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Effect of *Hamamelis virginiana*, *Persea americana*, *Cynara scolymus* L and *Stryphnodendron barbatiman* M plant extracts on the phenotypic expression of virulence factors in biofilms of the *Candida albicans*

Efeito de extratos vegetais de *Hamamelis virginiana, Persea americana, Cynara scolymus L e Stryphnodendron barbatiman M* sobre a expressão fenotípica dos fatores de virulência em biofilmes de *Candida albicans*

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ABSTRACT

Objective: Analyze the phenotypic expression of virulence factors in *Candida albicans* biofilms against plant glycolic extracts. **Material and Methods:** The biofilms of *Candida albicans* (ATCC 18804) obtained from incubation for 48 hours were exposed for 5 minutes and 24 hours to different concentrations of glycolic extracts of *Hamamelis virginiana* and *Persea americana*, *Cynara scolymus* L and *Stryphnodendron barbatiman* M, in order to verify the antifungal activity of the proteinase, phospholipase and hemolysin. **Results:** All extracts were effective in reducing biofilm. In contact for 5 minutes. the extracts reduced 50% of the biofilm. After 24 hours, the *Persea americana* extract showed the biofilm at 90%, followed by *Cynara scolymus*, which interrupted it at 85%, There was a change in proteinase intensity after 5 minutes and 24 hours. with an average enzymatic activity of 0.69 compared to the control of 0.49. *Cynara scolymus* was the extract with the highest mean concentration of 100 mg/ml; the phospholipase intensity was changed with *Stryphnodendron barbatiman* being more effective in 24 hours compared to the control ($p < 0.0001$). The hemolysin secretion was modified by *Hamamelis virginiana* (12.5 mg/ml) after 5 minutes of exposure, and in 24 hours. all extracts were capable to cause changes in secretion. **Conclusion:** The tested extracts have antifungal potential in *Candida albicans* biofilms, implying a significant reduction in virulence factors. Thus, these can be indicated as an alternative therapeutic tool to reduce the morbidity of these infections, as in both investigated exposure times. they were able to reduce the
enzymatic secretion of the fungus.

KEYWORDS

Antifungal agents; *Candida albicans*; Infection; Plant extracts; Virulence factors.

RESUMO

Objetivo: Analisar a expressão fenotípica de fatores de virulência em biofilmes de *Candida albicans* frente a extratos glicólicos de plantas. **Material e Métodos:** Os biofilmes de *Candida albicans* (ATCC 18804) obtidos a partir de incubação de 48 horas foram expostos por 5 minutos e 24 horas a diferentes concentrações de extratos glicólicos de *Hamamelis virginiana* e *Persea americana*, *Cynara scolymus* L e *Stryphnodendron barbatiman* M, a fim de verificar a ação antifúngica da proteinase, fosfolipase e hemolisina. **Resultados:** Todos os extratos foram eficazes na redução do biofilme. Em contato por 5 minutos. os extratos reduziram 50% do biofilme. Após 24 horas. o extrato de *Persea americana* apresentou o biofilme em 90%, seguido de *Cynara scolymus*, que o interrompeu em 85%. Houve mudança na intensidade da proteinase após 5 minutos e 24 horas, com uma atividade enzimática média de 0,69 em comparação com o controle de 0,49. Cynara scolymus foi o extrato

com maior concentração média de 100 mg/ml; a intensidade da fosfolipase foi alterada com Stryphnodendron barbatiman sendo mais efetivo em 24 horas em relação ao controle (p< 0,0001). A secreção de hemolisina foi modificada por *Hamamelis virginiana* (12,5 mg/ml) após 5 minutos de exposição e em 24 horas. todos os extratos foram capazes de causar alterações na secreção. **Conclusão:** Os extratos testados apresentam potencial antifúngico em biofilmes de *Candida albicans*, implicando em redução significativa dos fatores de virulência. Assim, estes podem ser indicados como uma ferramenta terapêutica alternativa para reduzir a morbidade dessas infecções, já que em ambos os tempos de exposição investigados, eles foram capazes de reduzir a secreção enzimática do fungo

PALAVRAS-CHAVE

Agentes anfifúngicos; *Candida albicans*; Infecção; Extratos vegetais; Fatores de virulência.

INTRODUCTION

Candida albicans is a dimorphic fungus that can change its commensal form to an invasive. opportunistic pathogenic form. This transition is an important example of their phenotypic expression. in which a switch between a unicellular and a multicellular gene expression program occurs [1]. Its prevalence in hemocompatible isolates is high. and it is mainly responsible for invasive fungal infections (IFI) that affect hospitalized and immunocompromised patients. especially those who make prolonged use of broad-spectrum antibiotics [2-4].

This species can cause superficial and invasive infections with the serious potential to enter the bloodstream and affect several organs. causing harm to the patient and even exposing him to the risk of death in the most severe forms of dissemination [5-7]. Mortality rates caused by *C. albicans* vary worldwide from 19.6% to 67%. In the United States, the pathogen is ranked as the 4th most prevalent microorganism in nosocomial infections that cause death. The ability to induce disease is directly related to host defense mechanisms and virulence factors associated with the pathogen [8-12].

The pathogenicity of *C. albicans* is due to its morphogenesis. its ability to form a biofilm. and tissue invasion by different mechanisms, including endocytosis, active penetration induced by hyphae, and the production and secretion of hydrolytic enzymes such as phospholipases and proteinases, in addition to hemolysin. which causes erythrocyte hemolysis [13-15].

Aspartyl-proteinases (SAP) are encoded by a family of 10 genes (SAP1–SSAP10) that play a vital role in the virulence of *C. albicans* as well as assist in the formation of hyphae, phenotypic exchange, adherence, and degradation of host tissue proteins. The enzyme acts on junction intracellular proteins, as well as components such as collagen and keratin, in addition to degrading proteins associated with host defense such as immunoglobulins and cytokines [16,17].

Phospholipases are a group of enzymes located on the surface of the yeast and at the end of the germ tube. They hydrolyze phospholipids in fatty acids and other lipophilic substances during the period of tissue invasion, causing damage to the host's epithelial cells [18-20].

In addition to acting on virulence. these enzymes secreted by *C. albicans* also help in the development of antifungal resistance to conventional antibiotics. Due to the continuous and early use of antifungals in the hospital environment. resistance to this pathogen associated with high mortality has been prevalent [21,22]. For this reason. alternative medicine has been sought for drugs capable of combating this species, acting mainly in its enzymatic production, in the control of infection, and in the inhibition of biofilm formation, using herbal medicines obtained from plant extracts, dry fruits, and essential oils.

A small plant, with golden yellow leaves. native to North America called *Hamamelis virginiana* has already been studied showing promising results in the treatment of gastrointestinal and inflammatory skin diseases, also helping in the healing of wounds and burns, as it is rich in tannins, flavonoids, and proanthocyanidins. Another study identified that it has resistance to degradation by bacterial collagenase [23-26]. Another fruit with several proven biological activities is *Persea americana*, popularly known as avocado. which acts as an antioxidant, antidiabetic, antihypertensive, antimicrobial, antinociceptive [27-30].

Cynara scolymus L, known worldwide as artichoke, is an herbaceous plant, native to the Mediterranean region, and it is used in medicine for the treatment of dyspeptic disorders and having high antioxidant and anti-aging properties [31,32]. *Stryphynodendron barbatiman* is a plant native to the Brazilian Amazon but can also be found in other Brazilian regions. Its properties perform anti-inflammatory, healing, astringent, hemostatic, antimicrobial, antifungal, and antiseptic functions [26].

The glycolic extracts of *H. virginiana* and *P. americana*, *C. scolymus* and *S. barbatiman* obtained excellent results regarding the antifungal action. When used in low concentrations, presenting low cytotoxicity for the host tissues. Thus, knowing the antifungal potential of these extracts, it becomes interesting to investigate their effect on the phenotypic expression of virulence factors in *C. albicans*. In particular, it is valuable to understand the mechanism of action of these extracts to inhibit the enzymatic and hemolytic activity of this fungus when organized in a biofilm.

MATERIALS AND METHODS

Standard strains of *C. albicans* (ATCC 18804) were used. kept in a freezer at -70°C. in the Microbiology and Immunology Laboratory of the Institute of Science and Technology of São José do Campos/UNESP. reactivated in Dextrose Sabouraud medium. and incubated for 48 hours at 37°C. The glycolic extracts of *H. virginiana* L, *P. americana* M, *C. scolymus* L and the bark of *S. barbatiman* M, were prepared in 20% propylene glycol. obtained from the company Mapric (São Paulo. SP. Brazil). with the due reports and specifications.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC)

To determine the MIC. the method of microdilution in broth was used by the Clinical and Laboratory Standards Institute (CLSI), standards M7- A6 and M27- A2. The inoculum was prepared from the culture for 24 hours. in sterile physiological solution (NaCl 0.9%) and standardized in a spectrophotometer (Micronal) with 0.380 absorbance at a wavelength of 530 nm.

The test was performed on a microplate (KASVI. Parana. Brazil), where 100 μl of culture medium (broth Mueller Hinton-Himedia, Mumbai, India) and 100 μl of the extracts were added only in the first column of wells, from where they departed a series of 12 serial dilutions. and the inoculants were added.

After incubation from 24 hours to 37 °C. the MIC was determined in the first well of the microplate. which did not present turbidity. indicative of microbial growth. For the determination of the MFC of the extract. they were inoculated into plates containing Sabouraud-dextrose culture medium (Himedia) and 20 μl of the MIC, as well as 20 μl of all other higher concentrations. After 48 h of incubation at 37 °C, the drop in which colony growth was not observed was determined as the MFC of the plant extract to *C. albicans*.

Biofilm formation

The biofilms were formed in the background of 24 well plates (KASVI. Parana. Brazil). For this. the inoculum of the microorganism in broth was prepared with yeast nitrogen base (YNB-Himedia) supplemented with 100 mM glucose. diluted 10 times in sterile distilled water. For this, the microorganism was incubated at 37°C for 16 h. After this period. the inoculum was centrifuged and washed twice with sterile physiological solution (NaCl 0.9%) and made into standard suspensions in a spectrophotometer (B582, Micronal, São Paulo. Brazil) containing 107 UFC/ml in YNB broth (10x). The microbial suspension (1 ml) was placed in the microplate wells, and it was incubated for 1 h and 30 min (37 °C under agitation of 75 rpm) for initial adhesion. After that time, the wells were washed twice with sterile physiological solution and placed in 1 ml of BHI broth. The plate was then incubated under the same conditions as the initial adhesion for 48 hours; however, after 24 hours of incubation, the culture medium was changed. After the formation period of the biofilm. this was put in contact with the extract.

Exposure to plant extracts

After the biofilm of 48 hours, this was exposed to the action of the glycolic extracts of *Hamamelis virginiana*, *Persea americana*, *Cynara scolymus L.*, and *Stryphnodendron barbatiman* M. Concentrations were determined by the CIM and CFM. as shown in Table I and tested for 5 minutes and 24 hours.

The wells were washed three times with PBS and received 1 mL of each extract in the three different concentrations. Five independent experiments were performed, with 3 repetitions each, totaling n = 15 for each experimental group. The control group was maintained in sterile saline. The plates with the different concentrations of the extract were incubated for 5 minutes at room temperature and 24 hours at 37°C under agitation.

After the exposure time, the supernatant was discarded, and the joined cells were washed three times with 2 ml of sterile physiological solution. The biofilm was added to 1 ml of sterile physiological solution, and the microorganisms were disengaged by friction with the aid of a disposable tip for 30 seconds.

Analysis of virulence factors

To evaluate the secretion of proteinase. the medium recommended by Rüchel et al. (1982) was prepared as follows: The first medium (a) is composed of 15 g of noble agar and 900 ml of distilled water. The medium (B), composed of 2.5 g of liquid vitamin (Protovit), 11.5 g of yeast carbon base (Sigma), and 2 g of bovine serum albumine (Sigma), was sterilized by filtration with a pore membrane of 0.22 μm diameter (Millipore, Sao Paulo, Brazil). The medium A was sterilized in an autoclave at 121 °C for 15 minutes and cooled to 50 °C. Then the medium B was added to the A, homogenized, and distributed in sterile Petri dishes.

The strains obtained from the biofilm, previously exposed to the extracts and incubated for 24 hours in *Agar Sabouraud Dextrose*, were sown, Minced, and equidistantly placed on the plates, which remained incubated at 37 °C for 5 days. The production of proteinase was verified by the formation of hyaline halo around the colony, resulting from the hydrolysis of the substrate.

For the evaluation of the production of phospholipase. the proposed medium was used by Polak. In 1000 ml of sterile distilled water, 10 g of peptone, 30 g of glucose, 57.3 g of sodium chloride, 0.55 g of calcium chloride, and 20 g of agar were dissolved. After sterilizing the medium in an autoclave (121 °C for 15 minutes) and cooling it to 50 °C, an emulsion of sterile egg yolk without potassium teluritum was added to it.

The strains obtained from the biofilm. previously exposed to the extracts. and incubated for 24 hours in *Sabouraud* dextrose medium were sown at equidistant points in the middle, and after 4 days of incubation at 37 °C, the formation of a zone of yellowish color was observed around the colonies.

To evaluate the hemolytic activity of the strains exposed to the extracts. 1000 ML of the dextrose *Sabouraud agar* culture medium supplemented with 30 g glucose, pH 5.6, after sterilization in an autoclave (121 °C for 15 minutes) were prepared, cooled to 50 °C, and added to 70 mL of ram blood.

The strains obtained from the biofilm and incubated for 24 hours in *Sabouraud* dextrose culture medium with chloramphenicol were sown in this medium and incubated at 37 °C for two days. After that time, the presence of a translucent halo around the colonies indicated positive hemolytic activity.

The enzyme activity of proteinase (Pz), phospholipase (PHz), and hemolysin (Hz) was evaluated by the ratio between the diameter of the colony and the diameter of the colony plus the precipitation zone. The lower the value of Pz, the greater the enzyme activity. The enzyme activity was classified as negative (PZ = 1), positive (0.64 ≥ PZ < 1), and strongly positive (Pz < 0.64).

Statistical analysis

The data that presented a normal distribution (Shapiro Wilks) were statistically analyzed by the ANOVA method complemented by the Tukey test, with a significance level of 5% (P ≤ 0.05).

Table I - Concentration of plant extracts used for control of growth in the biofilm of 48 hours of *C. albicans*

Glycolic Extract	Concentrations used in biofilm (5 minutes and 24 hours)		
Cynara scoymus	25 mg/mL	50 mg/mL	100 mg/mL
Hamamelis virginiana L.	3.13 mg/mL	6.25 mg/mL	12.5 mg/mL
Persea americana	6. 25 mg/mL	12.5 mg/mL	25 mg/mL
Stryphynodendron barbatiman	25 mg/mL	50 mg/mL	100 mg/mL

The results that did not present a normal distribution were analyzed by the Kruskal-Wallis test and the Dunns test, with a significance level of 5% ($P \leq 0.05$).

RESULTS

Determination of MIC and MFC

In the test of the microdilution in broth. it was possible to determine the most effective concentrations for controlling the growth of *C. albicans* in its planktonic form. The most effective extract was *H. virginiana*. which inhibited growth and was a microbicide when in concentrations above 6.25 mg/ml, followed by *P. americana* (avocado), which displayed inhibitory and microbicide capacity in concentrations above 12.5 mg/ml. When exposed to the extracts of *S. barbatiman* and *C. scolymus* (artichoke), no concentration was capable of inhibiting the growth of microorganisms.

To verify the action of the extract in biofilm, it was chosen to use the concentration before the MIC and a concentration after (Table I) so that the cells remain alive, and it is possible to check the effect of the extracts on the secretion of virulence factors.

Growth control in biofilm

The biofilms of 48 hours of *C. albicans* were kept in contact with the glycolic extracts for 5 min and 24 h, in different concentrations (Table I). When in contact for 5 minutes, all the tested extracts were able to reduce the biofilm by about 50% in relation to the control, as shown in Figure 1 (A, C, E, and G). There is no statistical difference being verified between the different concentrations tested for each extract. In 24 hours of contact. the extract of *C. scolymus* was able to reduce on average 55.3% in relation to the control; the concentration of 100 mg/ml showed a reduction near 85%, with a statistical difference between the groups ($p = 0.0008$) (Figure 1B).

The biofilms of *C. albicans*. when treated by the different concentrations of *H. virginiana*. presented average reduction percentages of 54.85% to 5 min and 58.53% to 24 h. When compared to the tested concentrations, there was no statistical difference observed between them for 5 min ($p = 0.8408$) and for 24 h ($p = 0.3527$) of contact, as shown in Figure 1 C and D.

The reduction percentage of the glycolic extract of *P. americana* in contact with the biofilms of *C. albicans* for 5 min was less than 50% in relation to the control (Figure 1 E), with no statistical difference between the tested concentrations ($P = 0.7644$). In the period of 24 hours, the average reduction in relation to the control was 55.17%, 88%, and 90% in the different concentrations of 6.25 mg/ml, 12.5 mg/ml, and 25 mg/ml, respectively. In the statistical analysis ($p = 0.0777$), no difference was verified between the concentrations tested (Figure 1F).

In 5-minute contact with the glycolic extract of *S. barbatiman*. the biofilms of *C. albicans* showed a 42.4% reduction in relation to the control, with no statistical difference between the different concentrations tested ($p = 0.8534$). Already after 24 hours of exposure to the extract, it was able to reduce the biofilm by 57.4% in relation to the control. When the concentrations were compared between them, there was no statistical difference ($p = 0.1392$).

Analysis of virulence factors

Table II brings the found values of expression of the virulence factors found after exposure to the different concentrations of the different plant extracts of 5 minutes of exposure. The most effective extract in reducing the expression of proteinase was that of *C. scolymus* in the concentration of 100 mg/ml. the production of phospholipase, in turn, was better controlled by the extract of *S. sarbatiman* Achieving the same efficiency in all the tested concentrations. The production of hemolysin. was only altered by exposure to the extract of *H. virginiana* at the concentration of 12.5 mg/ml.

Table III shows the results obtained after exposure to the extract for 24 hours, in which a decrease in the expression of the most effective virulence factors can be observed in relation to the control. All tested extracts were able to reduce the reduction of these factors, and the extracts of *S. barbatiman* at 100 mg/ml were more effective in reducing the reduction of proteinase and phospholipase. The production of hemolysin suffered more interference after exposure to a concentration of 12.5 mg/ml of *H. virginiana* extract.

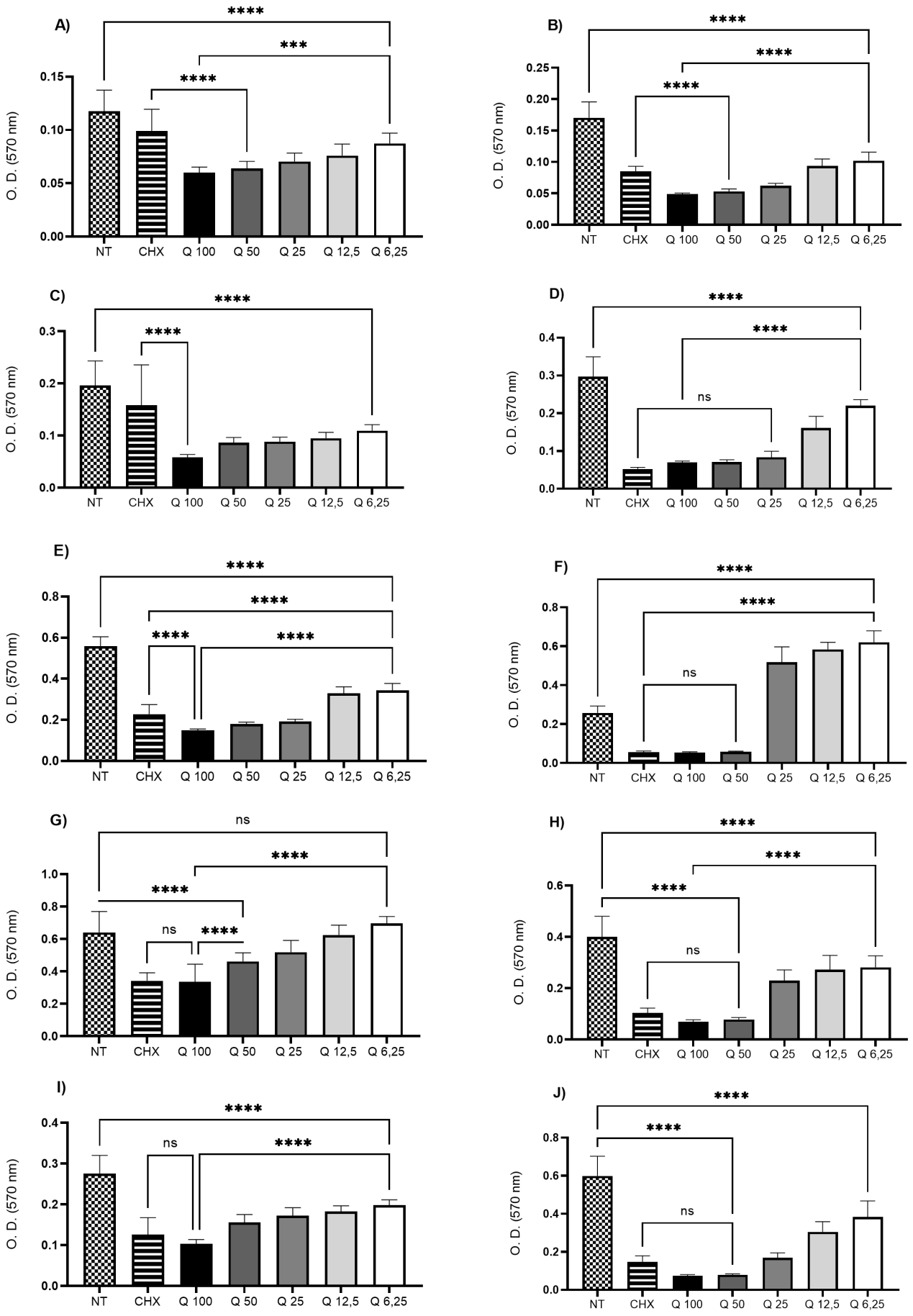

Figure 1 - A-F. Reduction percental of the mature biofilm of *C. albicans* after exposure to the glycolic extracts. for 5 minutes and 24 hours. Legend: A. C. E. and G are referring to exposure to the extract for 5 minutes; B. D. F. and H are referring to exposure for 24 hours.

Table II - Production of virulence factors of *C. albicans* after 5 minutes of exposure to the extracts

			Cynara scolymus		
	Control	**100 mg/ml**	**50 mg/ml**	**25mg/ml**	**Value of *P***
Proteinase (Pz)	0.51	0.74*	0.66**	0.53	<0.0001
Phospholipase (Phz)	0.53	0.57	0.62*	0.60*	<0.0001
Hemolysin (Hz)	0.46	0.50	0.47	0.43	0.0454
			Hamamelis virginiana		
	Control	**12.5 mg/ml**	**6.25 mg/ml**	**3.13 mg/ml**	**Value of *P***
Proteinase (Pz)	0.50	0.70*	0.68*	0.72*	<0.0001
Phospholipase (Phz)	0.44*	0.55**	0.46*	0.50***	<0.0001
Hemolysin (Hz)	0.49	0.66*	0.50	0.49	<0.0001
			Persea americana		
	Control	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**Value of *P***
Proteinase (Pz)	0.46	0.70*	0.65*	0.64**	<0.0001
Phospholipase (Phz)	0.44	0.43	0.46	0.53*	<0.0001
Hemolysin (Hz)	0.48	0.55	0.52	0.52	0.1365
			Sphyrodendron barbatiman		
	Control	**100 mg/ml**	**50 mg/ml**	**25mg/ml**	**Value of *P***
Proteinase (Pz)	0.51	0.72*	0.71*	0.54	<0.0001
Phospholipase (Phz)	0.52	0.62*	0.66*	0.62*	<0.0001
Hemolysin (Hz)	0.49	0.47**	0.47**	0.45*	<0.0001

*=p<0.5; **=p>0.5

Table III - Production of virulence factors of *C. albicans* after 24 hours of exposure to the extracts

			Cynara scolymus		
	Control	**100 mg/ml**	**50 mg/ml**	**25mg/ml**	**Valor of *P***
Proteinase (Pz)	0.45	0.78*	0.68**	0.71**	<0.0001
Phospholipase (Pz)	0.49	0.64*	0.67*	0.67*	<0.0001
Hemolysin (Hz)	0.46	0.64*	0.58*/**	0.55**	<0.0001
			Hamamelis virginiana		
	Control	**12.5 mg/ml**	**6.25 mg/ml**	**3.13 mg/ml**	**Valor of *P***
Proteinase (Pz)	0.52	0.71*	0.72*	0.72*	<0.0001
Phospholipase (Pz)	0.56	0.70*	0.67*	0.73*	<0.0001
Hemolysin (Hz)	0.50*	0.70**	0.61***	0.52*	<0.0001
			Persea americana		
	Control	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**Value of *P***
Proteinase (Pz)	0.54	0.75*	0.70*	0.70*	<0.0001
Phospholipase (Pz)	0.53	0.61*	0.63*	0.61*	<0.0001
Hemolysin (Hz)	0.50	0.68*	0.56**	0.57**	<0.0001
			Sphyrodendron barbatiman		
	Control	**100 mg/ml**	**50 mg/ml**	**25mg/ml**	**Value of *P***
Proteinase (Pz)	0.45	0.76*	0.71*/**	0.68**	<0.0001
Phospholipase (Pz)	0.50	0.74*	0.67*/**	0.64**	<0.0001
Hemolysin (Hz)	0.46	0.63*	0.69*	0.60*	<0.0001

*=p<0.5; **=p>0.5

DISCUSSION

All around the world, there has been observed a significant increase in the incidence of fungal infections, thus affecting global health. That is why many research groups have included in their studies objectives that aim to understand biochemical and molecular characteristics that interfere in the pathogenicity and fungal virulence, as well as verify susceptibility and resistance to antifungals, to propose new antimicrobial alternatives. With the species of *Candida* spp., it has not been different, mainly with *Candida albicans*, due to its high prevalence. Many studies prioritizing the mentioned items have already been reported in the literature [11,33-35].

According to the World Health Organization, plant extracts or their active principles are used by 80% of the world's population. Due to the growing use of herbal medicine, several studies are being done to assess the antimicrobial activity of these compounds [14,36,37]. The extracts were used in inhibitory concentrations; after all. the objective of the study was to evaluate the production of virulence factors associated with the invasiveness and perpetuation of the microorganisms after exposure to the extracts, allowing analysis later concerning the production of proteinase, phospholipase, and hemolysin.

The present study was not the first to evaluate the antifungal action of the extracts of *C. scolymus*, *H. virginiana*, *P. americana*, and *S. barbatiman*, and as expected, the glycolic extracts of these plants, were able to reduce the biofilm of *C. albicans*, corroborating with several authors. These results are promising because the main death rates related to this microorganism are in the production of biofilms [26,38-42]. However, no studies were found in the scientific literature that associated the action of the extracts tested in this study with the phenotypic expression of the virulence factors of *C. albicans*, mainly the hydrolytic enzymes produced by the different species of *Candida*, which have stood out as possible therapeutic targets, due to their decisive role in the pathological process [13].

The production of proteinase was affected by exposure to all the tested extracts; although they were not capable of negative production, there was a significant reduction compared to the control [43]. Different from this study, the exhibition of 50 strains of *C. albicans* (all of them producing proteinase and phospholipase), isolated from patients with oral candidiasis. after being subjected to liver transplant, to the crude extract of the leaves of *Eugenia uniflora* (Cherry), was able to zero the secretion of these enzymes, in 94% of the strains tested [44].

The effectiveness of the extract of the leaf of *Pluchea dioscoridis* was analyzed in the different expressions of proteinases; this presented high potential against this enzyme because the expression decreased by 90% and 40% for SAP1 and SAP10, respectively [45]. These characteristics attenuating the production of *C. albicans* proteinase have also been observed in studies with the oil of *Ocimun Sanctum* (Basil-Sacred), and similar results were observed in reducing the production of proteinase. It was also able to inhibit the expression of the SAP1 gene, responsible for encoding these enzymes [46]. The same was seen after contact for 18 hours with the methanolic extract of *Juglans regia*. in which a reduction in the production of proteinase to the control (Pz = 0.34) was observed in the concentrations of 175 μl/mL (PZ = 0.45) and 350 μl/mL (Pz = 0.62) [47].

Regarding phospholipases. it can be seen that *S. babatiman* extracts were more effective in reducing phospholipase production when compared to the highest tested concentrations. The same happened when strains of *C. albicans* were isolated from patients with prosthetic stomatitis for 30 min from the essential oil of *Origanum vulgare*. To evaluate the anti-enzymatic activity, it was verified that the oil was able to inhibit the production of phospholipase compared to the control [37]. Different from this work, which verified a statistically relevant reduction in the secretion of phospholipase in microorganisms that were exposed to extracts for 24 hours, the results were similar to those observed after exposure to the essential oil of *Junglans regia*, which presented a reduction in the production of phospholipase [47].

The flavones are part of the flavonoids group, compounds with widely known biological activity in traditional medicine. A flavone, 2-phenyl-4h-chromen-4-one, has been tested in the control of the growth and enzyme activity of *C. albicans*, as well as its cytotoxicity. Although the compound presents a significant reduction in the growth of *C. albicans* and low cytotoxicity, it wasn't able to alter the patterns of secretion of enzymes like proteinase and phospholipase [48-50].

Different concentrations of Dracorodim's percolation. a flavonoid extracted from a plant widely used in Chinese medicine, the *Daemonorops draco* (Dragon's Blood), were put in contact with strains of *C. albicans* for 24 hours and were able to inhibit the growth of the fungus as well as the formation of the biofilm and the morphological transition. The active principle was also able to inhibit the secretion of phospholipase concerning the control (PHz = 0.6), compared to the different concentrations tested (16, 32, and 64 mM), which exhibited PHz statistically inferior to the control (0.64, 0.68, and 0.69, respectively) [51]. Different from this, the cetonic fractions of the aqueous extract of *Buchenavia tomentosa* presented an antifungal effect, but exposure to the extract for 1 hour was not able to change the production of proteinase and phospholipase, with Pz avenge from 0.45 to all groups [14]. Like these plants, *H. virginiana* has flavonoid-rich properties as well.

About hemolysin secretion, it was possible to verify in this work that the glycolic extract of *H. virginiana* was the only one capable of altering the secretion of hemolysin at a concentration of 12.5 mg/mL during a 5-minute exposure. However, when the strains were exposed for 24 hours, it was possible to perceive a significant reduction in the production of hemolysin. Similar results were obtained when strains of *C. albicans*, resistant to different antifungals, were tested in sub-MIC oil concentrations of *Carum copticum* and *Thymus vulgaris* [52,53].

Essential oils of different plants, *Melissa citratus indica*, *Cymbopogon citratus*, *Pelargonium graveolens*, and *Eugenia caryophyllata*, were placed in contact with standard strains of *C. albicans* and subsequently evaluated for the secretion of hemolysin, among other factors of virulence, concluding that these oils have a negative impact on the pathogenicity of *C. albicans* acting in an inhibitory way in the production of these factors [54]. However, another study demonstrated that the essential oil of *Mentha piperita*, showed results in all virulence factors of *C. albicans* and the cinnamon bark oil exhibited high antifungal activity, active against a pre-formed *C. albicans* biofilm [55,56].

As well as other microorganisms, *Escherichia coli* and *Staphylococcus aureus* have already been evaluated, together with *C. albicans* exposed to *P. americana* extract and *Cynara scolymus* acting against *Porphyromonas gingivalis* [57]. Other studies have already shown that tea. including *H. virginiana*, can decrease oral bacterial bioadhesion and act as a photosensitizer in antimicrobial photodynamic therapy (aPDT) [40,41].

Work numbers have been used to determine the production of these virulence factors by *C. albicans* isolated from different infectious outbreaks. Different species of *Candida* were isolated from injuries associated with oral cancer, chronic candidiasis, and atypical infections, as well as from isolated species of asymptomatic patients. When some virulence factors were checked, you concluded that the isolated species of patients with symptoms, irrespective of the type of lesion, showed greater production of proteinase and phospholipase. That factors are directly linked to the capacity of this microorganism to invade the tissues of the host, which leads to an increase in the morbidity of the infectious process. Many of the samples isolated from infections, whether they come from blood or superficial infections, cause a lot of damage to the host [10,53,58-63]. Considering the above. these data reinforce that the presence of the production of these virulence factors is directly related to the capacity of these strains to cause disease.

These studies show the importance of knowing the standard of production of these virulence factors in different types of lesions since their production is associated with factors inherent to the host and the infectious site. Also, knowledge of the virulence factors involved in pathological processes can allow new therapeutic strategies to be created and can control the infections caused by this pathogen. Different factors are involved in the action of these extracts. such as the amount of tannins, flavonoids, and anthocyanidins, among other principles inherent to each plant. The use of total extracts allows synergism between these compounds. often enhancing your action.

CONCLUSION

This study demonstrated that the tested extracts have antifungal potential in Candida albicans biofilms, having the ability to influence the decrease in the phenotypic expression of virulence factors, reducing enzymatic secretion, and reaching the proposed objectives. which can be indicated as alternative therapeutic tools with the objective of reducing the morbidity of these infections. In both times tested, the secretion of phospholipases, proteases, and hemolyzines produced by *C. albicans* decreased.

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Author's Contributions

JGS, IA: Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft Preparation. ERLB: Writing – Review & Editing. GNBB: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft Preparation, Visualization, Supervision, Project Administration, Funding Acquisition. LDO: Conceptualization, Methodology, Software, Validation, Visualization, Supervision, Project Administration, Funding Acquisition

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions of ICT/CSJC – UNESP Ethical Committee Agency. It does not require approval from the ethics committee because the research does not involve humans or animals.

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