
**Investigation of antifungal activity from *Vismia guianensis* (Aubl.)
standardized extract**

**Investigação da atividade antifúngica de extrato padronizado de *Vismia guianensis*
(Aubl.)**

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ABSTRACT

In the Amazon, the use of medicinal plants by native populations is an important source of ethnopharmacological knowledge. *Vismia guianensis* (Aubl.) Choisy is widely used against diseases caused by fungi. Thus, the aim of this work was to evaluate the antifungal potential of the species *Vismia guianensis* (Aubl.) Choisy and the influence of extractive parameters on the antifungal activity. Dried extracts were prepared from leaves and tested for activity against the fungal species *Candida albicans*, *Malassezia furfur*, and *Trichophyton rubrum*. The most promising extract was obtained with polar solvents (methanol and ethanol), and no significant difference was measured between the activities exhibited by these extracts (MIC 410 µg/mL). The method of maceration and ethanol:water 1:1 (v/v) presented the lowest MIC and when the extracts were dried in a spray drier showed MIC for *C. albicans* 1.66 mg/mL; *M. furfur* 8.0 mg/mL and *T. rubrum* 1.0 mg/mL. The dried extract from leaves of *Vismia guianensis* demonstrated significant inhibitory activity against the growth of fungi tested, indicating that this plant has great potential to be an effective botanically derived drug for the treatment of cutaneous mycoses.

Keywords: *Vismia guianensis*, Spray dryer, Antifungal activity

RESUMO

Na Amazônia, o uso de plantas medicinais pelas populações nativas é uma importante fonte de conhecimento etnofarmacológico. *Vismia guianensis* (Aubl.) Choisy é amplamente utilizada contra doenças causadas por fungos. Assim, o objetivo deste trabalho foi avaliar o potencial antifúngico da espécie *Vismia guianensis* (Aubl.) Choisy e a influência de parâmetros extrativos na atividade antifúngica. Os extratos foram preparados a partir das folhas da espécie vegetal e testados quanto à atividade contra as espécies fúngicas *Candida albicans*, *Malassezia furfur* e *Trichophyton rubrum*. O extrato mais promissor foi obtido com solventes polares (metanol e etanol) e nenhuma diferença significativa foi encontrada entre as atividades exibidas por esses extratos (CIM 410 µg/mL). O método de maceração com etanol:água 1:1 (v/v) apresentou a menor CIM e quando os extratos foram secos em secagem por aspersão apresentara para *C. albicans* 1.66 mg/mL; *M. furfur* 8.0 mg/mL e *T. rubrum* 1.0 mg/mL). Os extratos secos das folhas de *Vismia guianensis* demonstraram significativa atividade inibitória contra o crescimento dos fungos testados, indicando que esta planta tem grande potencial para ser um medicamento eficaz de origem botânica para o tratamento de micoses cutâneas.

Palavras-chave: *Vismia guianensis*, Secagem por aspersão, atividade antifúngica

INTRODUCTION

Vismia guianensis (Aubl.) Choisy is a species that is native to South America and is found in Colombia, Venezuela, Guyana and Brazil, in the forests of secondary vegetation in the states of Amazonas, Pará, Maranhão, Bahia and Minas Gerais (Ewan, 1962). It is a small tree belonging to the Hypericaceae family and is popularly known as “lacre” (Lorenzi; Matos, 1997). The sap (a reddish resin) obtained from the bark, and the infusion of its leaves are widely used in folk medicine, primarily against fungi (Amorozo; Gély, 1988; Lorenzi; Matos, 1997), in the treatment of malaria (NCCLS, 2002), as an anti-rheumatic (Berg, 1971) and for gastric disorders (Berg, 1971), purging (Lee, 1939) and skin burns (NCCLS, 2002).

Several methods and tests using extracts of the genus *Vismia* have demonstrated the presence of important antibacterial, antifungal, antiparasitic, insecticidal and antiviral substances, among others (Vizcaya; Morales; Rojas, 2012; Oliveira et al. 2017; Motta et al, 2022).

The antimicrobial potential of the *Vismia* extracts has been evaluated and has been shown to be effective against a several of different microorganisms. Studies proved the antimicrobial power of these extracts against *Mycobacterium phlei*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* (Vizcaya; Morales; Rojas, 2012). The resin and hexane extract of the leaves showed antifungal activity against *Candida albicans* (Gonçalves; Mors, 1978)

This study aims to evaluate the antifungal potential of the species *Vismia guianensis* (Aubl.) Choisy and the influence of extraction parameters on the antifungal activity. The species *Vismia guianensis* was chosen for this study because it is widely used in folk medicine in the Amazonian region and has some scientifically proven biological activities, mainly for skin diseases, especially fungal infections (Santos et al, 2006; Seo et al, 2000; Silva et al, 2007). This species constitutes a promising candidate for the processing of plant material into a standardized product that can be used safely and with therapeutic efficacy.

METHODOLOGY

Plant Material

Vismia guianensis leaves were collected in EMBRAPA (Brazilian Company for Agricultural Research located in the city of Manaus-AM-Brazil (South Latitude 02°89'34'' and West Longitude 59°97'29'')). The botanical identification was performed at the Federal Institute of Education, Science and Technology of Amazonas (East campus), where the voucher specimen was deposited at the herbarium, under the registration number 6794. The plant material was subjected to drying in a circulating air oven at a temperature of 45 ± 2 °C until the residual humidity was stabilized. After drying, the leaves were subjected to milling in a knife mill using a 1 mm mesh.

Preparation of liquids extracts

The liquids extracts were prepared using three different solvents (hexane, methanol and dichloromethane). The technique of maceration consisted of the sequential extraction (for 1 min each, with agitation) of the same plant material with hexane, then dichloromethane, and then methanol. Posteriorly, two more extracts were prepared by maceration for 24 hours using pure ethanol or methanol as solvent. All extracts were initially obtained with a 5% (w/v) drug:solvent ratio; the solvent was eliminated on a rotary evaporator at a temperature of 40 °C, and the residue was placed in a laminar flow chamber until complete dryness.

Microorganisms tested

The microorganisms tested belonged to the Microbial Collection of the National Institute for Amazonian Research (INPA). Three different strains from each pathogenic species were tested: *C. albicans* ATCC 36232, *C. albicans* U1101, *C. albicans* Tp415, *M. furfur* PV401, *M. furfur* PV399, *M. furfur* PV697, *T. rubrum* ATCC28189, *T. rubrum* U136, and *T. rubrum* 470.

Evaluation of antifungal activity

Methodologies were performed as described by CLSI document M27-A2 (NCCLS, 2002) with some modification. Briefly, *C. albicans* was cultivated in Sabouraud dextrose agar at 37 °C, *T. rubrum* in the same culture medium at 28 °C and *M. furfur* in Sabouraud dextrose olive agar at 37 °C. The colonies were suspended in sterile culture medium (Sabouraud dextrose broth), and the cell density was adjusted to obtain a suspension containing $1-5 \times 10^3$ CFU/mL. The assay was performed in sterile 96-well disposable microdilution plates. The drugs were dissolved in Sabouraud dextrose broth.

The tests were performed using controls ensuring that there was no interference of the diluent, inoculum, or problems with the viability of the cells. The extracts were tested in concentrations ranging from 16.000 to 0.03 $\mu\text{g/mL}$, and ketoconazole (standard drug) was tested between 16 to 0.03 $\mu\text{g/mL}$ as a control. The plates were incubated at 35 °C for 24 hours, and a visual reading was taken. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that caused a 50% reduction in growth compared to the control.

Evaluation of extractive parameters

The best extraction procedure was investigated to obtain extracts able to be dried by the spray drying technique. Two solvents (ethanol:water 50:50 v/v and ethanol) and three extraction methods (maceration for 24 hours, ultrasound for 15 minutes using a Clear Unique ultrasonic washer (Brazil), or decoction for 15 minutes) were investigated. The drug:solvent ratio was fixed at 5% (w/v) in all extractive methods. Dried extracts were obtained using a Mini Spray Dryer (Model MSD 1.0, the Labmaq Brazil).

Characterization of the extracts

Total tannin assay

Total tannin was calculated through the difference between the total polyphenols content and the non-tannin fraction. Total polyphenols quantification was carried out by reading the absorbance at a wavelength of 278 nm on a spectrophotometer. The results were expressed using epicatechin as a reference substance with $A_{1\text{cm}}^{1\%}$ (specific absorption coefficient). The non-tannin fraction quantification was carried out by precipitating the polyphenols with casein and then reading the absorbance at a wavelength of 278 nm on a spectrophotometer (Hartke; Mutschler, 1987; Lins et al., 2016).

Dry residue determination

An aliquot of 20 mL of liquid extract was weighed on a tared flat-bottomed dish and evaporated to dryness. Then, it was placed in oven at 105 ± 1 °C for 2 hours and subsequently placed in a desiccator for 20 minutes and weighed. This procedure was repeated each hour until the sample reached a constant weight. The result was expressed

relative to 100 g of the extraction solution from the mean of three determinations (F. Bras. 6ED, 2022).

Determination of density

Density determination was performed using a pycnometer. The results were expressed as the mean of three determinations (F. Bras. 6ED, 2022).

Determination of pH

The pH was measured using a digital pH meter calibrated with buffer solutions of pH 4.0 and 7.0. The result was expressed as the mean of three determinations (F. Bras. 6ED, 2022).

RESULTS AND DISCUSSION

To evaluate the antifungal activity of *V. guianensis*, the antifungal activities of leaf extracts with different polarities (methanol, dichloromethane and hexane) were assessed against *C. albicans* ATCC 36232. The methanol extract inhibited *C. albicans* growth and demonstrated an MIC of 250 µg/mL. Ketoconazole (a standard substance) presented an MIC of 2 µg/mL. The hexane and dichloromethane extracts did not inhibit *C. albicans* growth.

Because the polar extract presented the inhibitory activity, a methanol extract and an ethanol extract (more polar solvents) obtained using 24 hours of maceration were evaluated to investigate the influence of the solvent. In this assay, the antifungal activity was tested against 3 strains of each of the following pathogenic species: *C. albicans*, *M. furfur* and *T. rubrum*, totaling 9 strains. There was no significant difference in antifungal activity displayed by the two extracts (Table 1). *C. albicans* was the species most sensitive to the action of the extracts, presenting an MIC between 250 and 500 µg/mL.

Table 1: MICs (µg/mL) of methanol and ethanol extracts of *V. guianensis* against strains of *C. albicans*, *M. furfur* and *T. rubrum*.

Isolates	Methanol extract 24 h		Ethanol extract 24 h		Ketoconazole	
	Mean	Range	Mean	Range	Mean	Range
<i>C. albicans</i>	410	250 – 500	410	250 – 500	3	1 - 4
<i>M. furfur</i>	660	500 – 1000	660	500 – 1000	6.16	0.5 - 16
<i>T. rubrum</i>	1000	NR	1000	NR	0,25	NR

Mean: MIC mean of three cultures; Range: MIC range of three cultures; NR- No Range

To obtain an extract with high antifungal activity able to be dried by a spray dryer apparatus, trials were carried out investigating the influence of two types of solvents (ethanol and ethanol:water, 1:1) and three extraction methods (ultrasound, decoction and maceration). All extraction solutions obtained were dried by spray drying, and the antifungal activities of the dried extracts obtained are shown in Table 2.

Table 2: MIC (mg/mL) of dried extracts obtained against isolates of *C. albicans*, *M. furfur* and *T. rubrum*.

Extraction method	Microorganism	Solvent			
		Ethanol		Ethanol: water	
		Mean	Range	Mean	Range
Ultrasound	<i>C. albicans</i>	8.0	NR	4.0	NR
	<i>M. furfur</i>	8.0	NR	8.0	NR
	<i>T. rubrum</i>	1.0	NR	1.0	NR
Decoction	<i>C. albicans</i>	8.0	NR	4.0	NR
	<i>M. furfur</i>	16.0	NR	4.0	NR
	<i>T. rubrum</i>	1.0	NR	1.0	NR
Maceration	<i>C. albicans</i>	1.660	1 - 2	1.160	0.5 – 2.0
	<i>M. furfur</i>	8.0	NR	3.330	2.0– 4.0
	<i>T. rubrum</i>	1.0	NR	1.0	NR

Mean: MIC mean of three cultures; Range: MIC range of three cultures; NR- No Range

The dried extract obtained from maceration extraction using the hydroalcoholic solvent (1:1) presented the lowest MIC for all pathogenic species. The spray drying technique decreased the antifungal activity of the liquid extracts. For example, maceration

using ethanol as the solvent (where the solvent was evaporated on a rotary evaporator and the residual solvent was removed in laminar flow until dryness) presented an MIC for *C. albicans* of 410 µg/mL, while the same extract dried by spray drying presented an MIC of 1660 µg/mL.

Considering these results, the extract solution obtained by maceration using ethanol:water as the solvent was chemically characterized, and the results are presented in Table 3.

Table 3: Characterization of hydroalcoholic extract obtained by maceration, using ethanol:water as the solvent.

Assay	Results
Total tannin content %	1.36 ± 0.03
Dry residue %	1.17 ± 0.003
pH	5.0 ± 0.0
Density	0.82±0.02
MIC µg/mL against <i>C. albicans</i>	500 – 2000

Traditional medicine, which is mainly based on the use of medicinal plants, enjoys a respected position, especially in developing countries where the availability of modern services is limited (Agra et al., 2007). In the Amazon, the use of medicinal plants by indigenous riverside populations represents an important source of knowledge of traditional therapy. One of the plants with medicinal properties that is widely used by Amazon populations in the treatment of skin diseases, especially those caused by fungi, is *Vismia guianensis* (Aubl.) Choisy (Motta et al, 2022).

Antifungal drugs available on the market have problems of therapeutic failure (high recurrence and resistance), which, combined with their high toxicity, have rendered it important to search for alternate antifungal agents that are more effective and less toxic (Fenner et al., 2006). Currently, researchers have turned their attention to natural antifungal agents, namely medicinal plants, which could circumvent the undesirable effects. In this case, *V. guianensis* demonstrated significant inhibitory activity against the growth of fungi tested and likely has great potential to be a candidate drug for the treatment of cutaneous mycoses.

The initial results of antifungal activity demonstrated that the substances with antifungal activity have polar characteristics, as only the methanol and ethanol extracts were able to inhibit the growth of *C. albicans*. The results showed no difference in the antifungal activity of three extracts against three strains of microorganisms tested. The extracts inhibited each microorganism with the same MIC.

The results presented herein, from examining the inhibitory activities of methanol and ethanol extracts, showed that there was no significant difference between the two solvents. It is thus more feasible to use ethanol extracts because ethanol is accessible, cheaper than methanol, and compatible with drying by a spray dryer, giving the extracts higher physical and chemical stability (Pasqua, 1995).

The analysis of the effects of extraction parameters on the antifungal activities of the extracts showed that the method of maceration with the 1:1 ethanol:water solvent presented the lowest MIC. However, the extracts dried by spray drying showed lower antimicrobial activity than those dried at room temperature. This result can be explained by the high temperature used in the spray drying process, which can degrade some substances present in extracts, leading to a decrease in antifungal activity. However, in the preparation of a formulation from this plant species, the mass percentage of the extract can be adjusted, thereby optimizing antimicrobial activity.

Many secondary metabolites have been identified in *Vismia guianensis*: β -sitosterol, vismione H (Botta et al., 1985), quinones (Gonzales et al., 1980), dianthrones (Santos et al., 2007), anthraquinones (Santos et al., 2007) and xanthone (Botta et al., 1986). Seo and colleagues (2000) succeeded in isolating the chloroform fraction from the roots of *V. guianensis* and identified five benzophenones (vismiaguianones A, B, C, D and E) and two benzocoumarines (vismiaguianines A and B). Four classes of secondary metabolites are present as main components of the leaves of *V. guianensis*: anthraquinones, tannins, flavonoids, xanthenes and benzophenones (Santos et al., 2007). Whereas plants are rich in chemicals, antimicrobial activity demonstrated by *V. guianensis* can be attributed to the presence of polyphenolic constituents, which are easily extractable by polar solvents such as ethanol, methanol and water, taking into account that this class of compounds already has proven antimicrobial activity (Soares et al., 1998). Other biological activities have also been demonstrated by *V. guianensis*. Some studies have demonstrated the activity of *V. guianensis* in fighting cancer cells. The metabolite vismione, which is present in *V. guianensis* and other species of the genus, has shown *in vitro* activity against the experimental tumor lineage M5076, ovarian

carcinoma, and B16 melanocarcinoma (Politi et al., 2004). Lethal activity of organic and aqueous extracts as a front line therapy against MCF-7 human breast adenocarcinoma was also identified (Tada et al., 1991).

CONCLUSION

The characteristics of the extract with the best antifungal activity with MIC range of 250 to 500 µg/mL were identified and standardized. The dry residue found for the solution was 11.7 g/L, a low soluble solids content. The density of the extract was 0.82, due to the liquid extract (a hydroalcoholic solution at 50% (v/v)). The pH of the extraction solution was 5.0, which is acidic. Tannins have many ionizable hydrogen groups, resulting in an extraction with acidic characteristics and the extract presented 1.36 g% of tannins total.

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