

Use of anatomy for the identification of vegetal drugs from two species of *Justicia* (Acanthaceae) marketed as “anador”

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Various medicinal plants used in Brazil are called by the name “anador”, including *Justicia pectoralis* Jacq. and *J. gendarussa* Burm. f. Both plants are used due to the anti-inflammatory and analgesic properties of their components. Their leaves are used, and they can be sold fragmented, making the precise identification difficult. This study aimed to verify the use of leaf epidermal anatomy of the species for the identification of the plant drugs. For the characterization of the epidermis, paradermic sections were obtained from the both sides and for the samples of fragmented leaves, these were dried and later fragmented. The sections and the fragments were clarified and mounted on temporary slides. The main features that allowed anatomical differentiation between the species under study and also the fragmented leaves were the sinuosity of periclinal cell walls, the presence of tector trichomes in *J. pectoralis* and the absence of stomata on the adaxial side and the presence of abnormal stomata in *J. gendarussa*. According to the results obtained, it has been found that due to the drying and fragmentation process used, the anatomical structures observed were still present, with anatomical differentiation between species being possible.

Keywords: *Justicia gendarussa*, *Justicia pectoralis*, medicinal plants.

Emprego da anatomia para a identificação da droga vegetal de duas espécies de *Justicia* (Acanthaceae) comercializadas como anador

Diversas plantas medicinais empregadas no Brasil são denominadas anador, dentre elas *Justicia pectoralis* Jacq. e *J. gendarussa* Burm. f. Ambas são empregadas pelas ações anti-inflamatória e analgésica, além de usos específicos. São empregadas principalmente as folhas, as quais podem ser vendidas rasuradas, dificultando a correta identificação. O trabalho teve por objetivo verificar o emprego da anatomia da epiderme foliar das espécies com o intuito da aplicação para a identificação das drogas vegetais. Para a caracterização da epiderme foram realizadas secções paradérmicas das faces adaxial e abaxial e para as amostras rasuradas as folhas foram secas e posteriormente fragmentadas. As secções e os fragmentos foram clarificados e montados em lâminas temporárias. As principais características que permitem a diferenciação anatômica entre as espécies em estudo e em relação ao material rasurado são a sinuosidade das paredes celulares periclinais da epiderme; a presença de tricomas tectores em *J. pectoralis* e a ausência de estômatos na face adaxial e presença de estômatos anormais em *J. gendarussa*. De acordo com resultados obtidos foi possível determinar que pelo processo de secagem e de fragmentação empregados às estruturas anatômicas observadas continuam presentes, sendo possível a diferenciação anatômica das espécies.

Palavras-chave: *Justicia gendarussa*, *Justicia pectoralis*, plantas medicinais.

1. Introduction

The popular designation of medicinal plant species is important because it provides evidence of their popular use and because it is a source of cultural information [1]. However, several species, often from different genera and families are designated by the same popular names, which leads to misunderstandings in the recognition and employment [2,3,4,5].

Many medicinal plant species used in Brazil receive medicine’s names [2], including two of the genus *Justicia* L. (Acanthaceae): *J. pectoralis* Jacq. and *J. gendarussa* Burm. f., which are popularly designated as “anador” and are mostly used due to the anti-inflammatory and analgesic properties of their components [6,7], being some of the most studied species of the genus [8].

Justicia gendarussa, native to southern and southeastern Asia, is widely cultivated [9] and used for the treatment such as arthritis and rheumatism [8]. In Brazil, this plant is employed for use in rituals and for pain and fever [8,10]. *J. pectoralis*, in turn, occurs in Tropical America and is used to treat stomach ache, muscle injuries, as well as nervous system problems [8] and respiratory diseases [11].

Reproductive structures are needed for accurate identification of botanical species, since vegetative structures are often similar. In the studied species, the leaves are used and marketed, many times fragmented, and they rarely have reproductive structures. Thus, other methods are needed for the proper identification of the species.

Microscopic evaluation, a tool used in botanical recognition and in the verification of a possible adulteration is performed through observation of the cells and tissues [12,13]. For the identification of species of Acanthaceae, plant anatomy has proven to be a very effective tool [14,15], although there are few studies of this type with native species in Brazil [16,17].

The present study aimed to verify the use of the anatomic features of epidermal leaf of two *Justicia*'s species for the identification of these plant drugs.

2. MATERIALS AND METHODS

The plants were grown in the Herbarium of Medicinal Plants of the University of Taubaté - UNITAU, Taubaté, São Paulo, Brazil. The geographic location is 23° 01' 51" S and 45° 30' 34" W and altitude of 565 m. It has a maximum temperature range of 32°C and minimum of 10°C, and average annual rainfall of 1,300 mm [18,19]. With respect to precipitation in Taubaté, seasonal distribution has two distinct and well-defined periods, and summer is very rainy (44 % of annual rainfall), with a dry winter (with only 7% of the annual rainfall). The soil in the planting area is classified as red-yellow latosol, sandy loam texture [20].

Samples were collected in August. Specimens were in the reproductive stage, and adult fully expanded 2nd and 3rd leaves of *Justicia pectoralis* and *J. gendarussa* were used. Vouchers were deposited at the Herbarium SP, under the following registers SP 442080 and SP 442081, respectively.

The collected leaves were divided into two batches: the first was fixed in FAA (formaldehyde: acetic acid: ethyl alcohol 50%, 2:1:18, v/v) [21], for 48 hours and later transferred to ethanol 70%. The second batch was packed in brown paper bags, kept at room temperature until dehydration and the leaves were later fragmented.

For anatomical characterization of the epidermis freehand paradermic sections of the middle region of the adaxial and abaxial sides of the fixed leaves were performed. The dehydrated samples were fragmented using a mortar and pestle to obtain fragments of 1 to 1.5 mm long.

The sections and the fragments were clarified in solution of sodium hypochlorite 50% [22] for 1 hour and mounted on temporary slides with glycerin 50% [23].

The slides were analyzed at photonic microscope and the images were obtained in photomicroscope, with projection micrometer scale.

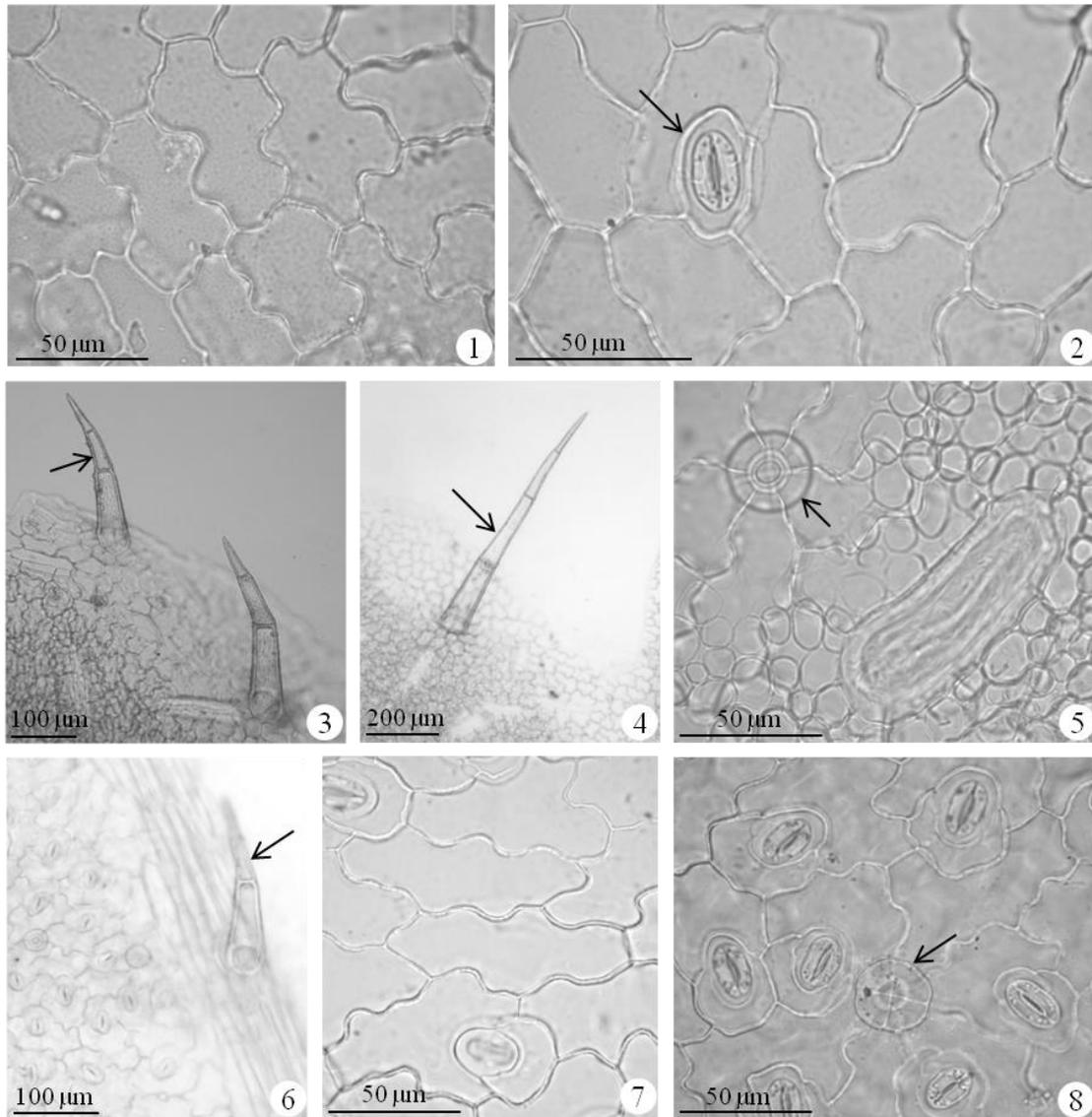
The descriptions and classification of stomata were made according to Inamdar [24] and Inamdar *et al.* [25] and of trichomes, according to Patil & Patil [26].

3. RESULTS AND DISCUSSION

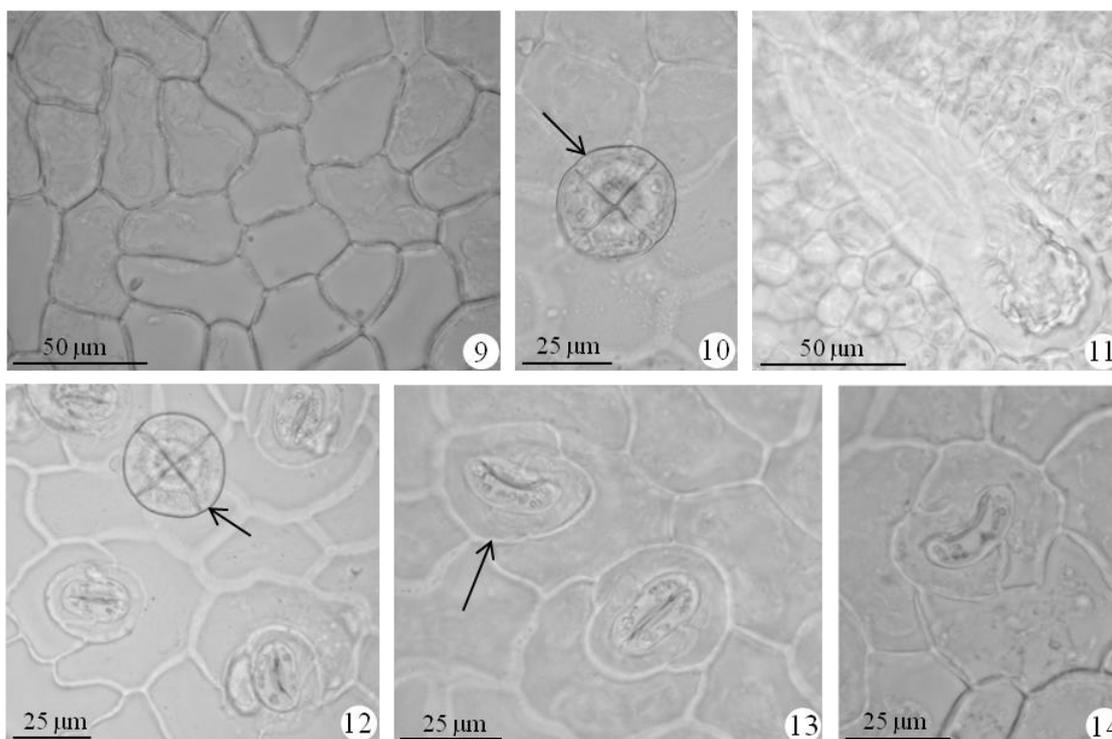
The results showed that *Justicia pectoralis* leaves have, in front view, epidermal cells irregularly shaped with sinuous periclinal walls (Figures 1, 2, 7, 8, 17, 19 and 21) (Table 1), whereas the epidermal cells of *J. gendarussa* have straight periclinal cell walls (Figures 9 and 13) (Table 1).

Another difference was observed in the pattern of distribution of stomata, with *Justicia pectoralis* being amphistomatic (Figures 2, 7 and 8) (Table 1), whereas *J. gendarussa* was hypostomatic (Figures 12-14) (Table 1). Unlike *J. gendarussa*, stomata were always found in

fragmented material of *J. pectoralis* (Figure 18), which corroborates with the distribution pattern observed in paradermal sections.



Figures 1 – 8. Paradermal leaves sections of *Justicia pectoralis* Jacq. 1-5. Frontal view of adaxial face. 1. Epidermal cells irregularly shaped with sinuous periclinal walls. 2. Stomate (arrow). 3-4. Multicellular tector trichomes and uniseriate (arrow). 5. Sessile glandular trichomes (arrow) and lithocyst within cystolith. 6-8. Frontal view of abaxial face. 6. Multicellular tector trichomes (arrow) under the ribs. 7. Stomata. 8. Sessile glandular trichomes (arrow) and stomata.

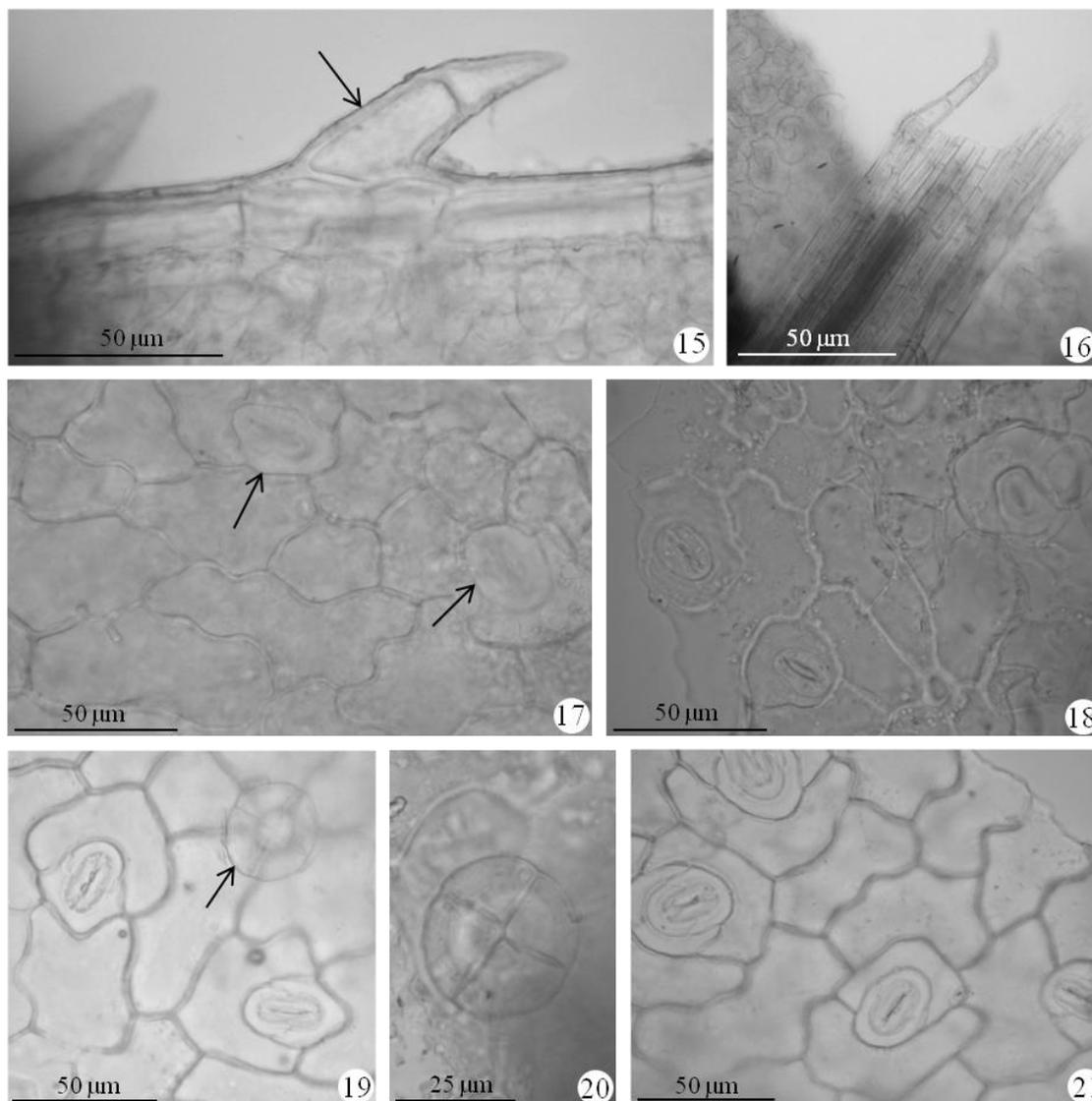


Figures 9 – 14. *Paradermical leaves sections of Justicia gendarussa* Burm. f. 9-11. Frontal view of adaxial face. 9. Epidermal cells irregularly with straight periclinal cell walls. 10. Sessile glandular trichomes (arrow). 11. Lithocyst within cystolith. 12-14. Frontal view of abaxial face. 12. Sessile glandular trichomes (arrow) and stomata. 13. Normal and abnormal stomata (arrow). 14. Abnormal stomate.

Table 1 - Anatomical characteristics of leaves epidermis *Justicia pectoralis* Jacq. and *J. gendarussa* Burm. f. (Acanthaceae).

Characteristics	<i>Justicia pectoralis</i>	<i>Justicia gendarussa</i>
Cellular walls sinuosity	+	-
Stomata position	amphistomatic	hypostomatic
Stomata type	diacytic	diacytic
Abnormal stomata	-	+
Lithocysts/cystoliths	adaxial	both surfaces
Sessile glandular trichomes	+	+
Unicellular tector trichomes	+	-
Multicellular tector trichomes	+	-

+ = presence and - = absence



Figures 15-21. Fragmented material. 15-18. *Justicia pectoralis* Jacq. 15. Multicellular tector trichomes at leaves margin (arrow). 16. Multicellular tector trichomes under the ribs. 17. Epidermal cells irregularly shaped with sinuous periclinal walls and stomata (arrows). 18. Stomata. 19-21. *Justicia gendarussa* Burm. f. 19. Stomata and sessile glandular trichomes (arrow). 20. Sessile glandular trichomes. 21. Stomata.

The stomata are of the diacytic type, positioned slightly above the other epidermal cells (Figures 7 and 12) (Table 1). In *Justicia gendarussa* we found some not fully formed, abnormal stomata (Figures 13 and 14) (Table 1).

In the studied species, differences were observed in the arrangement of lithocysts: in *Justicia gendarussa* they were found in both sides, whereas in *J. pectoralis* they were found only in the adaxial side (Table 1). The lithocysts are elongated, cylindrical, with one end thinner than the other, which was more rounded (Figures 5 and 11).

The species have sessile glandular trichomes in the two sides (Figures 5, 8, 10, 12 and 20) (Table 1). Unicellular tector trichomes were observed in *J. pectoralis* in the margins and multicellular and uniseriate under the ribs (Figures 3, 4, 6, 15 and 16) (Table 1).

The main characteristics that allow anatomical differentiation between the studied species are the differences between the sinuosity of epidermal periclinal cell walls in *J. pectoralis*, the absence of stomata in the adaxial side and the presence of abnormal stomata in *J. gendarussa*, besides the presence of unicellular tector trichomes in *J. pectoralis*.

The sinuosity of the cell wall may vary with environmental conditions, particularly luminosity [27,28,29], however, in the present study the plants are in the same environmental conditions, excluding the adaptive factor of this observed characteristic.

In regarding the distribution of the stomata, Inamdar et al. [25] in a study on the development and structure in Acanthaceae, mentioned *J. gendarussa* as amphistomatic. However, stomata are not seen in the illustration indicated by the authors (figure 3 E of the cited work). Also, the authors do not indicate which side of the leaf is represented in the illustration, which makes it difficult to understand the author's work [30]. Thus, in this study, the different distribution of stomata in the analyzed species, amphistomatic in *Justicia pectoralis* and hypostomatic in *J. gendarussa*, has a distinctive character.

The positioning of diacytic stomata observed in both species and the presence of abnormal stomata in *J. gendarussa* has already been observed in other studies [24,25,30].

Another structure very common in many Acanthaceae are the lithocysts [31]. Although the shape and the arrangement of the lithocysts have taxonomic significance for many taxa of the family [31,32], in the present study, due to the dimensions and thickness of the fragmented material, this observation was difficult.

The species have sessile glandular trichomes in both sides, a common characteristic in many Acanthaceae [30,33,34]. Ahmad [33] mentions the presence of sparse tector trichomes in *J. gendarussa*. In our study, in *J. pectoralis* only unicellular tector trichomes were observed in the margins and pluricellular and uniseriate trichomes were found under the ribs. The structural features observed in *J. pectoralis* leaves are consistent with the description of Tavares & Viana [16], which does not occur with studies of *J. gendarussa* [24,25,33].

4. CONCLUSIONS

According to the results obtained, anatomical differentiation of the species was possible. After the drying and fragmentation processes, the structures observed in plant drugs were kept. Among the main differences, showed up: sinuosity of epidermal periclinal cell walls in *J. pectoralis*, the absence of stomata in the adaxial side and the presence of abnormal stomata in *J. gendarussa*, besides the presence of unicellular tector trichomes in *J. pectoralis*.

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