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ORIGINAL ARTICLE

Chemical Composition, Antibacterial and Antifungal Potential of an Extract From the Leaves of *Guapira Graciliflora* Mart. Against Oral Microorganisms of Dental Interest

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Abstract

Objective: To perform an *in vitro* analysis of antibacterial and antifungal potential of an alcoholic extract from the leaves of *Guapira Graciliflora* Mart. against oral microorganisms and determine its chemical composition. **Material and Methods:** A hydroalcoholic extract of the leaves form *G. graciliflora* was obtained through maceration, vacuum concentration and freeze-drying. Antibacterial and antifungal activities were evaluated against *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus oralis*, *Streptococcus parasanguinis*, *Streptococcus mitis* and strains of *Candida albicans* using broth microdilution method. Phytochemical analysis determined the total phenolic compounds, protein concentration and total of sugars present in the extract. **Results:** *G. Graciliflora* demonstrated antifungal activity against the LM 11 and LM 410 clinical isolates of *C. albicans* (MIC 0.5 mg/mL and 2 mg/mL, respectively). The other microorganisms tested were resistant to the extract. The phytochemical analysis revealed 3% proteins, 13% total sugars and 17% phenolic compounds. **Conclusion:** *G. Graciliflora* has antifungal activity against clinical strains of *C. albicans* and exhibits proteins, sugars and phenolic compounds in its chemical composition.

Keywords: Plants, Medicinal; Plant Extracts; Phytotherapy; Anti-Infective Agents.

Introduction

The oral cavity is an important site regarding the complexity and diversity of its microbiome, it has a complex ecosystem with over 700 identified species [1,2] in which fungi and bacteria gained relevance due to its correlation with prevalent infections such as oral candidiasis [3], periodontal diseases [4], and dental caries [5]. However numerous strains of bacteria and fungi have developed resistance to the action of conventional antimicrobial agents, possibly as a consequence of the indiscriminate use of antibiotics by the population [6-8].

The interest in natural products, such as medicinal plants, has grown in the primary health care setting since plants represent an excellent source of biologically active agents for the development of products of medical and dental interest [9]. *Guapira Graciliflora* Mart. belongs to the family *Nyctaginacea* and is commonly found in the *Caatinga* semi-arid biome of northeastern Brazil [10,11]. This plant is used as a folk remedy and demonstrates antimicrobial activity [12,13] and anticandidal activity [14].

Identification of a novel antimicrobial agents can contribute in the treatment of oral conditions that are considered public health problems, such as dental caries and oral candidiasis. Therefor *in vitro* studies with microorganisms of oral interest are important and must be conducted. Additionally, this study proposes to use *G. graciliflora* leaves obtained in the semi-arid region of the state of Paraíba in northeastern Brazil in a winter time, studies has shown that geographic location and environmental conditions may have influence on the plant chemical composition [15,16].

Thus, the aim of the present study was to evaluate the antimicrobial potential of a hydroalcoholic extract from the leaves of *Guapira Graciliflora* Mart. against microorganisms related to these diseases and determine its chemical composition.

Material and Methods

Vegetal Material and Obtainment of Alcoholic Extract

Leaves of *G. Graciliflora* Mart. were collected from a rural area of the municipality of Queimadas in the semi-arid region of the state of Paraíba in northeastern Brazil between July and August 2012. The material was cleaned, placed in paper bags and dehydrated in a laboratory incubator (Fanem 330, Fanem Ltda., São Paulo, SP, Brazil) at 40°C. A voucher specimen was deposited in the collection of the Manuel de Arruda Câmara Herbarium of the State University of Paraíba, Campus I, Campina Grande, Brazil (n° 907/ ACAM). The extraction process involved maceration for five days, using a proportion of 200 grams of dried, ground leaves to one liter of 50% ethanol. The extract was vacuum concentrated in a rotary evaporator Q344M (Quimis Aparelhos Científicos, Diadema, SP, Brazil) at 40°C and the residual portion was freeze dried (Labconco Freezone 4.5, Labconco Corp., Kansas City, MO, USA) [16].

Antimicrobial Assays

Microbial Growth Conditions

Six bacterial strains - *Streptococcus mutans* (ATCC 25175), *Streptococcus salivarius* (ATCC 7073), *Streptococcus oralis* (ATCC 10557), *Streptococcus parasanguinis* (ATCC 903), *Streptococcus mitis* (ATCC 49456) and *Candida albicans* (ATCC 18804) and clinical isolates of the yeast *Candida albicans* (LM 11 and LM 410) were acquired from the Antimicrobial Research Laboratory of the State University of Paraíba. Bacteria and yeast were activated in brain heart infusion (BHI) and in sabouraud dextrose agar medium respectively; the plates were incubated at 37°C (SP Labor Equipamentos Para Laboratórios, São Paulo, SP, Brazil) for 24 h in either an aerobic or microaerophilic atmosphere (Anaerobic Jar Permution, Curitiba, PR, Brazil) [17,18].

Determination of Minimum Inhibitory Concentration (MIC)

The broth microdilution method was used for the determination of the minimum inhibitory concentration (MIC). Using a 96-well plate, 100 µl/well of culture medium (BHI agar for bacteria and sabouraud dextrose agar for yeasts) and 100 µl of *G. graciliflora* were added to the first well of each row to begin the serial microdilution process. Next, 100 µl/well of inoculum (5 x 105 colony forming units/mL for bacteria and 2.5 x 103 colony forming units/mL for yeasts) were added. The plates were incubated at 37ºC (SP Labor Equipamentos Para Laboratórios, São Paulo, SP, Brazil) for 24 h in either an aerobic or microaerophilic atmosphere (Anaerobic Jar Permution, Curitiba, PR, Brazil), depending on the microorganism [17,18]. The positive controls were chlorhexidine (Sigma-Aldrich Brasil, Merck KGaA, Darmstadt, Germany) for bacteria and nystatin (Sigma-Aldrich Brasil, Merck KGaA, Darmstadt, Germany) for yeasts. Individual wells of the microplates were used to determine the control growth of the microorganism as well as the sterility of the culture medium, vegetal material and vehicle.

After the incubation period, 50 mL/well of 0.01% resazurin solution (Sigma-Aldrich Brasil, Merck KGaA, Darmstadt, Germany) were added and the MIC was defined as the lowest concentration of extract capable of impeding the appearance of red coloration in the medium when the cells demonstrated respiratory activity. The following criteria were used for the classification of the antimicrobial activity of the extract: MIC < 100 µg/mL = strong activity; MIC between 100 and 500 µg/mL = moderate activity; MIC between 500 and 1000 µg/mL = weak activity; and MIC > 1000 µg/mL = no activity [19].

Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

For the determination of the MBC and MFC, a 50 µL aliquot of the MIC and a concentration above the MIC were sub-cultivated in BHI agar medium (bacteria) or sabouraud dextrose agar medium (yeasts) and incubated at 37°C for 24 h. The MBC and MFC were defined as the lowest concentration that inhibited visible growth in the medium.

Phytochemical Analysis

Total Phenolic Compounds

The content of total phenolic compounds was determined using the Folin-Ciocalteau spectrophotometric method, with gallic acid as the reference standard [20]. The reading was performed in a spectrophotometer 600S (FEMTO Indústria e Comércio de Instrumentos, São Paulo, SP, Brazil) at 765 nm.

Protein Concentration

The concentration of protein was determined using the Bradford reagent [21]. Bovine serum albumin was used as the standard. The reading was performed at 595 nm in a microplate reader (Epoch, Biotek, Gen5 Data Analysis Software, Winooski, USA).

Total Sugars

The quantification of sugars was determined using the Dubois method through the formation of furfural compounds following the dehydration of sugars by sulfuric acid (A.R.) [22]. The reading was performed in a spectrophotometer 600S (FEMTO Indústria e Comércio de Instrumentos, São Paulo, SP, Brazil) at 490 nm.

Results

The *G. graciliflora* extract exhibited antimicrobial activity against the clinical isolates of *Candida albicans*, with moderate potential regarding strain 11 (MIC: 0.5 mg/mL) and weak potential regarding strain 410 (MIC: 2 mg/mL), based on the classification described by some authors [19]. The other microorganisms tested were resistant to the extract (Table 1). Regarding the chemical composition it was revealed that the extract was 3% proteins, 13% total sugars and 17% phenolic compounds.

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration of *G. graciliflora* extract.

Microorganism	Extract (mg/mL^{-1})			Chlorhexidine[c]		Nystatin[c]	
	MIC	MBC	MFC	MIC	MBC	MIC	MFC
S. mutans	R	R	-	H	H	-	-
S. salivarius	R	R	-	H	H	-	-
S. oralis	R	R	-	H	H	-	-
S. parasanguinis	R	R	-	H	H	-	-
S. mitis	R	R	-	H	H	-	-
C. albicans	R	-	R	-	-	H	H
C. albicans (LM 11)*	0.5	-	1	-	-	H	H
C. albicans (LM 410)*	2	-	2	-	-	H	H

MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration; *Clinical strain; [c]Positive Control; R: Resistant; H: Concentration of Last Well.

Discussion

Based on the criteria established by another study [19] the antifungal activity of *G. graciliflora* extract was classified as moderate for the LM11 clinical strain of *C. albicans*. The antifungal activity of this extract was also established in a previous work [14], however different strains of *Candida* were used. *C. albicans* are considered the etiological factor of oral candidiasis, it has been isolated in about 50% of the cases, therefore considering the consequences of oral candidiasis this is an important finding in the search for effective antifungal agents against the progression of this infection, especially in immunocompromised patients [23-25].

G. graciliflora extract didn't exhibit antibacterial activity diverging from the literature. Some authors [13] investigated the potential of *G. graciliflora* using hydroalcoholic extracts of the leaves and bark and demonstrated antimicrobial activity against the following bacteria associated with endodontic infection: *S. aureus* (12.50 and 12.50 µL/µL), *E. faecalis* (12.50 and 6.25 µL/µL) and *E. coli* (12.50 and 6.25 µL/µL). The ethanol extract from the stems of *G. Graciliflora* Mart. demonstrated antimicrobial action against *S. aureus*, *E. coli*, *P. aeruginosa* (MIC ≥ 1024 µg/mL) and *K. pneumoniae* (MIC = 512 µg/mL) [12].

The divergences in the results may be explained by differences in the parts of the plant employed, the extraction method, and time of year in which the vegetal material was collected, which are factors that exert an influence on the compounds obtained and the bioactive activities of the extracts [26,27]. The antimicrobial activity of an extract occurs through the combined action of the chemical compounds in the plant rather than by the isolated activity of each compound [26].

Regarding the *G. graciliflora* extract used in this study, chemical analysis revealed proteins, sugars and phenolic compounds, additionally, phytochemical profile stablished in a previous study showed the presence of flavonoids rutin and kaempferol as well as the polyol pinitol [14]. The phytochemical characterization of vegetal species is fundamental, as such species are rich in bioactive substances that are useful to the development of therapeutic agents [28]. Studies involving the phytochemical characterization of plants from

the family *Nyctaginaceae* have also revealed the presence of secondary metabolites belonging to the flavonoid, carotenoid, betacyanin, alkaloid and saponin groups [10,29,30]

Rutin and kaempferol are known for their antioxidant, antitumor, antimicrobial and anti-inflammatory effects [31] and have demonstrated antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Bacillus subtilis* and *C. albicans* [32,33]. These compounds can inactivate enzymes and form complexes with proteins in the cell wall of microorganisms, which are likely the mechanisms of action for the present study [34,35]. Phenolic compounds, as phenols are also good antimicrobials [36,37] and polyols, such as pinitol, are products of the metabolism of carbohydrates and are associated with strong antioxidant activity [38].

The present results underscore the need for further biological assays involving *G. graciliflora*. The need for more tests to seek the confirmation of the antifungal activity must be conducted, and it is a limitation of this study, more species of *Candida* should be tested since it demonstrated a moderate potential against a fungal stain tested, and therefore give continuity to studies that seek bioactive substances for the development of novel antimicrobial drugs [39] since studies have already shown the growth in the resistance against conventional antimicrobial agents [6-8]. Additionally, toxicological studies must be done to assure its safety when used by the general population [40].

Conclusion

The extract from the leaves of *G. graciliflora* has proteins, sugars and phenolic compounds in its chemical composition and exhibits antifungal activity, capable of *in vitro* inhibition of the growth of clinical isolates of *C. albicans*.

Authors' Contributions

TKA	0000-0003-1178-4552	Methodology, Investigation and Writing - Original Draft.
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RFL	0000-0002-2818-7036	Methodology and Investigation.
GPG	0000-0002-7648-0683	Conceptualization and Supervision.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

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