

Full Length Research Paper

Phytochemical screening, toxicity and antimicrobial action of *Solanum paniculatum* Linn extract against dental biofilm bacteria

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The aims of study were to determine the phytochemical composition of hydroalcoholic extract of *Solanum paniculatum* Linn (jurubeba) root, to evaluate its *in vitro* antimicrobial action, as well as to determine the acute toxicity and potential cytotoxic effects of this extract. The extract was characterized by phytochemical screening and thin-layer chromatography. The following oral bacteria were used to determine the minimum inhibitory concentration (MIC): *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus salivarius* and *Lactobacillus casei*. Each assay was carried out in duplicate and the positive control (0.12% chlorhexidine digluconate) was subjected to the same procedure. Results were analyzed by Student t-test or Mann-Whitney test, with the level of significance set at 5%. Preclinical acute toxicity assays were performed using the median lethal dose of the extracts in animals. In addition, the cytotoxic effects of the extracts on human erythrocytes were evaluated. *Solanum paniculatum* showed MIC values of 7.81 mg/mL. The extract had no acute effects at concentrations of 0.97 to 500 mg/mL. The *S. paniculatum* extract was only cytotoxic at a concentration of 250 mg/mL. Phytochemical screening revealed predominance of phenolic compounds such as flavonoids and tannins. In conclusion, *S. paniculatum* Linn showed *in vitro* antimicrobial activity against bacterial monocultures. No toxicological effects were observed. The predominance of phenols may explain the pharmacological activity of this extract. However, randomized controlled clinical trials should be conducted to evaluate the effect of *S. paniculatum* added to mouthwash solution.

Key words: Microbiology, phytotherapy, chromatography, toxicity, *Solanum paniculatum* Linn.

INTRODUCTION

Diseases affecting the oral cavity are of infectious origin.

Depending on factors such as diet and regular

mechanical removal of plaque, the type of microbiota prevailing in the oral cavity may vary, having a high or low degree of pathogenicity. There are difficulties in achieving patients' adequate mechanical control of dental plaque accumulation, therefore, antimicrobial substances could compensate with regards to good teeth cleaning (Torres et al., 2000).

Many cultures commonly use medicinal plants for the treatment of various disorders and diseases affecting humans. From this perspective, the benefits of these herbal medicines are a source of promising research in countries like Brazil, known for its immense floral diversity, as well as for a high prevalence and variability of infectious diseases (Vieira et al., 2010).

Research involving herbs and plants in dentistry is increasing in order to elaborate new therapies against oral infections. In this context, there are many studies that relate the oral microbiota to the use of medicinal plants, with the objective of eliminating pathogenic agents (Majid and Omid, 2011).

The oral cavity often has a diverse microbiota and the eruption of dental elements leaves this microbiota even more complex. The pioneer organisms in the mouth are the *Streptococcus mitis*. After tooth eruption, other commensal species of *Streptococcus*, including *Streptococcus sanguinis* and *Streptococcus gordonii* are present as pioneers of the dental biofilm. From these species, it is possible that other opportunistic pathogens such as the *mutans* colonize, which is the primary species associated with dental caries. Thus, the state of oral health or disease suffers crucial influence of colonization by the commensal species of *Streptococcus* because the oral microbiota is occupied by them (Morales et al., 2014).

The "jurubeba" (*Solanum paniculatum* Linn) is a species found in America, in tropical regions, mainly in the Brazilian Cerrado. It is used not only in folk medicine for the treatment of liver and gastric diseases, but also in culinary practices (Vieira Junior et al., 2015; Vieira et al., 2010). In addition, as a highlight of its utility, "jurubeba" tea is great for hangovers and extracts are used for respiratory and stomach diseases. It has properties that prevent gastric secretion, and also anti-inflammatory and antioxidant characteristics (Vieira et al., 2010, 2013).

The *Solanaceae* family is constituted by species and toxic characteristics and pharmacological properties. Some previous studies with the ethanolic extract of the leaf or fruit of *S. paniculatum* have shown that they have no genotoxic activity in rats or bacterial strains, despite their cytotoxicity and antigenotoxicity in high doses. The active constituents of jurubeba have been reported as steroid saponins, glycosides, alkaloids and tannins in the roots, stems and leaves (Vieira et al., 2013).

The aim of the study was to evaluate the antimicrobial action, toxicity and cytotoxicity of the *S. paniculatum* and to analyze the plant material with respect to its phytochemical aspects.

MATERIALS AND METHODS

Preparation of the *Solanum paniculatum* Linn. extract

The roots of *S. paniculatum* were collected in the town of Teixeira, Paraíba, Brazil. The crude extract was prepared at the Laboratory of Chemical and Biological Sciences, Federal University of Campina Grande (UFCG), Center for Health and Rural Technology (CSTR). A voucher specimen of the plant was deposited at the Dárdano de Andrade Lima Herbarium, Regional University of Cariri (URCA), Crato, Ceará (Registration No. 4016).

After collection, the *S. paniculatum* root samples were desiccated in an oven under circulating air at an average temperature of 45°C and then ground to powder in a mechanical grinder. The dry and ground material was macerated with 2 L of 95% ethanol for 72 h. The resulting crude *S. paniculatum* extract was concentrated in a rotary evaporator under reduced pressure at a temperature that did not exceed 40°C. For phytochemical analysis, the crude extract was resuspended in distilled water to a final concentration of 10%.

Phytochemical analysis and thin layer chromatography (TLC)

The crude extract of *S. paniculatum* was resuspended in distilled water (10% solution) and then was submitted to various qualitative tests of color change, excitation by ultraviolet light and precipitation, to identify several classes of secondary metabolites. These tests investigated the presence of saponins, tannins, gums, mucilages, flavonoids, quinones, lactones, coumarins, steroids, triterpenoids, carotenoids, alkaloids, catechins and resins through specific chemical reactions.

Next, the extract of *S. paniculatum* was analyzed by thin layer chromatography (TLC) through the resuspension in methanol (1% solution) for application in chromatographic plates (Merck®, Darmstadt, Germany). Silica gel chromatoleaves were used as a fixed-phase and various mobile phases were assayed for the presence of specific phenols. The extract and standards were eluted in saturated chromatographic vats, using a methanol solution of ferric chloride to 5% for the revelation of tannins (catechin, epicatechin, tannic acid, ellagic acid and gallic acid), while the plates which researched flavonoids (quercetin, isoquercetina, rutin, vitexin and isovitexin) were used a natural reagent (Wagner and Bladt, 1996; Lionço et al., 2001).

Determination of minimum inhibitory concentration (MIC)

Antimicrobial activity on plates was determined according to the methodology proposed by Bauer et al. (1966), by diffusion in solid medium in Petri dishes for the determination of minimum inhibitory concentration (MIC) of extract of *S. paniculatum* Linn.

The bacterial strains (*S. mitis* -ATCC 903-, *S. mutans* -ATCC 25175-, *S. sanguinis* -ATCC 15300-, *S. oralis* -ATCC 10557-, *S. salivarius* -ATCC 7073- and *Lactobacillus casei* -ATCC 9595) were cultured in nutrient broth (BHI - brain heart infusion - Difco,

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Michigan, USA), incubated at 37°C for 18-20 h under microaerophilic conditions by the candle flame method. Mueller-Hinton agar plates (Difco, Michigan, USA) were prepared and after 24 h (sterility control), they were flooded with saline inoculated with each bacterial growth at a concentration of 10^{-1} , and below, and standard holes were prepared measuring approximately 6 mm in diameter. In each plate, five holes were made receiving numbering ranging from 1 to 5, which corresponded to the solution of the crude extract (CE), which were then diluted in distilled water (CE to 1:512). After the introduction of 50µL of the test substances, the plates were incubated inside a bacteriological greenhouse at 37°C for 24 h. Each assay was carried out in duplicate of each selected strain. The same procedure was used for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA.).

The lowest concentration of the extract that could inhibit growth was regarded as MIC, represented by the presence of an inhibition halo, measured in mm with the aid of the caliper (Digimess®, São Paulo, Brazil).

Determination of the lethal dose (LD₅₀)

For the LD₅₀, adult animals (Swiss albino mice) were used, weighing between 18 and 40 g, provided by Vivarium professor Thomas George LTF-UFPB. All were placed in polyethylene cages, with five animals per cage, maintained under temperature conditions of $27 \pm 2^\circ\text{C}$, without any medication, having free access to food (pellets type purine diet) and water. The animals were kept in light/dark cycles of 12 h before performing any experimental protocol. The animals were divided into 21 groups of five units and treated with the extract of *S. paniculatum* Linn (at different concentrations - CE to 1:512) intraperitoneally (I.P.) in a single dose of 0.1 mL/animal. The control group was given distilled water.

Then, the animals were observed for a period of 24 h with the objective of mapping possible behavioral changes, suggestive activity on the central nervous system (CNS) or on the autonomic nervous system (ANS). The animal observation method was based on the experimental protocol developed by the psychopharmacology sector LTF/UFPB (Almeida et al., 1999). After treatment, the general comparative effects on the animals in the control group were observed in intervals of 30, 60, 120, 180 and 240 min.

In the end, the number of dead animals was counted to determine the dose which killed 50% of the experimental animals (lethal dose = LD₅₀).

Evaluation of the cytotoxic potential of extract of *S. paniculatum* Linn in human erythrocytes

The human erythrocytes (A, B, O and AB) were derived from blood that could not be used for transfusion (blood being discarded), obtained in the Transfusion Unit of the University Hospital Lauro Wanderley/UFPB. The handling and disposal of blood was performed according to security norms followed by the unit.

A human blood sample was mixed with NaCl 0.9% in the ratio 1:30 and centrifuged (model 206, FANEM) at 2500 rpm for 5 min to obtain the erythrocytes. This procedure was repeated twice and the pellet from the last centrifugation was resuspended in 0.5%, at NaCl 0.9%. Samples of the test products (extract) at different concentrations were added to 2 mL of the red cell suspension to a final volume of 2.5 mL. The negative control was assembled with red cell suspension + NaCl 0.9% (0% hemolysis), and the positive control with red cell suspension + Triton X-100, 1% (100% hemolysis). Samples were incubated for 1 h at 25°C under slow and constant stirring (100 RPM). After this time, they were centrifuged at 2500 rpm for 5 min and hemolysis was quantitated by

spectrophotometry (DU 640 BECKMAN) 540 nm (Rangel et al. 1997). All experiments were performed in triplicate.

Ethical considerations

The research was approved by the Research Ethics Committee of UFCG (Protocol / CEP / UFCG No. 34/2010) to determine LD₅₀. The research was approved by the Ethics Committee in Research with Human Beings UFPB (Protocol / CEP / HULW No. 743/10), Cover Sheet 390665, CAAE 6396.0.000.126-10 for the evaluation of the cytotoxic potential of extracts in human erythrocytes.

RESULTS

Phytochemical analysis

Phytochemical screening of the *S. paniculatum* Linn. extract revealed the presence of alkaloids, gums and lactones, as well as a high content of phenols (flavonoids and tannins). Qualitative analysis of phenols by TLC showed a spot suggestive of isovitexin (flavonoid) and the presence of tannic acid (tannin) in the extract.

Determination of minimum inhibitory concentration

The *S. paniculatum* extract exhibited antibacterial activity against all bacteria tested. The extract was effective against *S. sanguinis* and *L. casei* up to a dilution of 1:64 (7.81 mg/mL). The antibacterial activity of the *S. paniculatum* extract was significantly higher than that of chlorhexidine at concentrations/dilutions of 1:4 (125 mg/mL) and 1:8 (62.5 mg/mL) (Table 1).

Determination of the lethal dose (LD₅₀)

This study observed that the mice showed behavioral changes such as piloerection and intense movement of the vibrissae up until the first 60 min for the *S. paniculatum* extract (up to 1:16 dilution- 31.25 mg/ml). There were no serious side effects and the animals showed behavioral changes, suggestive of stimulating the central nervous system.

Evaluation of the cytotoxic potential of extract of *S. paniculatum* Linn in human erythrocytes

The *S. paniculatum* extract at a concentration of 7.81 mg/mL (MIC) haemolysed 41.2% of human erythrocytes of type A, 45.1% of type B, 16.4% of type O and 24.8% of type AB. However, it showed cytotoxicity at a dilution of 1:2 (250 mg/ml).

DISCUSSION

The control of dental biofilm is the primary goal to be

Table 1. Student t-test or Mann-Whitney U test (MIC *Solanum paniculatum* extract vs. chlorhexidine digluconate).

Concentrations/dilutions of extract/chlorhexidine	MIC – Mean (rank [#])		Statistic (t ou U)	Significance p-value
	Extract	Chlorhexidine		
EB (500 mg/ml) vs. C	18.33 (7.33)	18.00 (5.67)	t=0.291. gl=10 ⁺⁺	0.777
E 1:2 (250 mg/ml) vs. C1:2	17.17 (7.58)	16.33 (5.42)	U=11.50	0.310
E 1:4 (125 mg/ml) vs. C1:4	16.50 (8.75)	14.33 (4.25)	U=4.50	0.026*
E 1:8 (62.5 mg/ml) vs. C1:8	14.50 (8.75)	13.33 (4.25)	U=4.50	0.026*
E 1:16 (31.25 mg/ml) vs. C:1:16	13.00 (7.67)	10.50 (5.33)	U=11.00	0.310
E 1:32 (15.65 mg/ml) vs. C1:32	8.17 (7.33)	5.67 (5.67)	U=13.00	0.485
E 1:64 (7.81 mg/ml) vs. C1:64	4.00 (7.00)	2.00 (6.00)	U=15.00	0.699

[#] Rank = mean of Student t-test or Mann-Whitney U test CE: crude extract CD: chlorhexidine digluconate; ⁺⁺⁺ Teste de Levene; * (p -value < 0,05) (significant results).

achieved in order to prevent caries and periodontal disease, which can be accomplished by employing methods of oral hygiene, using instruments that provide the removal and mechanical disruption of these biofilms such as toothbrushes and dental floss. Furthermore, the combined association of using these mechanical devices with chemical procedures have demonstrated their efficiency and highlighted them as being ideal for the control of dental biofilm, especially in patients with high potential for developing oral diseases (Filogônio et al., 2011).

Although, the joint use of mechanical and chemical methods is recommended as “ideal” with positive effects in controlling biofilm and for preventing or minimizing the development of diseases, herbal medicines represent an alternative that is gaining more space as a therapeutic option in fighting various pathologies. Obviously, the mere fact of being natural is not indicative of being totally free from side effects, but studies suggest that they present a considerable safety margin, as their medicinal properties do not represent a risk to human health (Queiroz et al., 2014).

Brazil has a very diverse flora, which is a result of the climatic and geological aspects of its extensive territory. This facilitated the development of several species of plants, which are the object of current experimental studies in order to identify the presence of antimicrobial, antiseptic, anti-inflammatory and antioxidant properties with healing characteristics. Thus, it is important to investigate the use of these leaves and roots of plants as substances that are capable of inhibiting the onset and progression of some oral and respiratory diseases.

The aim of the study was to evaluate *in vitro* antimicrobial action, toxicity and cytotoxicity of the *S. paniculatum*, and to analyze the plant material in relation to its phytochemical aspects. The results of the phytochemical analysis make it clear that high activity against various species of microorganisms occurs due to the presence of large quantities of phenols (among which flavonoids and traces of pyrogallol tannins can be found), gums, lactones and alkaloids (in the presence of

reactives of Dragendorff and Bertrand) in jurubeba extract, which is consistent with the findings of Mesia-Vela et al. (2002), Silva et al. (2005) and Cheng et al. (2008). Moreover, the chromatogram highlighted a stain which suggested the presence of isovitexin and tannic acid in the jurubeba, important finding to illustrate its pharmacological effect. The results of phytochemical screening and TLC of the extract were in accordance with those reported by Mesia-Vela et al. (2002), Silva et al. (2005) and Cheng et al. (2008). The antimicrobial potential of these compounds may explain the pharmacological activity of the extract studied. Also, the presence of sulfur compounds is interesting, since one of the main characteristics of these compounds, despite their structural differences, is the antimicrobial activity (Heinzmann, 2007).

The presence of products classified as polyphenols justified the analysis of *in vitro* antibacterial activity by determining the minimum inhibitory concentration (MIC) of the plant root extracts in question, against the activity of the microorganisms *S. mutans*, *S. mitis*, *S. sanguinis*, *S. oralis*, *S. salivarius* and *L. casei*. This demonstrates that there was homogeneous inhibition of growth of these bacteria, which were verified by the concentration of the extract in this study directly related to the diameters of halos, indicating that the progressive reduction of the extent of such halos thereby reduce the extract concentration.

The *S. paniculatum* extract showed an antibacterial effect in the dilution of 1:64 (7.81 mg/mL) for *Streptococcus sanguinis* and *L. casei*. Chlorhexidine had an antibacterial effect on all microorganisms as expected, and *S. mutans* bacteria was more sensitive to the extract, with zone of inhibition up to 1:64 dilution. The *S. paniculatum* extract had a higher antimicrobial activity than that of chlorhexidine digluconate at concentrations of 125 mg/mL (1:4) and 62.5 mg/mL (1:8). This data showed the potential bacteriostatic effect *in vitro* of *S. paniculatum* in all strains (Table 1).

Although, the beneficial effects *in vitro* are noticeable, it is necessary to investigate the toxic effects, to allow its

use *in vivo*. For this, pre-clinical trials were developed in mice by using a lethal dose (LD₅₀), did not cause mortality in any of the tested concentrations (500 mg/mL [undiluted] and 0.97 mg/ml [diluted]) after 1, 3 and 15 days. However, behavioral modification is seen at time intervals of 30, 60, 90 and 240 min, verifying piloerection and vibrissae movements within 60 min and even 31.25 mg/mL (1:16). There were no serious side effects and the animals showed only minor behavioral changes, suggestive of CNS stimulation, thus from these findings extracts could be applied clinically in the concentrations tested. Regarding *S. paniculatum*, there are no other tests to determine its lethal dose, however, Vieira et al. (2010) evaluated the mutagenic and cytotoxic activities of ethanolic extracts of leaves and fruits of *S. paniculatum*, using the micronucleus test in bone marrow of mice, and found that there was no mutagenic action in mouse bone marrow. Nevertheless, in higher doses, both extracts showed cytotoxic activity. This study did not show an increase in cytotoxicity dose-response at 200 and 300 mg.kg⁻¹.

The cytotoxicity of the extract of *S. paniculatum* in human erythrocytes induced a low hemolytic activity (Prokof'eva et al., 2004), rate lower than 50% in all four blood types in MIC 7.81 mg/mL, and cytotoxic at 250 mg/mL (1:2). Studies involving antimicrobial activity and the adverse effects of *S. paniculatum* are still little mentioned in the literature. Although, they do not report anything that would compromise human health, they require further research to verify the effectiveness of this present study in the prevention of oral diseases.

The results showed that *S. paniculatum* has a significant potential of having a bacteriostatic reaction *in vitro* and only presented deleterious effects toxicologically in high concentrations making possible, the performance of a randomized controlled clinical trial, and evaluating the effect of *S. paniculatum* incorporated in a mouthwash, solution over the oral bacteria and dental biofilm.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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