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

ORIGINAL ARTICLE

Anatomical Description and *in vitro* Evaluation of the Antibacterial Potential of *Aristolochia esperanzae* Kuntze (*Aristolochiaceae*) Extract on Oral Micro-organisms

Carlos Roberto Botelho-Filho, Gabriela Keiko Izumi[1], Juliana Campos Vieira[2], Fernanda Mara de Paiva Bertoli, Antônio Pedro Ricomini Filho[2], Leila Teresinha Maranho[3], Flares Baratto-Filho[4], Marilisa Carneiro Leão Gabardo

School of Health and Biological Sciences, Postgraduate Program in Dentistry, [1]School of Health and Biological Sciences, Undergraduate Program in Dentistry, Universidade Positivo, [2]Department of Physiological Sciences, Piracicaba Faculty of Dentistry, Universidade Estadual de Campinas, [3]School of Health and Biological Sciences, Postgraduate Program in Biotechnology, Universidade Positivo, [4]Department of Dentistry, Universidade da Região de Joinville, Santa Catarina, Brazil

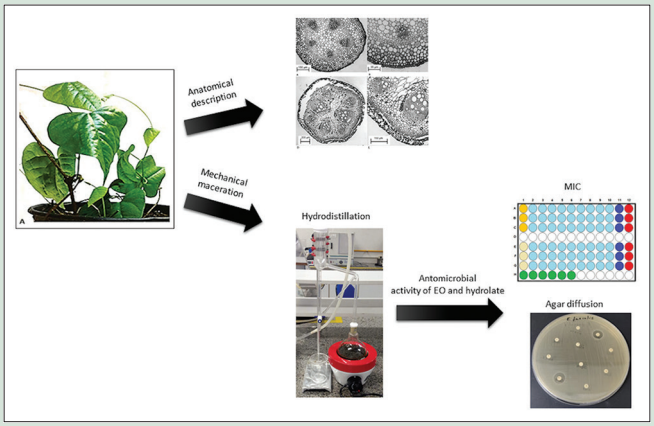
ABSTRACT

Background: *Aristolochia esperanzae* Kuntze (*Aristolochiaceae*) is a plant present in the Brazilian flora, with few studies in the dental area, but used in integrative therapies to treat some health problems. **Objectives:** The initial purpose was to make an anatomical description of *A. esperanzae* and then test its antibacterial activity and minimum inhibitory concentration (MIC) on specific oral micro-organisms-*Streptococcus mutans*, *Porphyromonas gingivalis* and *Enterococcus faecalis*. **Materials and Methods:** For the pharmacognostic study, the samples were fixed in formaldehyde, acetic acid and 70% ethyl alcohol and stored in 70% ethanol. For the *in vitro* study, to obtain the essential oil (OE) and hydrolate, the plant material was mechanically macerated and subjected to hydrodistillation in Clevenger and then frozen until the time of testing. The EO was diluted in dimethylsulfoxide and the tests performed were agar diffusion (disc-diffusion) with isolated bacteria and MIC. A 0.12% chlorhexidine digluconate solution was used as a positive control and 0.9% sodium chloride as a negative. **Results:** The anatomical study showed the presence of hook-shaped trichomes and stomatal complexes. Regarding the microbiological results, they were negative for the extracts used. **Conclusion:** The anatomical findings may guide future studies related to the pharmacognostic properties of *A. esperanzae* and both OE and hydrolate did not have an antibacterial effect against *S. mutans, P. gingivalis* and *E. faecalis*.

Keywords: Antimicrobial activity, dentistry, essential oil, minimum inhibitory concentration, phytotherapy

SUMMARY

- *Aristolochia esperanzae* Kuntze (*Aristolochiaceae*) is found in the Brazilian flora and used to treat some health problems
- Plants of the same genus have shown diuretic, analgesic, anti-inflammatory and anticancer activity in laboratory conditions
- The anatomical study of aspects showed important aspects for future pharmacognostic research
- There was no antibacterial effect of essential oil and hydrolate on the micro-organisms tested.



Abbreviations Used: BHI: Brain heart infusion, DMSO: Dimethyl sulfoxide, EO: Essential oil, FAE: Formaldehyde, acetic acid and ethyl alcohol, MH: Mueller Hinton broth, MIC: Minimum inhibitory concentration.

Correspondence:

Dr. Marilisa Carneiro Leão Gabardo,

Universidade Positivo, Rua Prof. Pedro Viriato Parigot De Souza 5300, Curitiba, Paraná 81280-330, Brazil.

E-mail: marilisagabardo@gmail.com

DOI: 10.4103/pr.pr_52_20

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

INTRODUCTION

Aristolochia is a genus of the family *Aristolochiaceae*, a plant commonly found in the tropical regions of South America, Africa, and Asia.[1] In Brazil, *A. esperanzae* Kuntze is known as "cipó mil-homens," "papo-de-perú" or "jarrinha," depending on the country region. Ethnopharmacological studies have ponted to the use of Aristolochia for the treatment of rheumatoid arthritis,[2] tumors,[3,4] respiratory infections,[4] dysentery,[4] malaria, and fever.[3] Furthermore, it can be used as an anthelmintic,[1] and with analgesic,[3] anti-allergic[5] and antiseptically properties, with its traditional use is through decoction or infusion of parts of the plant.[6]

Cite this article as: Botelho-Filho CR, Izumi GK, Vieira JC, Bertoli FM, Filho AP, Maranho LT, *et al*. Anatomical description and *in vitro* evaluation of the antibacterial potential of *Aristolochia esperanzae* kuntze (*Aristolochiaceae*) extract on oral micro-organisms. Phcog Res 2020;12:424-9.

Submitted: 02-Jun-2020 **Revised:** 15-Jul-2020
Accepted: 15-Sep-2020 **Published:** 23-Jan-2021

According to Lopes *et al*.[7] the genus *Aristolochia* and its representatives present 275 terpenoids, 57 lignoids, 167 alkaloids, and other 99 phenolic derivatives. These chemical constituents are responsible for the various pharmacological activities presented.

In microbiological terms, laboratory tests have already been carried out with its ethanolic extract of *A. esperanzae* at a concentration of 10%, and an antibacterial potential was identified against *Bacillus cereus*.[8] In this research, Asarinin was identified, as a compound with bactericidal effect for Gram-positive and negative bacteria. In addition, aristolochic acid had bactericidal action through *Staphylococcus aureus* and *Listeria monocytogenes*, both of which are relevant in terms of diseases in humans.[8]

Since there are few researches about *A. esperanzae* evaluating its antibacterial properties and there are no reports involving oral micro-organisms, nor any descriptions about its anatomy, this research is justified.

Furthermore, the current challenge is to obtain products that act against of the resistance generated by the prolonged use of certain compounds and that at the same time, have a wide spectrum of action so that it can be effective against various pathogens.[9]

Most oral diseases are related to changes in the oral environment, generating ecological imbalance that leads to dental biofilm dysbiosis. It favors the selection and prevalence of pathogenic microorganisms, such for dental caries, periodontal disease, and infectious processes from the canal root. Therefore, there has been a constant search for chemical agents, synthetic or natural, that promote the control of the microbiota, providing a return to the symbiotic relationship with the host, or eliminating pathogenic micro-organisms.[10,11]

The dental carie is a disease caused by the frequent consumption of sugars, which are fermented to acids by bacteria causing tooth demineralization. The acidic environment favors the selection of aciduric and acidogenic microorganisms, such as *Streptococcus mutans*, which is a gram-positive bacterium closely related to the cariogenic biofilm.[12] Periodontal disease, similar what happens with the dental carie, results from the accumulation of biofilm near the gingival margin that results in an inflammatory process.[12] The accumulated biofilm also allows the selection of periodonpatogenic micro-organisms, such as *Porphyromonas gingivalis*,[13,14] a strict and gram-negative anaerobic micro-organisms, highly virulent and with destructive potential.[13] Concerning micro-organisms involved in pathological endodontic processes, the most important is *Enterococcus faecalis*, a gram-positive bacterium,[15] which has factors of survival, virulence, resistance to nutritional deprivation, ability to penetrate dentinal tubules and also compete with other micro-organisms.[16]

Therefore, the present research had as objectives to first describe the morpho-anatomy of *A. esperanzae* and also evaluate its antibacterial activity on the oral micro-organisms *S. mutans, P. gingivalis,* and *E. faecalis.*

MATERIALS AND METHODS

Collection and methodology for pharmacognostic tests

The plant material was collected from a private property in the city of Curitiba, PR, Brazil, close to the geographical coordinates 25°35'01.2" S and 49°15'43.7" W. Three fully expanded leaves of *Aristolochia esperanzae* were collected and positioned from the fourth stem node, and three stem samples positioned 5 cm from the apex. These samples were immediately fixed in formaldehyde, acetic acid, and ethyl alcohol-FAA 70% for 48 h[17] and subsequently kept in 70% ethanol until final processing.[18]

To obtain the permanent slides, samples were selected from the median region of the leaves, which were included in methacrylatoaglycol (JB-4) using the procedure described by O'Brien *et al*.[19] and manufacturer recommendations (Polysciences Inc., Washington, USA). The sections were made in a Leica RM2125 rotation microtome (Leica Biosystems, Buffalo Grove, USA) with a thickness of 7 μm. After that, the samples were stained with 1% toluidine blue.[20] The slides were mounted with synthetic resin (Entellan®; Merck Millipore, Burlington, USA).

To view the trichomes, semi-permanent slides were prepared from cross-sections, with a razor blades and the freehand, from the median region of the leaf.

Subsequently, the slides were mounted with glycerin and the sealing was done with colorless nail polish. For the observation of stomatal complexes and other epidermal cells, in frontal view, paradermic sections were made on the adaxial and abaxial sides of the leaf. They were mounted with glycerin and sealed with colorless nail polish.

The total number of the slides was 11 permanent and 3 semi-permanent. The description of the transversal and paradermic sections was made from the visualization and interpretation of the images under a photonic microscope (Olympus® CX41RF; Olympus, Tokyo, Japan). The photomicroscope (Olympus® BX 41; Olympus, Tokyo, Japan), with image capture, was used to obtain the illustrations using the Pro-plus software, the scales being obtained in the same conditions as the photos.(Olympus® CX41RF; Olympus, Tokyo, Japan).

Collection and preparation of plant material for microbiological tests

The plant material was collected from a private property in Curitiba, PR, Brazil, close to the geographical coordinates 25°35'01.2" S and 49°15'43.7" W in November 2018. A desiccata was deposited in the Herbarium of the Positivo University under registration n.º 105.

The collection of leaves and stems of *A. esperanzae* Kuntze was carried out. Then a screening of the material, by the visual selection, allowed the exclusion of undesirable organic and inorganic materials and the nonhealthy parts attacked by insects and/or fungi or dried.[21] Then, fresh leaves and stem samples were crushed in a blender.

The extraction of essential oil (EO) was carried out with fresh and crushed material by steam dragging in the Clevenger apparatus (SPlabor, Presidente Prudente, Brazil) according to a pre-established protocol.[22] The crude product after uninterrupted operation of the device for around 2 h was recorded. The samples obtained from EO and hydrolate were frozen at a temperature of −10°C to later be used in the tests to assess antibacterial activity.

Bacterial activity assay

The antibacterial activities of EO and hydrolate were evaluated in two tests: Agar diffusion with isolated bacteria (inhibition halo) and minimal inhibitory concentration (MIC).

The evaluated bacteria were: *S. mutans* UA159 (ATCC 700610), *P. gingivalis* (ATCC 33277), and *E. faecalis* (ATCC 19433). The micro-organisms were grown in broth with the bacterial suspension adjusted in a spectrophotometer to an OD 600 nm from 1.0 ± 0.05 (ranging from 0.9737–1.0029).[23]

As a positive control in both tests, chlorhexidine digluconate was used at a concentration of 0.12%; for the negative control in both tests, sodium chloride solution, 0.9% NaCl was used.

After defrosting, the dilution of the EO was performed with the solubilizer dimethylsulfoxide (DMSO) 0.2% (Synth, Diadema, Brazil) in the proportion of 1: 1. To use it as a solvent, DMSO was also used as a

solution in the control group. The hydrolate did not need to be diluted because it is aqueous and easily dissipated.

Antibacterial activity: Agar diffusion test (inhibition halo)

The agar diffusion tests were performed according to the standardization of antimicrobial sensitivity tests by disc-diffusion.[24]

The tests with *S. mutans* and *E. faecalis* were performed using brain heart infusion (BHI) + agar (Laborclin. Pinhais, Brazil). For *P. gingivalis*, the blood agar was used (Laborclin. Pinhais, Brazil). The bacteria were spread on the agar with a disposable swab (Labor Import, Osasco, Brazil).

For the test, filter paper discs previously sterilized were used. In the Petri dishes with BHI, 10 paper disks were distributed in each one, two disks for each treatment (EO, hydrolate, NaCl, chlorhexidine, and DMSO) were positioned in an antagonistic way to each other. In the Petri dishes with blood agar, 4 paper discs were used per plate, one for each group (EO, hydrolate, NaCl, and chlorhexidine). Due to previous tests, DMSO was removed because it did not show an antibacterial response to any bacteria.

With the positioning of the discs performed, 20 μL of each solution was applied over each of them. Then the plates were incubated in an oven at 37°C and 10% CO_2 saturation for 24 h for all groups.

Minimal inhibitory concentration test

For this test, 100 μL of each bacterium inoculated and adjusted in a spectrophotometer were added to 9900 μL of new culture medium to obtain a standardization of the inoculum of 106 cells/mL. Furthermore, a 96-well plate was used, with each bacterium evaluated on a different plate. Serial dilutions of each extract were performed in wells with 1–10, while wells 11 and 12 were added with negative and positive control, respectively. The test was performed by a three times repetition for each extract with each bacterium.

Initially, 100 μL of each solution was deposited in one of the wells with the exception of number 1 (A-G), where the amount of EO and hydrolate was 200 μL. For serial dilution, 100 μL of each well 1 (A-G) was passed to well 2 (A-G), then from 2 to 3 (A-G) and so on up to well number 10 (A-G). To conclude, 100 μL of solution from wells number 10 were discarded to be used as a standardized final of 100 μL of each solution in each well.

The initial concentration was "x" in wells number 1, and a final concentration of "x/256" was reached in wells number 10, being possible to test 10 different concentrations. Then, 100 μL of the bacterial inoculums were placed in each well of the plates, which were incubated at 37°C for 24 h.

The results of the MIC test were evaluated by the turbidity of the wells under visual analysis. If the well did not become cloudy or its turbidity decreased according to the concentration, there was a positive result.

RESULTS

Morpho-anatomical analysis

A. esperanzae has an herbaceous and climbing habit; its stem is volatile and branched and its leaves are simple, cordiform, dark green, with alternating phyllotaxis and reticulated venation [Figure 1].

Regarding the structural organization in frontal view, the leaf of *A. esperanzae* has the epidermis on the adaxial face in a rectangular shape and on the abaxial face cells of irregular shape and in both different formats [Figure 2a], being hypoestomatic and their complexes stomatal type stomata [Figure 2b]. The presence of single-celled glandular trichomes, type filiform was also observed [Figure 2c and d].

In the cross-section, it was possible to verify the dorsiventral organization of the mesophile. The epidermis is uniseriate with a thin cuticle. The palisade parenchyma has one to two cell layers, while the lacunous parenchyma has four to five [Figure 2e]. The midrib of the leaf is prominent on both sides but more pronounced on the abaxial face.

The epidermis is uniseriate, and the cells are smaller when compared to cells in the mesophilic region, both on the adaxial and abaxial surfaces. Internally to the epidermis, there is a small portion of collenchyma followed by cells of fundamental parenchyma. Around the vascular bundle, there are two to five layers of cells with a different shape. The vascular bundle is collateral, formed by phloem cells and cell layers arranged in radial series that correspond to the xylemic region. The phloem is arranged in an arc shape, with the opening facing to the adaxial face. Both in xylem and phloem, it is possible to see cells with different stages of differentiation. So, it is possible to conclude there is a

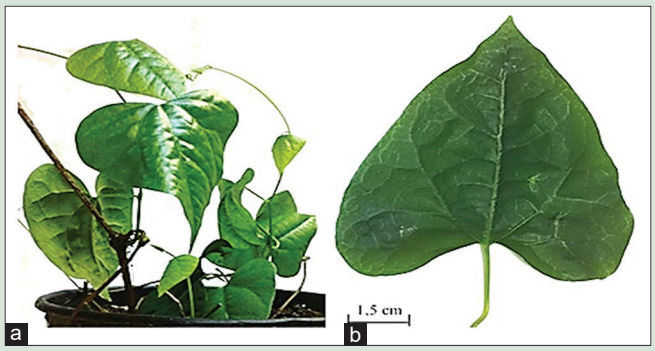


Figure 1: *Aristolochia esperanzae* Kuntze. (a) Vegetative aspects of a young plant. (b) Aspect of the leaves, cordiform shape and presence of accentuated veins

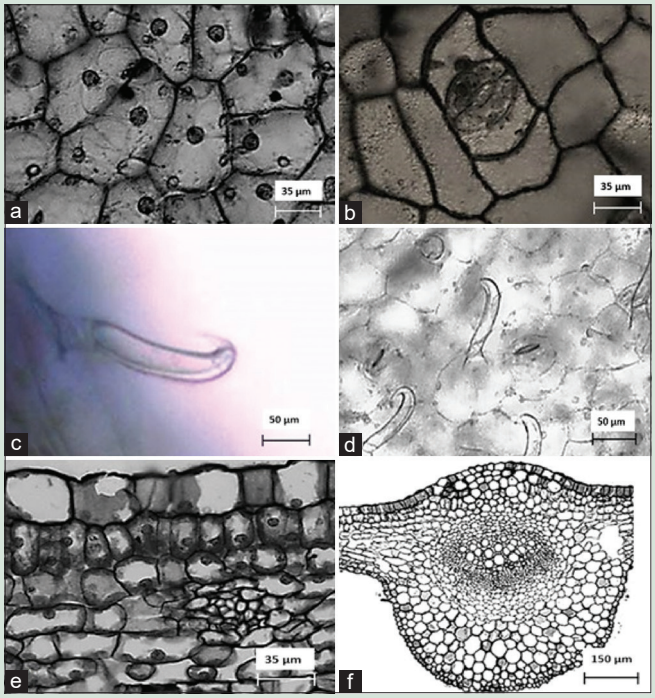


Figure 2: Aspects of the leaf blade of *Aristolochia esperanzae* Kuntze. (a) Epidermis in frontal view and on the adaxial face. (b) Epidermis in frontal view and on the abaxial face. (c and d) Filiform trichomes. (e) Detail of the mesophile region. (f) Detail of the mid rib

secondary growth in this region due to the formation of a thin layer of vascular bundle [Figure 2f].

The petiole, in cross-section, is characterized by a thin layer of cuticle, with a single layer epidermis of rounded cells and homogeneous appearance, followed by approximately four to five layers of collenchyma cells, which surround the entire petiole. The cortical parenchyma, located internally to the collenchyma, has three to four layers of cells of varied sizes. As for the arrangement of the vascular bundles, the collateral type was identified, where the phloem has small cells and irregular shape, while in the xylem, the vessels are rounded and varying from two to five. External to the vascular cylinders, a ring of cells of small size and irregular shape was found and when filling between these structures, is the medullary parenchyma, composed of cells of varying sizes and small intercellular space [Figure 3a and b].

Related to the structural organization of the stem blade, it has secondary development, but at an early stage. In the cross-section, the presence of an epidermis covered by a thin cuticle was noted, with irregular cells followed by the cortex, with collenchymatic cells with four to five layers of elongated cells. More internally, the cortical parenchyma with approximately six layers of rounded cells and a thin cell wall. It has secondary growth of the eustele type, being evident in the formation of the fascicular vascular bundle, with seven vascular cylinders and consisting of six to nine layers of cells with thin walls.

The xylem is well developed and is characterized by the presence of vessel elements with thick walls and variations in the size of the lumen. Hence, they can be narrow, intermediate, or wide, where five to 12 xylemic elements also be observed. Between the vascular regions, the presence of an intercalary region was seen, composed of thin-walled parenchymatic cells and small intercellular spaces that fill the entire central part of the stem. In the phloem, there was a distinction between protofloem and metafloem, formed by several layers of cells with irregular and juxtaposed shapes [Figure 3c and d].

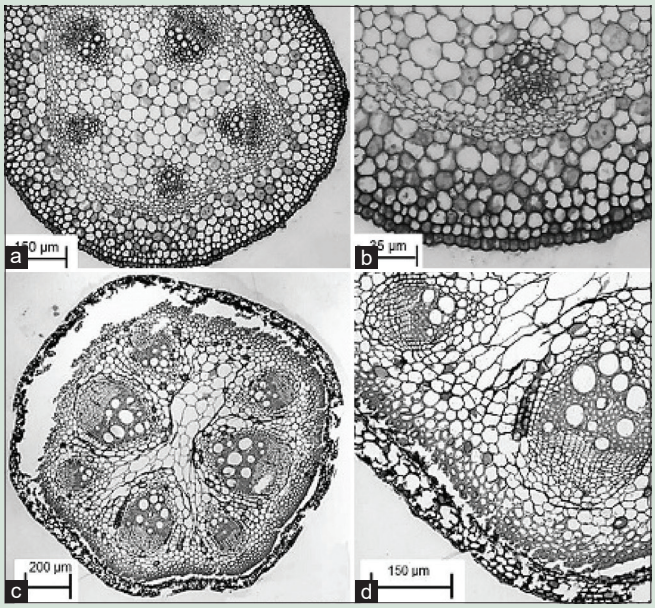


Figure 3: Aspects of the petiole and stem of *Aristolochia esperanzae* Kuntze in cross section. (a) General aspect of the petiole. (b) Detail of the petiole cells: Epidermal, collenchyma, cortical parenchyma, vascular cylinder and medullary parenchyma. (c) General aspect of the stem. (d) Details of stem tissues

Agar diffusion test

After the incubation period, the plates were submitted to visual analysis and measurement with a millimeter ruler. In the three bacteria analyzed, only one group had a positive effect, the 0.12% chlorhexidine group with an inhibition zone of 0.5 mm to 1 cm. The other groups showed negative results, confirmed by the absence of the inhibition zone, as shown in Figure 4.

Minimal inhibitory concentration test

In the visual analysis of the turbidity of the wells, all were cloudy, except those with the positive control (0.12% chlorhexidine), an expected result according to the outcome of the inhibition halo test.

DISCUSSION

The aim of this study was to make an anatomical description, emphasizing some markers, which lead to the determination of the botanical origin of *A. esperanzae* Kuntze. And then to evaluate its antibacterial activity on three oral micro-organisms *S. mutans*, *P. gingivalis* and *E. faecalis*, involved in relevant oral pathological processes. The anatomical study identified hook-shaped trichomes and stomatal complexes, which are important data for future pharmacognostic research. Otherwise, in the microbiological analysis, there was no antibacterial effect of EO and hydrolate on these microorganisms.

The use of integrative therapies, especially allelopathy, is being used normally with the improper use of its regional flora in search of alternative alternatives. As such procedures are not always scientifically based, the answers must be sought about the risks and benefits of using plants. The literature indicates the use the Aristolochia genus for the treatment of various health problems, such as rheumatoid arthritis,[2] tumors,[3,4] respiratory infections,[4] dysentery,[4] malaria and fever,[3] as anthelmintic,[1] analgesic,[3] anti-allergic,[5] and antiseptic.[6]

Although the medicinal use of its roots and leaves has been highlighted with relevant anthelmintic power, several parts, for example, from

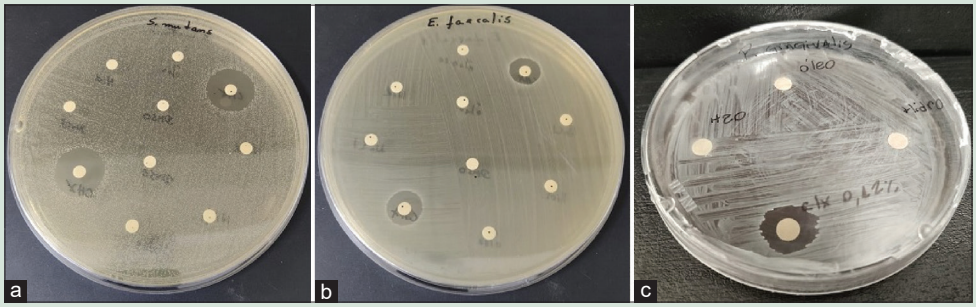


Figure 4: Results of the agar diffusion tests for *Streptococcus mutans* (a), *Enterococcus faecalis* (b) and *Porphyromonas gingivalis* (c). Note: The halo of inhibition means no bacterial proliferation, showed around the disks of the control group with chlorhexidine

A. bracteata can be used, according to Kalpana *et al*.[25] In this context, *A. esperanzae* Kuntze is a plant whose scientific investigation starting from the popular use and still needs to be further explored. In this way, the first part of the present research arose from the need of an anatomical investigation of the plant. Hence, its antibacterial potential could be assessed and until now, that was investigated only in the presence of some micro-organisms, which are not present in the oral cavity.[8]

Regarding its morphology or stem of the *Aristolochiaceae* family of plants, it is generally cylindrical, but rarely, can also be quadrangular, and the herbaceous species have annuals stems.[26] The stem of climbing plants tends to be green, fragile, and flexible,[27] an aspect observed in m *A. esperanzae*.

In the same genus, Pattar and Jayaraj[28] also reported the paracitic stomatal complex in *A. indica* L., which, different from *A. esperanzae*, are located on both faces, abaxial and adaxial. According to Sangwan *et al*.[29] the trichomes have great phytoaccumulation capacity of secondary metabolites related to plant defenses. Trichomes storing the four largest classes of monoterpenes have been reported by Croteau and Johnsons[30] in *Salvia officinalis*. Moreover, Duke and Paul[31] showed the presence of sesquiterpenoids with antimalarial properties in trichomes of *Artemisia annua*. Corroborating the hypothesis that trichomes store secondary metabolites and, based on this information, suggest that the glandular trichomes of *A. esperanzae* may be related to the reported pharmacological properties.

In comparison, the petiole of *Ricinus communis* L., a specie of the same class as *A. esperanzae*, is organized in a uniseriate epidermis covered by a cuticle, with approximately eight layers of collenchyma with irregularly shaped cells and about six layers of cortical parenchyma. Regarding to vascular bundles, the phloem is composed by small cells, while the xylem has large and round cells,[27] which shows the similarity between both species. The *Piper hispidum* petiole, of the same order as *A. esperanzae*, has a uni-stratified epidermis, covered by multicellular and uniseriate cuticles and trichomes. There are discontinuous bands of angular collenchyma, with eight to 11 layers of cells,[32] structures similar to those of *A. esperanzae*.

As for the stem, the genus *Holostylis*, also belonging to the *Aristolochiaceae* family, has erect or slightly curved stems, with broad leaves. The stem of *P. arboreum*, the same order as *A. esperanzae*, has a uniseriate epidermis, with cells in the shape of cubes or tabular. The cortex has discontinuous bands of collenchyma, and the central cylinder is formed by a ring of vascular bundles that surround the medullary parenchyma.[33] *P. hispidum*, Piperales genus, same order as *A. esperanzae*, has a cylindrical stem, constituted by the uni-stratified epidermis, chlorophyll, and covered by a thin cuticle, with discontinuous collenchyma strips.[32] Therefore, the anatomical features found here are encountered in other species in the same family, *A. elegans*, *A. fimbriata Cham*. and Schltdl., *A. gigantea* Mart. and Zucc. and *A. melastoma*.[27]

According to the second part of this study, about the microbiological analysis, a theoretical background had as a starting point, scientific evidence found in the literature in researches that evaluated the action of natural compounds on micro-organisms currently related to oral diseases, such as *S. mutans*,[34,35] *P. gingivalis*,[36,37] and *E. faecalis*.[38,39] These micro-organisms were chosen in the present study since they could guide the action compared to the others that are also present in the cariogenic, periodontopathogenic and/or endodontic biofilm. In addition, the entire context must be analyzed so that cause and effect can be established in relation to oral diseases. For example, regarding dental caries, a chronic disease of acknowledged multifactorial origin, the statement that a natural product can prevent it is a complex condition to affirm. In the same way, this can be extended to a series of other oral diseases, including those that have a strict relationship with the micro-organisms evaluated in this study.

In a previous study, a concentrated stem extract and a heartwood extract from *A. esperanzae*, had an effect against *B. cereus*, with a similar methodology used in this research.[8] However, the difference was that these authors made the use of a concentrated ethanolic extract, and the studied bacteria, sensitive to this extract, is not found in the oral environment, instead in meat and cereals. For two other bacteria, *S. aureus* and *L. monocytogenes*, an active ingredient of *A. esperanzae* proved to be promising, the aristolochic acid, although with action only when isolated.[8] Nevertheless, some compounds found did not show antibacterial action or are not cataloged and may still be effective on other micro-organisms, suggesting new studies with extracts of *A. esperanzae*.[8]

The use of ethanolic extracts indicates a difference in the MIC when compared to aqueous extracts, so the dilution in ethanol can alter the antibacterial potential.[40] These findings suggest that other extraction methods, different solvents, and isolation of active ingredients show different results than those found in this study, for example, the antifungal action found in the aqueous extract of *A. bracteolata*.[41]

Concerning the method used in the tests, there is a standardization that was followed in this research.[24] Still, the greenhouse configurations were standardized for the three bacteria, where they remained at 37°C with 10% CO_2 saturation for 24 h because all samples showed relevant proliferation in the evaluated time. No other method or other research to date has been similar to the one proposed here. Furthermore, no studies with specific oral micro-organisms were found, and other techniques and bacteria may be tested in the future to look for new results.[24]

The results may even serve as a warning in relation to popular use as an antiseptic, since its action is not completely clear. Still, other ways of using the plant, such as cooking and infusion, have not been tested, and it may be that the results with them are different.

CONCLUSION

The anatomical study allowed obtaining information on the structural organization of *A. esperanzae*, which can facilitate its identification, emphasizing the most evident characteristics, such as stomatal complexes of the paracitic type and hook-shaped trichomes, that are relevant data to search for their pharmacological activities. Regarding microbiological tests, the plant had no effect on the micro-organisms evaluated. In this context, it is a plant whose scientific investigation started from popular use and which still needs to be better investigated, even in other areas of the health area.

Acknowledgements

The authors are grateful to Ana Lúcia Aires Monteiro, from the Laborclin Laboratory Products for providing some material for the conduction of this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Samia HA, Elmalik KH, Khalid HS. Therapeutic effect of *Aristolochia bractealata* extract against experimental Trypanosoma evansi infection. Int J Trop Med 2006;1:170-2.
2. Chitme HR, Patel NP. Antiarthritis activity of *Aristolochia bractealata* extract in experimental animals. Open Nat Prod J 2009;2:6-15.
3. El-Tahir A, Satti GM, Khalid SA. Antiplasmodial activity of selected sudanese medicinal plants with emphasis on Acacia nilotica. Phytother Res 1999;13:474-8.

4. Thirumal M, Vadivelan R, Kishore G, Brahmaji VS. Aristolochia bracteolata: An overview on pharmacognostical, phytochemical and pharmacological properties. Crit Rev Pharmac Sci 2012;1:70-82.
5. Chitme HR, Malipatil M, Chandrashekhar VM, Prashant PM. Antiallergic activity of *Aristolochia bracteolata* Lank in animal model. Indian J Exp Biol 2010;48:46-52.
6. Rodrigues VEG, Carvalho DA. etnobotanical survey of medicinal plants in the dominion of meadows in the region of the alto rio grande - minas gerais. Ciênc Agrotec 2001;25:102-23.
7. Lopes LM, Nascimento IR, Silva T. Phytochemistry of the *Aristolochiaceae* family. Kerala Res Adv Phytochem 2001;2:19-108.
8. Pacheco AG, Silva TM, Manfrini RM, Sallum WST, Duarte LP, Piló-Veloso D, *et al*. Chemical study and antibacterial activity of stem of *Aristolochia esperanzae* Kuntze (Aristolochiaceae). Quím Nova 2010;33:1649-52. [Portuguese].
9. Beloin C, Renard S, Ghigo JM, Lebeaux D. Novel approaches to combat bacterial biofilms. Curr Opin Pharmacol 2014;18:61-8.
10. Albuquerque CA, Vieira L, Bucal S. Antiadherent effect of the extract of the *Matricaria recutita* Linn on microrganisms of dental biofilm. Rev Odontol UNESP 2010;39:21-5.
11. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, *et al*. The oral microbiome An update for oral healthcare professionals. Br Dent J 2016;221:657-66.
12. Sanz M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H, *et al*. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol 2017;44 Suppl 18:S5-11.
13. Carvalho C, Cabral CT. Papel da *Porphyromonas gingivalis* na doença periodontal. Rev Port Estomatol Cir Maxilofac 2007;48:167-71. [Portuguese].
14. Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T, *et al*. *Porphyromonas gingivalis*: Major periodontopathic pathogen overview. J Immunol Res 2014;2014:476068.
15. Rôças IN, Siqueira JF Jr., Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. J Endod 2004;30:315-20.
16. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: It's role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8.
17. Johansen DA. Plant Microtechnique. New York: Mc Graw Hill Book; 1940.
18. Berlyn GP, Miksche JP. Botanical Microtechnique and Cytochemistry. Iowa: Ames; 1976.
19. O'Brien TP, Feder N, McCully ME. Polycromatic staining of plant cell walls by toluidine blue. Protoplasma 1990;59:368-73.
20. Sakai WS. Simple method for differential staining of paraffin embedded plant material using toluidine blue O. Stain Technol 1973;48:247-9.
21. NCCLS. Performance standards for antimicrobial disk susceptibility tests. Approved Standard. 8th ed. Wayne: NCCLS; 2003.
22. Santos AS, Alves SM, Figueirêdo FJ, Rocha Neto OG. Descrição de Sistema e de Métodos de Extração de Óleos Essenciais e Determinação de Umidade de Biomassa em Laboratório. Comunicado Técnico 99 Embrapa INFOTECA-E; 2004. p. 1-6.
23. Manoil D, Filieri A, Schrenzel J, Bouillaguet S. Rose bengal uptake by *E. faecalis* and *F. nucleatum* and light-mediated antibacterial activity measured by flow cytometry. J Photochem Photobiol B 2016;162:258-65.
24. Clinical and Laboratory Standards Institute. Clinical and Laboratory Standards Institute/NCCLS. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI/NCCLS Document M100-S15. Pennsylvania: Clinical and Laboratory Standards Institute; 2005.
25. Kalpana DB, Kanimozhi S, Suganyadevi P. Phytochemical screening and biological property of *Aristolochia bracteata*. J Pharm Res. 2011;4:1509-14.
26. Ahumada LZ. Revision of the Argentine Aristolochiaceae. Opera Lilloana 1968;16:1-145.
27. Capellari L Jr. *Aristolochiaceae*. In: Rizzo JA, edtor. Flora dos Estados de Goiás e Tocantins. Vol. 27. Goiânia: Coleção Rizzo; 2001. p. 1-34.
28. Pattar PV, Jayaraj M. Pharmacognostic and phytochemical investigation of whole plant of *Aristolochia indica* L. – A threatened medicinal plant. Int J Pharm Sci Rev Res 2012;15:95-8.
29. Sangwan NS, Farooqi AH, Shabih F, Sangwan RS. Regulation of essential oil production in plants. Plant Growth Regul 2001;34:3-21.
30. Croteau R, Johnson MA. Biosynthesis of terpenoids in glandular trichomes. In: Rodriguez E, Healey PL, Mehta I, editors. Biology and Chemistry of Plant Trichomes. New York: Plenum Press; 1984. p. 135-85.
31. Duke OS, Paul NR. Development and fine structures of the glandular trichome in *Artemisia annua* L. Int J Plant Sci 1993;154:107-18.
32. Albiero AL, Paoli AA, Souza LA, Mourão KS. Morphology and anatomy of vegetative organs of Piper crassinervium H.B. & K. (Piperaceae). Acta Bot Brasil 2005;19:305-12.
33. Cronquist A. An Integrated System of Classification of Flowering Plants. New York: Columbia University Press; 1981.
34. Shetty SB, Mahin-Syed-Ismail P, Varghese S, Thomas-George B, Kandathil-Thajuraj P, Baby D, *et al*. Antimicrobial effects of Citrus sinensis peel extracts against dental caries bacteria: An *in vitro* study. J Clin Exp Dent 2016;8:e71-7.
35. Swadas M, Dave B, Vyas SM, Shah N. Evaluation and comparison of the antibacterial activity against *Streptococcus mutans* of grape seed extract at different concentrations with chlorhexidine gluconate: An *in vitro* study. Int J Clin Pediatr Dent 2016;9:181-5.
36. Solmaz G, Ozen F, Ekinci Y, Bird PS, Korachi M. Inhibitory and disruptive effects of shiitake mushroom (Lentinula edodes) essential oil extract on oral biofilms. Jundishapur J Microbiol 2013;6:e9058.
37. de Medeiros AK, de Melo LA, Alves RA, Barbosa GA, de Lima KC, Porto Carreiro AD. Inhibitory effect of cranberry extract on periodontopathogenic biofilm: An integrative review. J Indian Soc Periodontol 2016;20:503-8.
38. Sponchiado EC, Pereira JV, Marques AA, Garcia LR, França SC. *In vitro* assessment of antimicrobial activity of Pothomorphe umbellate extracts against *Enterococcus faecalis*. Indian J Dent Res 2014;25:64-8.
39. Nazemisalman B, Vahabi S, Yazdinejad A, Haghghi F, Jam MS, Heydari F. Comparison of antimicrobial effect of *Ziziphora tenuior*, *Dracocephalum moldavic*a, *Ferula gummos*a, and *Prangos ferulace*a essential oil with chlorhexidine on *Enterococcus faecali*s: A*n in vitro* study. Dent Res J (Isfahan) 2018;15:111-6.
40. Vieira DS, Peixoto RM, Costa MM, Freire DP, Silva TM, Silva TM. *In vitro* antimicrobial activity of ethanolic extract of Hymenaea martiana Hayne leaf on strains of Staphylococcus spp. and evaluation of its potential as a disinfectant in goats. Pesq Vet Bras 2018;38:462-9.
41. Kavitha D, Nirmaladevi R. Assessment of *Aristolochia bracteolat*a leaf extracts for its biotherapeutic potential. Afr J. 2009;8:4242-4.