Where is the reward?
Floral nectaries in *Aspidosperma australe* flowers

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Key words:
- Apocynaceae
- Flower
- Nectary
- Rauvolfioid

Abstract

Nectaries are secretory structures that produce a solution rich in sugars, which are involved in animal-plant interactions. Such secretory structures can be externally distinguishable (recognizable), as in glands or discs, or not, as in some gynoecial ones. The absence of floral nectaries has been reported as a characteristic of the genus *Aspidosperma* (Apocynaceae), except for one species with records of nectariferous tissue in the ovary wall. We observed some ants visiting the flowers of *Aspidosperma australe* in the field, which could indicate the occurrence of nectaries in these flowers. So, we aimed to verify the presence of nectar and nectaries in the flowers of *A. australe* in this study, using morphoanatomical analysis. Our results showed a subepidermal differentiated tissue at the base of the ovary, composed of tiny cells with a secretory aspect, rich in starch and polysaccharides. The epidermis cells in this region are smaller, presenting dense content and non-glandular trichomes. We have also achieved positive outcomes through glucose tests conducted on flower nectar. These findings support the presence of an inconspicuous ginoecial nectary at the base of the ovary for *A. australe* and mark the initial confirmation of nectar secretion for this species.

Palavras-chave:
- Apocynaceae
- Flor
- Nectário
- Rauvolfioide

Resumo

Nectários são estruturas secretoras que produzem uma solução rica em açúcares envolvida nas interações animais-plantas. Estas estruturas secretoras podem ser distinguíveis externamente, como no caso das glândulas ou discos nectaríferos, ou não, como nos nectários septais ou não septais do gineceu. A ausência de nectários florais tem sido reportada para espécies do gênero *Aspidosperma* (Apocynaceae), exceto por uma espécie em que se foi registrado tecido nectarífero na parede do ovário. Nós observamos formigas patrulhando/visitando as flores de *A. australe* no campo, o que poderia indicar a presença de néctar e nectários nessas flores. Assim, objetivamos verificar a presença de nectários nas flores de *A. australe* nesse estudo, através de uma análise morfoanatômica. Nossos resultados mostram um tecido subepidérmico diferenciado na base do ovário, composto por células diminutas com aspecto secretor, ricas em amido e polissacarídeos. As células da epiderme dessa região são menores, apresentam conteúdo denso e tricomas não-glandulares. Além disso, tivemos resultados positivos através do teste de glicose conduzidos no néctar das flores. Esse conjunto de características corrobora a presença de nectário inconspícuo na base do ovário para *A. australe* e traz a primeira confirmação da secreção de néctar para essa espécie.

Short Communications

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Nectaries are secretory structures that produce and secrete a solution rich in sugar called nectar (Roshchina & Roshchina 1993) and are related to animal-plant interactions (Pacini *et al*. 2003). The definition of nectary is based on the secretory structure function and secretion of the secretory structure, as the morphology and anatomy are quite variable characters (Pacini *et al.* 2003). There are several types of nectaries in terms of position, origin, structure, and nectar chemical composition (Ray *et al*. 2017). Such secretory structures can be externally distinguishable (recognizable), as in glands or discs, or not, as in some gynoecial ones (Castro & Machado 2013).

In the Apocynaceae family, ring-shaped nectaries are reported, lobed or not (Gomes 2006), as in *Mandevilla* Lindl. (Galetto 1997), *Secondatia* A.DC. (Martins *et al*. 2013), and *Rauvolfia* L. (Gomes 2006). A nectariferous zone at the base of the ovary is reported in species of *Condylocarpon* Desf. (Morokawa *et al.* 2015), *Himatanthus* Willd. (Gomes 2006), and *Hancornia* Gomes (Darrault & Schlindwein 2005). In *Aspidosperma* Mart. & Zucc., there would be no floral nectaries, according to Marcondes-Ferreira (2005). However, a nectariferous tissue at the base of the ovary of *A. quebracho-blanco* Schltdl. was reported by Lin & Bernardello (1999). The sporadic presence of a nectary for *A. macrocarpon* Mart. has also been reported (Gomes & Cavalcanti 2001), and field observations reported ants in flowers of *Aspidosperma australe* Mull.Arg., which may indicate the occurrence of nectar (Koch I., personal communication), despite the absence of any morphologically distinguishable structure identifiable as a nectary.

Anatomical studies could help to corroborate the hypothesis that there are floral nectaries in *Aspidosperma*. So, in this study, we aim to verify the presence of nectaries in the flowers of *A. australe* and describe them.

In order to conduct this study, we collected flowers in pre-anthesis and anthesis of three individuals of *Aspidosperma australe* Müll.Arg., on the campus of the University of Campinas, in the city of Campinas/SP (Tab. 1). To confirm the presence of sugar release, we conducted a sugar test (ST) using urinalysis test strips. Flowers in anthesis and pre-anthesis buds were collected and dissected to expose the interior of the floral tube. Test strips were placed inside the floral tube and held in contact with the base of the tube, near the ovary region, for approximately 30 seconds. The change in color on the strip indicates the presence of sugar and provides an approximate concentration. We also fixed pre-anthesis buds in FAA 50 (Johansen 1940), stored it in 70% ethanol, dehydrated it in an increasing ethylic series, and included it in historesin following the manufacturer's protocol. We cut the blocks in a rotary microtome at 8 μm thickness, stained the slides with Toluidine Blue 0.05% pH 4.7 (O'Brien *et al*. 1964, modified by using acetate buffer), counterstained with ruthenium red, and mounted in water for observation.

We performed histochemical tests using material sectioned in a microtome and subjected to the following reagents and/or dyes: Sudan IV for lipid substances; Lugol for starch; ferric chloride plus sodium carbonate for phenolic compounds (Johansen 1940); bromophenol mercury blue, for proteins (Mazia *et al.* 1953) and PAS (Schiff reagent/periodic acid) (McManus 1948) for general polysaccharides.

We used light microscopy to analyze the slides and photo-documented the results using an Olympus microscope (BX 53) coupled to a photographic camera (Olympus SC 30).

The flower of *A. australe* is complete (Fig. 1a) and pentamerous (Fig. 1b-c). The calyx is dialyssepalous (Fig. 1c), while the corolla is gamopetalous (Fig. 1a,c). The gynoecium is bicarpelar, with free carpels in the region of the ovaries and a united style, with a single style-head (Fig. 1a). The ST indicated the release of a sugary solution around the ovary, and the coloration of the urinalysis strip indicated a sugar concentration of approximately 250 mg/dL. Anatomically, the ovary is formed by a uniseriate outer epidermis, an ovarian mesophyll divided into two regions, and a uniseriate inner epidermis. At the ovary's base, a tissue formed by cells with denser content is observed in the region of the external ovarian mesophyll (Fig. 1d-i). This tissue is continuous throughout the base of the ovary, forming a ring (Fig. 1e), which is not visible to the naked eye. In this region, the outer epidermis consists of smaller cells with denser content compared to the rest of the ovary (Fig. 1g), with numerous non-glandular trichomes (Fig. 1f,h-i), and no stomata were observed. The cells of the outer ovarian mesophyll in this region are tiny, with thin walls, a secretory aspect (Fig. 1d-g), many starch grains (Fig. 1h), and polysaccharides (Fig. 1i). No vascular bundle occurs in the external ovarian mesophyll, the bundles being restricted to the region of the internal ovarian mesophyll (Fig. 1g). Based on the results, we conclude that the nectary of *A. australe* is inconspicuous and located at the base of the ovary.

The Apocynaceae family members are positioned in two paraphyletic grades, rauvolfioids and apocynoids, and tree monophyletic subfamilies, Secamonoideae, Periplocoideae, and Asclepiadoideae (Simões *et al*. 2007; Endress *et al.* 2014, 2019). The flowers of these lineages present gradually more complex characteristics (See Fallen

Table 1 – List of voucher material from individuals collected from *Aspidosperma australe*.

Voucher	Collector e collector number	Localization
UEC124194	*S.M. Gomes 658*	22.8999996185303 S 47.060001373291 W
UEC037501	*S.M. Gomes 656*	22.8999996185303 S 47.060001373291 W
UEC037500	*S.M. Gomes 495*	22.8999996185303 S 47.060001373291 W

1986). The nectary found in *Condylocarpon isthmicum* (Vell.) A.DC. is very similar to the nectary of *A. australe*, with a discreet nectary at the base of the ovary, with small cells and dense cytoplasm, with polysaccharides, starch, and proteins; stomata on the ovary wall through which nectar is secreted during anthesis (Morokawa *et al.* 2015). Floral nectaries are also reported in another species of rauvolfioid grade (Fallen 1983; Simões *et al.* 2007, 2016). They can look like a ring at the base of the ovary, as in *Allamanda* L., *Thevetia* L., *Anechites* Griseb. (Fallen 1983), and *Rauvolfia* (Simões *et al.* 2016), or lobed as in *Vinca* L., *Catharanthus* G.Don., and *Laxoplumeria* Markgr. (Simões *et al.* 2007).

In species of the apocynoid grade, the nectary usually presents as a ring fused at the base and divided at the apex into five lobes, with vascular tissue, nectariferous parenchyma, and epidermis with stomata (Galetto 1997; Martins *et al.* 2013). In *Periploca* L. species, five nectaries surround the upper part of the ovaries and the styles and are covered by unicellular trichomes (Heneidak & Yougasphree 2015).

In species of Asclepiadoideae, the nectaries are presented as a stigmatic nectariferous chamber, covered by the style-head in its upper part, where the secretory tissue corresponds only to the cells of the epidermis (Monteiro & Demarco 2017.).

Despite nectaries being widely recorded in the rauvolfioid grade, the genus *Aspidosperma* has been described as lacking nectaries (Marcondes-Ferreira 2005). This idea is reinforced by Gomes & Cavalcanti (2001) results, who analyzing the floral morphology of nine species of the genus, indicated the sporadic presence of nectaries in only one of them (*A. macrocarpon*), and by Demarco (2005) results, who found no nectariferous tissue in the flowers of *A. australe*. The only unequivocal record of a nectary in the genus was made by Lin & Bernardello (1999) for *A. quebracho-blanco*, who emphasizes that it is a non-functional nectary. In this species, the nectary is disc-shaped and located at the base of the ovary, containing a row of stomata on its external surface. Still, they are constantly closed, with no nectar release, and therefore are considered as a non-functional nectary (Lin & Bernardello 1999).

Our results show that the outer epidermis at the base of the ovary is thinner with non-glandular trichomes. These characteristics, besides the presence of sugary solution around the ovary and the presence of ants as floral visitors, indicate that nectaries found in *A. australe* are functional, at least from the secretory standpoint. We could not observe stomata in the nectary epidermis nor any other obvious structure that could function as a pathway for nectar release. However, we observe small ruptures present in the cuticle wall and the trichomes located around the ovary in *A. australe*, both of which could serve as potential exit routes for nectar since the presence of trichomes is a significant difference between the nectary structure found in this study and that found in *A. quebracho-blanco* (Lin & Bernadello 1999). Nectaries that secrete through trichomes have already been documented in previous literature (Evert 2006; Fahn 1988); however, elucidating the nectar exit pathway is not the focus of this study, and future research should be undertaken to address this aspect.

The confirmation of the presence of nectary in the flower of *A. australe* was possible through anatomical and histochemical analyses that showed the presence of a region in the external ovarian mesophyll with tiny cells, with a thin wall, dense content, and a high presence of starch grains and polysaccharides, differentiating it from the other cells of the mesophyll. These characteristics and its secretory aspect conclude that it is an inconspicuous nectary. We also observed a small protuberance at the base of the ovary with secretory cells in the region. The sporadic presence of these structures, as reported for *A. megalocarpon* (Gomes & Cavalcanti 2001), can explain why our study found a different result than Demarco's (2005). Studies with other species could bring more elements to understand the evolution of nectaries in the family.

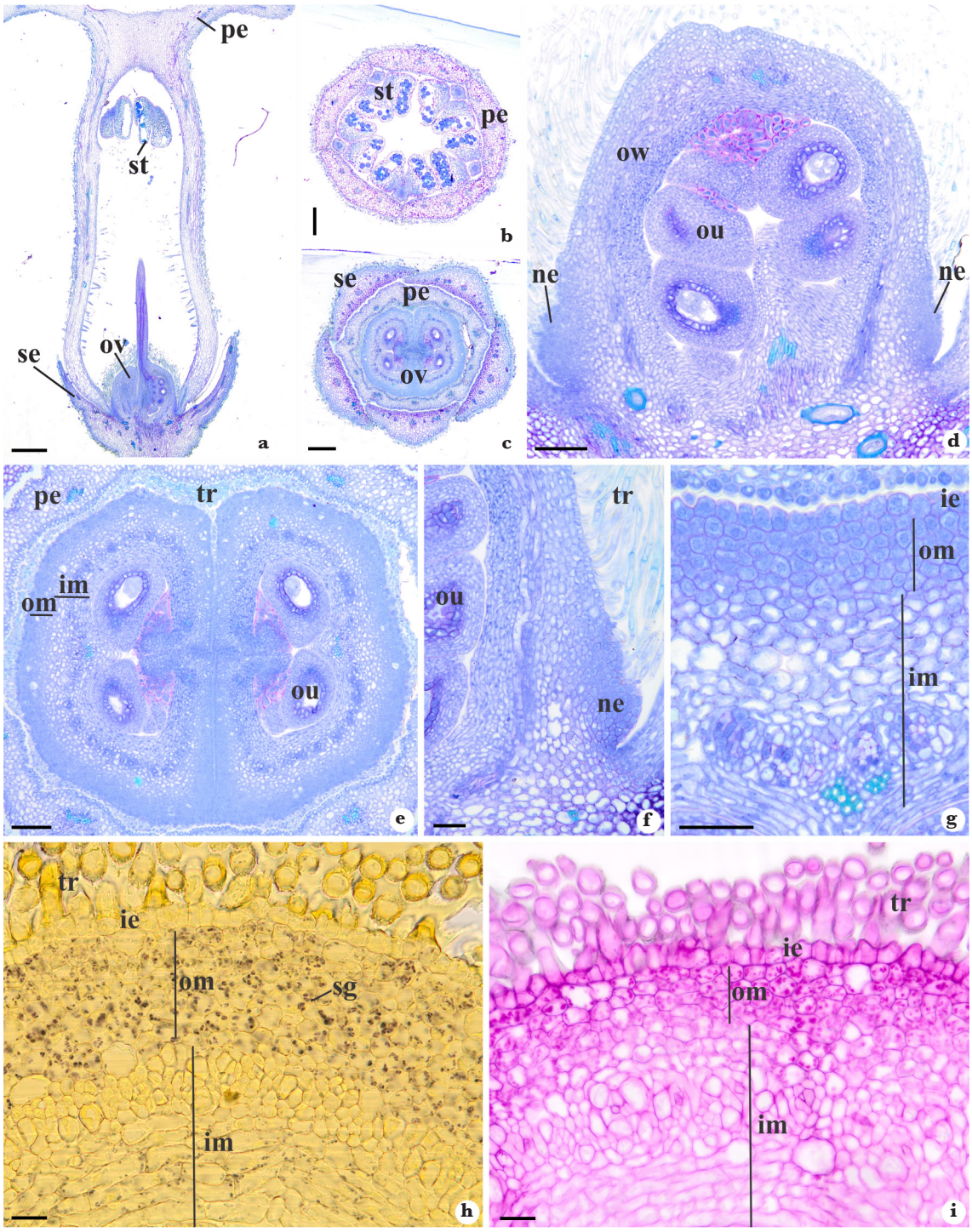

Figure 1 – a-i. Flowers of *Aspidosperma australe* in longitudinal (a, d, f) and transverse (b-c, e, g-i) sections – a. overview of the flower showing gamopetalous corolla and the superior ovary; b-c. flowers in anthesis cut at the level of the anthers and the ovary, respectively; d-g. details of ovary showing the region nectary in the outer ovarian mesophyll, evidenced by tiny cells with dense content, thin walls, and secretory appearance; h. nectary stained with Lugol's solution, highlighting the starch grains (in black); i. nectary stained with PAS, highlighting the polysaccharides (in magenta). ie = inner epidermis; im = inner mesophyll; ne = nectary; om = outer mesophyll; ou = ovule; ov = ovary; pe = petal; se = sepal; sg = starch grain; st = stamen; tr = trichome. Scale bars: a = 500 μm; b-c = 250 μm; d-e = 100 μm; f-g = 50 μm; h-i = 20 μm.

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Data Availability Statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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