo et al. 2012, 2014; Oliveira et al. 2013; Silva et al. 2014). The secretory structures represent an important taxonomic character among the anatomical features in Asteraceae due to their position and variety (Castro et al. 1997; Appezzato-da-Glória et al. 2008).

Phytochemical analyses also emphasize the use of secondary metabolites in the phylogeny and chemotaxonomy of *Aldama* (Schilling et al. 2000; Da Costa et al. 2001) as well as their pharmacological potential (Tirapelli et al. 2002; Valério et al. 2007; Canales et al. 2008). It is worth highlighting the essential oils among such metabolites, which are a complex mixture of lipophilic substances mostly composed of volatile low molecular weight terpenes (Fahn 2000). Essential oils have been already reported

in several Asteraceae genera (Heinrich et al. 2002; Alvarenga et al. 2005; Agostini et al. 2005; Maia et al. 2010; Chagas-Paula et al. 2012); however, only three species have been reported in *Aldama* (Bombo et al. 2012, 2014).

The strong vegetative similarities between *Aldama nudibasilaris* (S.F. Blake) E.E. Schill. & Panero and *Aldama pilosa* (Baker) E.E. Schill. & Panero as well as the possible formation of hybrids between *Aldama anchusifolia* (DC) E.E. Schill. & Panero and *Aldama megapotamica* (Malme) Magenta & Pirani highlighted the difficulty in identifying them. Therefore, the aim of the current study is to point out some anatomical and phytochemical features that can help circumscribing these *Aldama* species.

## Materials and methods

**Plant species and study area** – The aerial organs of *A. anchusifolia*, *A. megapotamica*, *A. nudibasilaris* and *A. pilosa* which have derived from plants in the flowering stage were collected in grasslands and in the borders of highways in the Brazilian Southern and Southeastern regions between 2012 and 2013. A total of nine specimens from three distinct populations, spaced at least 30 km from each other, were sampled for each species. The species were identified by a specialist, and the vouchers were deposited at the ESA Herbarium (Luiz de Queiroz College of Agriculture).

**Structural analyses** – The middle region and the petiole of fully expanded leaves and stems were analyzed for each specimen collected. The thinner and the median size diameters, as well as the internode near to the ground, were sampled in the stem.

The samples were fixed in FAA 50 (formaldehyde, acetic acid and 50% ethanol) (Johansen 1940) or in Karnovsky solution (Karnovsky 1965). Subsequently, they were subjected to vacuum to remove the air from the tissues and dehydrated in an ethanol series up to 70% ethanol, wherein they were stored until the time to be processed. A fraction of each material was embedded in Leica Historesin® plastic resin (Heraeus Kulzer, Hanau, Germany). The blocks were sectioned by means of Leica RM 2245 rotary microtome at 6 µm. The sections were stained with 0.05% toluidine blue O in a citrate–phosphate buffer, pH 4.5 (Sakai 1973) and mounted on glass slides in Entellan® synthetic resin (Merck, Darmstadt, Germany). Thicker sections (20–60 µm) of fixed samples were also prepared in Leica SN 2000 R sliding microtome. The sections were clarified in 20% sodium hypochlorite, washed in distilled water, stained with safranin and astra blue (Bukatsch 1972) and mounted in 50% glycerin. The classification of the glandular trichomes was based on Castro et al. (1997).

Leaf and stem surfaces were also analyzed through the epidermal dissociation technique using the Jeffrey's solution (Johansen 1940). The epidermis was also analyzed through scanning electron microscopy (SEM). The samples were dehydrated in ethanol series up to absolute ethanol, dried according to the $CO_2$ critical point method (Horridge and Tamm 1969), mounted on aluminum stubs and coated with a gold layer (30–40 nm). The observations and photomicrographs were obtained in a LEO 435 VP SEM (Zeiss, Oberkochen, Germany) operated at 20 kV.

**Histochemical analysis** – The histochemical analyses were performed in sections obtained from the material embedded in historesin and from the fixed material that had not been embedded in historesin. The following reagents and dyes were used: NADI reagent, to identify the essential and resin oils (David and Carde 1964); zinc chloride-iodide, to detect the starch grains (Strasburger 1913); phloroglucinol in acid medium, to detect lignin; ferric chloride, for the phenolic compounds (Johansen 1940); Sudan IV for the lipophilic substances (Jensen 1962); Sudan black B for the total lipids (Pearse 1968); and ruthenium red for the pectic substances (Johansen 1940).

The digital photomicrographs were obtained in Leica DM LB microscope equipped with Leica DC 300F camera.

**Essential oil extraction** – The fresh material was subjected to hydrodistillation for 3 h, in Clevenger-type apparatus. The aqueous phase was collected after cooling, and the setup was washed with dichloromethane (50 ml) to obtain the essential oils. Each solution was dried over anhydrous sodium sulfate, weighed on an analytical scale to set the yield and stored at −5 °C in sealed amber glass flasks.

**Essential oil analysis: gas chromatography** – The essential oil constituents were set in a gas chromatography coupled to a mass spectrometer (GC–MS) using HP 5890 chromatograph Series II (Palo Alto, CA, USA) equipped with the Hewlett-Packard 5971 mass selective detector and the HP-5 capillary column (25 m × 0.20 mm × 0.33 µm). The GC–MS was performed through split/splitless injection by using the injector at 220 °C; the detector at 280 °C; the column, at 60 °C, with increments of 3 °C $min^{-1}$ up to the final temperature of 240 °C. The constituents were also set in a flame ionization detector (FID/DIC/ULTRA FAST) coupled to the Thermo Scientific TRACE GC Ultra gas chromatograph with AS 3000 autosampler, split/splitless injection, HP-5 capillary column (30 m × 0.25 mm × 0.25 µm), temperatures equal to the aforementioned ones and final temperature of 250 °C. Helium at 1 mL $min^{-1}$ was used as carrier gas. The samples were dissolved in ethyl acetate at 20 mg $mL^{-1}$ concentration.

The constituents of the essential oils were identified through the comparison of their mass spectra and the NIST-05 library data, by co-injection of hydrocarbons patterns in order to calculate the Arithmetic index and through data described in the literature (Adams 2007).

**Statistical analyses** – The values found for essential oil yield were submitted to variance analysis (ANOVA), and the means were compared through Tukey's test ($P < 0.05$).

## Results

**Leaf anatomy** – The epidermal cells have sinuous walls on both leaf sides of *A. anchusifolia*, *A. nudibasilaris* and *A. pilosa* from the front side view (Table 1; Fig. 1). *A. megapotamica* has straight cell walls (Fig. 2). The stomata were anomocytic and occurred on both leaf sides, except for *A. nudibasilaris*, which has hypostomatic leaves.

The indumentum of the four species comprised two glandular trichomes (types II and IV) and a non-glandular trichome. The non-glandular type (Figs. 1, 3) occurred on both leaf sides and consisted of three cells: two cone-shaped basal cells and a terminal cell with an acute apex. The walls were thickened in pectin; the verrucous ornamentations (humps) were more commonly found in the basal cells; and they were absent in the apical cells. The basis of each non-glandular trichome was delimited by concentric series of epidermal cells (Fig. 1). The amount of series may vary from one species to another (Table 1). These cells had mucilaginous content, and their walls were thickened in pectin. The type II glandular trichome occurred on both leaf sides and accumulated phenolic substances; it was linear and uniseriate; and its terminal cell was spatulate (Fig. 3). The type IV trichome just occurred on the abaxial surface of the epidermis (Fig. 4); its exudate had lipophilic nature; it was capitate and biseriate and comprised of two basal cells and 3–8 pairs of secreting cells, which corresponded to the trichome head.

Both sides of the leaf presented uniseriate epidermis with thickened outer periclinal cell walls covered with a thin cuticle (Figs. 5, 6, 7). The mesophyll was dorsiventral in all species (Figs. 5, 6). *A. megapotamica* was the only one to present tiny prismatic crystals in this tissue. Lipid droplets were found in the palisade cells of *A. anchusifolia* and *A. megapotamica* (Fig. 6, inset).

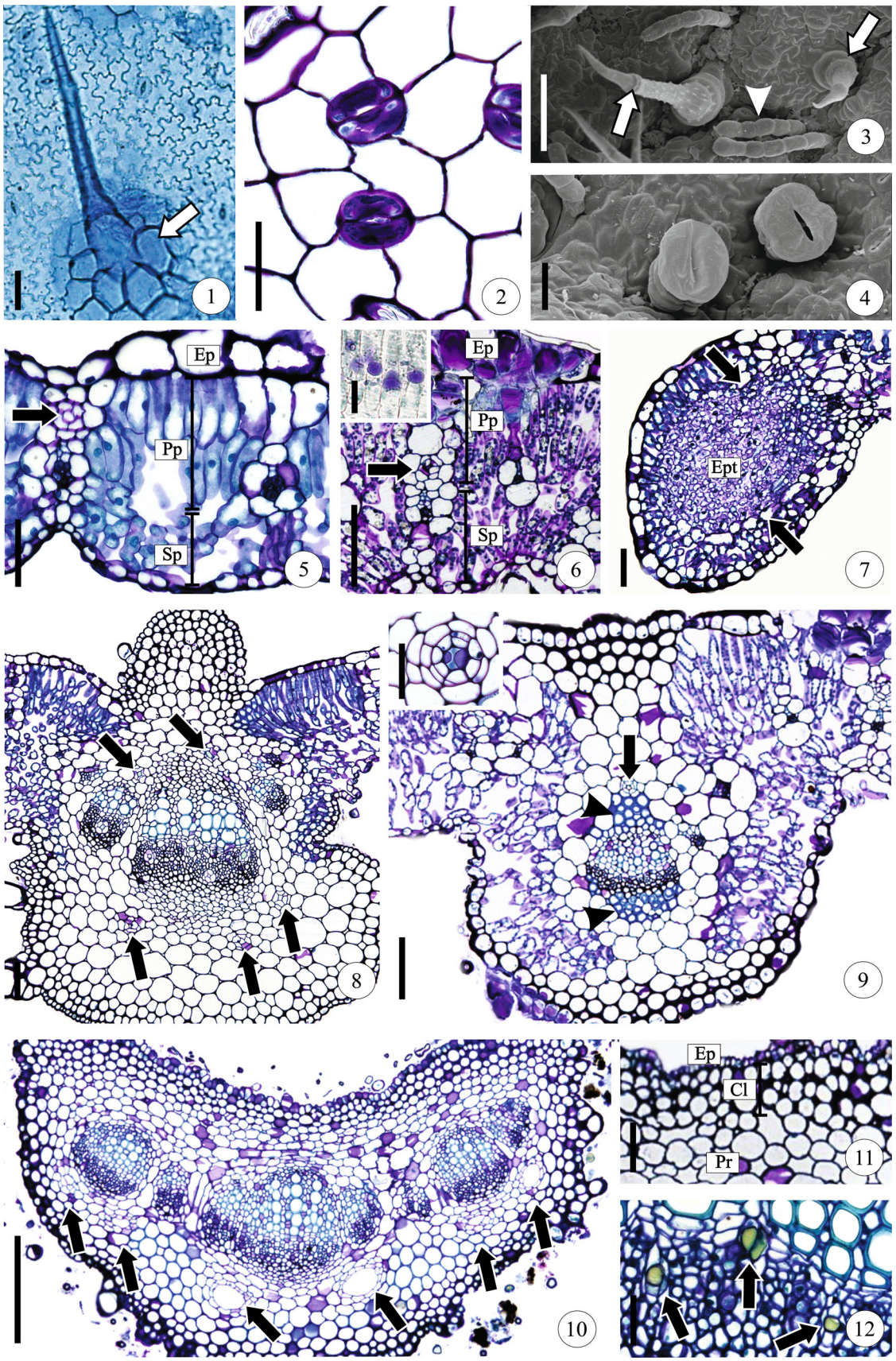
Revolute leaf margins were found in the four species. Their tissue organization was similar to that visualized in the rest of the leaf blade. The ornamentation regions in *A. nudibasilaris* and *A. pilosa* leaves may have hydathodes (Fig. 7), which are constituted by water pores, incomplete parenchymatous sheaths that surround the thin-walled cells of the epithem, and the terminal tracheids of the vascular bundle.

The mid-rib had a conspicuous projection filled with collenchyma cells in adaxial side of *A. nudibasilaris* and *A. pilosa* leaves (Fig. 8). This projection was less prominent in *A. anchusifolia*, and it was missing in *A. megapotamica* (Fig. 9). The fundamental parenchyma surrounded the vascular bundles (Fig. 8), but the palisade parenchyma cells extend to the lateral side of the mid-rib in *A. anchusifolia* and in *A. megapotamica* (Fig. 9). The number

**Table 1** Main anatomical features distinguishing *Aldama anchusifolia* (Aa), *A. megapotamica* (Am), *A. nudibasilaris* (An) and *A. pilosa* (Ap)

| Trait | Aa | Am | An | Ap |
|---|---|---|---|---|
| Leaf | | | | |
| Stomata position | Amph | Amph | Hyp | Amph |
| Anticlinal walls of epidermis | Sinuous | Straight | Sinuous | Sinuous |
| Number of cells surrounding the non-glandular trichome | 1–2 Ad, 0–1 Ab | 1–3 Ad, no | 0 Ad, 1–3 Ab | 1 Ad, 1–3 Ab |
| Hydathode on leaf margin | Absent | Absent | Present | Present |
| Palisade parenchyma extended to the abaxial side of the mid-rib | Present | Present | Absent | Absent |
| Number of secretory ducts on the mid-rib | 0 Ad, 2 Ab | 1 Ad, 0–2 Ab | 2 Ad, 2–3 Ab | 0–2 Ad, 2 Ab |
| Fiber caps on the mid-rib bundle | Absent | Present | Absent | Absent |
| Phloem secretory duct on the mid-rib | Absent | Absent | Present | Present |
| Secretory ducts on abaxial region of the petiole | Absent | – | Present | Present |
| Phloem secretory duct on the petiole | Absent | – | Present | Present |
| Stem | | | | |
| Secretory duct on the primary phloem | Absent | Absent | Present | Present |
| Phloem cells interrupting the pericycle | Absent | Absent | Absent | Present |
| Hypertrophy and hyperplasia of medulla cells | Conspic | Inconspic | Conspic | Conspic |
| Sclerified medulla | Absent | Present | Absent | Absent |
| Inulin crystal | Absent | Absent | Absent | Present |

*Amph* amphistomatic, *Hyp* hypostomatic, *Ab* abaxial region, *Ad* adaxial region, *no* not observed, *conspic* conspicuous, *inconspic* inconspicuous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◄**Figs. 1–12** Surface view and cross sections of the leaf blade and petiole in *Aldama anchusifolia* (**4**, **5**, **10**), *A. megapotamica* (**2**, **6**, **9**), *A. nudibasilaris* (**3**, **7**, **8**, **11**, **12**) and *A. pilosa* (**1**). **1**, **2** Sinuous (**1**) and straight (**2**) epidermal cell walls. See the thickening of cells surrounding the basis of the non-glandular trichomes (*A*, *arrow*) and of the anomocytic stomata (**2**). **3**, **4** Scanning electron micrograph of the non-glandular (**3**, arrows), glandular type II (**3**, arrowheads) and glandular type IV (**4**) trichomes. **5**, **6** Uniseriate epidermis (*Ep*), palisade parenchyma (*Pp*), spongy parenchyma (*Sp*), lateral bundles and secretory ducts (*arrow*). Detail of the lipid droplets in chlorophyllous parenchyma stained in NADI reagent (inset in **6**). **7** Hydathode consisting of tracheids, epitema (*Ept*) and incomplete sheath (*arrows*). **8**, **9** Mid-rib with secretory ducts (*arrows*) and fiber caps (**9**, *arrowheads*). Detail of the secretory duct (inset in **9**). **10**, **12** Petiole with secretory ducts (**10**, *arrows*). Detail of the epidermis (*Ep*), collenchyma (*Cl*), parenchyma (*Pr*) (**11**) and ducts in the primary phloem (**12**, *arrows*). *Bars* = 10 µm (**10**, *inset*); 25 µm (**2**, **4**, **6** *inset*, **7**–**9**, **12**); 50 µm (**1**, **3**, **5**, **11**); 100 µm (**6**); 200 µm (**10**)

of ducts in the fundamental parenchyma can vary from one leaf to another in the same individual and also between individuals in different populations in *A. megapotamica*, *A. nudibasilaris* and *A. pilosa* (Table 1). Such variation was not observed in *A. anchusifolia*, which only had two secretory ducts facing the abaxial surface. The ducts had different sizes, secreted lipophilic substances and occurred in the adaxial and abaxial regions of the fundamental parenchyma (Figs. 8, 9). Some parenchyma cells around the vascular bundles exhibited starch grains in all the species analyzed. The vascular system was collateral, arranged in a larger central bundle and in two smaller lateral bundles in *A. anchusifolia*, *A. nudibasilaris* and *A. pilosa* (Fig. 9, inset). *A. megapotamica* had one large central bundle, which was associated with fiber caps (Fig. 9). The secretory ducts were found in the primary phloem just in *A. nudibasilaris* and *A. pilosa.* The lateral veins may present parenchymatous sheaths that can reach the epidermis on both sides of the leaf; however, the secretory ducts just occurred in the adaxial bundle extension (Fig. 5).

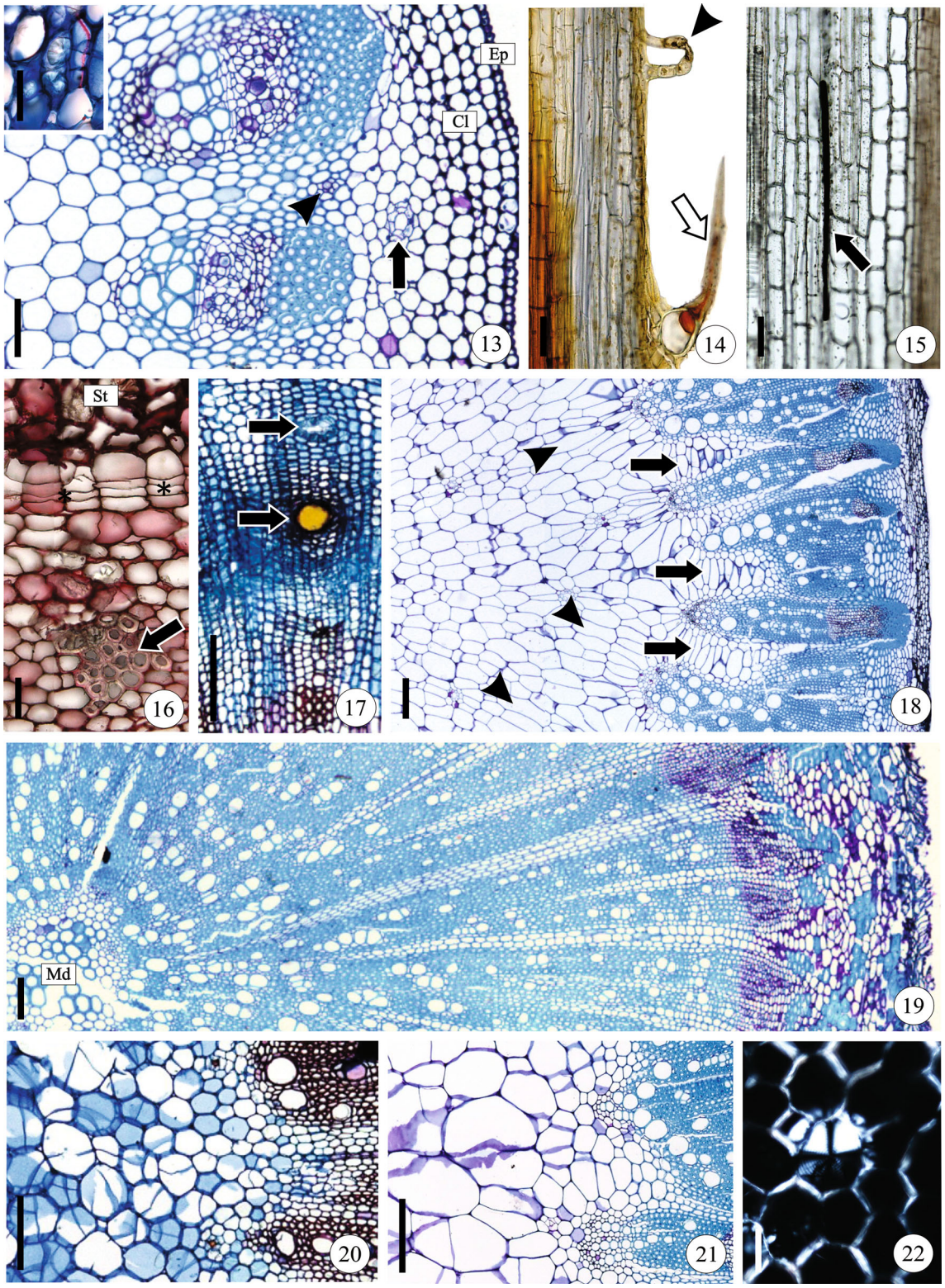
The description of petiole was held herein for the first time to the genus selected. Only *A. anchusifolia*, *A. nudibasilaris* and *A. pilosa* presented petiole, which had an epidermis structure similar to that one observed in the leaf blade (Fig. 10). The indumentum was formed by the non-glandular trichome and by the type II glandular trichome. The collenchyma and the subjacent fundamental parenchyma are arranged in layers immediately below the epidermis. The layers may vary in number, depending on the sample analyzed (Fig. 11). Secretory ducts of different sizes are immersed in this parenchyma (Fig. 10). Ducts in *A. anchusifolia* only developed toward the abaxial region, whereas those in *A. nudibasilaris* and *A. pilosa* also occurred in the adaxial region. The vascular system consisted of three major bundles and of a varying number of smaller lateral bundles, which may be surrounded by cells containing starch grains. The secretory ducts only occurred in the phloem of *A. nudibasilaris* and *A. pilosa* (Fig. 12).

**Stem anatomy** – In an incipient secondary structure, the epidermis of all studied species was uniseriate (Fig. 13) and exhibited non-glandular trichomes and glandular trichomes type II, similarly to that described in the leaves (Fig. 14). There were secretory ducts in the cortical parenchyma (Figs. 13, 15) and the endodermal cells presented Casparian strips and starch grains (Fig. 13, inset). The pericycle region opposite to the primary phloem formed fiber cap, and it was interrupted by phloematic cells in *A. pilosa* (Table 1; Fig. 13). The vascular bundles were collateral, and only *A. nudibasilaris* formed secretory ducts in the primary phloem. The medulla was parenchymatic, and its ducts were distributed in the perimedullary zone (Fig. 13), which rarely occurred in *A. pilosa*.

In stems with established secondary structure, the epidermis was replaced by suberized thick-walled cells, which were originated by periclinal divisions of subepidermal cells (Fig. 16). Sclereids emerged in the cortical region either alone or in small groups (Fig. 16). In the fascicular and interfascicular regions of the vascular cylinder, the cambium produced secondary xylem and phloem with all elements of the axial and radial systems (Figs. 17, 18, 19). Secretory ducts occurred in the secondary phloem in all studied species (Fig. 17).

The stem thickening in *A. anchusifolia*, *A. nudibasilaris* and *A. pilosa* mainly resulted from the expansion of the medulla. In the first two species, the vascular ray cells elongated in periclinal direction, whereas the cells belonging to the perimedullary zone divided and elongated in anticlinal direction (Fig. 18). On the other hand, the expansion of the medulla in *A. pilosa* resulted from cellular hyperplasia (Fig. 20) and hypertrophy (Fig. 21). The stem thickening was not very pronounced in *A. megapotamica* and the medulla became sclerified at the end of the development (Fig. 19). There were inulin crystals in the cortical and medullary parenchyma cells (Fig. 22), in the cambium and in other vascular tissues, often inside the tracheary elements, only in thicker stems of *A. pilosa.*

**Essential oils** – The yield of essential oils extracted from the aerial organs was different among the populations analyzed (Table 2). However, there was no significant difference between the mean values of the leaves and stems in each species (Fig. 23). In *A. anchusifolia,* the mean yield of leaf EO was 0.26 ± 0.07 for leaves and 0.26 ± 0.03 for stems, and it was almost four times higher than the mean value for *A. megapotamica* leaves (0.07 ± 0.07) and five times higher for stems (0.05 ± 0.06). Furthermore, the mean values of *A. pilosa* (0.26 ± 0.03 and 0.19 ± 0.06)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◀**Figs. 13–22** Cross sections and longitudinal section of stems of *Aldama anchusifolia* (**14**, **15**), *A. megapotamica* (**19**), *A. nudibasilaris* (**17**, **18**) and *A. pilosa* (**13**, **16**, **20**–**22**). **13** Internode in an incipient secondary structure. Epiderm (*Ep*), collenchyma (*Cl*), secretory duct in the parenchyma (*arrow*) and phloem cells interrupting the pericycle (*arrowhead*). Detail of the Casparian strips (inset in **13**). **14** Non-glandular (*arrow*) and type II glandular (*arrowhead*) trichomes. **15** Longitudinal section of the secretory duct (*arrow*). **16** Periclinal divisions (*asterisk*) of the outmost cortical region that originates the suberized tissue (*St*) in the secondary structure and in the sclereids in the cortex (*arrow*). **17** Secreting ducts (*arrows*) in the secondary phloem. **18** Secondary structure of the internode. Periclinal elongation of the ray cells (*arrows*) and anticlinal elongation and division of the cells in the perimedullary zone (*arrowheads*). **19** Secondary structure of the thickened stem, with sclerified medulla (*Md*). **20**, **21** Hypertrophy (**20**) and hyperplasia (**21**) of the medullary cells. (**22**) Inulin crystals observed under polarized light. *Bars* = 50 μm (**13** *inset*, **14**–**16**); 100 μm (**13**, **19**, **22**); 200 μm (**17**, **18**, **20**, **21**)

were also higher than those of *A. megapotamica*, and similar to those of *A. nudibasilaris* (0.18 ± 0.06 for leaves and 0.05 ± 0.12 for stems).

The essential oils from the aerial organs were featured by the presence of monoterpenes, sesquiterpenes and diterpenes and by the prevalence of sesquiterpenes in all four species (Table 3). Sixty-two constituents were identified (59 terpenes and three phenylpropanoids), totaling approximately 85% of the total amount.

Carotol was the major compound in *A. anchusifolia*, although α- and β-pinene (monoterpenes) and 5-neo-cedranol (sesquiterpene) also stood out in more than one population due to the high relative percentage of them both organs (Table 3). Germacrene D (sesquiterpene) presented the highest relative percentage of essential oil in *A. megapotamica* leaves in the three populations, whereas *t*-caryophyllene showed the highest value in the EO's from stems (24.91%; Table 3). Eugenol (phenolic derivative) also stood out in this species since it achieved 63.26% in one of the populations of *A. megapotamica*. As for *A. nudibasilaris*, the constituents showing significant relative percentage were caryophyllene oxide and bicyclogermacrene, respectively, on the leaves and stems of the three populations. Bicyclogermacrene (sesquiterpene) was detected as the major compound in the leaves and stems of *A. pilosa* (Table 3). The following constituents were identified in all four species: *t*-caryophyllene, germacrene D and bicyclogermacrene (in both organs), caryophyllene oxide (only in the leaves) and spathulenol (only in the stems; Table 3).

When the constituents were found in leaf and stem EOs in all analyzed populations of a single species, they were considered unique. Three constituents were unique in *A. anchusifolia* (δ-amorphene, carotol and 5-neo-cedranol), one in *A. megapotamica* (isodaucene) and one constituent in *A. nudibasilaris* (copaene). No constituent was considered unique in *A. pilosa* (Table 3).

**Table 2** Fresh matter (M) and essential oil yield (Y) of leaves and stems of *Aldama* populations (Pop)

| Species | Organ | Pop | M (g) | Y (% w/w) |
|---|---|---|---|---|
| *A. anchusifolia* | Leaf | 1 | 194.8 | 0.24 |
| | | 2 | 152.35 | 0.34 |
| | | 3 | 240.83 | 0.21 |
| | Stem | 1 | 549.61 | 0.48 |
| | | 2 | 122.48 | 0.19 |
| | | 3 | 308.11 | 0.12 |
| *A. megapotamica* | Leaf | 1 | 9.60 | 0.0025 |
| | | 2 | 40.83 | 0.12 |
| | | 3 | 19.76 | 0.11 |
| | Stem | 1 | 21.46 | 0.0025 |
| | | 2 | 70.95 | 0.12 |
| | | 3 | 78.57 | 0.05 |
| *A. nudibasilaris* | Leaf | 1 | 99.90 | 0.15 |
| | | 2 | 156.56 | 0.25 |
| | | 3 | 71.04 | 0.16 |
| | Stem | 1 | 210.45 | 0.03 |
| | | 2 | 341.7 | 0.05 |
| | | 3 | 102.7 | 0.07 |
| *A. pilosa* | Leaf | 1 | 124.11 | 0.28 |
| | | 2 | 126.25 | 0.23 |
| | | 3 | 139.44 | 0.28 |
| | Stem | 1 | 117.02 | 0.12 |
| | | 2 | 131.55 | 0.21 |
| | | 3 | 153.83 | 0.24 |

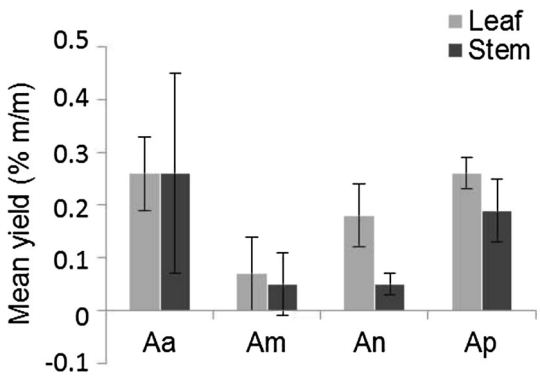


**Fig. 23** Essential oil yield of leaves and stems of *Aldama anchusifolia* (*Aa*), *A. megapotamica* (*Am*), *A. nudibasilaris* (*An*) and *A. pilosa* (*Ap*). *Bars* represent mean ± standard deviation ($n = 3$). Values are not significantly different ($p > 0.05$) under the same organ

## Discussion

The vegetative anatomy of *Aldama* has helped in morphologically differentiating of similar species that pose taxonomic problems (Bombo et al. 2012; Oliveira et al. 2013; Silva et al. 2014). The available data suggest that

**Table 3** Chemical profile and relative percentage area (%) of the essential oils extracted from leaves and stems of *Aldama anchusifolia*, *A. megapotamica*, *A. nudibasilaris* and *A. pilosa*

| Constituent | $AI_{lit}$ | $AI_{cal}$ | *A. anchusifolia* | | | | | | *A. megapotamica* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Leaf | | | Stem | | | Leaf | | | Stem | | |
| | | | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 |
| Monoterpene hydrocarbons | | | 29.63 | 30.07 | 31.74 | 57.48 | 35.84 | 49.48 | – | 1.39 | 4.64 | – | – | 13.45 |
| α-Thujene | 924 | 926 | – | – | 1.20 | 0.62 | 0.28 | 1.99 | – | – | – | – | – | – |
| α-Pinene | 932 | 933 | 6.13 | 2.68 | 5.67 | 24.40 | 8.90 | 8.78 | – | – | – | – | – | – |
| Camphene | 946 | 948 | 0.51 | 0.52 | – | 0.71 | 0.48 | – | – | – | – | – | – | – |
| Sabinene | 969 | 973 | 0.64 | 7.75 | 3.82 | 1.14 | 6.20 | 6.19 | – | – | – | – | – | – |
| β-Pinene | 974 | 976 | 8.52 | 7.01 | 8.28 | 16.74 | 9.29 | 12.60 | – | – | – | – | – | 5.35 |
| Myrcene | 988 | 990 | 6.55 | 4.12 | 1.26 | 10.05 | 5.41 | 3.70 | – | – | – | – | – | – |
| 3-Carene | 1008 | 1010 | – | – | – | – | – | – | – | – | – | – | – | – |
| D-Limonene | 1024 | 1027 | 2.65 | 3.80 | 8.20 | 2.89 | 4.17 | 14.5 | – | – | – | – | – | 4.33 |
| (*Z*)-β-ocimene | 1032 | 1035 | – | – | – | – | – | – | – | – | – | – | – | – |
| (*E*)-β-ocimene | 1044 | 1046 | 3.75 | 3.83 | 2.67 | 0.93 | 0.69 | 1.19 | – | 1.39 | 4.64 | – | – | 3.77 |
| γ-Terpinene | 1054 | 1057 | 0.88 | 0.36 | 0.64 | – | 0.42 | 0.53 | – | – | – | – | – | – |
| Oxygenated monoterpenes | | | 0.84 | 1.42 | – | – | 1.42 | 1.38 | – | – | – | – | – | – |
| (*E*)-pinocarveol | 1135 | 1138 | – | – | – | – | – | – | – | – | – | – | – | – |
| 4-Terpineol | 1174 | 1176 | – | 0.77 | – | – | 1.42 | 1.38 | – | – | – | – | – | – |
| α-Terpineol | 1186 | 1189 | – | – | – | – | – | – | – | – | – | – | – | – |
| Bornyl acetate | 1287 | 1285 | 0.84 | 0.65 | – | – | – | – | – | – | – | – | – | – |
| Sesquiterpene hydrocarbons | | | 17.36 | 17.61 | 29.83 | 12.39 | 18.39 | 11.23 | 60.75 | 84.77 | 55.84 | 38.29 | 21.05 | 52.99 |
| δ-Elemene | 1335 | 1337 | – | – | – | – | – | – | 0.88 | 1.80 | – | – | – | – |
| β-Elemene | 1389 | 1391 | – | – | – | – | – | – | 3.85 | 1.73 | – | – | – | – |
| Copaene | 1374 | 1375 | – | – | – | – | – | – | – | – | – | – | – | – |
| 1,7-Di-epi-β-cedrene | 1411 | 1413 | – | – | – | – | – | – | – | 1.38 | – | 3.31 | 12.66 | – |
| *t*-Caryophyllene | 1417 | 1418 | 1.34 | 0.93 | 1.42 | – | 0.60 | 0.52 | 5.38 | 2.72 | 4.16 | 9.21 | – | 24.91 |
| β-Gurjunene | 1431 | 1431 | – | – | – | – | – | – | 0.64 | – | – | 1.43 | 3.24 | – |
| α-(*E*)-bergamotene | 1432 | 1435 | – | – | – | – | – | – | – | – | – | – | – | – |
| α-Humulene | 1452 | 1452 | 0.39 | – | 1.30 | – | – | – | 1.33 | – | – | 1.67 | – | 11.27 |
| β-Chamigrene | 1476 | 1474 | – | – | – | – | – | – | – | – | – | – | – | – |
| Germacrene D | 1484 | 1480 | 6.72 | 3.40 | 7.88 | 4.80 | 2.94 | 2.43 | 27.94 | 32.84 | 24.65 | 9.46 | 1.21 | – |
| β-Selinene | 1485 | 1482 | – | – | – | – | – | – | – | – | – | – | – | – |
| Bicyclogermacrene | 1500 | 1495 | 6.51 | 12.11 | 17.42 | 6.10 | 13.2 | 6.66 | 16.74 | 41.10 | 22.38 | 9.12 | 3.94 | 16.81 |
| Isodaucene | 1500 | 1504 | – | – | – | – | – | – | 1.59 | 3.20 | 4.65 | – | – | – |
| β-Bisabolene | 1505 | 1507 | – | – | – | – | – | – | – | – | – | – | – | – |
| δ-Amorphene | 1511 | 1514 | 0.83 | 0.50 | 1.18 | – | 0.67 | 0.86 | – | – | – | – | – | – |
| γ-Cadinene | 1513 | 1514 | – | – | – | – | – | – | – | – | – | – | – | – |
| δ-Cadinene | 1522 | 1522 | 1.57 | 0.67 | 0.63 | 1.49 | 0.98 | 0.76 | 0.96 | – | – | 3.24 | – | – |
| (*E*)-iso-γ-bisabolene | 1528 | 1526 | – | – | – | – | – | – | – | – | – | – | – | – |
| Germacrene B | 1559 | 1556 | – | – | – | – | – | – | 1.44 | – | – | 0.85 | – | – |
| Oxygenated sesquiterpenes | | | 48.60 | 47.75 | 34.04 | 25.37 | 19.75 | 29.87 | 8.98 | 4.97 | – | 9.61 | – | 23.02 |
| Germacren D-4-ol | 1574 | 1575 | 13.85 | 7.42 | 2.19 | – | 2.15 | – | 3.29 | 3.27 | – | – | – | – |
| Spathulenol | 1577 | 1576 | 6.73 | 4.10 | 3.41 | 7.67 | 5.64 | 6.71 | – | – | – | 3.51 | – | 9.24 |
| Caryophyllene oxide | 1582 | 1582 | 1.64 | 1.32 | 1.50 | – | – | – | 1.14 | – | – | 4.75 | – | 13.78 |
| Globulol | 1590 | 1590 | – | – | – | – | – | – | – | – | – | – | – | – |
| Carotol | 1594 | 1594 | 15.93 | 21.13 | 18.70 | 12.11 | 2.86 | 17.26 | – | – | – | – | – | – |
| β-Atlantol | 1608 | 1607 | – | – | – | – | – | – | – | – | – | – | – | – |
| Juneno | 1618 | 1618 | – | – | – | – | – | – | – | – | – | – | – | – |

**Table 3** continued

| Constituent | $AI_{lit}$ | $AI_{cal}$ | *A. anchusifolia* | | | | | | *A. megapotamica* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Leaf | | | Stem | | | Leaf | | | Stem | | |
| | | | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 |
| Isolongifolan-7-α-ol | 1618 | 1617 | – | – | – | – | – | – | 0.81 | – | – | – | – | – |
| Caryophylla-4(12),8(13)-dien-5α-ol | 1639 | 1634 | – | – | – | – | – | – | – | – | – | – | – | – |
| Alloaromadendrene epoxide | 1639 | 1636 | – | – | – | – | – | – | – | – | – | – | – | – |
| *ent*-Espatulenol | n.i. | 1636 | – | – | – | – | – | – | 1.50 | – | – | – | – | – |
| Epi-α-cadinol | 1638 | 1639 | 2.80 | 1.75 | 1.90 | 2.15 | 2.91 | 2.10 | 0.78 | 1.70 | – | – | – | – |
| Epi-α-muurolol | 1640 | 1640 | – | – | – | – | – | – | – | – | – | | – | – |
| α-Muurolol | 1644 | 1644 | – | – | – | – | – | – | – | – | – | | – | – |
| β-Eudesmol | 1649 | 1648 | – | – | – | – | – | – | – | – | – | | – | – |
| α-Cadinol | 1652 | 1652 | 3.41 | 1.50 | – | 2.49 | 1.88 | 0.90 | 0.84 | – | – | 1.35 | – | – |
| 14-Hydroxy-9-epi-(β)-caryophyllene | 1666 | 1668 | – | – | – | – | – | – | – | – | – | – | – | – |
| Khusinol | 1679 | 1682 | – | – | – | – | – | – | – | – | – | – | – | – |
| Germacra-4(15), 5,10-trien-1-α-ol | 1685 | 1683 | – | – | – | – | – | – | 0.62 | – | – | – | – | – |
| Eudesma-4(15),7-dien-1 β-ol | 1687 | 1683 | – | – | – | – | – | – | – | – | – | – | – | – |
| 5-Neo-cedranol | 1684 | 1689 | 4.24 | 10.53 | 7.84 | 0.95 | 4.31 | 2.90 | – | – | – | – | – | – |
| Diterpene hydrocarbon | | | – | – | 1.40 | – | – | 0.85 | 2.53 | – | – | 2.66 | – | – |
| Kaurene | n.i. | n.i. | – | – | 1.40 | – | – | 0.85 | 2.53 | – | – | 2.66 | – | – |
| Oxygenated diterpenes | | | – | – | 3.14 | – | – | 3.41 | – | – | – | 4.37 | – | – |
| Manool | 2056 | 2054 | – | – | – | – | – | – | – | – | – | – | – | – |
| Pimaral | n.c. | 2154 | – | – | 1.74 | – | – | 2.56 | – | – | – | 4.37 | – | – |
| *Ent*-8(14),15pimaradien-3β-ol | n.c. | 2195 | – | – | 1.40 | – | – | 0.85 | – | – | – | – | – | – |
| Phenylpropanoids | | | 0.67 | – | – | – | – | – | 8,54 | 2.14 | – | 0.94 | 63.26 | – |
| *o*-Cymene | 1022 | 1023 | 0.67 | – | – | – | – | – | – | – | – | – | – | – |
| 4-Vinylguaiacol | 1309 | 1312 | – | – | – | – | – | – | 1.28 | – | – | – | – | – |
| Eugenol | 1356 | n.i. | – | – | – | – | – | – | 7.26 | 2.14 | – | 0.94 | 63.26 | – |
| Total identified (%) | | | 97.10 | 96.85 | 96.25 | 95.24 | 75.40 | 96.22 | 80.80 | 93.27 | 60.48 | 55.87 | 84.31 | 89.46 |
| Constituent | $AI_{lit}$ | $AI_{cal}$ | *A. nudibasilaris* | | | | | | *A. pilosa* | | | | | |
| | | | Leaf | | | Stem | | | Leaf | | | Stem | | |
| | | | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 |
| Monoterpene hydrocarbons | | | 13.61 | 22.94 | – | 6.55 | 35.26 | – | 10.30 | 29.19 | 31.73 | 2.20 | 49.96 | 36.59 |
| α-Thujene | 924 | 926 | – | 0.94 | – | – | 0.71 | – | – | 1.81 | 0.62 | – | 3.68 | 4.19 |
| α-Pinene | 932 | 933 | 1.25 | 0.94 | – | – | – | – | 0.38 | 7.59 | 3.60 | 2.20 | 1.25 | – |
| Camphene | 946 | 948 | – | – | – | – | – | – | – | – | – | – | – | – |
| Sabinene | 969 | 973 | 0.67 | 1.99 | – | – | – | – | 0.38 | 3.92 | 0.73 | – | 5.81 | 1.81 |
| β-Pinene | 974 | 976 | 5.24 | 0.75 | – | 2.29 | 1.56 | – | 2.30 | 1.45 | 3.11 | – | 14.57 | 3.86 |
| Myrcene | 988 | 990 | 1.88 | 0.27 | – | 2.32 | 0.61 | – | 0.49 | 1.75 | 1.75 | – | 5.45 | – |
| 3-Carene | 1008 | 1010 | 1.00 | 0.30 | – | – | – | – | – | 0.59 | 0.94 | – | – | – |
| D-Limonene | 1024 | 1027 | 2.67 | 16.39 | – | 1.94 | 32.38 | – | 4.56 | 7.93 | 11.64 | – | 19.20 | 26.73 |
| (*Z*)-β-ocimene | 1032 | 1035 | 0.90 | 1.36 | – | – | – | – | – | 2.58 | 4.12 | – | – | – |
| (*E*)-β-ocimene | 1044 | 1046 | – | – | – | – | – | – | 1.72 | 1.57 | 4.00 | – | – | – |
| γ-Terpinene | 1054 | 1057 | – | – | – | – | – | – | 0.47 | – | 1.22 | – | – | – |
| Oxygenated monoterpenes | | | 3.56 | – | – | 4.22 | 4.94 | 18.57 | – | – | – | 5.43 | 1.64 | 3.15 |
| (*E*)-pinocarveol | 1135 | 1138 | 1.16 | – | – | – | – | – | – | – | – | – | – | 3.15 |

**Table 3** continued

| Constituent | $AI_{lit}$ | $AI_{cal}$ | *A. nudibasilaris* | | | | | | *A. pilosa* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Leaf | | | Stem | | | Leaf | | | Stem | | |
| | | | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 |
| 4-Terpineol | 1174 | 1176 | – | – | – | 4.22 | 4.94 | 18.57 | – | – | – | 5.43 | 1.64 | – |
| α-Terpineol | 1186 | 1189 | 2.40 | – | – | – | – | – | – | – | – | – | – | – |
| Bornyl acetate | 1287 | 1285 | – | – | – | – | – | – | – | – | – | – | – | – |
| Sesquiterpene hydrocarbons | | | 26.10 | 23.44 | 43.28 | 34.85 | 26.65 | 43.30 | 58.66 | 48.62 | 41.52 | 72.70 | 25.14 | 20.87 |
| δ-Elemene | 1335 | 1337 | – | – | – | – | – | – | – | – | – | – | – | – |
| β-Elemene | 1389 | 1391 | – | – | – | 2.15 | 0.90 | – | – | – | – | – | – | – |
| Copaene | 1374 | 1375 | 0.87 | 0.32 | 5.11 | – | – | – | – | – | – | – | – | – |
| 1,7-Di-epi-β-cedrene | 1411 | 1413 | – | – | – | – | – | – | – | – | – | – | – | 2.39 |
| *t*-Caryophyllene | 1417 | 1418 | 6.10 | 1.39 | 12.15 | 1.68 | 1.39 | – | 13.72 | 7.98 | 7.20 | 16.77 | 1.25 | – |
| β-Gurjunene | 1431 | 1431 | – | – | – | – | – | – | – | – | – | – | – | – |
| α-(*E*)-bergamotene | 1432 | 1435 | 1.30 | – | – | – | – | – | – | – | – | – | – | – |
| α-Humulene | 1452 | 1452 | – | 1.15 | – | – | – | – | 1.20 | 1.00 | 0.62 | – | – | – |
| β-chamigrene | 1476 | 1474 | 1.78 | – | – | – | – | – | 1.90 | – | – | 4.40 | – | – |
| Germacrene D | 1484 | 1480 | 4.79 | 4.10 | 5.53 | 12.49 | 8.58 | 11.40 | 17.46 | 14.68 | 17.44 | 16.77 | 7.19 | 9.72 |
| β-Selinene | 1485 | 1482 | 1.28 | 1.99 | 12.58 | 2.93 | 1.61 | 15.20 | 1.30 | 0.34 | – | – | – | 8.76 |
| Bicyclogermacrene | 1500 | 1495 | 7.29 | 12.45 | 7.91 | 13.35 | 14.17 | 16.70 | 25.19 | 23.27 | 15.58 | 33.76 | 16.70 | – |
| Isodaucene | 1500 | 1504 | – | – | – | – | – | – | – | – | – | – | – | – |
| β-Bisabolene | 1505 | 1507 | – | 1.40 | – | – | – | – | – | – | – | – | – | – |
| δ-Amorphene | 1511 | 1514 | – | – | – | – | – | – | – | – | – | – | – | – |
| γ-Cadinene | 1513 | 1514 | 1.79 | 0.64 | – | – | – | – | – | 0.97 | 0.68 | – | – | – |
| δ-Cadinene | 1522 | 1522 | – | – | – | 2.25 | – | – | 0.99 | 0.38 | – | – | – | – |
| (*E*)-iso-γ-bisabolene | 1528 | 1526 | 0.90 | – | – | – | – | – | – | – | – | – | – | – |
| Germacrene B | 1559 | 1556 | – | – | – | – | – | – | – | – | – | – | – | – |
| Oxygenated sesquiterpenes | | | 48.60 | 47.75 | 52.92 | 30.26 | 28.55 | 20.70 | 25.53 | 13.29 | 24.34 | 20.02 | 10.13 | 17.51 |
| Germacren D-4-ol | 1574 | 1575 | – | – | – | – | – | – | 11.52 | 3.62 | – | – | – | 16.35 |
| Spathulenol | 1577 | 1576 | 13.21 | 9.53 | 6.29 | 19.29 | 15.60 | 2.70 | 5.43 | 3.15 | 11.70 | 16.17 | 6.52 | – |
| Caryophyllene oxide | 1582 | 1582 | 26.30 | 12.38 | 38.6 | 4.16 | 4.16 | 18.00 | 2.53 | 1.19 | 2.25 | 3.85 | – | 1.16 |
| Globulol | 1590 | 1590 | – | – | 4.20 | – | – | – | 1.81 | 2.81 | 6.97 | – | 2.15 | – |
| Carotol | 1594 | 1594 | – | – | – | – | – | – | – | – | – | – | – | – |
| β-Atlantol | 1608 | 1607 | 2.10 | 1.26 | – | – | 2.20 | – | – | – | – | – | – | – |
| Juneno | 1618 | 1618 | – | – | – | – | – | – | 1.94 | – | – | – | – | – |
| Isolongifolan-7-α-ol | 1618 | 1617 | – | – | – | – | – | – | – | 0.60 | 1.45 | – | – | – |
| Caryophylla-4(12),8(13)-dien-5α-ol | 1639 | 1634 | 6.22 | 1.10 | – | – | – | – | – | – | – | – | – | – |
| Alloaromadendrene epoxide | 1639 | 1636 | – | 1.20 | – | 2.37 | 1.80 | – | – | – | – | – | – | – |
| *ent*-Espatulenol | n.i. | 1636 | – | – | – | – | – | – | – | – | – | – | – | – |
| Epi-α-cadinol | 1638 | 1639 | – | – | – | – | – | – | – | 0.51 | 1.29 | – | – | – |
| Epi-α-muurolol | 1640 | 1640 | 2.21 | – | – | – | – | – | – | – | – | – | – | – |
| α-Muurolol | 1644 | 1644 | – | – | – | – | – | – | 2.30 | – | – | – | – | – |
| β-Eudesmol | 1649 | 1648 | – | – | – | – | – | – | – | 1.41 | – | – | 1.46 | – |
| α-Cadinol | 1652 | 1652 | 1.70 | 3.13 | 3.83 | 3.13 | 2.32 | – | – | – | – | – | – | – |
| 14-Hydroxy-9-epi-(β)-caryophyllene | 1666 | 1668 | 2.69 | 0.83 | – | – | – | – | – | – | – | – | – | – |
| Khusinol | 1679 | 1682 | – | – | – | – | – | – | – | – | 0.68 | – | – | – |
| Germacra-4(15), 5,10-trien-1-α-ol | 1685 | 1683 | – | – | – | – | – | – | – | – | – | – | – | – |
| Eudesma-4(15),7-dien-1 β-ol | 1687 | 1683 | 3.18 | 2.21 | – | 1.31 | 2.47 | – | – | – | – | – | – | – |

**Table 3** continued

| Constituent | $AI_{lit}$ | $AI_{cal}$ | *A. nudibasilaris* | | | | | | *A. pilosa* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Leaf | | | Stem | | | Leaf | | | Stem | | |
| | | | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 | P1 | P2 | P3 |
| 5-Neo-cedranol | 1684 | 1689 | – | – | – | – | – | – | – | – | – | – | – | – |
| Diterpene hydrocarbon | | | – | – | – | – | – | – | – | – | – | – | – | – |
| Kaurene | n.i. | n.i. | – | – | – | – | – | – | – | – | – | – | – | – |
| Oxygenated diterpenes | | | – | – | – | – | – | – | – | – | 4.77 | – | – | – |
| Manool | 2056 | 2054 | – | – | – | – | – | – | – | – | 4.77 | – | – | – |
| Pimaral | n.c. | 2154 | – | – | – | – | – | – | – | – | – | – | – | – |
| *Ent*-8(14),15pimaradien-3β-ol | n.c. | 2195 | – | – | – | – | – | – | – | – | – | – | – | – |
| Phenylpropanoids | | | 3.38 | 1.85 | – | 9.45 | – | – | – | – | 0.88 | – | – | – |
| *o*-Cymene | 1022 | 1023 | – | – | – | – | – | – | – | – | 0.88 | – | – | – |
| 4-Vinylguaiacol | 1309 | 1312 | – | – | – | – | – | – | – | – | – | – | – | – |
| Eugenol | 1356 | n.i. | 3.38 | 1.85 | – | 9.45 | – | – | – | – | – | – | – | – |
| Total identified (%) | | | 96.46 | 79.87 | 96.20 | 85.33 | 95.40 | 82,57 | 94.49 | 91.10 | 87.94 | 92.75 | 86.87 | 78.12 |

*P* population, $AI_{lit}$ arithmetic index from literature, $AI_{cal}$ calculated arithmetic index

certain leaf and stem characteristics, such as the shape of epidermal cells, positioning of the stomata and the presence of secretory structures, play a decisive role in differentiating species. Indeed, the evaluation herein provided a set of useful leaf and stem characteristics for distinguishing four closely related *Aldama* species.

Epidermal features such as the distribution of stomata on the leaf surface (to distinguish *A. nudibasilaris*) and the outline of the anticlinal walls of epidermal cells (to distinguish *A. megapotamica*) have been of important taxonomic value. Both characteristics have also been used to contrast other *Aldama* species (Bombo et al. 2012; Oliveira et al. 2013; Silva et al. 2014).

The non-glandular trichome described herein is common among Asteraceae (Cornara et al. 2001; Adedeijo and Jewoola 2008) and has already been reported in *Aldama* species (Bombo et al. 2012; Oliveira et al. 2013; Silva et al. 2014). However, the presence of epidermal cells with pectin-thickened walls surrounding the non-glandular trichome was reported only by Silva et al. (2014) in the four *Aldama* species analyzed herein. The number of rings in the arrangement of these mucilage-containing cells proved to be an important distinguishing feature for the species. Apart from the role, these cells play as supports (socket cells, Evert 2006); they are also important for storing carbohydrate (Clifford et al. 2002), moisture uptake (Westhoff et al. 2009), reducing transpiration (Zimmermann et al. 2007) and protection against herbivory (Thompson et al. 2014).

The existence of secretory structures of various types and in varying positions in plant body is useful in identifying Asteraceae species (Metcalfe and Chalk 1979; Kelsey 1984; Castro et al. 1997; Appezzato-da-Glória et al. 2008). The presence of glandular trichomes, hydathodes, ducts and cavities has already been reported in *Aldama* (Bombo et al. 2012; Oliveira et al. 2013; Silva et al. 2014) and is confirmed for the species analyzed in this study.

Hydathodes were identified in the leaf margin of *A. nudibasilaris* and *A. pilosa*, with structures similar to those reported for other *Aldama* (Oliveira et al. 2013; Silva et al. 2014). These hydathodes are responsible for guttation, which occurs under conditions of low transpiration and high soil moisture content (Fahn 1979), but this phenomenon has, to date, not been reported for *Aldama* species.

Secretory ducts have already been used for differentiating *Aldama* species (Bombo et al. 2012; Oliveira et al. 2013; Silva et al. 2014). In the leaves, these ducts occur on the midrib parenchyma, primary phloem and also in the sheath extensions of lateral bundles. Nevertheless, those authors did not examine whether the number and distribution of these structures are constant from one leaf or specimen to another. Variations in secretory ducts have already been reported in Burseraceae leaves of different sizes (Kakrani et al. 1991) and in *Pinus taiwanensis* Havata needles from distinct populations (Sheue et al. 2003). For the *Aldama* species examined herein, these characteristics are constant only in *A. anchusifolia*. In the other three species, the number of secretory ducts can vary from one leaf to another in the same individual. Therefore, we recommend that this characteristic should be used carefully, or combined with other characteristics, for differentiating *Aldama* species.

This is the first study to describe the anatomy of the *Aldama* petiole, which helped in differentiating three of the

species selected. According to Magenta (2006), the presence or absence of the petiole in *Aldama* leaves may also play a part in differentiating species. Among the 35 Brazilian *Aldama*, *A. megapotamica* is one of 11 species that develop sessile leaves. In contrast, *A. nudibasilaris* and *A. pilosa*, together with another nine species, have leaves with petioles. The leaves of the remaining 13 taxa can be either sessile or petiolate. In this case, the petioles are usually tiny, ranging from 1 to 3 mm (Magenta 2006), as in *A. anchusifolia*. The presence of secretory ducts in the petiole is useful for identifying the species mentioned.

In terms of stem anatomy, despite close similarities, some features were important in distinguishing species: phloem cells interrupting the pericycle and the presence of inulin crystals (only in *A. pilosa*), inconspicuous hypertrophy and hyperplasia of medulla cells and sclerified medulla (only in *A. megapotamica*), and the presence of secretory ducts in the primary phloem (*A. nudibasilaris* and *A. pilosa*). The last two features have already been reported for *Aldama* and proved useful in delimiting species with very similar morphologies (Bombo et al. 2012; Oliveira et al. 2013; Silva et al. 2014).

Inulin crystals (fructans) were detected only in the stem of *A. pilosa*. This carbohydrate is typical in the underground organs of Asteraceae species (Figueiredo-Ribeiro 1993; Silva et al. 2015; Abdalla et al. 2016; Moraes et al. 2016) and has already been reported, but only in tuberous roots of *Aldama* (Oliveira et al. 2013; Silva et al. 2014; Bombo et al. 2014). It is well known that fructan storage enables plants to tolerate stress conditions, such as drought (Valluru and Van den Ende 2008; Vilhalva et al. 2011; Oliveira et al. 2013) and low temperatures (Hendry 1987; Pontis 1989; Vijn and Smeekens 1999; Portes et al. 2008), both common environmental conditions in the regions in which *A. pilosa* is usually found.

Secretory ducts proved to be the main essential oil secretion sites in all the species studied. The yield of essential oil, as well as its constituents, varied from species to species and from one organ to another in the same species. These variations from one organ to another may be related to the number of secretory structures (Fahn 1979), plant genotype (Gershenzon et al. 2000) and the environmental conditions they are subjected to (Erdtman 1963). The highest yield values were observed for leaves and stems of *A. anchusifolia*, with *A. pilosa* in second place. In contrast, low yield values were found for stems of *A. nudibasilaris* and for the aerial organs of one population of *A. megapotamica*.

In terms of chemical composition, the predominance of sesquiterpenes was noteworthy in oils from leaves and stems of the selected species, in contrast to the predominance of monoterpenes reported for another three species of *Aldama* (Bombo et al. 2012). Diterpenes were also detected in the four *Aldama* species studied. However, they were not useful in differentiating the species, since they occurred in only one population of *A. anchusifolia*, *A. megapotamica* and *A. pilosa*. The presence of these high molecular weight terpenes has already been reported in essential oils from other Asteraceae (Heinrich et al. 2002; Meragelman et al. 2003; Chagas-Paula et al. 2012). Furthermore, the medicinal importance of some diterpenes, such as the Kaurene and Pimarane, has already been reported for *Aldama* (=*Viguiera*) (Ambrosio et al. 2002; 2006; Carvalho et al. 2011). However, the authors identified these constituents by analyzing root extracts rather than essential oils.

Several components identified herein occur widely in Asteraceae (Agostini et al. 2005; Godinho et al. 2014) and in other *Aldama* species (Canales et al. 2008; Bombo et al. 2012). Some of them are highlighted in phytochemical studies as being active biological agents. The antibacterial agents reported in these studies are spathulenol, bornyl acetate, *trans*-caryophyllene, myrcene, germacrene D, bicyclogermacrene and α- and β-pinene (Constantin et al. 2001; Canales et al. 2008; Souza et al. 2007; Carvalho et al. 2011). Bicyclogermacrene and α- and β-pinene also have antifungal effects (Constantin et al. 2001; Silva et al. 2007), whereas α- and β-thujene have anthelmintic effects (Godinho et al. 2014).

As well as being of considerable phytochemical importance, the identification of potential chemical markers to lend support to taxonomic studies is also widely recognized in *Aldama* (Da Costa et al. 1996, 2001; Spring et al. 2003; Ambrosio et al. 2004; Carvalho et al. 2011; Bombo et al. 2012, 2014). In the present study, determining unique constituents in three of the four selected species provides very useful information for chemical differentiation.

In conclusion, this work determined that despite the strong morphological similarities shared by the *Aldama* studied herein, some anatomical features of leaves and stems can be decisive to distinguish these species. Furthermore, we also succeeded in identifying the unique chemical constituents for each *Aldama* species analyzed. Our results indicated that the anatomical and chemical features have potential to elucidate the taxonomical issues raised by this genus.

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