Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Anatomical and Phytochemical Characterization of *Physalis angulata* L.: A Plant with Therapeutic Potential

Léa Maria dos Santos Lopes Ferreira[1], Ademir Evangelista do Vale[2], Amancio José de Souza[1], Kelly Batista Leite[3], Cássia Sacramento[3], Maria Lucia Vieira Moreno[4], Thessika Hialla Araújo[1], Milena Botelho Pereira Soares[4], Maria Fernanda Rios Grassi[1,4]

[1]Bahian School of Medicine and Public Health, [2]Faculty of Pharmacy, Federal University of Bahia, [3]Biology Institute, Federal University of Bahia, [4]Gonçalo Moniz Institute, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil

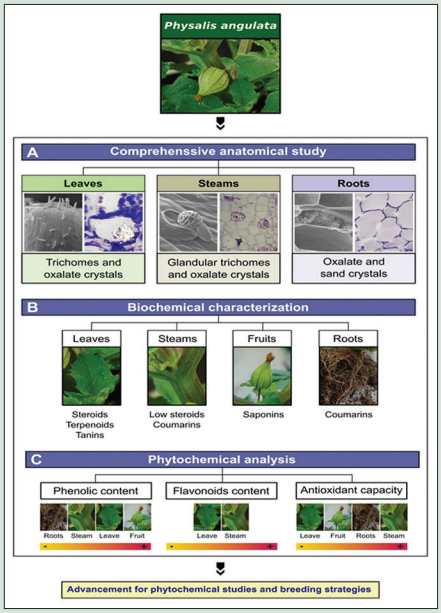
ABSTRACT

Background: *Physalis angulata* L. is widely used in folk medicine. Secondary metabolites with pharmacological potential, including physalins that exhibit anti-inflammatory/immunomodulatory and antiparasitic activities, have been identified in this specie. To date, few studies have investigated storage sites for secondary metabolites in *P. angulata*. **Objective:** The objective of the study is to characterize the anatomical structures and determine the phytochemical composition of the vegetative organs of *P. angulata*. **Materials and Methods:** Electron and conventional optical microscopy was used for the anatomical characterization of *P angulata* organs (leaves, roots, stems, and fruits). Methanolic extracts from leaves, roots, stems, and fruits were chemically characterized for the presence of steroids, terpenoids, tannins, alkaloids, saponins, flavonoids, anthraquinones, coumarins, and phenolic compounds. Phenolic compounds, flavonoid contents, and antioxidant capacity of these extracts were determined by 2,2-diphenyl-1-picrylhydrazyl-free radical scavenging activity. **Results:** Abaxial leaf stomata were more abundant than the adaxial stomata. Trichomes were more abundant along veins in the petioles and stems, beyond the margin in the sepals and petals, and dispersed in the ovary. Steroids and terpenoids were present in leaves, stems, and fruits of *P. angulata*. Saponins were exclusive to fruits. Phytochemical screening did not detect flavonoids, anthraquinones, and alkaloids in all tested plant parts. The highest antioxidant capacities were identified in leaf and fruit extracts, possibly due to the presence of phenolic compounds in these organs. **Conclusion:** This study describes anatomical and biochemical features from *P angulata* that will assist in future phytochemistry and pharmacological studies, particularly pointing toward organs abundant in antioxidants (leaves and fruits) and steroids (possibly physalins; leaves).

Key words: Anatomy, botany, electron microscopy, photochemistry, *Physalis angulata*

SUMMARY

- This work describes the anatomy and chemical composition of *Physalis angulata* organs and serves as a springboard for future phytochemical studies on physalins, assisting a range of fields including plant breeding and pharmacognosy.



Abbreviations Used: DPPH: 2,2-diphenyl-1- picrylhydrazyl, HTLV-1: Human T-lymphotropic virus type 1, TPC: Total phenolic content, TFC: Total flavonoid content, SEM: Scanning electron microscopy, BHA: Buthylated hydroxyanisole.

Correspondence:
Dr. Léa Maria dos Santos Lopes Ferreira,
Av. Dom João Vi, No. 275, Brotas, Salvador, Bahia, Brazil.
E-mail: lmslferreira@bahiana.edu.br

DOI: 10.4103/pr.pr_97_18

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Physalis angulata L., a member of the Solanaceae family, is an annual herbaceous species distributed in tropical and subtropical areas worldwide. In Brazil, *P. angulata* is found in all regions and is widely used in popular medicine to treat chronic rheumatism, kidney, bladder, liver, and skin diseases, as well as for its sedative, antipyretic, and antiemetic properties.[1]

The wide range of biological activities presented by the genus *Physalis* is possibly due to the vast metabolic and structural diversity of compounds present in these plants. Several secondary metabolites with pharmacological properties have been identified in the genus *Physalis*, such as alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, physalins, and withanolides, especially a series of C28 steroidal lactones.[2,3] Studies with secosteroids purified from extracts of *P. angulata* (physalins B, F, or G) or extracts prepared from roots showed potent anti-inflammatory/immunomodulatory[4] and antineoplastic[5,6] activities. Particularly, physalin F has been identified as a substance with

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Ferreira LM, do Vale AE, de Souza AJ, Leite KB, Sacramento C, Moreno ML, *et al*. Anatomical and phytochemical characterization of *Physalis angulata* L.: A plant with therapeutic potential. Phcog Res 2019;11:171-7.

pharmacological potential, presenting an immunosuppressive effect on the proliferation of human T-lymphotropic virus type 1-infected cells[7] and *Trypanosoma cruzi*,[8] as well as antileishmanial[9] and antiplasmodial[10] activities.

Few studies have characterized the anatomical structure of *P. angulata* and the tissular distribution of secondary metabolites in the species. Glandular trichomes located in the epidermis of plants are important sites of synthesis, secretion, and/or storage of compounds such as terpenoids, alkaloids, and tannins.[11,12]

To determine the secondary metabolites with pharmacological properties in *P. angulata,* comprehensive anatomical description and phytochemical characterizations of the vegetative organs were conducted. This work sheds light on functional aspects of the secretory structures and might contribute to improve the bioprospecting process and consequently the plant's pharmacological potential.

MATERIALS AND METHODS

Plant materials

Adult *P. angulata* individuals [Figure 1a-c] were collected in summer (November, 2017) at the Horto Florestal Experimental Unit of the Universidade Estadual de Feira de Santana (UEFS), Bahia, Brazil. Specimens were harvested at physiological maturity. A voucher specimen was deposited in the Herbarium of UEFS (Voucher number: 110448).

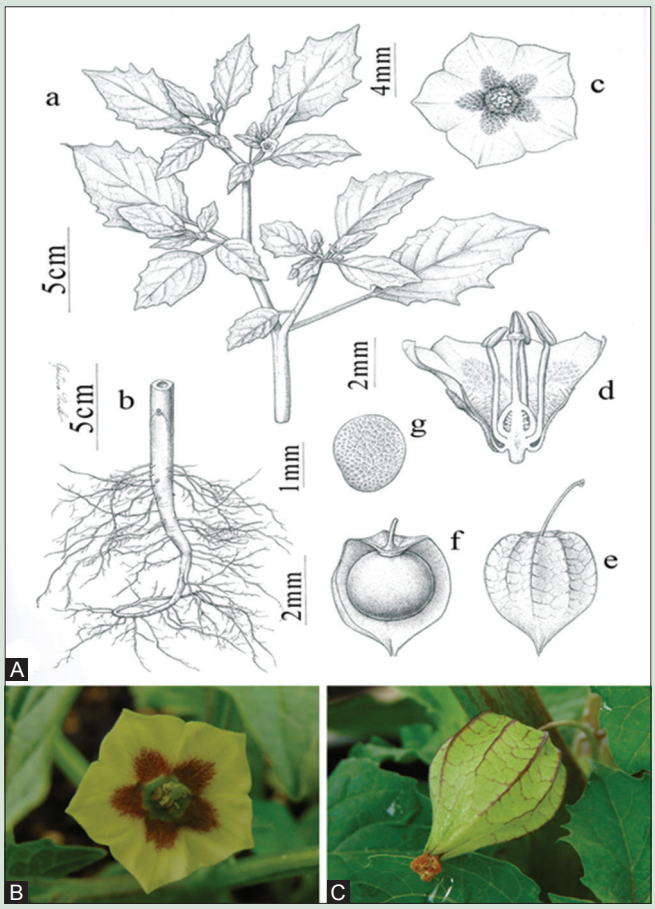


Figure 1: Structure of *Physalis angulata* L. (A) Plant parts, a: General aspect of the branch; b: Primary and secondary roots; c: Corolla in front view; d: Reproductive structures: Stamens and pistil (ovary and stigma); e: Fruit wrapped in the fruitful cup; f: Fruit; g: Seed. (B) Flower, (C) Fruit. *P. angulata* growing in an experimental field at the Horto Florestal Experimental Unit of the Universidade Estadual de Feira de Santana, Bahia, Brazil

Anatomical analysis

Tissues from three *P. angulata* individuals were fixed in FAA70 (formaldehyde, acetic acid, and ethanol) and stored in 70% alcohol. The leaves (apex, base, and central portion), petioles, stems, and roots were embedded in methacrylate (Historesin; Leica Biosystems, Nussloch, Germany) in accordance with manufacturer's instructions and then sectioned on a rotary microtome using disposable steel razors. Sections were stained with 0.05% toluidine blue to detect cellulose and lignin in cross-sections and slides mounted with Entellan (Merck, Darmstadt, Germany). The description of anatomical structure was done according to Metcalf and Chalk.[13] The measurement of stomatal density was performed using the ANATI QUANTI software (Federal University of Viçosa, Viçosa, Minas Gerais, Brazil).[14] The paradermal sections were obtained by dissociation and stained with safranin to give contrast in the paradermal cut. The material was photographed on a QImaging Go-3 camera coupled to the Olympus BX 41 optical photomicroscope.

Crystal tests

Histochemical acid solubility was employed to characterize the anionic saline nature of the crystals, by subjecting the crystals of the test material to acetic acid A. R. (Analytical grade reagent) and hydrochloric acid 10% (v/v) aqueous in roots, stems, and leaves of *P. angulata*. The calcium oxalate crystals were insoluble in acetic acid and soluble in hydrochloric acid, without producing effervescence.

Phytochemical tests

Roots, stems, leaves, and fruits were separated, dried to a constant weight in a 36 ± 2°C oven (Fanem, Mod. 320-SE), and then ground in a Wiley mill (Tecnal, TE 650). Powdered samples (30 g) were extracted by maceration with 300 ml of methanol for 72 h at room temperature (30°C). The extracts were filtered (using Whatman no. 1 filter paper) and concentrated under reduced pressure using a rotate evaporator (IKA RV 10 digital) at 40 ± 2°C. The yields of the methanol extracts were between 3.1% and 9.6% by dry weight. All dry crude extracts obtained were stored at 8°C in airtight containers until analysis. A stock solution of crude extract (3.0 mg/mL) was dissolved in 10 mL of methanol for phytochemical screening. Qualitative screening for secondary metabolites such as flavonoids, coumarins, tannins, saponosides, steroids and terpenoids, anthraquinones, and alkaloids was carried out according to Trease and Evans, 1983, and Harborne, 1998.[15,16]

Antioxidant activity

The antioxidant activity (AC) of extracts was determined using the *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging radical method.[17] In short, serial dilutions of the extracts of parts of *P. angulata* were prepared in methanol (0.625, 1.25, 2.5, and 5.0 mg/mL), and 50 µl of the samples was added to 200 µl DPPH (0.2 mM) in methanol in 96-well microtiter plates. Quercetin was used as a reference/standard compound. DPPH solution and methanol served as blank. Absorbance was determined at 517 nm using a microtiter plate reader (Bio-Rad Elx 800), and the percentage of DPPH radical scavenging activity (% RSA) was calculated according to the following equation:

%RSA = 100 × ([absorbance of control-absorbance of sample]/absorbance of control).

Determination of total phenolic content

The determination of the phenolic compounds was performed with the Folin–Ciocalteu method using gallic acid as standard.[18] Briefly,

20 µL of each extract (300 mg L in methanol) was mixed with 100 µL of the Folin–Ciocalteu reagent in a microplate and shaken for 4 min followed by the addition of 75 µl of sodium carbonate (100 g/L). After a 2-h incubation in the dark at room temperature, the absorbance was measured at 750 nm on the Bio-Rad Elx 800 microplate reader. The reaction blank was prepared with distilled H_2O. All assays were performed in triplicate.

Determination of total flavonoid content

Total flavonoid content (TFC) was measured using a modified spectrophotometric method, as proposed by Chatatikun and Chiabchalard.[19] Briefly, 50 µL of extracts (1000 mg/L) or quercetin was added to 10 µL of a 10% aluminum chloride solution followed by the addition of 150 µL of 95% ethanol. The blank was prepared with 80% ethanol. About 10 µL of 1 M sodium acetate was added to the mixture in a 96-well microplate and incubated for 40 min at room temperature protected from light. The absorbance was measured at 415 nm on a Bio-Rad Elx 800 microplate reader. The TFC was calculated using a quercetin standard curve. The results were expressed as mg quercetin equivalent gram of extract. All samples were analyzed in triplicate.

Scanning electron microscopy analysis

Fragments of the vegetative and reproductive parts of *P. angulata* were fixed with 2.0% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) in sodium cacodylate buffer (0.1 M, pH 7.2) for 1 h at room temperature. After fixation, samples were washed three times with sodium cacodylate buffer (0.1 M, pH 7.2). Cells were then postfixed with a solution of osmium tetroxide for 30 min and dehydrated in increasing concentrations of ethanol (30%, 50%, 70%, 90%, and 100%). The samples were dried until the critical point, method with CO_2, mounted on aluminum stubs, metallized with gold, and analyzed in a JEOL JSM-6390 LV scanning electron microscope.

Statistical analyses

A linear regression was used for calculating IC_{50} values. Results were considered statistically significant when $P < 0.05$. All analyses were performed using Analyse-it software regression method (Leeds, United Kingdom).

RESULTS

Leaf anatomy

Stomata

The leaf epidermal surfaces of *P. angulata* exhibit uniseriate cells with sinuous walls. The species is amphistomatic (stomata on both surfaces) with anisocytic-type stomata (surrounded by three subsidiary cells) in the epidermis presenting striated cuticle [Figure 2a]. Higher concentration of stomata was observed in the abaxial face (370.1 ± 152.8 mm^2) compared to the adaxial surface (269.2 ± 167.2 mm^2).

Trichomes

A comprehensive analysis of types and quantities of trichomes in the different plant tissues was conducted. Multicellular tectorial and glandular capitate trichomes with single-celled stalks were more abundant along veins in the petioles [Figure 2b and c] and stems [Figure 2d and e], beyond the margin in the sepals [Figure 2f] and petals [Figure 2g] and dispersed in the ovary [Figure 2h]. Scattered tectorial [Figure 2i] and glandular [Figure 2j] trichomes were observed in adaxial and abaxial surfaces of the leaf blade.

Mesophyll

The mesophyll structure is characterized by a dorsiventral organization (palisade and spongy parenchyma), comprising one layer of palisade parenchyma and three layers of spongy parenchyma [Figure 3a and b]. The vascular bundles were bicollateral, i.e., the xylem is found between layers of phloem [Figure 3c]. Numerous crystals of calcium oxalate, druses, were found in the mesophyll (palisade and spongy parenchyma) [Figure 3d].

Stem anatomy

The collenchyma was angular with two layers of cells, the cortex presents three layers of cells, the stem was hollow [Figure 4a], and numerous crystals of prismatic calcium oxalate were observed in the parenchyma [Figure 4b].

Root anatomy

The cortex exhibits about 3–4 layers of cells, and the medulla was filled with parenchyma cells [Figure 4c]. Numerous crystalline sands and crystals of prismatic calcium oxalate were observed in the cortex [Figure 4d-f].

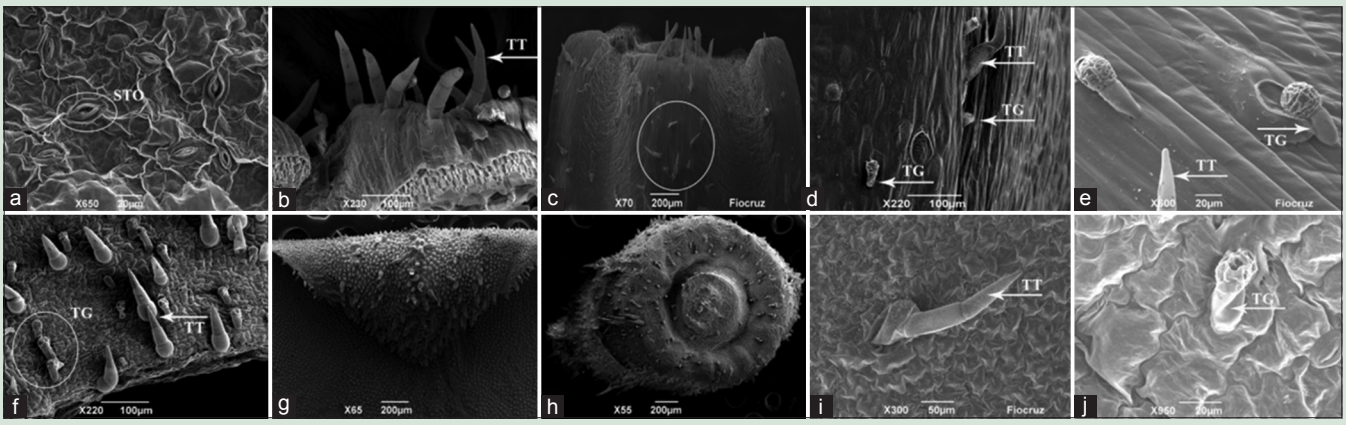


Figure 2: Transmission electron microscopy images of the epidermis of *Physalis angulata* L. Stomata in leaf (a). Tectorial trichomes in petiole (b). Trichomes aggregated on the mid-veins of petioles (c) and in stems (d). Detail of tectorial and capitate glandular trichomes (e); Distribution of trichomes on sepal (f), petal (g), and ovary (h). Detail of tectorial (i) and glandular (j) trichomes in leaf blade. STO: Stomata; TT: Tectorial trichome; GT: Glandular trichome

Phytochemical composition

The phytochemical screening of leaf, stem, root, and fruit of *P. angulata* revealed the presence of steroids, terpenoids, and tannins mainly in the leaf. Saponins were found only in the fruits. Flavonoids, anthraquinones, and alkaloids were absent in all parts of the plant [Table 1].

The maximum amount of total phenolic content (TPC) was observed in the root (30.63 mg GAE/g) and the lowest in the fruit (26.28 mg GAE/g) extracts [Table 2]. The equation for the gallic acid standard calibration curve was y = 0.0051x +0.0802 (R^2 = 0.9988). The content of TPC followed the pattern: Root > stem > leaf > fruit.

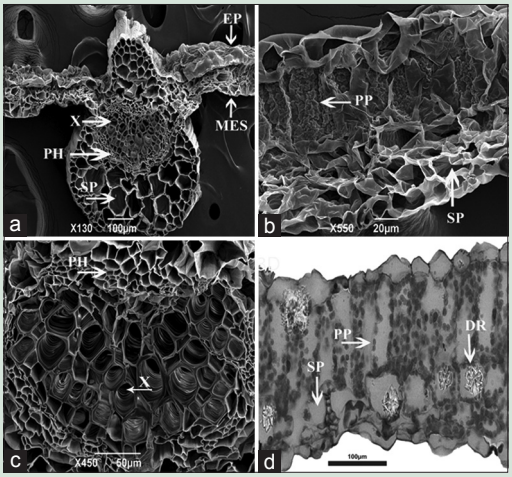


Figure 3: Anatomical details of the leaf of *Physalis angulata* L. Transmission electron microscopy images of palisade and spongy parenchyma with xylem and phloem (a); detail of parenchyma (b) and central vein (c). Optical microscopy of druses in mesophyll (d). EP: Epidermis; MES: Mesophyll; PP: Palisade parenchyma; SP: Spongy parenchyma; X: Xylem; Ph: Phloem; DR: Druse

The leaf extract yielded the highest amounts of TFC (32.41 mg QE/g extract), whereas none was detected in roots and fruits [Table 2]. The equation for the quercetin standard calibration curve was y = 0.0056x + 0.0473 (R^2 = 0.9982).

The highest AC was found in leaf and fruit extracts of *P. angulata* [Table 3].

DISCUSSION

To date, only a few anatomical studies have been reported on *P. angulata* and these are exclusively based on optical microscopy.[20-22] In this work, a comprehensive anatomical description of *P. angulata* was performed using scanning electron microscopy analysis. In agreement with previous reports on *P. angulata*, stomata were found in both faces of the leaf epidermis, with a higher number in the abaxial face.[22,23] The stomata was anisocytic and protrusive, diverging from other reports that found sporadically anomocytic stomata.[20]

Glandular trichomes are crucial in the production of chemicals. In this study, glandular capitate and tectorial trichomes were observed on the mid-veins of petioles and the stems. This pattern of distribution is present in the Solanaceae *Withania somnifera* and has been associated with the protection of the underlying vasculature[24] and to an improved access to nutrients and chemicals originated from the vascular bundles. Within Solanaceae, alkaloids and phenolic compounds are synthesized in the roots and transported to the leaves. Glandular trichomes are therefore located on mid-veins for easy sequestration of these compounds.[24]

The parenchyma of leaf, stem, and root was similar to that previously described for *P. angulata*.[20] Regarding the vascular structures, bicollateral bundles were found in leaves and stems, as reported previously for Solanaceae.[25] However, the stem was hollow in all evaluated parts (apex, middle, and base), which contradicts a previous report of solid stem.[21]

Several oxalate crystals in the form of druses were found in the parenchyma of leaves and in the cortex of *P. angulata* stems, while crystals with a prismatic shape were present mainly in the root cortex. The formation of crystals is a physiological process that regulates the

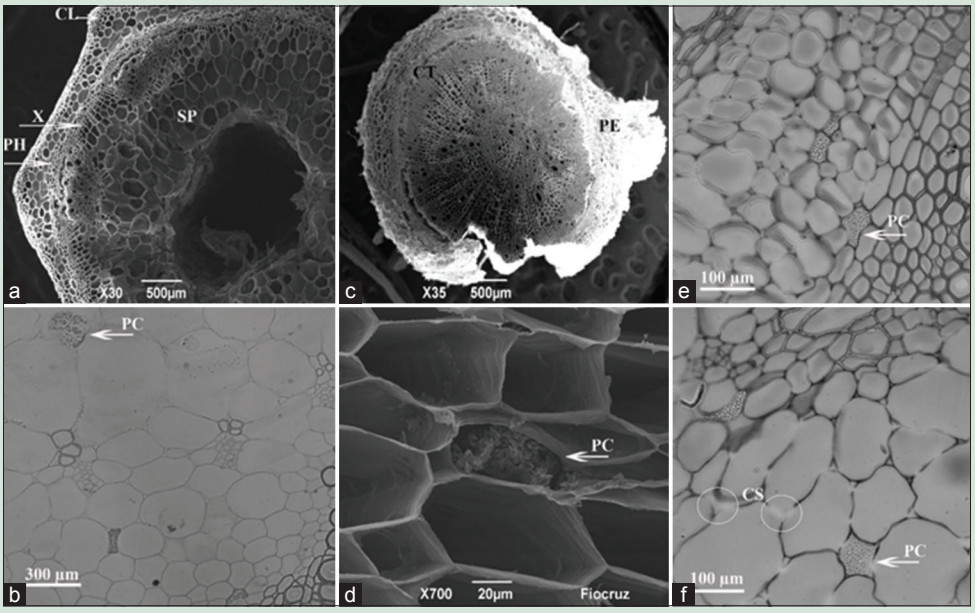


Figure 4: Anatomical details of *Physalis angulata* L. Transmission electron microscopy image of collenchyma, cortex (spongy parenchyma), conducting vessels (xylem and phloem), and hollow stems (a). Prismatic crystals in the stem parenchyma (b). Transmission electron microscopy image of root (c). Prismatic crystals (d and e) and crystal sands (f) in root. EP: Epidermis; CT: Cortex; Ph: Phloem; X: Xylem; SP: Spongy parenchyma; PC: Prismatic crystals; CS: Crystal sands, PE: Periderm

Table 1: Phytochemical constituents of methanolic extracts from different parts of *Physalis angulata*

Part	Steroids	Terpenoids	Tannins	Alkaloids	Saponins	Flavonoids	Anthraquinones	Coumarins
Leaves	+++	+++	++	–	–	–	–	–
Stems	+	+	–	–	–	–	–	+
Roots	–	–	–	–	–	–	–	++
Fruits	+	–	–	–	++	–	–	–

–: Negative; +: Mild positive; ++: Positive; +++: Highly positive

Table 2: Total phenolic and flavonoid content present in *Physalis angulata*

Part	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Leaves	27.39±0.08	32.41±0.015
Stems	30.24±0.056	9.91±0.003
Roots	30.63±0.058	Nd
Fruits	26.28±0.055	Nd

Values were expressed as mean±SD. (*n*=5). Nd: Not determined; GAE: Equivalent of gallic acid; QE: Quercetin equivalent; SD: Standard deviation; TPC: Total phenolic content; TFC: Total flavonoid content

Table 3: Half maximal inhibitory concentration values of 2,2-diphenyl-1-picrylhydrazyl-free radical scavenging activity of extracts of *Physalis angulata*

Part	DPPH (IC_{50} mg/mL)
Leaves	7.24±0.012
Stems	38.05±0.022
Roots	15.25±0.04
Fruits	8.34±0.014

Values were expressed as mean±SD. (*n*=5). DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: Half maximal inhibitory concentration; SD: Standard deviation

calcium dynamics in plants and has an additional role in the protection against herbivores and in the detoxification of several heavy metals.[26] Prismatic crystals have been reported in the leaves of other species of Solanaceae, such as tomato and tobacco.[27]

The chemical composition of plant extracts found among species of Solanaceae varies both in quality and in quantity.[28] Determining the phytochemical tissue, composition of a species improves compound isolation procedures and bioprospection. The presence of secondary metabolites in parts of *P. angulata* was determined. Steroids, terpenoids, and tannins were present mainly in leaves of *P. angulata* [Table 1].

These results corroborate phytochemical studies that revealed withanolides (steroids) and chemotaxonomic markers in Solanaceae.[29] The withanolides are the main secondary metabolites in *Physalis* spp. with almost 60 types synthesized, especially physalins, ixocarpalactones, and acnistins.[28,30] The biological proprieties of withanolides include action against tumor cells,[5,6,31] parasites such as *T. cruzi*[8] and *Leishmania*,[9] and in addition to antimicrobial,[32] immunomodulatory/anti-inflammatory activities.[4,33] Other common steroids such as β-sitosterol, stigmasterol, campesterol, and methylene-cholesterol can also be found in *Physalis* spp.[34]

The alkaloids are abundant in Solanaceae, particularly tropane, steroidal, indole, pyrrolidine, and imidazole variants.[29] The isolation and identification of alkaloids in *Physalis* can be challenging. The isolation and identification of the alkaloids such as N-trans-feruloyltyramine, N-p-coumaroyltyramine, and phygrine have been reported in at least one species of the genus (*P. alkekengi var. franchetii*).[4,28,35] In the present study, however, alkaloids were not detected in any investigated parts [Table 1]. This suggests that the concentrations of alkaloids in *P. angulata* might be very low and thus not detectable by the method employed or, alternatively, that these plants do not synthesize this class of chemicals.

The present study identified terpenoids in leaves of *P. angulata*. Terpenoids are essential for plant survival. These compounds have been associated with antimicrobial, antifungal, antiparasitic, antiviral, antihyperglycemic, antihypoglycemic, anti-inflammatory, and immunomodulatory properties.[36] Except for carotenoids, terpenoids are rare in *P. angulata*; in contrast, two studies have isolated labdane diterpenoids from *Physalis coztomatl* and *Pitcairnia sordida*.[37,38]

The presence of terpenoids in trichomes has been well documented in Solanaceae[39-41] as well as other plant families including Lamiaceae[42] and Asteraceae.[43] Other species such as *Petunia hybrida* accumulate functional (insecticidal) steroidal compounds in trichomes.[44] Therefore, it is possible that glandular trichomes in *P. angulata* harbor the site for the synthesis of terpenoid-derived physalins.[20] This study found that trichomes in *P. angulata* are in lower numbers as compared with other species of *Physalis* (*Physalis peruviana* and *Physalis pubescens*) or other Solanaceae.[20,21] Genetic engineering would be alternative to increase trichome density on *P. angulata*[40,45] and consequently physalin production. This approach has been successfully used in transgenic canola.[45,46] This highlights the importance of anatomical studies coupled to phytochemical characterization in the plant with therapeutic potential.

Phenolic compounds (including flavonoids) may react with free radicals in the cell, conferring antioxidant properties that have the potential to inhibit pathological and degenerative processes, such as cancer. The amount of total phenolic and flavonoid compounds, as well as the antioxidant capacity found in the present study, was slightly lower than those reported previously [Table 4].

A comparison of the absolute values among these studies is limited due to methodological differences in compound quantification, extract production, as well as types of plant parts studied. Previously, *P. angulata* extracts were shown to have relevant AC, presenting an antitumor effect on human oral cancer cells[50] and on intestinal inflammation in a rat model.[51] A mechanism for the possible inhibition of carcinogenesis by phenolic compounds remains unclear; however, the antioxidant properties might play an important role blocking the molecular events involved in all stages of cancer development.[52] In this study, the highest antioxidant capacity was identified in extracts obtained from leaves and fruits of *P. angulata*. The antioxidant capacity is positively associated with the amount of phenolic, flavonoid, and aromatic compounds present in a given extract. Interestingly, flavonoids were not detected in fruits and roots, and this is consistent with findings from Medina–Medrano *et al*.[47] which were also unable to detect flavonoids in fruits of *P. angulata* [Table 4]. Thus, other compounds might be responsible for the antioxidant capacity observed in these extracts. The extracts that presented the greatest antioxidant activities were precisely those that show greater phenolic content. Phenolic contents or antioxidant capacity in species of *Physalis* has been mainly focused on fruits. For example, goldenberry (fruit of *P. peruviana)* is one of the most promising tropical fruits for its medicinal and edible uses.[47-49,53]

CONCLUSION

In summary, the anatomical analysis of *P. angulata* presents new and relevant information regarding the distribution of tectorial and glandular trichomes in the petioles, stems, sepals, and petals. These structures might be the site of production and accumulation of physalins, potentially the most useful compound in this plant. *P. angulata* antioxidant properties

Table 4: Values of phenolic and flavonoid contents and antioxidant capacity found in reference studies

Part	TPC*	TFC	Antioxidant capacity	References
Leaves	40.51 mg equivalents/g dry tissue	23.036 mg/g (dry tissue)	2.44 mg of ascorbic acid equivalents/mL	[47]
Leaves	0.47 mg equivalent/g (water extract)	Not performed	2.875 mg of water extract/g of BHA equivalent	[48]
	0.49 mg equivalent/g (ethanolic extract)		4.311 mg of ethanolic extract/g of BHA equivalent	
Fruits	36.92 mg equivalents/g (dry tissue)	Absent	0.92 mg of ascorbic acid equivalents/mL	[47]
Fruits	0.46 mg equivalent/g (water extract)	Not performed	1.575 mg of water extract/g of BHA equivalent	[48]
	0.51 mg equivalent/g (ethanolic extract)		2.258 mg of ethanolic extract/g of BHA equivalent	
Calyces	33.18 mg equivalents/g (dry tissue)	8.829 mg/g (dry tissue)	2.10 mg of ascorbic acid equivalents/mL	[47]
Tender stem, leaves and fruits	0.20 mg equivalent per gram (fresh weight)	Not performed	0.41 µmol Trolox equivalent antioxidant capacity per gram of fresh weight	[49]

*Gallic acid. TPC: Total phenolic content; TFC: Total flavonoid content; BHA: Buthylated hydroxyanisole

confirm the therapeutic potential of this plant. The current findings may support future breeding programs and biotechnological approaches for the optimization of useful compound production from *P. angulata*.

Acknowledgement

This study was supported by the Fundação de Amparo a Pesquisa da Bahia (FAPESB). The authors are grateful for the electronic microscopy platform of the Gonçalo Moniz Institute (Fiocruz) for technical assistance and to Professor Claudinéia Regina Pelacani Cruz (UEFS) for assigning the individuals of *P. angulata* and Romeu da Silva Leite, Gustavo Surlo Nascimento for providing the photos and illustration of *P. angulata*., respectively We also thank Dr. Fred Luciano Neves Santos for pictorial abstract.

Financial support and sponsorship

This study was financially supported by the FAPESB.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Lorenzi H, Matos FJ. Plantas Medicinais no Brasil: Nativas e Exóticas Cultivadas. 2nd ed. Nova Odessa, São Paulo: Instituto Plantarum; 2008.
2. Lashin II, Elhaw MH. Evaluation of secondary metabolites in callus and tissues of *Physalis peruviana*. J Mod Bot 2016;6:10-7.
3. Lan YH, Chang FR, Pan MJ, Wu CC, Wu SJ, Chen SL, *et al*. New cytotoxic withanolides from *Physalis peruviana*. Food Chemistry 2009;116:462-9.
4. Soares MB, Bellintani MC, Ribeiro IM, Tomassini TC, Ribeiro dos Santos R. Inhibition of macrophage activation and lipopolysaccaride-induced death by seco-steroids purified from *Physalis angulata* L. Eur J Pharmacol 2003;459:107-12.
5. Wu SJ, Ng LT, Chen CH, Lin DL, Wang SS, Lin CC, *et al*. Antihepatoma activity of *Physalis angulata* and *P. peruviana* extracts and their effects on apoptosis in human hep G2 cells. Life Sci 2004;74:2061-73.
6. Chiang HC, Jaw SM, Chen PM. Inhibitory effects of physalin B and physalin F on various human leukemia cells *in vitro*. Anticancer Res 1992;12:1155-62.
7. Pinto LA, Meira CS, Villarreal CF, Vannier-Santos MA, de Souza CV, Ribeiro IM, *et al*. Physalin F, a seco-steroid from *Physalis angulata* L. has immunosuppressive activity in peripheral blood mononuclear cells from patients with HTLV1-associated myelopathy. Biomed Pharmacother 2016;79:129-34.
8. Meira CS, Guimarães ET, Bastos TM, Moreira DR, Tomassini TC, Ribeiro IM, *et al*. Physalins B and F, seco-steroids isolated from *Physalis angulata* L. strongly inhibit proliferation, ultrastructure and infectivity of *Trypanosoma cruzi*. Parasitology 2013;140:1811-21.
9. Guimarães ET, Lima MS, Santos LA, Ribeiro IM, Tomassini TB, Ribeiro dos Santos R, *et al*. Activity of physalins purified from *Physalis angulata* in *in vitro* and *in vivo* models of cutaneous leishmaniasis. J Antimicrob Chemother 2009;64:84-7.
10. Sá MS, de Menezes MN, Krettli AU, Ribeiro IM, Tomassini TC, Ribeiro dos Santos R, *et al*. Antimalarial activity of physalins B, D, F, and G. J Nat Prod 2011;74:2269-72.
11. Levin DA. The role of trichomes in plant defense. Q Rev Biol 1973;48:3-15.
12. Schilmiller AL, Last RL, Pichersky E. Harnessing plant trichome biochemistry for the production of useful compounds. Plant J 2008;54:702-11.
13. Metcalf CR, Chalk L. Anatomy of the Dicotyledons. Oxford: Claredon Press; 1950.
14. Aguiar TV, Sant'anna-Santos BF, Azevedo AA, Ferreira RS. ANATI QUANTI: quantitative analysis software for plant anatomy studies. Planta Daninha 2007;25:649-59.
15. Harborne JB. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. Germany: Springer Science & Business Media; 1998.
16. Trease G, Evans W. Pharmacognosy. 12th ed. London: Bailliere Tindall; 1983.
17. Malterud KE, Farbrot TL, Huse AE, Sund RB. Antioxidant and radical scavenging effects of anthraquinones and anthrones. Pharmacology 1993;47 Suppl 1:77-85.
18. Bobo-García G, Davidov-Pardo G, Arroqui C, Vírseda P, Marín-Arroyo MR, Navarro M, *et al*. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. J Sci Food Agric 2015;95:204-9.
19. Chatatikun M, Chiabchalard A. Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts J Chem Pharm Res 2013;5:97-102.
20. Silva KN, Agra MF. Comparative pharmacobotanical study on *Nicandra physalodes* and *Physalis angulata* (Solanaceae) Rev Bras Farmacognosia 2005;15:344-51.
21. da Silvia DF, Strassburg RC, Villa F. Stem morphoanatomy of species of the genus *Physalis* Rev Ci Agrovet 2015;14:38-45.
22. Wahua C, Sam SM. Comparative chemotaxonomic investigations on *Physalis angulata* Linn. and *Physalis micrantha* Linn. (Solanaceae). Asian J Appl Sci 2013;1:220-8.
23. Thepsithar C, Thongpukdee A. Comparative micro-morphology, anatomy and architecture of leaf of *Physalis*. Int J Bioeng Life Sci 2013;7:806-10.
24. Munien P, Naidoo Y, Naidoo G. Micromorphology, histochemistry and ultrastructure of the foliar trichomes of *Withania somnifera* (L.) dunal (Solanaceae). Planta 2015;242:1107-22.
25. Fahn A. Plant Anatomy, 4th. ed. Oxford [England]; New York: Pergamon Press; 1990.
26. Franceschi VR, Nakata PA. Calcium oxalate in plants: Formation and function. Annu Rev Plant Biol 2005;56:41-71.
27. Bouropoulos N, Weiner S, Addadi L. Calcium oxalate crystals in tomato and tobacco plants: Morphology and *in vitro* interactions of crystal-associated macromolecules. Chemistry 2001;7:1881-8.
28. Zhang WN, Tong WY. Chemical constituents and biological activities of plants from the genus *Physalis*. Chem Biodivers 2016;13:48-65.
29. Pigatto AG, Blanco CC, Mentz LA, Soares GL. Tropane alkaloids and calystegines as chemotaxonomic markers in the *Solanaceae*. An Acad Bras Cienc 2015;87:2139-49.
30. Maldonado E, Hurtado NE, Pérez-Castorena AL, Martínez M. Cytotoxic 20,24-epoxywithanolides from *Physalis angulata*. Steroids 2015;104:72-8.
31. Chiang HC, Jaw SM, Chen CF, Kan WS. Antitumor agent, physalin F from *Physalis angulata* L. Anticancer Res 1992;12:837-43.
32. Silva MT, Simas SM, Batista TG, Cardarelli P, Tomassini TC. Studies on antimicrobial activity, *in vitro*, of *Physalis angulata* L. (Solanaceae) fraction and physalin B bringing out the importance of assay determination. Mem Inst Oswaldo Cruz 2005;100:779-82.
33. da Silva BJ, Rodrigues AP, Farias LH, Hage AA, Do Nascimento JL, Silva EO, *et al*. *Physalis angulata* induces *in vitro* differentiation of murine bone marrow cells into macrophages. BMC Cell Biol 2014;15:37.
34. Tomassini TC, Barbi NS, Ribeiro IM, Xavier DC. Genus Physalis – A Revision of withasteroids. Química Nova 2000;23:47-57.

35. Basey K, McGaw BA, Woolley JG. Phygrine, an alkaloid from *Physalis* species. Phytochemistry 1992;31:4173-6.
36. Singh B, Sharma RA. Plant terpenes: Defense responses, phylogenetic analysis, regulation and clinical applications 3 Biotech 2015;5:129-51.
37. Pérez-Castorena AL, Martínez M, Maldonado E. Labdanes and sucrose esters from *Physalis sordida*. J Nat Prod 2010;73:1271-6.
38. Pérez-Castorena AL, Oropeza RF, Vazquez AR, Martínez M, Maldonado E. Labdanes and withanolides from *Physalis coztomatl*. J Nat Prod 2006;69:1029-33.
39. Bergau N, Bennewitz S, Syrowatka F, Hause G, Tissier A. The development of type VI glandular trichomes in the cultivated tomato *Solanum lycopersicum* and a related wild species S. Habrochaites. BMC Plant Biol 2015;15:289.
40. Glas JJ, Schimmel BC, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR, *et al.* Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 2012;13:17077-103.
41. Balcke GU, Bennewitz S, Bergau N, Athmer B, Henning A, Majovsky P, *et al.* Multi-omics of tomato glandular trichomes reveals distinct features of central carbon metabolism supporting high productivity of specialized metabolites. Plant Cell 2017;29:960-83.
42. Trikka FA, Nikolaidis A, Ignea C, Tsaballa A, Tziveleka LA, Ioannou E, *et al.* Combined metabolome and transcriptome profiling provides new insights into diterpene biosynthesis in *S. pomifera* glandular trichomes. BMC Genomics 2015;16:935.
43. Aschenbrenner AK, Amrehn E, Bechtel L, Spring O. Trichome differentiation on leaf primordia of *Helianthus annuus* (Asteraceae): Morphology, gene expression and metabolite profile. Planta 2015;241:837-46.
44. Sasse J, Schlegel M, Borghi L, Ullrich F, Lee M, Liu GW, *et al. Petunia hybrida* PDR2 is involved in herbivore defense by controlling steroidal contents in trichomes. Plant Cell Environ 2016;39:2725-39.
45. Alahakoon UI, Taheri A, Nayidu NK, Epp D, Yu M, Parkin I, *et al.* Hairy canola (*Brasssica napus*) re-visited: Down-regulating TTG1 in an atGL3-enhanced hairy leaf background improves growth, leaf trichome coverage, and metabolite gene expression diversity. BMC Plant Biol 2016;16:12.
46. Bloomer RH, Lloyd AM, Symonds VV. The genetic architecture of constitutive and induced trichome density in two new recombinant inbred line populations of *Arabidopsis thaliana*: Phenotypic plasticity, epistasis, and bidirectional leaf damage response. BMC Plant Biol 2014;14:119.
47. Medina-Medrano JR, Almaraz-Abarca N, González-Elizondo MS, Uribe-Soto JN, González-Valdez LS, Herrera-Arrieta Y, *et al.* Phenolic constituents and antioxidant properties of five wild species of *Physalis* (Solanaceae). Bot Stud 2015;56:24.
48. Kusumaningtyas RW, Laily N, Limandha P. Potential of Ciplukan (*Physalis angulata* L.) as source of functional ingredient. Procedia Chem 2015;14:367-72.
49. Tukun AB, Shaheen N, Banu CP, Mohiduzzaman M, Islam S, Begum M. Antioxidant capacity and total phenolic contents in hydrophilic extracts of selected Bangladeshi medicinal plants. Asian Pac J Trop Dis 2014;7:S568-73.
50. Lee HZ, Liu WZ, Hsieh WT, Tang FY, Chung JG, Leung HW, *et al.* Oxidative stress involvement in *Physalis angulata*-induced apoptosis in human oral cancer cells. Food Chem Toxicol 2009;47:561-70.
51. Almeida Junior LD, Quaglio AE, de Almeida Costa CA, Di Stasi LC. Intestinal anti-inflammatory activity of ground cherry (*Physalis angulata* L.) standardized CO2 phytopharmaceutical preparation. World J Gastroenterol 2017;23:4369-80.
52. Olivares-Tenorio ML, Dekker M, Verkerk R, van Boekel MA. Health-promoting compounds in cape gooseberry (*Physalis peruviana* L.): Review from a supply chain perspective. Trends Food Sci Technol 2016;57:83-92.
53. Valdenegro M, Fuentes L, Herrera R, Moya-León MA. Changes in antioxidant capacity during development and ripening of goldenberry (*Physalis peruviana* L.) fruit and in response to 1-methylcyclopropene treatment. Postharvest Biol Technol 2012;67:110-7.