Effect of Schinus terebinthifolius on Candida albicans growth kinetics, cell wall formation and micromorphology

Article *in* Acta odontologica Scandinavica · January 2013

DOI: 10.3109/00016357.2012.741694 · Source: PubMed

6 authors, including:

Livia Alves
University of Campinas
16 PUBLICATIONS **21** CITATIONS
SEE PROFILE

Irlan Almeida Freires
University of Campinas
46 PUBLICATIONS **57** CITATIONS
SEE PROFILE

Acta Odontologica Scandinavica, 2013; 71: 965–971

informa
healthcare

ORIGINAL ARTICLE

Effect of *Schinus terebinthifolius* on *Candida albicans* growth kinetics, cell wall formation and micromorphology

LÍVIA ARAÚJO ALVES[1], IRLAN DE ALMEIDA FREIRES[1], TRICIA MURIELLY PEREIRA, ANDRADE DE SOUZA[1], EDELTRUDES DE OLIVEIRA LIMA[2] & RICARDO DIAS DE CASTRO[1]

[1]*Department of Clinics and Social Dentistry, School of Dentistry, and* [2]*Department of Pharmaceutical Sciences, Federal University of Paraiba, Joao Pessoa, Paraiba, Brazil*

Abstract

Objective: To evaluate the anti-fungal activity of a tincture from *Schinus terebinthifolius* (Brazilian pepper tree) on *Candida albicans* (ATCC 289065), a micro-organism associated with fungal infections of the oral cavity. ***Materials and methods:*** Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined through microdilution technique, as well as the microbial growth curve of *C. albicans* promoted by *S. terebinthifolius.* In addition, this study investigated a possible activity of the product on the fungal cell wall and its biological activity on fungal morphology. Nystatin was used as control and all tests were performed in triplicate. ***Results:*** *S. terebinthifolius* showed MIC of 312.5 μg/mL and MFC of 2500 μg/mL upon the strain tested, while Nystatin showed MIC and MFC of 6.25 μg/mL. As regards the microbial growth curve, *S. terebinthifolius* was able to significantly reduce the number of CFU/mL when compared to growth control until the time of 60 min. In the times 120 and 180 min there was no statistically significant difference between the growth control and the experimental product. *S. terebinthifolius* possibly acts on the fungal cell wall, once the sorbitol test indicated a MIC of 1250 μg/mL. In the fungal morphology, a reduction was observed of pseudo-hyphae, chlamydoconidia and blastoconidia in the presence of the experimental product. ***Conclusion:*** *S. terebinthifolius* showed anti-fungal activity against *C. albicans,* inhibiting, probably, the fungal cell wall formation.

Key Words: *anacardiaceae, biological products, Candida albicans*

Introduction

Oral candidiasis is an infection caused by *Candida* genus yeasts, which commonly inhabit healthy individuals' mouths. *Candida* is a saprophyte micro-organism that, depending on pre-disposing factors that change organic integrity, may modify its yeast-like conformation into a fusiform shape, becoming pathogenic [1].

Candida strains have been associated to superficial and systemic mycotic infections and may be found in 60% of adults' oral cavity. In this profile, *Candida albicans* is considered to be the most prevalent species [2].

Such a micro-organism presents virulence factors involved in the biofilm formation [3] and that ability is closely related to its aptitude in causing infections and therefore an increasing resistance to anti-fungal drugs and to host defense mechanisms [4,5].

Considering the shortcomings related to the use of synthetic anti-fungals (increased resistance, undesirable effects, among others), new agents have been proposed in an attempt to minimize those problems. In this respect, products originating from natural sources with potential anti-microbial activity have been investigated [6–12]. Among those is *Schinus terebinthifolius*, popularly known as Brazilian pepper tree.

S. terebinthifolius is native to Brazil and belongs to the plant kingdom, division Tracheophyta, class agnoliopsida, order Sapindales and family Anacardiaceae. The principal morpho-histological and chemical characteristics of the species in view of recognizing it as a drug are leaves and barks rich in tannins and essential oil [13].

Correspondence: Dr Ricardo Dias de Castro, 475 Cajazeiras St, Apt. 102, Coral Gables, Manaira, Joao Pessoa, Paraiba, ZIP CODE: 58038-040, Brazil. Tel: + 83 9317 1071. E-mail: ricardodiasdecastro@yahoo.com.br

(Received 9 November 2011; revised 9 August 2012; accepted 2 September 2012)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2012.741694

This species has presented anti-inflammatory and anti-ulcerogenic effects and has been used as an antiseptic and in the treatment of stomatitis. Its leaves have been commonly used worldwide in the management of venereal diseases, womb inflammation, urinary tract infections, skin wounds, diarrhoea and gastroduodenal ulcer [14,15]. Furthermore, *S. terebinthifolius* has been found to be a promising anti-microbial agent against gram-positive and negative bacteria [15,16] and some species of *Candida* [16,17].

Thus, it was aimed to investigate the anti-fungal effects of *S. terebinthifolius* on planktonic *Candida albicans*.

Materials and methods

Research centre and fungal strains

Microbiological assays were performed in the Oral Microbiology Laboratory of the Nucleus of Tropical Medicine and in the Mycology Laboratory of the Department of Pharmaceutical Sciences, Center for Health Sciences, Federal University of Paraiba, Joao Pessoa, Brazil.

Strains of *Candida albicans* (ATCC 289065) were provided by the National Institute of Quality Control in Health at Oswaldo Cruz Foundation (NIQCH).

Experimental product

The test product was a tincture from the stem bark of *Schinus terebinthifolius*, which was provided by a compounding pharmacy in Joao Pessoa, Paraiba, Brazil. The product, detailed in Table I, met all required specifications concerning its quality control according to the technical survey provided by the supplier.

Determination of the minimum inhibitory concentration (MIC)

The MIC determination of the S. *terebinthifolius* tincture was performed by microdilution technique proposed by Ellof [18], using 96-well U-bottom microtiter plates (ALAMAR®, Diadema, Sao Paulo, Brazil). Initially, 100 µL of Sabouraud Dextrose Broth (SDB, HIMEDIA®, Sao Paulo, Brazil) doubly concentrated were distributed into the plate's wells. Then, 100 µL of the *S. terebinthifolius* tincture were distributed at an initial concentration of 5000 µg/mL. From these concentrations were conducted serial dilutions by withdrawing an aliquot of 100 µL from the most concentrated well and inserting it into the following well. Concentrations then ranged between 5000 µg/mL and 39 µg/mL. Finally, aliquots of 10 µL of inoculum ($1–5 \times 10^6$ CFU/mL) were dispensed into the wells of each column. In parallel, a yeast viability control and also a susceptibility control were conducted by using powdered nystatin at an initial concentration of 100 µg/mL, ranging up to 0.78 µL/mL.

Tests were performed in triplicate and the plates were incubated at 35°C for 48 h. The reading to determine the tincture MIC on the yeast strain was made by visual method. The formation or non-formation of cellular clusters ('button') at the bottoms of the wells were taken into consideration. MIC was considered as the lowest concentration of the product under test capable of producing visible inhibition on the growth of yeast strains used in the microbiological assay [18].

Determination of the minimum fungicidal concentration (MFC)

After MIC determination, the inhibitory and two following higher concentrations as well as the positive controls were sub-cultivated on plates containing Sabouraud Dextrose Agar, in triplicate. Then, after 24 h of incubation at 35°C, the readings of MFC were carried out based on the growth controls. MFC was considered as the lowest drug concentration that hindered visible growth of the sub-culture.

Effect of S. terebinthifolius on yeasts growth kinetics

The study of interference of the test product (*S. terebinthifolius* tincture) on the viability of fungal strains was performed by means of the method of counting viable cells. In this assay, the behaviour of the yeasts strains submitted to the MIC found in the microdilution technique was observed. Initially, fungal suspension in 0.9% saline solution was prepared in accordance with the 0.5 tube of McFarland scale, which corresponds to ~$1–5 \times 10^6$ CFU/mL. Test tubes measuring 150 × 150 mm were prepared to contain 4.5 mL of sterilized DSB. Then, the experimental product or control anti-fungal was added at

Table I. Characterization of the experimental product containing *Schinus terebinthifolius*.

Scientific name	Schinus terebinthifolius
Popular name	Brazilian pepper tree
Origin	Stem bark
Pharm. Form	Tincture
Initial concentration	100 mg/mL[1]
Physical-chemical characteristics	pH: 4.99 Soluble in water Density: 0.910 g/mL Extractor liquid: hydroalcoholic solution Alcohol strength: 60° GL Dry residue: 2.0%
Major active components*	Alkaloids and tannins

*According to phytochemical analysis performed by the supplier.

the following concentrations: MIC, MIC × 2 and MIC × 4. Posteriorly, an aliquot of 0.5 mL of fungal suspension (2.5×10^6 cells) was transferred to all the test tubes. A growth control (culture medium plus inoculum) was also prepared to allow for comparisons and method assurance. Solutions were kept incubated at 35°C during the whole assay (180 min).

In the previously set times (0, 30, 60, 120 and 180 min), an aliquot of 10 µL of the test and control solutions was inoculated on plates containing Dextrose Sabouraud Agar (HIMEDIA®, Sao Paulo, Brazil) in order to determine the counting of colonies. All tests were performed in triplicate [19].

After the incubation period, reading of results was conducted by counting of grown colonies. Colony-number average ($\log_{10}$ CFU/mL) was set vs time period and the average and strength of anti-fungal activity was compared in several concentrations. Data analysis for the test product was considered as showing fungicidal activity when reduced microbial death ≥ 99.9% (≥ 3 $\log_{10}$) in CFU/mL in relation to the initial inoculum [19].

Effect of S. terebinthifolius on fungal cell wall

MIC determination of *S. terebinthifolius* tincture in the presence of sorbitol (0.8 M) was performed by micro-dilution technique [18], in triplicate. In each well of the plate were added 100 µL of SDB previously supplemented with sorbitol presenting a molecular weight of 132.17 g (Compounding pharmacy, Joao Pessoa, Paraiba, Brazil), both doubly concentrated. Subsequently, 100 µL of the tincture (100 mg/mL) were also dispensed into the wells in the first row of the plate. Also, through a serial dilution at a ratio of 2, were obtained concentrations ranging between 5000 µL/mL and 39 µL/mL of the *S. terebinthifolius* tincture and, in relation to sorbitol, a final concentration of 0.8 M in each well. Finally, 10 µL of inoculum ($1–5 \times 10^6$ CFU/mL) was added into the wells.

A micro-organism control was conducted by placing 100 µL of DSB plus sorbitol (0.8 M) doubly concentrated and 10 µL of inoculum ($1–5 \times 10^6$ CFU/mL) into the wells. In addition, a sterility control was also done by placing the same culture medium and sorbitol without fungal suspension. Plates were incubated at 35°C for 48 h and read afterwards [20].

Interference on fungal micromorphology

In view of observing morphological changes in the *C. albicans* strain, this study employed the technique of microculture for yeasts, using cornmeal agar in a moist chamber [21–24].

Cornmeal agar was prepared (100 mL) and the total amount was divided into three parts containing the same volume. Then, the test product at MIC concentration, standard anti-fungal and other third part were added. The trial was performed using Petri dishes of 90 × 15 mm containing a glass slide on a supporter for the microcultive and filter paper (30 × 30 mm) soaked in water (moist chamber) properly sterilized.

Posteriorly, the culture medium containing test product, anti-fungal and the part without anti-microbial agents was molten and 1 mL transferred and spread on the slider, creating a thin layer. After cornmeal agar solidification, the yeast was seeded by a needle-like bacteriological loop, making two parallel strias. A coverslip was added to cover the microcultive and 1 mL distilled and sterilized water was included to keep system humidity. Each plate was closed and incubated at 35°C for 48 h.

After the incubation period, each preparation was examined under optical microscopy in view of observing formation or non-formation of yeast-typical structures like blastoconidia, pseudo-hyphae and chlamydoconidia.

Data analysis

Data gathered in the kinetics assay were analysed by inferential statistics by means of ANOVA test followed by Tukey's post-test (Graphpad Prism, version 5.0, San Diego, CA). Type I error (α error) was set at 0.05. For other tests, descriptive statistics were employed.

Results

Table II includes the anti-fungal activity of *S. terebinthifolius* upon *Candida albicans* according to the assays of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration.

Microbial growth kinetics and growth curve of *C. albicans* under activity of *S. terebinthifolius* tincture and nystatin at different concentrations (MIC,

Table II. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Schinus terebinthifolius* tincture on *Candida albicans* (values expressed in µg/mL).

	S. terebinthifolius tincture		Nystatin	
Strain	MIC	MFC	MIC	MFC
Candida albicans (ATCC 289065)	312.5 µg/mL	2500 µg/mL	6.25 µg/mL	6.25 µg/mL

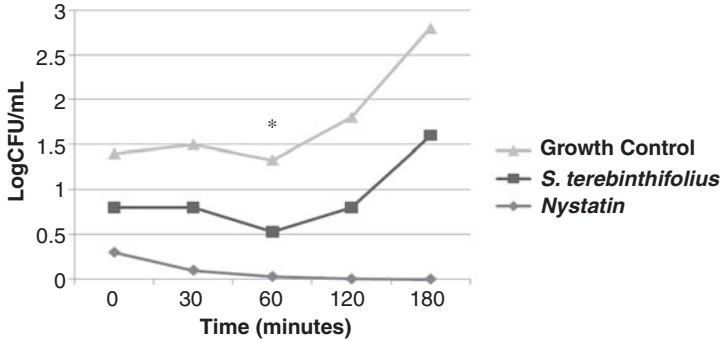

Figure 1. Effect of the MIC of *S. terebinthifolius* tincture on *C. albicans* (ATCC 289065) growth kinetics.

MIC × 2 and MIC × 4) are disposed in Figures 1,2,3, respectively. Data showed that *S. terebinthifolius* was able to reduce significantly ($p < 0.05$) the number of CFU/mL when compared to growth control from 0 up to 60 min. In the times 120 and 180 min no statistically significant difference ($p < 0.05$) was found between growth control and the tincture.

MIC values of *S. terebinthifolius* in presence and absence of sorbitol (0.8 M)—an osmotic protector—were 312.5 and 1250 μg/mL, respectively. As can be seen, such a test suggests that sorbitol protects cells from the inhibitory effects promoted by the tincture, once there were changes in the MIC of the experimental product upon the strain assayed.

The control with sorbitol guaranteed reliability of the methods employed and outcomes obtained, considering that the strain was able to grow in the presence of sorbitol and absence of the tincture. These results indicate the anti-fungal effect of the *S. terebinthifolius* tincture somehow involve a direct interaction with the *C. albicans* cell wall.

C. albicans micromorphology test under optical microscopy revealed the presence of structures indicating fungal growth: pseudo-hyphae, blastoconidia and chlamydoconidia (Figure 4). In the growth control, the presence of pseudo-hyphae, blastoconidia and chlamydoconidia was observed. In the presence of the tincture were seen blastoconidia, a few pseudo-hyphae and rare chlamydoconidia. Under nystatin activity, only blastoconidia and rare pseudo-hyphae were visualized.

Discussion

There has been an increasing interest in studying medicinal plants as sources of agents to treat infectious diseases. In this respect, experimental scientific studies are needed to be carried out in order to confirm possible antibiotic properties of a great number of plants and their derivates [24].

The Brazilian Ministry of Health released, in February 2009, a list containing 71 medicinal plants which may be used as phytotherapic drugs by the Brazilian Health Unified System in order to guide studies and researches. Among those plant species is *Schinus terebinthifolius* [25].

The present investigation assessed the anti-fungal activity of *S. terebinthifolius* tincture and found a MIC of 312.5 μg/mL and MFC of 2500 μg/mL upon *C. albicans*. Some studies evaluating the MIC of *S. terebinthifolius* can be found in the literature [15,16], but there is no mention of MFC, which makes this study unique in such an assessment.

A study evaluated the susceptibility of strains of *C. albicans*, *C. tropicalis* and *C. krusei* to nystatin, and MIC and MFC were found ranging from 0.5–8 μg/mL and from 8–64 μg/mL, respectively [26]. Wingeter et al. [27] investigated the sensitivity of isolated and

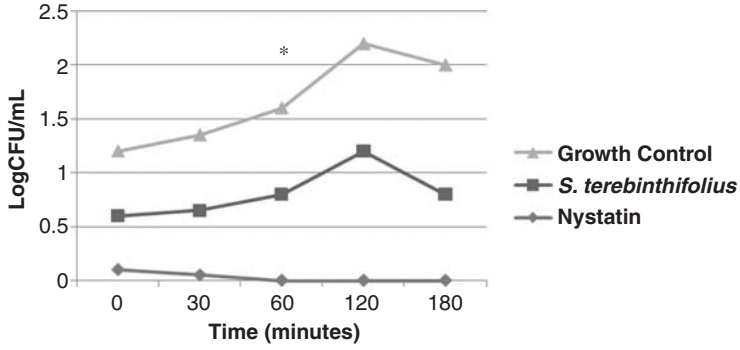

Figure 2. Effect of the MIC × 2 of *S. terebinthifolius* tincture on *C. albicans* (ATCC 289065) growth kinetics.

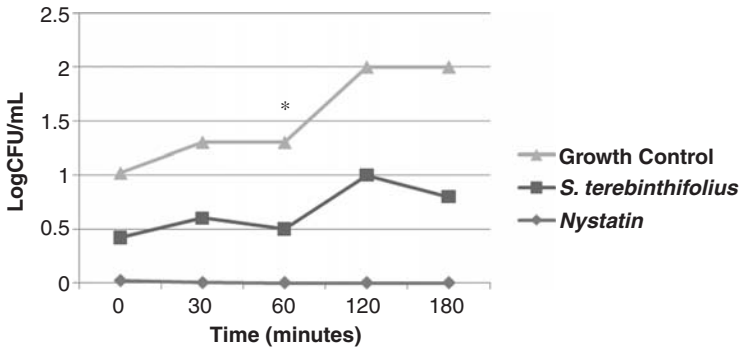

Figure 3. Effect of the MIC × 4 of *S. terebinthifolius* tincture on *C. albicans* (ATCC 289065) growth kinetics.

non-isolated strains of *C. albicans* from patients presenting prosthetics stomatitis and observed Nystatin MIC values ranging between 2–64 μg/mL. Similar results were found in the present study, in which Nystatin showed MIC and MFC of 6.25 μg/mL upon *C. albicans*.

An extract from *S. terebinthifolius* leaves (ethanol, 30%) was tested by the agar diffusion method, in different concentrations, against some gram-positive and gram-negative micro-organisms and also upon *C. albicans* yeasts. Results revealed that, in the lowest test concentration (10%), there was no microbial growth inhibition. However, in the 50% and 100% concentrations there was inhibition of gram-positive and gram-negative bacteria, but the yeast was not affected [15]. That differs from our findings, once *S. terebinthifolius* tincture presented anti-fungal activity with MIC of 312.5 μg/mL on *C. albicans*.

Guerra et al. [25] assessed the anti-microbial activity of the extract from *S. terebinthifolius* leaves (ethanol, 80%) against gram-positive and gram-negative micro-organisms and *C. albicans* yeast. It was verified that such an extract presented an anti-microbial effect in the concentrations 80, 60, 40, 30, 15, 5 and 1% on all tested micro-organisms. In the present investigation, *S. terebinthifolius* tincture was found to exhibit activity up to the concentration of 0.3125%.

Some methodological approaches are important to be distinguished between the studies by Martínez et al. [15] and Guerra et al. [16] and the present investigation. Those researches employed an agar diffusion method to determine MIC; their experimental product contained active compounds from *S. terebinthifolius* leaves in an extract form, and micro-organisms ATCC were distinct. In this study, MIC was obtained by a micro-dilution technique; the test substance had its origin in *S. terebinthifolius* stem bark and it is in tincture form. In this respect, it is noteworthy to point out the solvent used for preparing the pharmaceutical form (extract, tincture, etc.) rather influence the quantity and type of extracted

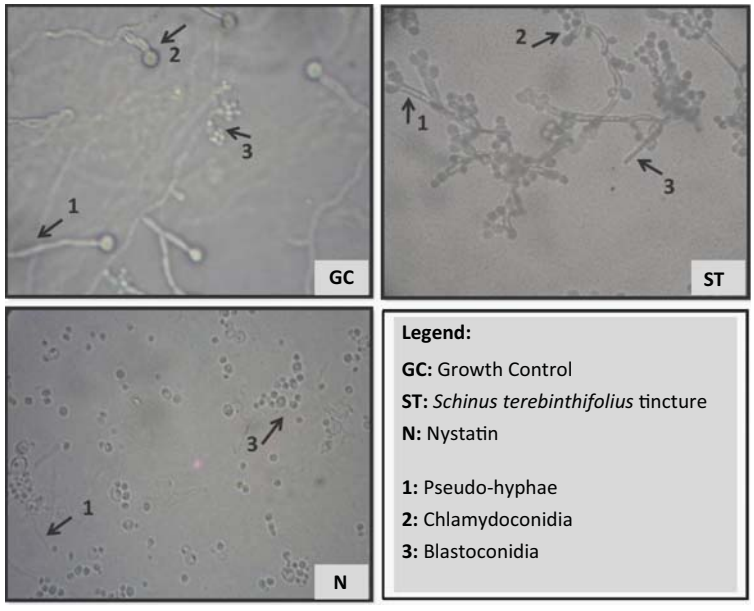

Figure 4. Micromorphology of *Candida albicans* cultivated in presence of *Schinus terebinthifolius* tincture and Nystatin.

phytochemicals (e.g. tannins) responsible for biological activity. Among other reasons, this may contribute to the differences found between extracts and tinctures.

In another study, Freires et al. [28] evaluated the anti-fungal activity of a 10% tincture from *S. terebinthifolius* stem bark upon *C. albicans*. The authors found mean diameters of growth inhibition halos of 25.32 mm promoted by the Brazilian pepper tree against 33.32 mm corresponding to the control (Nystatin).

The findings of this work complement what was found by Freires et al. [28], because it also assessed anti-fungal activity of Brazilian pepper tree tincture (10%) on *C. albicans* yeast (MIC and MFC), in addition to microbial growth curve, action on fungal cell wall and interference with fungal micromorphology.

This research brings a quite relevant contribution to fungal microbiology, seeing that until now no study has deeply evaluated the anti-fungal potential of the *S. terebinthifolius* tincture, according to the literature, because research is more often conducted to assess this species anti-bacterial activity.

In the kinetics assay, analysis of the microbial growth curve under *S. terebinthifolius* activity revealed that it was able to significantly reduce the number of CFU/mL when compared to growth control up to the time of 60 min. In the times 120 and 180 min there was no statistically significant difference between growth control and test product. These results show that up to 1 h the Brazilian pepper tree may inhibit fungal growth, but its action decreases over time and the yeast may have growth returned to normal. This situation, therefore, implies a possible low substantivity of *S. terebinthifolius*, ~ 1 h.

Accordingly, it is suggested to associate the tincture (natural product economically feasible and with proven antifungal property) with a synthetic antifungal so as to provide this new product a longer action. As such, this combination seems to be a worthy alternative, considering that a synergic effect achieved by both products may be responsible for an increased antifungal action, compared to the use of the agents by themselves. Thus, studies on this sort of association may contribute to improve care related to prevention and treatment of oral candidiasis.

The test with sorbitol performed in this study is based on the extent and capacity of the injuries that substances with anti-fungal activity generally promote on the fungal cell wall. Whether the agent somehow acts on the cell wall it will cause cell lysis when in the absence of an osmotic stabilizer. So, this test compares anti-fungals MIC in the absence and presence of 0.8 M sorbitol, which is an osmotic protector used to stabilize fungal protoplasts [29].

Based on the results of the present study, *S. terebinthifolius* was found to have a possible mechanism of action on the fungal cell wall, since MIC in the absence of sorbitol was 312.5 μg/mL and in the presence of the osmotic stabilizer was 1250 μg/mL. That implies that the tincture action was diminished in the presence of sorbitol, which precluded test product action on the fungal cell wall.

Fungal micromorphology assay indicated that the test product was able to inhibit formation of chlamydoconidia and pseudo-hyphae. According to Romani et al. [30], the formation of pseudo-hyphae is related to virulence factors expressed by *C. albicans*, since these structures represent a barrier for phagocytosis and allow liquidation of yeasts in the epithelial tissue. Morphological changes are associated to microorganism pathogenicity and environmental factors are believed to influence physiological status of commensal yeasts.

This is the first study to demonstrate activity of *S. terebinthifolius* tincture on fungal micromorphology, which hinders comparison with other research. In the study by Castro [31], the micromorphology of *C. albicans* was evaluated in the presence of *Cinnamomum zeylanicum* essential oil, Nystatin and a combination of both substances. The results indicated these products were able to inhibit formation of pseudo-hyphae, chlamydoconidia and blastoconidia.

It is important to consider that the trials performed in the present investigation represent an initial assessment to determine anti-fungal activity of *S. terebinthifolius* tincture, being therefore necessary to carry out further studies including analysis in *Candida* biofilms and toxicological assays.

Lima et al. [32] evaluated acute and sub-acute toxicity of *S. terebinthifolius* used orally for 45 days in Wistar rats of both genders. No toxic signal or deaths were observed relating to acute and sub-acute administration of that species extract.

Paulo et al. [33] conducted a phase I toxicological clinical trial with 28 volunteers assessing a phytotherapic formulation containing: *Schinus terebinthifolius* Raddi, *Plectranthus amboinicus* Lour and *Eucalyptus globulus* Labill. In that study, participants took orally 15 mL of the test product 3-times a day for 8 weeks. Acute toxicity was evaluated from the 1st to the 7th days of ingestion. After that, up to the 8th week, chronic toxicity was inspected. The authors concluded that oral administration of a 45 mL maximum per day for 2 months was well tolerated and neither clinical and laboratorial changes nor relevant adverse effects were observed.

The current study outcomes provide the continuity of the researches about *S. terebinthifolius*, taking into consideration its potential anti-fungal activity upon *C. albicans*. Clinical trials should be carried out in order to confirm the therapeutic efficacy of this species against oral candidiasis.

Hence, according to the present findings it may be concluded that:

- *Schinus terebinthifolius* tincture (Brazilian pepper tree) was found to present *in vitro* anti-fungal activity (fungistatic and fungicidal) on *Candida albicans*;
- As regards microbial growth kinetics of *C. albicans*, tincture was able to diminish fungal growth up to the time of 60 min;
- *S. terebinthifolius* presents a possible mechanism of action on the fungal cell wall in the presence of sorbitol; and
- Concerning fungal micromorphology, the test product was able to decrease the number of blastoconidia and chlamydoconidia.

The results obtained point to the possibility to clinically use this species in Dentistry as a low-cost, accessible and efficient alternative in treating oral candidiasis.

***Declaration of Interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Alves PM, Leite PHAS, Pereira JV, Pereira LF, Pereira MSV, Higino JS, et al. Antifungal activity of the extract of *Psidium guajava* Linn. ("goiabeira") upon leavens of *Candida* of the oral cavity: an *in vitro* evaluation. Braz J Pharmacognosy 2006; 16:192–6.

[2] Marsh P, Martin P. Oral microbiology. 5th ed. Edinburgh: Churchill Livingstone Elsevier; 2009.

[3] Thein ZM, Samaranayake YH, Samaranayake LP. Effect of oral bacteria on growth and survival of *Candida albicans* biofilms. Arch Oral Biol 2006;51:672–80.

[4] Henriques M, Azeredo J, Oliveira R. Adhesion of *Candida albicans* and *Candida dubliniensis* to acrylic and hydroxyapatite. Biointerfaces 2004;33:235–41.

[5] Ramage G, Saville SP, Thomas DP, López-Ribot JL. Candida biofilms: an update. Eukaryot Cell 2005;4:633–8.

[6] Alves LA, Freires IA, Jovito VC, Almeida LFD, Castro RD. Interference of essential oils on antibiotics used in the treatment of infections of the oral cavity. Int J Dent 2011;10:26–32.

[7] Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigates* and A. niger. World J Med Sci 2008;3:81–8.

[8] Bonjar GH, Aghighi S, Karimi NIKA. Antibacterial and antifungal survery in plants used in indigenous herbal-medicine of south east regions of Iran. Int J Biol Sci 2004;4:405–12.

[9] Konning GH, Agyare C, Ennison B. Antimicrobial activity of some medicinal plants of Ghana. Fitoterapia 2004;75:65–7.

[10] Lima IO, Oliveira RAG, Lima EO, Farias NMP, Souza EL. Antifungal activity of essential oils on *Candida* species. Braz J Pharmacognosy 2006;16:197–201.

[11] Srinivasan D, Nathan S, Suresh T. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharmacol 2001;74:217–20.

[12] Viuda-Martosa M, Ruiz-Navajasa Y, Fernández-López J, Pérez-Álvarez J. Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Control 2008;19:1130–8.

[13] Ribas MO, Sousa MH, Sartoretto J, Lanzoni TA, Noronha L, Acra LA. Effect of the *Schinus terebinthifolius* Raddi in the process of tissular repair in ulcers induced in mucosa oral of the rat. J Dent Sci 2006;21:245–52.

[14] Diniz MFFM, Oliveira RAG, Medeiros ACD. Herbal medicines: plants as a pharmaceutical alternative: scientific and popular aspects. Joao Pessoa: EDUFPB 1997; :205.

[15] Martínez MJ, Alonso GN, Badell JB. Actividad antimicrobiana del *Schinus terebinthifolius* Raddi (Copal). Rev Cuba Plantas Med 1996;1:37–9.

[16] Ellof JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Plant Med 1998;64:711–13.

[17] Rassoli I, Mirmostafa SA. Antibacterial properties of *Thimus pubescens* and *Thymus serpyllum* essential oil. Fitoterapia 2002; 73:244–50.

[18] Frost DJ, Brandt KD, Cugier D, Goidman R. A whole-cell Candida albicans assay for the detection of inhibitors towards fungal cell wall synthesis and assembly. J Antibiot 1995;28:306–9.

[19] Gungi S, Arima K, Beppu T. Screening of antifungal according to inducing morfological abnormalities. Agric Biol Chem 1983;47:2061–9.

[20] Lacaz CS, Porto E, Martins JEC. Medical mycology. 8th ed. Sao Paulo: Sarvier; 1991. p 695.

[21] Kurtzman CP, Fell JW. The yeats, a taxonomie study. Amsterdam: Elsevier Science B. V; 1998.

[22] Sidrim JJC, Moreira JLB. Clinical and laboratorial basis of medical mycology. 1st ed. Rio de Janeiro: Guanabara Koogan; 1999.

[23] Lima EO, Farias NMP, Souza EL, Santos BHC. Antibacterial properties of essential oils from medicinal plants. Rev Bras Ciênc Saúde 2003;7:251–8.

[24] Brazilian Ministry of Health. List of plants that may be used as phytotherapics in the Brazilian Unified Health System. Sao Paulo: Folha de Sao Paulo; 2009.

[25] Guerra MJM, Barreiro ML, Rodriguez ZM, Rubalcaba Y. Actividad antimicrobiana de un extracto fluido al 80% de *Schinus terebinthifolius* raddi (copal). Rev Cuba Plantas Med 2000;5:23–5.

[26] Alves SH, Cury AE. *Candida* from cancer patients: susceptibility *in vitro* to polyene antifungal agents. Rev Inst Med Trop Sao Paulo 1992;34:251–4.

[27] Wingeter MA, Guilhermetti E, Shinobu CS, Takaki I, Svidzinski TIE. Microbiological identification and *in vitro* sensitivity of *Candida* isolates from the oral cavity of HIV-positive individual. Rev Soc Bras Med Trop 2007;40:272–6.

[28] Freires IA, Alves LA, Jovito VC, Castro RD. Antifungal activity of *Schinus terebinthifolius* (Aroeira) on *Candida* strains. Revista Odontológica do Brasil Central 2011;20:68–72.

[29] Pereira FO. Antifungal activity of Cymbopogon winterianus Jowitt ex Bor essential oil on Trichophyton dermatophytes. Doctorate Thesis]. Joao Pessoa: Department of Pharmaceutical Sciences, Federal University of Paraiba; 2009. p 33.

[30] Romani L, Bistoni F, Puccetti P. Adaptation of *Candida albicans* to the host environment: the role of morphogenesis in virulence and survival in mammalian hosts. Curr Opin Microbiol 2003;6:338–43.

[31] Castro RD. Antifungal activity of Cinnamomum zeylanicum Blume (cinnamon) essential oil alone and combined with synthetic antifungals on Candida species. [Doctorate Thesis]. Joao Pessoa: Department of Pharmaceutical Sciences, Federal University of Paraiba; 2010.

[32] Lima LB, Vasconcelos CFB, Maranhão HML, Leite VR, Ferreira PA, Andrade BA, et al. Acute and subacute toxicity of *Schinus terebinthifolius* bark extract. J Ethnopharmacol 2009; 126:468–73.

[33] Paulo PTC, Diniz MFFM, Medeiros IA, Morais LCSL, Andrade FB, Santos HB. Phase I clinical toxicological assays of a complex herbal medicine (*Schinus terebinthifolius* Raddi, *Plectranthus amboinicus* Lour and *Eucaliptus globulus* Labill). Braz J Pharmacognosy 2009;19:68–76.