Diversitas Journal
ISSN 2525-5215
Volume 8, Number 2 (abr./jun. 2023) p. 886 – 895
https://diversitasjournal.com.br/diversitas_journal

Pharmacobotanical study and phytochemical prospection of *Mirabilis jalapa* L.

Estudo farmacobotânico e prospecção fitoquímica de *Mirabilis jalapa* L.

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A B S T R A C T

Mirabilis jalapa L., popularly known as "Maravilha", belonging to the family Nyctaginaceae Juss., is used in folk medicine to treat leukorrhea, itching, swelling, among other diseases. The study aimed to describe anatomical characteristics of root, stem and leaf; identify the sites of accumulation of metabolites in the leaves and perform phytochemical prospecting of the leaves of *M. jalapa*. In the study, the usual methods in plant anatomy were used in the preparation of the analyses of semi-permanent slides containing cross-sections of the root, stem, petiole and leaf blade and paradermal leaf blade of *M. jalapa*. Histochemical tests were also performed in order to locate the metabolites in the leaf blade through cross-sections and phytochemical prospection of methanolic extracts of the leaves, by means of Thin Layer Chromatography. For the root, layers of periderm and pheloderm were observed; on the stem the presence of phloem both near the xylem and the sclerenchyma; in the leaf blade, raffide-type crystals were observed only in the mesophyll region, and presenting amphistomatic leaf blade. For histochemistry it was observed that the crystals are calcium oxalate, in addition to observing phenolic compounds, alkaloids, steroids, lipophilic compounds and lignin. In the phytochemistry was identified mono and sesquiterpenes, triterpenes and steroids, flavonoids, cinnamic derivatives and reducing sugars. The results presented are fundamental for the quality control of the plant drug and the pharmacobotanical standardization of the studied species.

ARTICLE INFORMATION

Article process:
Submetido: 26/11/2022
Aprovado: 31/03/2023
Publicação: 10/04/2023

Keywords:
Anatomy, Histochemistry, Maravilha

RESUME

Mirabilis jalapa L., conhecida popularmente como "Maravilha", pertencente à família Nyctaginaceae Juss., é utilizada na medicina popular para tratar leucorreia, coceiras, inchaço, dentre outras enfermidades. O estudo teve o objetivo de descrever características anatômicas de raiz, caule e folha; identificar os locais de acúmulo de metabolitos nas folhas e realizar a prospecção fitoquímica das folhas de *M. jalapa*. No estudo realizado, foram utilizados métodos usuais em anatomia vegetal na preparação das análises de lâminas semipermanentes contendo secções transversais da raiz, caule, pecíolo e lâmina foliar e paradérmicos da lâmina foliar de *M. jalapa*. Também foram realizados testes histoquímicos, a fim de localizar os metabólitos na lâmina foliar através de secções transversais e prospecção fitoquímica de extratos metanólicos das folhas, por meio da Cromatografia em Camada Delgada. Para a raiz foram observadas camadas de periderme e feloderme; no caule a presença de floema tanto próximo ao xilema quanto ao esclerênquima; na lâmina foliar foram observados cristais do tipo ráfide apenas na região do mesofilo, e apresentando lâmina foliar anfiestomática. Para histoquímica foi observado que os cristais são de oxalato de cálcio, além de observar compostos fenólicos, alcaloides, esteroides, compostos lipofílicos e lignina. Na fitoquímica foi identificado mono e sesquiterpenos, triterpenos e esteroides, flavonoides, derivados cinâmicos e açúcares redutores. Os resultados apresentados são fundamentais para o controle de qualidade da droga vegetal e a padronização farmacobotânica da espécie estudada.

Keywords:
Anatomia, Histoquímica, Maravilha

DOI: 10.48017/dj.v8i2.2510

Introduction

The family Nyctaginaceae Juss has about 30 genera and 400 species, occurring in any region of the tropics (Lorenzi and Souza, 1999). In Brazil, about 11 genera and 65 species are distributed. This family has specimens of variable habits, ranging from large trees to small shrubs and herbs (Flora do Brasil, 2020). Many species of Nyctaginaceae are ornamental, such as *Mirabilis jalapa* L, a perennial herbaceous species, 50-100 cm tall, which has large flowers with a characteristic odor (may be yellow, pink, white or multicolored), leaves arranged oppositely, and protruding tuberous roots (Selvakumar; Kaniakumari; Loganathan, 2012; Gogoi et al., 2016).

Popularly known in Brazil as “batata-de-purga”, “belas-noites”, “jalapa”, “maravilha” or “bonina” (Lorenzi and Souza, 1999; Fenner et al., 2006), the roots of *M. jalapa* are used in folk medicine for the treatment of leukorrhea (Oliveira, 1854) and the juice of the rhizome used as an aphrodisiac. The leaves of the species are applied topically in the itching and to reduce swelling due to fracture or bone torsion (Sharma; Chhangte; Dolui, 2001). Its flowers are used as a food dye (Selvakumar; Kaniakumari; Loganathan, 2012), while the decoction of the entire plant is administered in the treatment of renal and urinary infections and as a diuretic (Sharma; Chhangte; Dolui, 2001).

In addition, extracts from the roots of the species have hypoglycemic and antidyslipidemic activity (Sadiq et al., 2018), stem and leaf extracts have antinociceptive activity (Walker et al., 2008), and leaf extracts have anti-inflammatory and antibacterial activity (Nath el al., 2010; Mohammed, 2012). Watthanachaiyingcharoen et al. (2010) aiming to purify proteins from *M. jalapa* seeds by precipitation with ammonium sulfate found the potential of these compounds as anticarcinogens.

According to Aher et al. (2016) and Zhou et al. (2012), the use of different parts of the species for the treatment of diseases is justified by the presence of different bioactive compounds. Also according to the authors, in the roots and leaves, in addition to proteins, a variety of flavonoids is found, compounds that have a great effect on the treatment of diseases. Thus, in addition to phytochemical and pharmacological studies, studies that characterize the species pharmacobotanyally are necessary. In view of this, the study aimed to describe anatomical characteristics of root, stem and leaf; identify the sites of accumulation of metabolites in the leaves and perform phytochemical prospecting of the leaves of *M. jalapa*.

Materials e methods

Adult specimens of *Mirabilis jalapa* L. were collected in Arapiraca - Alagoas. Exsicata No. 94080 was deposited in the Dárdano de Andrade Lima Herbarium, of the Agronomic Institute of Pernambuco (*Instituto Agronômico de Pernambuco* - IPA) for botanical identification.

Anatomical characterization

For anatomical characterization through light microscopy adult specimens were fixed in FAA 50 (formaldehyde, acetic acid and ethyl alcohol 50%; 1:1:18 v/v) (Johansen, 1940). Main roots, stem and leaves located between the third and fifth nodes were used. Cross-sections were performed in the secondary growth zone of the root, in the stem located between the third and fifth nodes and in the median region of the petiole and leaf blade freehand, using steel slides and medulla of the petiole of "embaúba" (*Cecropia sp.*) as support material. Then, all sections were submitted to a solution of sodium hypochlorite (50%) for discoloration process (Kraus and Arduin, 1997) and washed in distilled water. The cross-sections were stained according to the technique described by Bukatsch (1972), with safranin and Astra blue. Subsequently, all sections were mounted on semi-permanent blades, following usual procedures in plant anatomy (Johansen, 1940; Sass, 1951).

Histochemical characterization

Histochemical characterization was performed in cross-sections of fresh leaf blades, obtained freehand, using steel slides and embaúba petiole marrow as support material. The following reagents were used to indicate the presence of the substances: potassium dichromate (10%) for phenolic compounds (Gabe, 1968); hydrochloric vanillin for tannins (Mace and Howell, 1974); antimony trichloride for triterpenes and steroids (Mace; Bell; Stipanovic, 1974); Dragendorff for alkaloids (Brasil, 2010); Sudan III for lipophilic compounds (Sass, 1951); phrooglucinol for lignin (Johansen, 1940); Lugol for starch (Johansen, 1940) and hydrochloric acid (10%) to establish the nature of crystals (Jensen, 1962). Controls were performed in parallel to the histochemical tests and semi-permanent slides were prepared containing the cross-sections (Johansen, 1940; Sass, 1951). For the analysis of the slides, an optical light and polarization microscope (Leica DM750M) was used, coupled with a digital camera (Leica ICC50W), through which images processed in software (LAS EZ) were obtained.

Phytochemical prospecting

For phytochemical characterization, chromatographic analyses were used. The methanolic extract of *M. jalapa* leaves was analyzed by Thin Layer Chromatography - CCD (Merck silica gel plates). The chromatographic plates were performed using 5 µL of the sample, in chromatographic systems and specific developers for each group according to chart 1.

Chart 1.
Methodology used for the phytochemical analysis of leaf extracts of *Mirabilis jalapa* L.

METABOLITE	MOBILE PHASE	PATTERN	DEVELOPER	REFERENCE
Triterpenes and Steroids	Toluene: AcOEt (90:10)	β-sitosterol Ursoic Acid	Liebermann/ Burchard Δ	Harbone, 1998
Mono and sesquiterpenes	Toluene: AcOEt (97:3)	Carvacrol	Sulfuric Vanillin Δ	Wagner and Bladt, 1996
Alkaloids	AcOEt: Acetic Acid: Formic Acid: Water (100:11:11:27)	Atropine	Draggendorf	Wagner and Bladt, 1996
Coumarins	Hexane: AcOEt (3:2)	Umbeliferona	KOH 5% + EtOH + UV	Neu, 1956; Wagner and Bladt, 1996
Phenylpropaneglycosides	AcOEt: Acetic Acid: Formic Acid: Water (100:11:11:27)	Verbascoside	NEU + UV	Neu, 1946; Wagner and Bladt, 1996
Flavonoids	AcOEt: Acetic Acid: Formic Acid: Water (100:11:11:27)	Kekertine and rutin	NEU + UV	Neu, 1956; Markhan, 1982
Cinnamic Derivatives	AcOEt: Acetic Acid: Formic Acid: Water (100:11:11:27)	Caffeic Acid	NEU + UV	Neu, 1946; Wagner and Bladt, 1996
Hydrolyzable tannins	AcOEt: Acetic Acid: Formic Acid: Water (100:11:11:27)	Gallic acid	NEU + UV	Stiasny, 1912
Condensed tannins (Protoanthocyanidins and leucoanthocyanidin)	AcOEt: Acetic Acid: Formic Acid: Water (100:11:11:27)	Epicatechin	Hydrochloric Vanillin	Roberts et al., 1957
Anthraquinones	AcOEt: Propyl N Alcohol: Water: Acetic Acid (40:40:30:1)	Senosideo A+B	Nitric acid 25% (aqueous) + Δ	Brasil, 2010
Sugars	n-buOH: Me2CO: phosphate buffer pH 5.0 (40:50:10)	Glucose	Triphenyltetrazolium	Metz, 1961

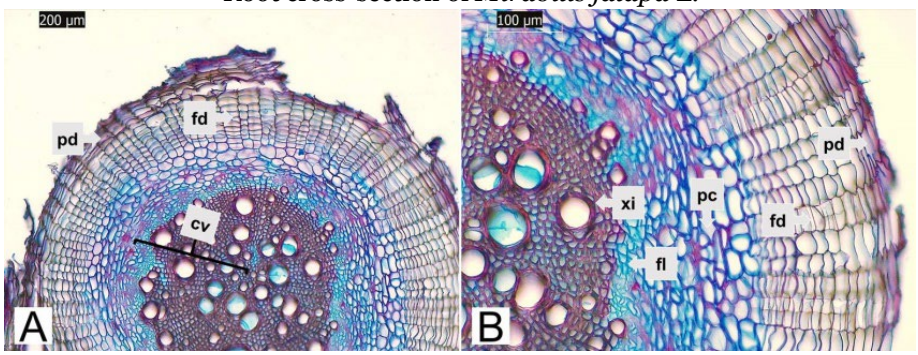
Source: The author.

Results and Discussion

Anatomical Characterization

In cross-section, the root of *Mirabilis jalapa* L. has a circular contour (Figure 1A). The development of secondary growth is observed with the presence of 8-10 layers of periderm, followed by 9-10 layers of pheloderm (Figures 1A and 1B). There are 10-11 layers of cortical parenchyma (Figure 1B). The vascular cylinder occupies the central region of the root with phloem involving the xylem (Figures 1A and 1B).

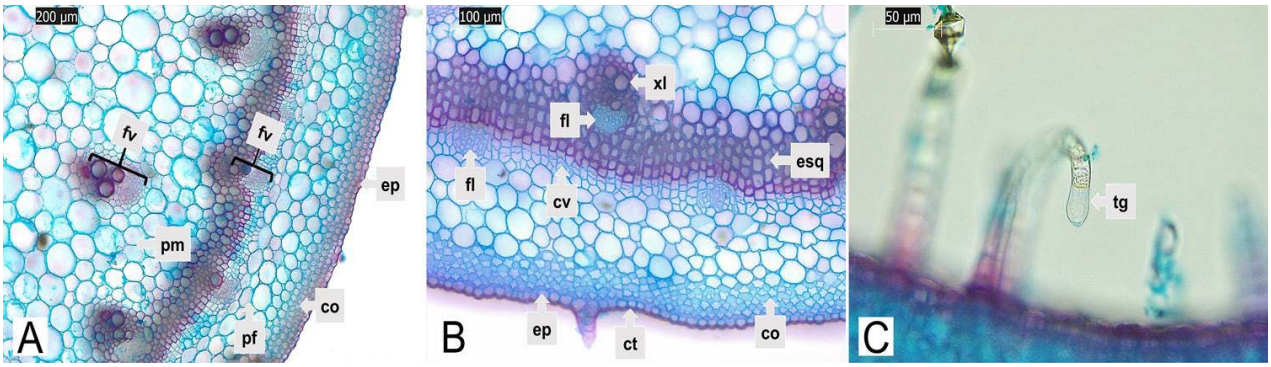
Figure 1.
Root cross-section of *Mirabilis jalapa* L.



A and B: Aspects of the root. cv = vascular cylinder, fd = phelodermis, fl = phloem, fv = vascular bundle, pc = cortical parenchyma, pd = peridermis, xi= xylem.
Source: The author.

The stem in transition to secondary growth, in transverse view, exhibits circular contour (Figure 2A). The epidermis is uniseriate, covered by a thick cuticle layer (Figures 2A and 2B). Adjacent to the epidermis there are 3-4 layers of angular colenchyma (Figures 2A and 2B) and then 4-5 layers of fundamental parenchyma (Figure 2A). Vascular cambium (Figure 2B) and then sclerenchyma (Figures 2B) are observed. The vascular bundles are collateral and are dispersed both in the medullary parenchyma and near the sclerenchyma (Figures 2A). The phloem was observed both adjacent to the xylem and solitary near the sclerenchyma (Figure 2B). In the central region, medullary parenchyma is observed (Figure 2A). Glandular trichomes were observed in the epidermis (Figure 2C).

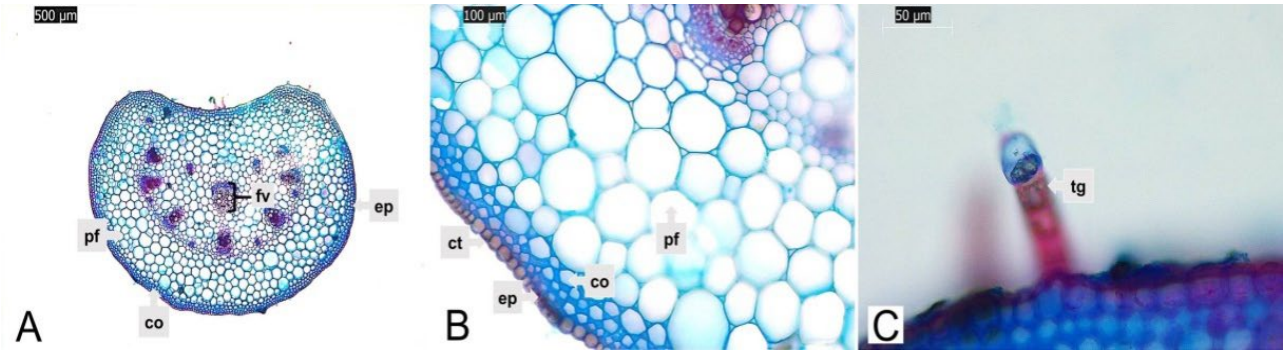
Figure 2.
Cross-section of stem of *Mirabilis jalapa* L.



A and C: Details of the stem in cross-section. co = colenchyma, ct = cuticle, cv = vascular cambium, ep = epidermis, esc = sclemenchyma, fv = vascular bundle, fl = phloem, pf = fundamental parenchyma, pm = medullary parenchyma, xi = xylem, tg = glandular trichomes.
Source: The author.

The petiole of *M. jalapa*, in transverse view, exhibits a biconvex contour (Figure 3A). The epidermis is uniseriate, covered by a slightly thick cuticle layer (Figures 3A and 3B). Next, 3-5 layers of angular cholenchyma are observed (Figures 3A and 3B). The fundamental parenchyma fills the entire petiole (Figures 3A and 3B). The vascular bundles are collateral and are arranged heterogeneously in the fundamental parenchyma, presenting a larger bundle in the central region (Figure 3 A). Glandular trichomes are observed throughout the epidermis (Figure 3 C).

Figure 3.
Cross-section of the petiole of *Mirabilis jalapa* L.



A: General aspect of the petiole. B and C: Details of aspects of the petiole. co = cholenchyma, ct = cuticle, ep = epidermis, fv = vascular bundle, pf = fundamental parenchyma, tg = glandular trichomes.

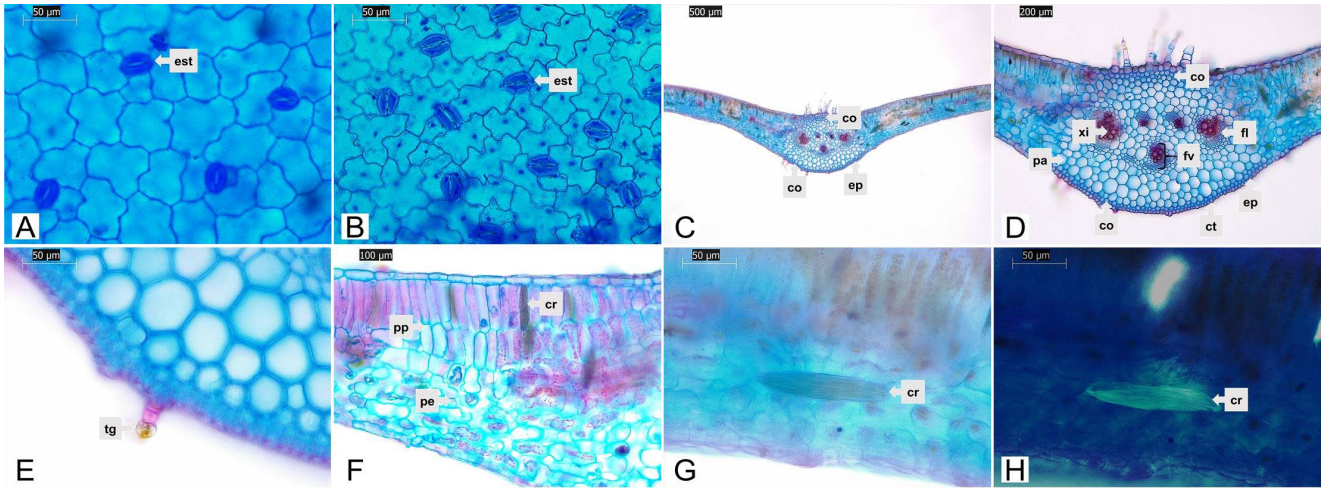
Source: The author.

In the paradermal sections, the leaf blade of *M. jalapa* presents epidermal cells on the adaxial surface with slightly sinuous walls (Figure 4A) and on the abaxial face with sinuous walls (Figure 4B). The leaf blade is classified as amphistomatic with anisocytic and tetracytic stomata on the adaxial face (Figure 4A) and tetracytic and anomocytic stomata on the abaxial face (Figure 4B).

The midrib, in cross-section, has a concave-convex shape (Figures 4C and 4D). The epidermis is composed of a layer of rounded cells, covered by a thin cuticle (Figures 4C and 4D). Adjacent to the epidermis of the adaxial region is 1-3 layers of angular cholenchyma, while in the abaxial region there is only one layer of angular colenchyma (Figures 4C and 4D). After the colenchyma, the fundamental parenchyma is observed, filling the entire rib (Figure 4D). The vascular bundles of the open collateral type are arranged in the center and form three main bundles and two accessory bundles (Figure 4D). Glandular trichomes are observed in the leaf blade (Figure 3E).

The mesophyll is classified as dorsiventral with two layers of palisade parenchyma and about six layers of spongy parenchyma (Figure 4F). Raffid-type crystals are observed scattered in the mesophyll parenchyma (Figure 4F) and it´s possible to observe in light microscopy and polarized light (Figures 4G and 4H).

Figure 4.
Cross-sections and paradermal leaf blade of *Mirabilis jalapa* L.



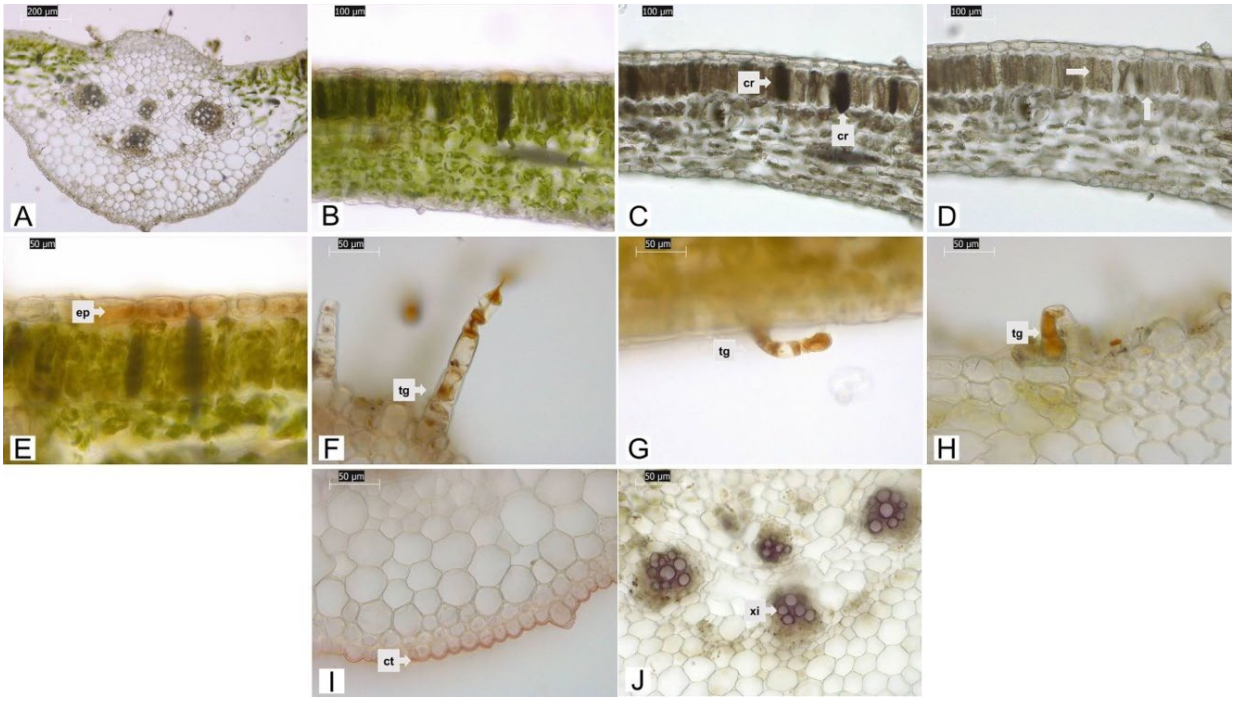
A and B: Paradermal sections of the leaf blade. C: General appearance of the leaf blade. D and E: Leaf blade details. F: Detail of the mesophyll. G and H: Detail of crystals in light and polarized light and light microscopy. co = colenchyma, ct = cuticle, ep = epidermis, est = stomate, fv = vascular bundle, pa = parenchyma, pe = spongy parenchyma, pp = palisade parenchyma, tg = glandular trichomes, xi = xylem, fl = phloem, cr = raffid crystal.
Source: The author.

Histochemical Characterization

Figure 5 A and 5B show in cross-section the leaf blade of *M. jalapa* without the addition of a reagent. The hydrochloric acid test (10%) showed that the raffids are calcium oxalate, after

their dissolution, without blistering (Figures 5C and 5D). Phenolic compounds were found in epidermal cells (Figure 5E) and glandular trichomes (Figure 5F). Alkaloids were identified in glandular trichomes (Figure 5G), as well as triterpenes and steroids (Figure 5H). Lipophilic compounds were found in the cuticle (Figure 5I) and lignin present in the xylem (Figure 6J). Tests for tannins and starch were negative.

Figure 5.
Histochemistry of the leaf blade of *Mirabilis jalapa* L.



A and B: Control, C and D = Hydrochloric acid 10%; E and F = Potassium dichromate; G = Dragendorff; H = Antimony trichloride; I = Sudam III; J = Fluoroglucinol; cr = radid crystal; ep = epidermis; ct = cutícula; tg = glandular trichomes; xi = xylem.
Source: The author.

Phytochemical prospecting

After development of the chromatographic plates, the presence and absence of the metabolites described in Chart 2 were identified:

Chart 2.
Identification of metabolites in ethanolic extract of the leaves of *Mirabilis jalapa* L.

METABOLITE GROUPS	RESULTS
Mono and sesquiterpenes	++
Triterpenes and steroids	+++
Alkaloids	-
Flavonoids	+++
Cinnamic Derivatives	+
Phenylpropaneglycosides	-
Coumarins	-
Condensed tannins	-
Hydrolyzed tannins	-
Anthraquinones	-
Reducing sugars	+

Up to 1 band = +, From 2 to 5 bands = ++, Over 5 bands = +++, absence = -

Source: The author.

The anatomical studies observed in the literature to discuss the results of the present study are from other countries, not finding any correlated study conducted in Brazil.

In the genera *Bougainvillea* (Nyctaginaceae), Chew (2010) reports for root the presence of periderm, phloem involving the xylem, and cortical parenchyma, characteristics similar to those found in the species under study.

In the stem, Al-Garaawi, Ali and Abu-serag (2021) observe in *M. jalapa* found in Iraq, epidermis composed of a single layer of cells, presence of glandular and non-glandular trichomes, region below the epidermis composed of 5-8 layers of colenchyma and 5-8 layers of parenchyma. These characteristics are similar to those found in the study, with the exception of the presence of non-glandular trichomes that were not found and the number of cholenchyma layers that were lower.

As described by Hanani, Prastiwi and Arlina (2017) for *M. jalapa* found in Indonesia, the current study also indicated the presence of trichomes on both sides of the leaf, vascular bundles of the open collateral type, rounded epidermis cells and the presence of calcium oxalate crystals of the raffide type. The authors also describe the presence of calcium oxalate crystals of the cubic and raffide type for the species, but only the raffids were found in the present investigation.

From the anatomical descriptions reported in this study for *M. jalapa*, it´s verified that many of the structures observed are common to descriptions carried out in previous research with the species found in other countries. Thus, these microscopic characteristics can be used for recognition and identification.

Of the metabolite groups investigated, the qualitative phytochemical analysis by colorimetric test of ethanolic extract of *M. jalapa* leaves performed by Mohammed (2012) indicated the presence of glycosides, tannins, phenolic compounds, and alkaloids, and the absence of flavonoids. Another colorimetric phytochemical determination of methanolic extract from all parts of the plant performed by Hanani, Prastiwi and Arlina (2017) identified the presence of carbohydrates, alkaloids, flavonoids, glycosides, tannins, phenols, saponins, terpenes and steroids; also expressed absence of proteins and anthraquinones.

The divergent results obtained in this study, when compared with the results of previous studies, can be justified by the time and place in which the species was collected, water availability, solar incidence, among other reasons, since these are factors that influence the content of secondary metabolites (Gobbo-Neto; Lopes, 2007), in addition to the employed technique.

Comparing the results obtained in histochemistry and phytochemistry, the presence of alkaloids was verified histochemically, but not phytochemically. These conflicting results show the possibility of a false-positive for alkaloids in the histochemical tests, which can be explained

because the Thin Layer Chromatography (*Cromatografia em Camada Delgada*) technique is more specific for the detection of metabolites or due to the fact that CCD for alkaloids was not performed with acid extract.

Final considerations

The use of microscopy techniques for the analysis of the anatomical characters of *M. jalapa* L. allowed the confirmation of data previously described for the species, as well as brought new information, especially about the types of trichomes present in it. With the histochemical and phytochemical tests, the metabolites produced by the leaf of the species were evidenced, with emphasis on the phenolic compounds, which are indicated in the literature as one of the main pharmacologically active groups. The information found in this study provides a basis for the quality control of this plant raw material of great biological importance.

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