Effect of denture liners surface modification with *Equisetum giganteum* and *Punica granatum* on *Candida albicans* biofilm inhibition

Background: This study investigated the effect of denture liners surface modification with *Equisetum giganteum* (EG) and *Punica granatum* (PG) on *Candida albicans* biofilm inhibition supposing its usage as a sustained-release therapeutical delivery system for *Candida*-associated denture stomatitis (CADS). **Methods:** *C. albicans* biofilm (SC5314 or ATCC 90028) was formed on soft liners superficially modified by a primer mixed to drugs at minimum inhibitory concentrations (0.100 g for EG and PG, or 0.016 g for nystatin per mL of primer). After 24 h, 7 or 14 days, anti-biofilm activity was evaluated by CFU counts. **Results:** Not all groups were equi-efficient to nystatin after 24 h and 7 days. After 14 days, EG and PG efficacies were not different from nystatin (almost 100% inhibition). **Conclusion:** The proposed protocol presents a promising option to allopathic drugs for CADS treatment.

Keywords: denture liners; denture stomatitis; *Candida albicans;* antimicrobial agents; natural products

Introduction

Removable denture wearers are often affected by oral candidiasis, most frequently the chronic atrophic type, known as denture stomatitis [1,2]. This infection is considered the most common form of oral candidiasis [3,4] and the most frequent oral lesion among the elderly [4], affecting up to 70% of otherwise healthy denture wearers [1,2,5]. It is characterized by inflammatory lesions, erythematous and edematous regions on oral mucosa in contact with removable dentures [6]. Clinically, DS is classified in many stages of severity affecting the basal area tissues, from multiple hyperemic pinpoints to erythematous and diffuse areas with papillary hyperplasia [6]. The main etiological factor of this condition is the overgrowth of *Candida* species, especially *Candida albicans*, in lesions, saliva, and denture biofilm. Therefore, the literature describes this condition as *Candida*-associated denture stomatitis (CADS) [1,7].

CADS treatment, even though effective, remains challenging due to high recurrence levels observed even two weeks after therapy end [8–12]. The constant salivary flow, the cleansing action of the associated musculature [8], and the colonization of denture bases by complex biofilms make the antifungal agents concentration inferior to that considered as therapeutic [10,13]. Conventional therapy failure is also related to the lack of patient adherence to the treatment, strict medication dosage [14], and to the microbial resistance of denture biofilm [7].

A potential alternative for CADS therapy is the association of temporary soft denture liners with antimicrobial/antifungal drugs once these components are uniform and gradually released from a modified polymeric matrix to the infected sites [14–18] . Bueno *et al.* [15] observed that nystatin, chlorhexidine diacetate, and ketoconazole incorporated at minimum inhibitory concentrations (MICs) in two temporary soft liners were able to inhibit 90% or more of *C. albicans* biofilm in up to 14 days of incubation. Therefore, such approach is able to maintain an effective drug concentration [18] throughout the useful life of these liner materials (14 days) [19–22], which is a period comparable to the duration of conventional treatment with topical antifungal agents [23]. An advantage of associating medication with the soft material is that the latter provides a new inner surface to the denture that is already being used by the patient. This avoids the acrylic base contacting and reinfecting the oral mucosa while drugs maintain their therapeutic effect over 14 days, preventing microbial adhesion [10]. Furtherly, this protocol favors the recovery of the injured mucosa due to cushioning effect of the soft material that promotes comfort to the patient, in addition to the readaptation of the denture base to supporting tissues, minimizing the trauma that exacerbates the infection [14]. To adhere the treatment,

the patient should only wear the relined denture, eliminating the necessity of compliance to the strict drug regimen of topical antifungal agents [14]. Therefore, the proposed drug-delivery protocol promotes, at the end of 14 days, tissue health recovery that permits denture replacement or relining with long-term materials [14,24].

Despite of temporary denture soft liners being benefited by modification with allopathic drugs, adverse effects as toxicity and drug interactions are to be considered [25,26]. Although such effects are minimized with the direct release of the drug on the infection site, Hotta *et al.* [27] observed a reduction in the thickness and area of the stratum corneum of the palatal mucosa of rats treated with a temporary soft denture liner modified by ketoconazole at MIC. Thus, there is an increasing interest in antimicrobial agents derived from medicinal plants as an alternative to synthetic substances [12,13,28–30], also relevant for microbial resistance to the conventional drugs [26]. Unlikely to allopathic substances, phytotherapics (phytocomplex or crude extract) have an abundance of active compounds with remarkable pharmacological properties that result in a less intense therapeutic reaction. As a consequence, undesired side effects are reduced or even eliminated [13,31]. Mice *in vivo* studies indicated that EG orally administered caused no acute toxicity with no behavioral, neurological, anatomical, weight and vital organs function alterations detected [32]. Treatment doses of EG extracts also maintained similar cell viability to unexposed control cells with no cytotoxicity on lymphocytes [33]. PG peel ethanol extract was classified as safe by *in silica* and *in vivo* studies based on aquatic toxicity with zebra fish [34]. Moreover, no toxicity was detected in rats with oral candidiasis after PG administration [35], and repeated administrations of this extract in mice did not alter or cause local irritation of the oral mucosa, presenting clinical and histopathological tolerability [36].

A wide variety of natural products [13,37,38], including plant extracts [28–30] has shown antifungal activity against *C. albicans*, suggesting a potential treatment for CADS. The genus *Equisetum* belongs to *Equisetaceae* family and consists of 15 species often named as “horsetail”. The alcoholic extract of *Equisetum giganteum* (EG) showed an antimicrobial effect against several oral pathogens as *Staphylococcus aureus* and *C. albicans* [28,39]. The hydroethanolic extract of EG presented antiadhesive properties for *C. albicans* on acrylic denture base resins and anti-inflammatory reactions on human monocytes activated by this fungus, but maintaining the viability of human palate monocyte and epithelial cells [28]. Its aqueous extract presented anti-inflammatory potential in a model of acute arthritis inflammation induced by antigens, and promoted immunomodulation of lymphocytes B and T [33]. Recently, it was also shown that hydroethanolic extract of EG affected *C. albicans* biofilm on acrylic denture base resin by reducing the biomass and the number of living cells [29].

Another alternative to synthetic drugs is *Punica granatum* (PG) for its anti-inflammatory, antioxidant, and antimicrobial properties. PG belongs to *Punicaceae* family, commonly known as pomegranate tree, a small tree native to Asia, which has been historically used by ancient cultures as a treatment for many diseases. The fruit (pomegranate) is mainly consumed fresh or with drinks, and owns a high source of phenolic compounds, including hydrolysed tannins, with high antioxidant activity. Pomegranate has a potential to prevent and treat inflammations and cancer, presenting antioxidative [40] and antiproliferative effects against tumours from oral cavity, colon, prostate, and cell lines of breast cancer [41]. Moreover, PG may inhibit viral replication, including SARS-CoV-2 [42], and has antimicrobial properties, inhibiting the growth of several bacteria, such as corynebacterial, staphylococci, streptococci, *Bacillus subtilis, Shigella, Salmonella, Vibrio cholera,* and *Escherichia coli* [43,44], also demonstrating potential effects against *Candida* spp. [30,44,45]. Hydroethanolic extracts of PG and EG combined with denture adhesive were significantly effective in affecting the development of *C. albicans* biofilm on acrylic denture base resin [39]. This anti-biofilm action on acrylic resins was also observed when a fibrin biopolymer was combined with PG as a drug delivery system [46]. The antifungal activity of nystatin was enhanced when this synthetic drug was associated with punicalagin, an ellagitannin isolated from PG [31]. In addition, a gel containing the PG extract presented a promising effects for the clinical treatment against CADS [47].

Since the modification of denture soft liners by allopathic drugs was effective against *C. albicans* biofilm [15] and considering the antifungal activity of EG and PG, it would be pertinent to evaluate the potential of these herbal products in a drug-delivery system. Thus, the aim of this study was to investigate the anti-*Candida albicans* biofilm effect of the superficial modification of temporary denture soft liners by EG and PG throughout the lifespan of these materials, as an alternative therapy for CADS. The hypothesis tested was that the superficial application of the phytotherapic drugs would reduce fungal biofilm on denture soft liners.

Materials & Methods

Preparation of plant material extract

The acquirement of plants and preparation of extracts followed the methodology described by Almeida *et al*. [39]. The aerial parts of EG were collected in March 2019 at 'Jardim Botânico Municipal de Bauru', SP, Brazil (22°20'30''S, 49°00'30''W). Fresh specimens of approximately 5 kg were prepared, identified, and included in the Herbarium collection of UNESP (UNBA) in Bauru at São Paulo State University 'Júlio de Mesquita Filho' under code number 5795. PG fruits were purchased at 'Boa Fruta' Fruit and Seedling Distributor Supermarket in July 2019. The fruit was cultivated in Petrolina, Pernambuco, Brazil (9°46'30''S, 24°21'30''W). Pruning was carried out every two months by scaling, and batch drip irrigation was provided.

The aerial parts of EG and fresh fruit peels of PG were completely dried in an air circulating oven at 45 ºC. Both extracts were obtained by percolation in 70% organic ethanol (EtOH:H2O 7:3 v/v) at room temperature. The solvent was evaporated, and the extracts were lyophilized, yielding 8.3% and 13% of crude hydroalcoholic extracts (70% EtOH) of EG and PG, respectively [39].

Specimen fabrication

Two resin-based temporary denture soft liners were selected for this study: Coe-Comfort (CC) (GC Europe, Leuven, Belgium) as a tissue conditioner, and Coe-Soft (CS) as a short-term resilient liner (GC America Inc, Chicago, IL, USA). Specimens were aseptically fabricated in a laminar flow chamber as described by Bueno *et al.* [15]. Each disk-shaped specimen was obtained from round stainless-steel molds with a breakaway compartment. The materials were prepared following the manufacturer's instructions. The resulting mixture was poured in each mold, which was pressed between two sterile glass plates previously sandblasted to simulate the inner portion of the denture base with a mean surface roughness (Ra) of 3 µm (Surftest SJ-301; Mitutoyo Corporation, Kanagawa, Japan) [48]. After final plastification, each specimen was removed from the mold, and the excess material was removed with a sterile scalpel [15] without no finishing and polishing processes to simulate the surface condition in a clinical situation as previously described [42].

Surface treatment of specimens

Nystatin (Nys) (CAS Number: 1400-61-9, Sigma-Aldrich Co., St. Louis, MO, USA) was tested as a control for phytotherapics since it is the chosen drug for conventional CADS treatment [16]. For surface treatment of the specimens, a vehicle was needed to allow the drugs to be gradually released from the soft materials. Several primers used to improve the adhesion between soft liners and denture base acrylic resins were tested for this purpose. Among them, primer of the tissue conditioner set from Rite-Lite (Rite-Dent Mfg Corp, Hialeah, FL, USA) proved to be the most suitable vehicle to remain on the material surface and gradually release the drugs over 14 days. Therefore, for surface treatment of the specimens, the powder of each antimicrobial agent was mixed with the Rite-Lite primer, following the

manufacturer's recommendations.

Nys, EG and PG were tested in concentrations ranged from 0.016 to 0.358 g/mL (gram of antimicrobial powder per primer milliliter) [15] to reach their MICs against two *C. albicans* strains (SC 5314 and ATCC 90028) using dilution method in agar described below [15]. These concentrations were tested at 37°C in individually fabricated specimens after 24 h, 7 days, and 14 days incubation. Hence, each experimental condition was designed according to the material, drug type, drug concentration, fungal strain, and period. For each period, specimens without any treatment were obtained for both materials. The MIC was considered as the drug concentration able to inhibit fungal growth during the evaluation period (14 days) in percentages ≥ 90 [15].

The antimicrobial agents at MICs were aseptically mixed to 1 mL of the primer and brushed onto the surface of each sample, which was individually placed in a 24-well cell culture plate for 2 min, to allow for the complete dryness of the product. For control groups, no drugs were added to the applied primer.

Microorganism and biofilm growth

Each strain of *C. albicans* (SC5314 and ATCC 90028) frozen culture stocks (−80 °C) was grown in YEPD broth (Clontech laboratories Inc., Mountain View, CA, EUA) at 37°C for 24 h. To obtain only the yeast-form, cells were inoculated into Sabouraud dextrose broth (Difco Laboratories Inc., Detroit, MI, USA) at 30°C for 24 h [39]. Afterwards, the suspension was centrifuged at 5000 rpm for 10 min at 22 °C and the cells were harvested and washed with phosphate-buffered saline (PBS, pH 7.2) and standardized at a concentration of 10^7 cells mL^{-1} in PBS using a hemocytometer [15,46]. After surface treatment (Nys, EG, PG or control), each specimen was individually transferred to a 24-well cell culture plate containing 2 mL of inoculum. For fungal adhesion, the culture plates were incubated for 90 min at 37 ºC under agitation (75 rpm). Then, the specimens were transferred to other wells containing 2 mL of PBS to wash non-adherent fungal cells. Finally, they were immersed in 2 mL of culture medium RPMI-1640 (Sigma-Aldrich Inc., St. Louis, MO, EUA), and were left for biofilm formation for 24h, 7 or 14 days [15,46]. Culture wells were daily topped up with RPMI-1640 up to 2 mL during the experimental periods [15].

Biofilm quantification by counting colony forming units (CFU mL^{-1})

After incubation on determined periods, biofilm adhered to the specimens previously accommodated in the 24-well cell culture plate, was gently detached using a cell scraper (Costar® 3010, Corning Incorporated, Corning, NY, USA) [39]. Then, the suspension containing fungal cells was recovered and serially diluted. Aliquots (25 µL) of each dilution were seeded, in duplicate, in Sabouraud Dextrose Agar (Difco) plates containing 0.05% chloramphenicol and incubated at 37 ºC for 24 h [39]. Afterwards, viable colonies of *Candida* were visually quantified by counting the colony forming units (CFU ml^{-1}).

Independent samples were prepared for each experimental condition designed according to the material (CC and CS), *Candida* strain (ATCC and SC), treatment (Nys, EG, PG and control) and incubation period (24 h, 7 and 14 days). As a parameter of fungal inhibition for the methods proposed in this study, *Candida* strains were grown directly in agar throughout the proposed periods (24 h, 7 and 14 days). Three independent experiments were performed, with each condition tested at least in triplicate.

Statistical analysis

Firstly, a 3-way independent measures ANOVA evaluated not only the antifungal potential of the primer without associations (with the reline materials and antimicrobials) but also the temporary soft liners CC and CS, isolated (without drug or primer) or associated alone to the primer (without drug). Those situations were compared to the superficially modified materials with one of the tested antimicrobial

agents.

Due to the obtained results, a second statistical analysis was performed, considering the percentages of fungal biofilm inhibition by the drugs applied to the surface of the soft liners (treatments) compared to those of primer only (which became the control of the second analysis). Data of percentages of fungal growth reduction was determined according to the following equation:

$$\text{Inhibition \%} = \text{CFU mL}^{-1} \text{ values (treatment)} / \text{CFU mL}^{-1} \text{ values (control)}$$

The inhibition percentages were statistically analyzed by 4-way independent measures ANOVA: "material", "strain", "treatment" and "period", followed by Tukey test (α=0.05).

Results

The MICs for both materials and for both strains after 14 days of incubation were 0.100 g/mL for EG, 0.100 g/mL for PG and 0.016 g/mL for Nys.

The 3-way independent measures ANOVA results showed that, for both fungal strains and all the evaluation periods, the primer and the soft liners tested alone presented CFU mL^{-1} values significantly higher to those materials modified by antimicrobial agents in all experimental conditions (P<0.05; supplemental material – Tables S1-S3). Considering the antifungal inefficacy of the primer and the materials without antimicrobial drugs, such conditions were excluded from the second analysis and the control group became the denture liner covered with primer (without drug). Thus, a 4-way independent measures ANOVA was performed considering inhibition percentages of fungal biofilm inhibition on soft materials associated to primer compared to control (0% inhibition).

Statistically significant differences were observed for all the factors: "material" (P<0.001), "treatment" (P<0.001), "strain" (P<0.001), and "period" (P<0.001). Differences were noticed between the interaction for all the factors (P=0.004) and in the following interactions: "material" x "strain" (P=0.001), "treatment" x "strain" (P<0.001), "material" x "period" (P=0.011), "treatment" x "period" (P<0.001), "strain" x "period" (P<0.001), "material" x "treatment" x "strain" (P=0.013), "material" x "treatment" x "period" (P<0.001), "material" x "strain" x "period" (P=0.009) and "treatment" x "strain" x "period" (P<0.001).

Minor percentages of fungal inhibition (73.26 to 77.74%) were observed with *C. albicans* (SC strain) biofilm formed on CS modified by EG in 24 h and by PG in 24 h and 7-day evaluation periods (P<0.05; Figure 1). For all the other experimental conditions, at 24 h and 7-day periods, EG and PG presented the same efficacy when compared to Nys, for both soft liners (P>0.05; Figure 1).

At 14-day period, no significant statistically differences in inhibition percentages were observed between both tested phytotherapics and Nys (approximately 100%), compared to control and regardless of the tested soft liners or *C. albicans* strains (P>0.05; Figure 1).

Discussion

The research hypothesis tested by this study was accepted. The results presented a progressive fungal inhibition of both *C. albicans* strains by the surface modification of both denture soft liners by EG and PG throughout the 14 days. That was shown by biofilm inhibition percentages of the phytotherapics compared to control in the same evaluation periods, being similar to Nys, regardless of the soft materials and strains. Therefore, the factors that influenced fungal inhibition considered in this discussion were surface treatment (EG, PG, and Nys) and incubation periods (24 h, 7 and 14 days).

Surface application of EG in both soft liners in 24 h resulted in an average percentage inhibition of 89,06% (77,74-95,92%), which was progressively increased by 93,85 % (91,10-96,62%) and 97,03% (95,92-99,98%) at 7 and 14 days of incubation, respectively. On the last period, EG was equi-effective to

Nys for fungal inhibition when compared to control and those results could be related to the antimicrobial effectiveness of its compounds.

EG composition consists mainly of flavonoids and glycosylated flavones of apigenin, quercetin and kaempferol [49] and phenolic compounds [28]. Flavonoids are produced by secondary metabolism of the plants, recognized by its medical efficacy as antiviral, antibacterial, anti-inflammatory, anticancer, and hepatoprotective agents [50]. It was shown that kaempferol-derived substances were the most abundant flavonoids found in EG samples [51]. Also, plants containing kaempferol or its glycosides are related to antibacterial, antiviral, antifungal, and antiprotozoal activities [52,53]. Furthermore, antioxidant, anti-inflammatory, and antitumor effects were positively correlated with the amount and composition of phenolics present in EG [51].

Surface application of PG in denture soft liners also resulted in an average percentage inhibition of 87.38% (75.38-97.62%) in the first 24 h, which progressively increased to 89.64% (73.26-99.88%) and 96.26% (89.14-99.99%) at 7 and 14-day periods of incubation, respectively. When compared to control, PG presented the same effectiveness of Nys in the last period of incubation for both fungal biofilms, and this could be related to the antimicrobial activity of some of its compounds.

The main components of the crude extract from PG peels are the ellagitannins [54], with high doses of punicalagin [45,54,55] and ellagic acid [54,56], known by its antimicrobial, antioxidant, anticancer, and anti-inflammatory effects [45]. Even with an unknown mechanism of action, some authors suggest that ellagitannins can cross the cell wall of fungal species, which contains polysaccharides and proteins, and bind to its surface [44]. Therefore, these compounds would precipitate proteins and inactivate enzymes responsible for microbial adherence [43]. It has been suggested that ellagitannins precipitate many vital proteins related to biofilm formation [57], affecting adhesive capacity and co-aggregation of bacteria on surfaces [47], fungal cell growth [58] and its interaction for biofilm development [57]. Bakkiyaraj *et al.* [56] addressed the antifungal effect of PG peel extracts to ellagic acid, capable of changing the architectural structure of *C. albicans*, methicillin-resistant *S. aureus,* and *E. coli* biofilms, decreasing their coverage area and thickness. Other authors identified punicalagin as the main compound of PG peel extracts and, therefore, the greatest responsible of antifungal activity, capable of damaging *C. albicans* structure [45].

The advantages to test a phytotherapic product (phytocomplex or crude extract) as performed in this investigation, is that there is no isolated action of a single active substance, reducing or even eliminating its adverse effects [13]. In a recent study [31], punicalagin alone (Punicalagin - CAS Number 65995-63-3, Sigma- Aldrich, St. Louis, MO, USA) was considered an ineffective fungistatic agent, once *C. albicans* metabolism was reduced to values lower than 60%. However, when associated to Nys, a synergic effect was noticed, resulting in a significant increase in antifungal efficacy [31]. Therefore, it can be assumed that the antifungal effect of a natural product depends on several and not an isolated active substance.

Despite of the lower efficacy of Nys in oral suspension for oral and oropharyngeal candidiasis in babies and HIV/AIDS individuals, this drug is still considered the first choice for topical treatment of uncomplicated cases, including CADS, also for patients with normal systemic conditions [16]. Therefore, Nys was selected in this study as a comparative drug with EG and PG for effectiveness analysis of the proposed treatment associated with soft liners. The results showed that, throughout the 14-day evaluation period, Nys presented fungal inhibition percentages close to or equal to 100%. Those findings were expected, since substances in polyene class such as nystatin present the widest spectrum of action among the available antifungal agents, considered as fungicides. The inhibitory effect of Nys is a result of the interaction from its polyenes with ergosterol, which leads to increased membrane permeability and consequent loss of potassium and other cytoplasmic components, resulting in cell death [55].

Initial percentages close to 90% of fungal biofilm inhibition with EG and PG were progressively increased throughout the experimental period, suggesting that the application of the phytotherapics on soft materials through the tested primer allowed a gradual, desired (MIC) drug release. These results

positively reaffirm the therapy with a drug delivery system in comparison to conventional therapy with antifungal agents for CADS treatment. This system allows to maintain a therapeutic concentration of the drug on the infected mucosa and the internal surface of the denture throughout the lifespan of the temporary soft denture liners [18], which corresponds to the duration of conventional CADS treatment with topical antifungal agents (14 days) [23]. By modifying the internal denture surface, mucosal injuries such as biofilm infection and an eventual lack of adaptation of the denture base are discontinued. Furthermore, the mucosa reinfection cycle is broken, favoring the supporting tissue recovery and restoring comfort to denture wearers [10].

There is a lack of information in Material Safety Data Sheet (MSDS) about Rite-Line tissue conditioner, from which the primer of this research was used, and no previous studies were found using this material. Similarly, no information is described in package insert on the chemical composition of the Rite-Line primer. MSDS assures that most of the primers accompanying those kits consist exclusively in monomers and/or solvents. Since no data was found about mixing drugs to primers applied on soft liners, only indirect comparisons with such products on denture base acrylic resins may be stablished to explain the results of this study. The primers contain solvents that may dissolve the surface of the polymer, since they promote penetration of the reliner into the acrylic resin of the denture base, thus enhancing this interface bonding [59]. It is possible that the surface alteration of the soft liners with the primer associated with the drugs have changed the penetrability of these antimicrobial agents to pores and channels created by the dissolution caused by the solvents of its composition [59]. This effect allowed to maintain an effective concentration of the three drugs (EG, PG, and Nys) on the material surface throughout the 14-day period of evaluation, being gradually released in desired inhibitory concentrations against *C. albicans* biofilms.

Based on the potential of slow and gradual release of EG and PG as their similar *C. albicans* anti-biofilm efficacy to nystatin confirmed by the results of this study, the superficial modification of soft liners with these phytotherapics is suggested for further investigation as an option to the conventional antifungal therapy for CADS. An important advantage of this protocol is reduced need of patient compliance during treatment [14], who may benefit even more by using phytotherapics, as EG and PG, instead of allopathic drugs. The CADS recurrence in clinical situations has led to an uncontrolled use of conventional synthetic antifungal drugs, increasing the chances of fungal resistance [55]. Yet, even if these drugs are released on the infection site, it is possible that, during the treatment, side effects occur such as toxicity and drug-to-drug interactions. These conditions may affect the individuals differently, depending on the disease's condition, age, gender, weight, among other factors [25,26].

The limitations of the present study include testing only one commercial brand of each material (tissue conditioner and temporary soft liner); therefore, these results may be inapplicable to other brands. The hydroethanolic extracts of EG and PG were tested in a crude form, and further research should describe which component (s) is/are responsible for the observed antifungal reaction. Additional experiments evaluating the biofilm after the proposed treatment, such as quantifying biomass cell by crystal violet assay or measuring cellular metabolic activity by XTT reduction assay could not be performed once the suspensions containing modified samples by the phytotherapics presented color alteration associated to their components, which would affect the methodologies. Although it has been reported that propolis orabase gel was able to induce changes in the surface properties of denture-based acrylic resins [60], there is no information on such effects for EG and PG extracts. Thus, prior to clinical testing of the effectiveness of the proposed protocol, additional studies are needed on fundamental properties of temporary soft liners modified by PG and EG, including those related to their surface. Also, further investigations should be conducted considering *in vitro* cytotoxicity and/or cell viability tests on human cells (such as epithelial cells, palate fibroblasts) to evaluate cellular response to the proposed treatment. Clinical factors with oral environment and the denture base conformation were not considered in this investigation. Therefore, a final evaluation of CADS treatment with

temporary denture soft liners with superficial modification by EG and PG should only be determined by clinical study with patients.

Conclusion

After 14 days of incubation, the proposed sustained drug-delivery system by the superficial treatment of soft denture liners by hydroethanolic extracts of EG and PG was as effective as Nys in fungal biofilm inhibition, regardless of the *C. albicans* strain or materials tested, reaching inhibitory percentages close to 100%.

Future Perspective

The promising results presented by the proposed drug-delivery system can be considered preliminary to the clinical evaluation of the surface modification of temporary denture liners by phytotherapic agents in patients with denture stomatitis.

Summary Points

- A direct and reproducible protocol for surface modification of commercially available temporary denture liners by phytotherapic extracts was presented.
- The anti- *C. albicans* biofilm effectiveness of a drug-delivery system by surface modification of denture liners was demonstrated with sustained release of herbal agents used during the period of conventional topical antifungal therapy for denture stomatitis.
- A promising therapy for denture stomatitis was proposed as an alternative to allopathic drugs usually prescribed for its management, but associated with fungal resistance and treatment failure within 2 weeks of follow-up.

Disclosure statement

The authors declare that they have no conflict of interest.

Data availability statement

The authors declare that the data supporting the findings of this study are available within the article and its supplementary materials.

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Figure legend

Figure 1: Means and standard deviation (error bars) of the of *C. albicans* (SC5314 - SC and ATCC 90028 - ATCC) biofilm inhibition by treatments (*Equisetum giganteum* - EG, *Punica granatum* - PG and Nystatin - Nys) compared to control for both soft materials (Coe-Comfort - CC and Coe-Soft - CS) in different periods of incubation (24 h, 7 and 14 days). For each material, different upper case letters indicate statistically different values between the experimental conditions (strain, treatment, and period) (P<0.05).