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ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF THE FLORAL ETHANOLIC EXTRACT OF *MANDEVILLA POHLIANA* (STADELM.) A. H. GENTRY

ATIVIDADE ANTIFÚNGICA E ANTIBACTERIANA DO EXTRATO ETANÓLICO FLORAL DE *MANDEVILLA POHLIANA* (STADELM.) A. H. GENTRY

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Resumo

Mandevilla pohliana é uma espécie pertencente à família Apocynaceae encontrada em ambientes de Cerrado. Objetivou-se avaliar neste estudo, a ação antifúngica e antibacteriana do extrato etanólico floral de *M. pohliana*. Flores frescas de *M. pohliana* foram coletadas e o extrato etanólico produzido por maceração. Em diferentes concentrações de extrato, foram determinadas as atividades antifúngicas sobre o gênero Candida, e antibacterianas sobre Escherichia, Salmonella, Enterococcus, Pseudomonas e Staphylococcus. Foi observada atividade de antibiose apenas para *C. guilliermondii* com 9-2 mm, para *P. aeruginosa* com 12-3 mm e para *E. faecalis* entre 10-3 mm nas maiores concentrações entre 50-500 mg mL-1. O extrato etanólico floral de *Mandevilla pohliana* demonstrou potencial como agente antifúngico e antibacteriano natural.

Abstract

Mandevilla pohliana is a species belonging to the Apocynaceae family found in Cerrado environments. The objective of this study was to evaluate the antifungal and antibacterial action of the floral ethanolic extract of M. pohliana. Fresh flowers of *M. pohliana* were collected and the ethanolic extract was produced by maceration. In different concentrations of extract, antifungal activities on the genus Candida were determined, and antibacterial activities on Escherichia, Salmonella, Enterococcus, Pseudomonas and Staphylococcus. Antibiosis activity was observed only for *C. guilliermondii* with 9-2 mm, for *P. aeruginosa* with 12-3 mm and *E. faecalis* between 10-3 mm at the highest concentrations between 50-500 mg mL-1. The floral ethanolic extract of *Mandevilla pohliana* showed potential as a natural antifungal and antibacterial agent.

INTRODUCTION

The Apocynaceae family has numerous botanical genera, among which, *Mandevilla* Lindl stands out. belonging to the *Mesechiteae* Tribe, widely distributed in zones of neotropical climate, presenting 150 species distributed from Central America to South America. The species inserted in the genus Mandevilla, are found in very varied natural environments such as desert, forests, tepuis, open pastures, and in particular *M. pohliana* (Fig. 1) in areas the cerrado domain, rupestrian fields, and restingas in Brazil, flourishing between September to July (SIMÕES et al., 2006; MONGUILHOTT; MELLO-SILA, 2008; DUARTE; LARROSA, 2011).

In general, the *Mandevilla* genus is composed of shrubs, sub-shrubs or lianas, which produce whitish latex, sometimes presenting a root system (xylopodium); the branches are glabrous or hairy;

opposite leaves, rarely verticilated, petiolate or sessile; lateral and axillary or terminal inflorescences, simple racemes, rarely composed; and fruits with comosa seeds (MONGUILHOTT; MELLO-SILA, 2008).

Phytochemical researches with *Mandevilla* were gathered and described in the study by Duarte and Larrosa (2011) where they include the considerable presence of groups of phytomolecules such as cardiac glycosides, steroids, and triterpenoids, oleic and linoleic acids, acetylilustrol, pregnanos glycosides, velutinoside A and velutinol A. However, there are few studies with *M. pohliana* (MARTINS; ALVES, 2008; VITAL, 2019), and there are no studies with biological activities from the flower of *M. pohliana*, making it necessary to know and elucidate the phytochemical profile and potentials biological activities.

The aim of this work was to evaluate the floral ethanolic extract of *M. pohliana* with antifungal and antibacterial activity in different concentrations.

Figure 1. *Mandevilla pohliana* in flowering period in the Cerrado domain, phytophysiognomy restricted sense. Source: Authors, 2020.

MATERIAL AND METHODS

Plant material

Fresh flowers of *M. pohliana* were collected in the municipality of Rio Verde (17° 47'10.9" S and 50° 58'02.0" W), at the University of Rio Verde, Goiás, Brazil. The species was identified and deposited at the Herbarium of the Goiano Federal Institute, Goiás, Brazil, with the voucher (number HRV 10090).

Extract production

Fresh flowers of *M. pohliana* were added in 98% ethanol. The extract was produced by maceration for five days. After this period, the solvent was extracted in a rotary evaporator with negative pressure and a temperature of 50 °C. The extract was then kept at -12 °C and lyophilized.

Antifungal and antibacterial activity

The microbiological test followed as described by Vieira et al. (2019) modified, using the paper disk diffusion technique, and the results expressed in (mm). Strains of *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 4563), *Candida guilliermondii* (ATCC 90877), *Candida krusei* (ATCC 34135), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (LB29212), *Salmonella serovar* Enteritidis (ATCC 13076) and *Salmonella serovar* Typhimurium (ATCC 14028).

The activation of the microorganisms was carried out in a sterile solution of NaCl conc. 0.85% until reaching the degree of 0.5 on the scale MacFarland conc. ($1x10^4$ CFU mL^{-1}). 10 cm *Petri* dishes were prepared with sabouraud dextrose agar (SDA) for the antifungal assay, and for the bacterial assay with mueller hinton agar (MHA) after sterilization.

The *Petri* dishes containing a specific medium were inoculated using a sterile swab soaked with the microbial suspension and spread throughout the plate. Filter paper discs with a diameter of 7 mm were impregnated with 100 µL of the extract in different concentrations (500, 200, 50, 25, and 5 mg mL-1), as a negative control, saline solution with 10% DMSO (*v*/*v*) and a disk containing a 10% (*v*/*v*) DMSO solution, and as a positive control disc with antimicrobial agents, for

bacteria Azithromycin (15 μg), Cephalexin (30 μg), Tigecycline (15 μg) and Amikacin (30 μg), and fungal ketoconazole (50 μg).

The *Petri* plates were incubated at 36 °C with an interval between 24-36 hours, after that period, the halo of antibiosis when present was measured with a digital caliper. The tests were carried out in triplicate.

Statistical analysis

The arithmetic mean was determined followed by ± standard deviation. The Tukey's test ($P \leq 0.05$) was used when there is a significant difference between the concentrations and patterns of antibacterials and antifungal agents, using the *PAST* 3 program.

RESULTS AND DISCUSSISON

Numerous fungi and pathological bacteria have acquired resistance to the main antimicrobial agents. And this is a serious problem since the widely used antifungals and antibacterials no longer have the effect of antibiosis. Evaluating the floral ethanolic extract of *M. pohliana* (Table 1), it was found that only the strain of *C. guilliermondii* was sensitive to the extract in the two highest concentrations of 500-200 mg mL^{-1} with an inhibitory effect of 9 and 2 mm. For the antibacterial assay, the extract demonstrated potential bacteriostatic activity on *P. aeruginosa* between 12-3 mm between concentrations 500-50 mg mL^{-1}, a similar effect was observed for *E. faecalis* with inhibition of 10-3 mm in the three highest concentrations.

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All results when positive showed a statistical difference between them and the three antibacterial agents and a reference antifungal agent using the Tukey's test with ($P \leq 0.05$).

Table 1. Antimicrobial activity of the floral ethanolic extract of *Mandevilla pohliana*.

Microorganisms	Growth inhibition zone (mm)								
	500	200	50	25	5	Disc 15 μg[a]	Disc 30 μg[b]	Disc 15 μg[c]	Disc 50 μg[d]
C. albicans	-	-	-	-	-				26
C. krusei	-	-	-	-	-				28
C. guilliermondii	9[b]	2[c]	-	-	-				24[a]
C. tropicalis	-	-	-	-	-				29
P. aeruginosa	12[b]	8[b]	3[c]	-	-	20[a]	22**[a]	nd	
E. coli	-	-	-	-	-	19	20*	nd	
S. aureus	-	-	-	-	-	24	27*	nd	
E. faecalis	10[b]	7[b]	3[cb]	-	-	20[a]	nd	22[a]	
S. Enteritidis	-	-	-	-	-	28	25*	nd	
S. Typhimurium	-	-	-	-	-	29	28*	nd	

Extract concentration determined in mg mL^{-1}. (-) There was no halo formation. Nd No determined. Azithromycin disc[a]. Cephalexin disc[b]*. Amikacin disc[b]**. Tigecycline disc[c]. Ketoconazole disc[d]. Source: Authors, 2021.

There are no studies in the literature on antifungal and antibacterial activity for *M. pohliana*, the few studies like that by Carpinella et al. (2010) describe brief essays on both biological actions, although they have not verified an effective fungistatic action on *Fusarium verticillioides* from the extracts of *M. laxa* and *M.*

pentlandiana. Moderate antibacterial activity on *P. aeruginosa*, *Mycobacterium phlei*, *Bacillus subtilis*, *E. faecalis* and *S. aureus* were observed from the leaf ethyl acetate extract of *M. veraguasensis* in the study by Abad-Reyes et al. (2006).

According to Sampaio et al. (2021), the *Mandevilla* genus has several pharmacological applications as venom inhibition, anti-edematogenic and anti-inflammatory activity (BIONDO et al., 2003; MATTOS et al., 2006; ALMEIDA et al., 2017). Thus, it is important to highlight the main biological and pharmacological properties described in the literature was antibacterial and bacteriostatic activity like flavonoids, organic acids, tannins, triterpenoids, phenols, and alkaloids (DUARTE et al., 2014; BARCELOS et al., 2017; CARVALHO et al., 2020; SANTOS et al., 2020).

Subsequent studies should be carried out by assessing the minimum inhibition concentration (MIC) assay for pathological agents that have been shown to be sensitive at the usual doses in this study. It is also suggested, that even if there is no inhibition to the other agents by the concentrations evaluated, it does not mean that the extract does not present such an inhibitory action, and should be evaluated in concentrations higher than those recommended in this work, as well as an analysis of the phytochemical profile of this floral extract.

CONCLUSION

It is concluded that the floral ethanolic extract of *M. pohliana*, has a potential antifungal and antibacterial activity for most of the tested strains.

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