Thymus vulgaris and Allium sativum essential oils showed inhibitory effects on Candida albicans biofilms

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Abstract

This study aimed to screen for essential oils with antibiofilm effect on *Candida albicans*. The antifungal effect of 15 essential oils was evaluated on *C. albicans* planktonic cells, and the most active essential oils were tested for anti-biofilm property. Toxicity to Vero cells was also assessed. *Thymus vulgaris* and *Allium sativum* essential oils showed higher fungistatic effects on *C. albicans* MYA-2876 and *C. albicans* ATCC 18804. Both essential oils also showed an anti-biofilm effect. *Thymus vulgaris* and *Allium sativum* essential oils showed low and moderate cytotoxicity, respectively. The results obtained in this study open promising possibilities for the elaboration of mouthwashes and topical formulations to improve the conventional treatment of oral candidiasis.

Highlights

- *Thymus vulgaris* and *Allium sativum* essential oils showed inhibitory effect on *Candida albicans* planktonic cells and promising anti-biofilm effect.
- Both essential oils were less cytotoxic than 0.12% chlorhexidine digluconate.

Background

Candida spp. and other species are part of the human oral commensal microbiota, but might cause infections when predisposing factors are present (Williams & Lewis, 2017), particularly in cases of immunosuppression. Oral candidiasis is one of the most commonly found oral lesion among patients with AIDS and *C. albicans* is the most frequently involved species (Ghadwal LT et al 2022).

Nystatin and miconazole are frequently used to treat oral candidiasis. However, the long duration is considered a limitation of this therapeutic protocol (Quindós et al., 2019). Besides, the number of refractory cases to conventional treatment is increasing, especially due to the rise in drug resistance among fungal pathogens worldwide (Ashrit et al., 2022). In fact, evidences suggest that *C. albicans* can be considered an emerging multidrug-resistant fungal pathogen (Dadar et al., 2018). Moreover, relapse cases after cessation of the antifungal therapy have been also reported among patients with HIV/AIDS (Nittayananta, 2016).

Due to this scenario, alternative therapies to treat oral candidiasis are needed (Rodrigues et al., 2019). Unfortunately, there are very few new antifungal drugs currently under development (Campoy & Adrio, 2017; Marquez & Quave, 2020). Traditional knowledge of plants as medicines or ethnobotanical knowledge are considered promising sources for the discovering of new therapeutic agents (Atanasov et al., 2015; Arockianathan et al., 2019; Marquez & Quave, 2020). Among the alternatives associated with plants, the essential oils stand out (Winska et al., 2019).

Essential oils can be important sources of antifungal molecules. Essential oils are composed of many volatile molecules, such as monoterpenes, sesquiterpenes, ketones, alcohols, ethers, and aldehydes (Winska et al., 2019). Besides the biological activity, these active principles are responsible for the aroma

as well (Mendonça 2006). For instance, in Brazil, *Artemisia vulgaris, Cupressus sempervirens, Citrus limon*, and *Litsea cubeba* showed antifungal properties on *C. albicans* (Malik et al., 2019; Pedroso et al., 2019). In China, He et al. (2019) reported the antifungal potential of *Clausena lansium* essential oil against *Candida* spp. Herrera-Rodriguez et al. (2018) reported the anticandidal activity of Mexican oregano essential oil. The essential oil of *Cinnamomum tonkinense*, a plant from north central Vietnam, also demonstrated antifungal activity against *C. albicans* (Dai et al., 2020). Furthermore, some oils such as cinnamon essential oil can also modulate *C. albicans* virulence factors, such as biofilm formation and adherence (Veilleux & Grenier, 2019).

The association of essential oils with traditional antifungals was also evaluated. Tullio et al. (2019) observed synergistic interaction between *Mentha piperita* essential oil and itraconazole against *Candida* spp. This synergistic effect was also observed for *Thymus vulgaris* essential oil (Jafri & Ahmad, 2019). Ngo-Mback et al. (2020) studied essential oils of *Aeollanthus heliotropioides, Cymbopogon citratus* and *Syzygium aromaticum*, aromatic plants used in Camerron, and detected antifungal activity. Mendoza-Juache et al. (2017) reported the effect of *A. sativum* essential oil against biofilm and planktonic cells of *Candida* spp. clinical isolates from acrylic-based dentures.

Considering the need for alternative therapies for oral candidiasis and that essential oils may be source of bioactive molecules, this study aimed to evaluate the antifungal activity of fifteen essential oils, searching for candidates to be incorporated into alternative products for prevention and treatment of oral candidiasis.

Materials & Methods

Essential oils and fungal strains

Thymus vulgaris, *Mentha pulegium, Foeniculum vulgare, Tagetes minuta, Ruta graveolens, Pinus ponderosa, Petroselinum sativum, Coffea arabica, Pinus sylvestris* and *Allium sativum* essential oils were commercially obtained from Laszlo Aromaterapia Ltda. (Belo Horizonte, MG, Brazil). *Cordia curassavica* essential oil was purchased from and Quinari Fragrances and Cosmetics Ltda. (Ponta Grossa, PR, Brazil). These commercial oils were prepared by the steam distillation. These commercial oils were prepared by the steam drag extraction method.

Tagetes minuta, Cordia curassavica, Eugenia uniflora, Matricaria recutita essential oils were prepared from the ex-situ germplasm bank collection of the medicinal and aromatic plants collection (CPMA) from the multidisciplinary center for agricultural, biological and chemical research – UNICAMP. This center is situated at Paulínia, São Paulo, Brazil (22° 45'00 " south and 47° 10'21 " West). The plants were collected and processed between August and September 2016. In collaboration with the present work, the essential oils were available to the research group of the Institute of Science and Technology (ICT) of the São Paulo University State (UNESP), São José dos Campos, from the postgraduate program in oral biopathology through Prof. Dr. Glyn Mara Figueira, who owns the permits and licenses for collection of

plant species, which after being transformed into essential oils, were analyzed. For *T. minuta*, we received the voucher CPMA 104of50, proving that it was collected by Narciso Ramos and deposited in the herbarium of the collection of medicinal and aromatic plants - Pluridisciplinary Center for Biological and Agricultural Chemical Research (CPQBA), Unicamp, with registration number 606. *C. curassavica* received the UEC 112744 voucher, collected and deposited at the State University of Campinas (UEC) by Pedro Mellino Magalhaes from the Institute of Biology, with registration number UEC042593. *Also, E. uniflora* received the UEC 184186 voucher, collected and deposited at the UEC herbarium by Henrique Santos of the Institute of Biology, with registration number UEC 107420. Finally, for *M. recutita*, as we did not have this species in the university's germplasm bank, the leaves were purchased commercially and identification was done by chromatography, however, we have no informations.

The work was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge – SisGen (A93EDB1). The essential oils were extracted using hydro distillation as described by Albuquerque et al. (2018). The oils were stored protected from light at -20°C. 100 µl of each essential oil were weighed, the value was noted, then 900 µl of glycerol were added to reach the initial working concentration. Ten different concentrations were analyzed.

The chemical characterization of *T. vulgaris* essential oil was previously performed by CG-MS and showed the presence of carvacrol, p-cimene and α- pinene as major constituents (Santos et al., 2010).

C. albicans ATCC 18804 and *C. albicans* MYA-2876 reference strains were included in the study. The microorganisms were kept frozen (-80°C) in Yeast Extract Peptone Dextrose (YPD - Himedia) broth supplemented with 16% glycerol.

Antifungal effect on C. albicans **planktonic cells**

As a screening test, the minimum inhibitory concentrations (MIC) of the 15 essential oils were determined by the broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI) (M27A3, 2008). First, strains were cultured on Sabouraud dextrose agar and standardized suspensions (1 x 10^6 cells/mL) were prepared in sterile saline (0.9% NaCl), with the aid of a spectrophotometer (1 x 10^6 cells/mL) (Micronal, São Paulo, Brazil; A_{530} = 0.138). RPMI 1640 (Himedia) broth with glutamine, with absence of bicarbonate and with phenol red indicator, buffered at pH 7.0 ± 0.1 with MOPS [3- (N-morpholino) propanesulfonic acid] (Sigma-Aldrich, St. Louis, EUA) was used in these assays. Serial dilutions (1:2) of the essential oils were obtained in 96-well plates (TPP, Trasadingen, Switzerland). Using a micro tube, the essential oils were weighed with the aid of an analytical balance, and the essential oil were diluted 10 times with glycerol and the concentration of each essential oil was determined using an analytical balance. A volume of 100 µl of essential oil were added in the first well, then from the second well were added a volume of 100 µl of culture medium. The serial dilution were made 1/10 to tenth dilution. In this way the essential oil were diluted 10 times. Growth (RPMI broth plus microorganism) and negative (RPMI broth only) controls were included. The MIC values were determined after 24 h incubation by visual reading, considering 50% of inhibition in comparison to growth control.

Anti-biofilm effect evaluation

The essential oils with lowest MIC values (*T. vulgaris* and *A. sativum*) were tested for anti-biofilm activity against *C. albicans*. The same biofilm formation methodology was used for the two reference strains (*C. albicans* ATCC 18804 and *C. albicans* MYA-2876). The microorganisms were first grown in Sabouraud dextrose agar and then transferred to a broth (RPMI 1640 (Himedia) with glutamine, with absence of bicarbonate and with phenol red indicator, buffered at pH 7.0 ± 0. 1 with MOPS [3- (N-morpholino) propanesulfonic acid] (Sigma-Aldrich, St. Louis, EUA) for 24 h at 37°C. The fungal suspension was centrifuged at 448 ×*g* for 10 min (MPW-350, Warsaw, Poland). After, the supernatant was discarded and the pellet was resuspended in NaCl (0.9%). The same procedure was performed twice. Subsequently, the turbidity of this suspension was adjusted to 10^7 cells/mL with the aid of a spectrophotometer (Micronal; A_{530} = 0.760 and 0.560 for *C. albicans* ATCC 18804 and *C. albicans* MYA-2876, respectively). Next, 250 µL/well of this suspension was distributed in 96-well plates. After that, the plates were incubated under shaking (37°C; 75 rpm) for 90 min for fungal adhesion. Then, the supernatant was discarded and 250 µL of RPMI 1640 (Himedia) broth with glutamine, with absence of bicarbonate and with phenol red indicator, buffered at pH 7.0 ± 0. 1 with MOPS [3- (N-morpholino) propanesulfonic acid] (Sigma-Aldrich, St. Louis, EUA) was added. Plates were incubated for 48 h. Spent culture medium was refreshed after 24 h incubation. After 48 h, the biofilms were exposed to the two most active essential oils (10 x MIC) and 0.12% chlorhexidine digluconate (positive control) for 1 and 5 min. Treatment time were chosen based on the mean contact time recommended for mouthwashes (1 min) and on the possibility to incorporate the essential oils in sustained-release formulations (5-min). As negative control, sterile saline solution (0.9% NaCl) was used. All experiments were performed in triplicate in three different occasions (n = 9). Biofilms treated with essential oil or chlorhexidine were washed twice with 0.9% NaCl solution. Then, the biofilm was dispersed with the aid of the pipette tip and homogenized with suction and dispersion movements. The suspension obtained was serially diluted (1:10) and 100 µl was plated on Sabouraud dextrose agar (SD). After 24 h of incubation, CFUs were counted and values of CFU/mL were determined.

Cytotoxicity of Thymus vulgaris **and** Allium sativum **essential oils**

Vero cells (ECACC 84113001) were grown in Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% of fetal bovine serum, 100 IU ml^{-1} of penicillin, and 100 µg ml^{-1} of streptomycin at 37 ºC and 5% CO_2). The cells (3.6×10^4 cells/ml, 100 µl cells/well) were cultured for 24 h. After this period, the culture medium was removed. Next, cells were exposed to *Thymus vulgaris* and *A. sativum* essential oils (at 10 x MIC; 200 µl DMEM/well) in 96-well plates. Plates were maintained at 37°C and 5% CO_2 for 48 h. After 48 h, cell viability was evaluated using the MTT colorimetric method and readings were performed at 570 nm. Untreated cells and DMEM were used as the negative control. The experiments were performed in triplicate in two different occasions.

Statistical Analysis

To check data's normal distribution, the Shapiro–Wilk test was used. Also, the homogeneity of variance was confirmed by the Levene test (α = 0.05). All analyses cited before, were performed with the aid of IBM SPSS statistical software package (version 25) for Windows (IBM Corp., New York, NY, USA). Mean values (± standard deviation) were compared by Analysis of Variance (ANOVA) and Tukey's post hoc test, considering α = 0.05. To analyze those data GraphPad Prism 5.0 for Windows (GraphPad Software, La Jolla, CA, USA) was used.

Results

T. vulgaris **and** A. sativum **were detected as the most effective essential oils against** C. albicans **planktonic cells**

The minimum inhibitory concentrations (MIC) of 15 different types of essential oils were determined for two *Candida albicans* reference strains (ATCC MYA-2876 and ATCC 18804) and the results are shown in Table 1. *T. vulgaris* essential oil showed fungistatic effect on *C. albicans* MYA-2876 and *C. albicans* 18804 at 0.3375 mg/mL and 0.1687 mg/mL, respectively. In addition, *Allium sativum* essential oil also demonstrated good fungistatic effect on *C. albicans* MYA-2876 and *C. albicans* ATCC 18804 at 0.832 mg/mL e 1.664 mg/mL, respectively.

T. vulgaris **and** A. sativum **showed anti-biofilm effect on pre-formed** C. albicans **monospecies mature biofilms**

Treatment with *T. vulgaris* essential oil, for 1 and 5 min, reduced significantly the number of *C. albicans* MYA-2876 and 18804 viable cells when compared to control ($p < 0.0001$). The reduction was similar to that observed for the positive control (0.12% chlorhexidine digluconate) ($p < 0.0001$) (Fig. 1).

Similarly, *A. sativum* essential oil (1 and 5 min) also reduced significantly the counts of *C. albicans* MYA-2876 and 18804 when compared to control ($p < 0.0001$). Also, the reduction was similar to that observed for the positive control (chlorhexidine digluconate 0.12%) ($p < 0.0001$) (Fig. 1). Final counts were similar to those observed for the positive control (0.12% chlorhexidine digluconate) ($p < 0.0001$).

T. vulgaris was more effective than *A. sativum* after 5 min of exposure against *C. albicans* 18804 biofilms ($p < 0.0001$) (Fig. 1).

Cytotoxicity of Thymus vulgaris **and** Allium sativum **essential oils**

Results of cell viability detected for groups treated at concentrations of 1.687 and 3.375 mg/ml of *T. vulgaris* essential oil and *A. sativum* (1.664 and 8.32 mg/ml) are shown in Figs. 2A and B. After exposure, *Thymus vulgaris* was classified as slightly cytotoxic and *A. sativum* as moderately cytotoxic.

Discussion

There are historical reports on the use of many oils and plant extracts as topical antiseptics with antimicrobial properties. Nowadays, it is recognized the importance of studying more deeply substances that are extracted from plants that have great potential as sources of new antimicrobial compounds. In addition to the antimicrobial potential that medicinal plant oils may have, these compounds have the advantage of forming a complex of active principles that can act directly on microorganisms (Brighenti et al., 2014, Girondi et al., 2017, Angane M et al., 2022, Swamy Mk et al., 2022). In the present work, the action of fifteen different essential oils from eight different families were analyzed. Those that shared a family are: i) *Tagetes minuta* and *Matricaria recutita*, both from Asteraceae and rich in terpenoids and flavonoids (Dantas JBL et al., 2022, Kumar A et al., 2022); ii) *Foeniculum vulgare* and *Petroselinum sativum* from Apiaceae, widely used as a condiment and also implemented in natural medicine (Elmadawy AA et al., 2022, Lauricella M et al., 2022); iii) *Mentha pulegium* and *Thymus vulgaris* (Lamiaceae); iv) *Pinus sylvestris* and *Pinus ponderosa* (Pinaceae). Other distinct family species were also used in the present work: Amaryllidaceae, Boraginaceae, Rubiaceae, Myrtaceae and Rutaceae (Table 1). Interestingly, the two most active essential oils are from different families. Previous studies also show a great inter-species variation in biological activity (Brighenti et al., 2014; Brighenti et al., 2017; Teodoro et al., 2015). The two essential oils that had the best results in the evaluated MIC were *Allium sativum* and *Thymus vulgaris* and these were chosen for further analysis in biofilms formed by *C. albicans*. *Allium sativum* essential oil has been used for culinary and health purposes (Rybak et al., 2004). Its composition is formed by organosulfur, potent anti-inflammatory and chronic disorders (Dewi et al., 2017; Ruhee et al., 2020). Other biological effects, such as antimicrobial and also antioxidant, were also reported. Besides that others interesting properties such as antiobesity and even antidiabetic properties was described as well (Shang et al., 2019). Its main biocompound is 4-methyl-2-phenylpyrimidine, 2-(([2-ethylhexyl]oxy)carbonyl)benzoic acid, (9-Hexacosene and clionasterol (Oh KK 2022). Thyme has been largely used in folk medicine (Kuete V 2017). Six *T. vulgaris* essential oils biocompounds are known: carvacrol, α-terpineol geraniol, α-terpineol, thujanol-4 and thymol. Besides, *T. vulgaris* essential oils are known to have anti-inflammatory, immune response modulator action (Mahboubi M et al., 2018, Segvić Klarić M et al., 2007, De Oliveira et al., 2017).

Minimal inhibitory concentration (MIC) determination of an antimicrobial agent is usually performed to find the ideal concentration capable of controlling microorganism's proliferation (Levison ME Levison JH 2009). In this sense, studies of MIC of essential oils in the present work provided results of the ideal concentration to obtain fungicidal and fungistatic actions. Among all essential oils studied, *T. vulgaris* and *Allium sativum* showed effect at a lower concentration in comparison to the other essential oils and were used for the treatment of mature biofilms. Pharmaceutical preparations based on essential oils are, in general, safe if the right amount and concentrations are used (Tisserand R et al., 2013). These threshold concentrations are usually based on literature evidence from previous studies performed to determine the threshold concentration to be used in formulations for both topical use and inhalation solutions (Tisserand R et al., 2013). Because essential oils are liquid, pure substances that contain a wide variety of phytocompounds that may have different pharmacological activities. There is a safe range of concentration that can be used in the formulations. In this sense, the concentrations of the

essential oils chosen for biofilm treatment in the present work are within this safety margin (Oliveira et al 2017, Zhou et al 2020).

Allium sativum and *T. vulgaris* showed anti-biofilm effect on biofilms of *C. albicans* (ATCC MYA-2876 and ATCC 18804) in all tested scenarios, both after 1 and 5 min treatment periods.

Our findings corroborate with previous work that evaluated the anti-biofilm activity of the same plants in other microbial species. These studies showed better antimicrobial effects against *Bacillus subtilis*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis, Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Candida albicans*, T*rypanosoma brucei brucei*, *Leishmania tarentolae* when compared to chlorhexidine 0.2% at the same exposure time (Krstin S et al., 2018, et al., 2018). In addition, another group reported the inhibitory effect of *A. sativum* extract and the synergism of this extract with bakuchiol (an compound isolated from the seeds of *Psoralea corylifolia*) on *C. albicans* mono- and multispecies species biofilms (*C. albicans, S. sanguinis and S. mitis*) (Fahim A et al., 2020). Besides, Mendoza-Juache A et al., 2017 reported that *Candida albicans*, *Candida glabrata, Candida tropicalis*, and *Candida krusei* clinical isolates were more susceptible to *A. sativum* essential oil when compared to fluconazole in both planktonic and in biofilm forms. The MIC of the *A. sativum* oil for *C. albicans* was 236.2 µg/ml, 321 µg/ml for *C. glabrata*, 188 µg/ml for *C. tropicalis*, and, 503 µg/ml for *C. krusei*. The antibiofim effect of *A. sativum* essential oil against *Candida* spp. biofilms were reached at 603.1 µg/ml (*C. albicans*); 640.6 µg/ml (*C. glabrata*) and 667 µg/ml (*C. tropicalis*). The concentrations in this present work were higher than previous work (Mendoza-Juache A et al., 2017). Moreover, in our study we performed cytotoxicity analyses which can contribute even more to the dentistry field and to the studies involving oral infections by *Candida* spp..

T. vulgaris essential oil inhibited *C. albicans* (ATCC MYA-2876 and ATCC 18804) biofilms after 1 and 5 min treatments with similar effects when compared to 0.12% chlorhexidine digluconate. Antibacterial and antifungal effect of *T. vulgaris* and its main active compounds have been studied (Marchese A et al., 2016). Jafri H & Ahmad I 2019) showed that association of *T. vulgaris* essential oil with thymol or these agents individually significantly reduced the viability of *C. albicans* and *C. tropicalis* biofilms. Time kill curves (0–50 hours) for the essential oil against *C. albicans* and *C. tropicalis* biofilms were determined. Microscopy and SEM analyses revealed disaggregation of biofilm and reduced hyphae formation after treatments with *T. vulgaris* or thymol (Jafri H & Ahmad I 2020).

T. vulgaris essential oil contains high percentage of phenolic compounds, such as thymol (Jafri H & Ahmad I 2020, Bogavac M et al., 2015). Thymol showed a lower MIC value for *C. albicans* (39 µg/mL; de Castro et al., 2015) in comparison to the results of the present study (337.5 µg/ml for *C. albicans* MYA-2876 and 168.7 µg/ml for *C. albicans* 18804). While it is expected that an improvement in MIC values is achieved with isolated compounds, the present work is justified by the fact that this is not a consensus in the literature: previous studies showed higher MIC values of the isolated compounds in comparison to the crude extract, which might be explained by the loss of biological activity due to loss of synergy

between different compounds that might be observed in the crude extract (Brighenti et al., 2016; Teodoro et al., 2015). Recently, the anti-inflammatory and healing activity of thymol was detailed described (Gabbai-Armelin PR et al., 2022).

Investigation of the cytotoxicity effect of the oils is crucial to support future clinical application. For cytotoxicity analyses, our results were expressed as percentage of viable cells (%) in relation to non-treated negative control (100% viability). Percentages of cell viability was classified as non-cytotoxic (> 90%), slightly cytotoxic (60–90%), moderately cytotoxic (59 – 30%) and severely cytotoxic (29% – 0%) (Sletten, Dahl, 1999). In the present study, the cytotoxicity of essential oils at 10 times MIC was assessed. *Thymus vulgaris* was classified as slightly cytotoxic. This finding is in accordance to previous study that analyzed the cytotoxic activity of *T. vulgaris* essential oils against other cell lines, obtaining lower cytotoxicity values than doxorubicin used as a reference drug in the trial (Hassan et al 2019).

A. sativum was moderately cytotoxic. Previous studies on the cytotoxicity of *A. sativum* at the concentrations used in this study were not detected. The moderate cytotoxicity observed for *A. sativum* in this study can be solved by the manipulation of these oils in the forms of nanocarriers or nanoemulsions. de Bastiani et al, 2020. used alginate-based nanocarriers to encapsulate antifungals from the azo and polyene groups, promoting sustained release and decreasing of their toxicity. In other studies, formulations were developed to treat candidiasis oral in the form of nanoemulsions, such as amphotericin nanoemulsion, avoiding systemic absorption and reducing side effects (Sosa et al 2017, Butani et al, 2016). These preparations allowed a sustainable release of the drug, making it possible to decrease the concentration used and, consequently, to decrease the toxicity (de Bastini et al 2020, Butani et al, 2016, Sosa et al 2017).

It is important to highlight that both *A. sativum* and *T. vulgaris* essential oils were less toxic than chlorhexidine digluconate, as a previous study classified it as highly toxic (Albuquerque 2019) such as the data used as a reference in this study (Borges et al. 2017).

Future studies may contribute even more to the understanding the action of these essential oils on biofilms formed by strains different from those studied in this work, such as *Candida glabrata, Candida tropicalis* and *Candida krusei*, both clinical and standard strains. It will also be very important to understand the synergistic action of these essential oils on biofilms formed by *Candida* sp. In addition, studies analyzing the action of these essential oils in induced infections in vivo and how these essential oils can modulate the immune response during these infections may also contribute a lot to the area of dentistry that studies diseases caused by oral infections, such as candidiasis.

The results obtained in this study for *T. vulgaris* and *A. sativium* essential oils open promising possibilities for the elaboration of mouthwashes and topical formulations to improve conventional treatment of oral candidiasis and the quality of life of patients.

Abbreviations

Thymus vulgaris (T. vulgaris), Allium sativum (A. sativum), Candida albicans (C. albicans), Medicinal and aromatic plants collection (CPMA), Yeast Extract Peptone Dextrose (YPD), Minimum inhibitory concentrations (MIC), Clinical and Laboratory Standards Institute (CLSI), Dulbecco's Modified Eagle's medium (DMEM).

Declarations

Information pertaining to writing assistance: "Not applicable" for these studies.

Ethics approval and consent to participate: "Not applicable" for studies not involving humans or animals. All experimental studies on plants were complied with relevant institutional, national, and international guidelines and legislation".

Consent for publication: "Not applicable"

Availability of data and materials: Data and all the materials are owned by the authors. The data that support the findings of this survey are available from the corresponding author upon reasonable request.

Competing Interesting: "The authors declare that they have no competing interests" for these studies.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

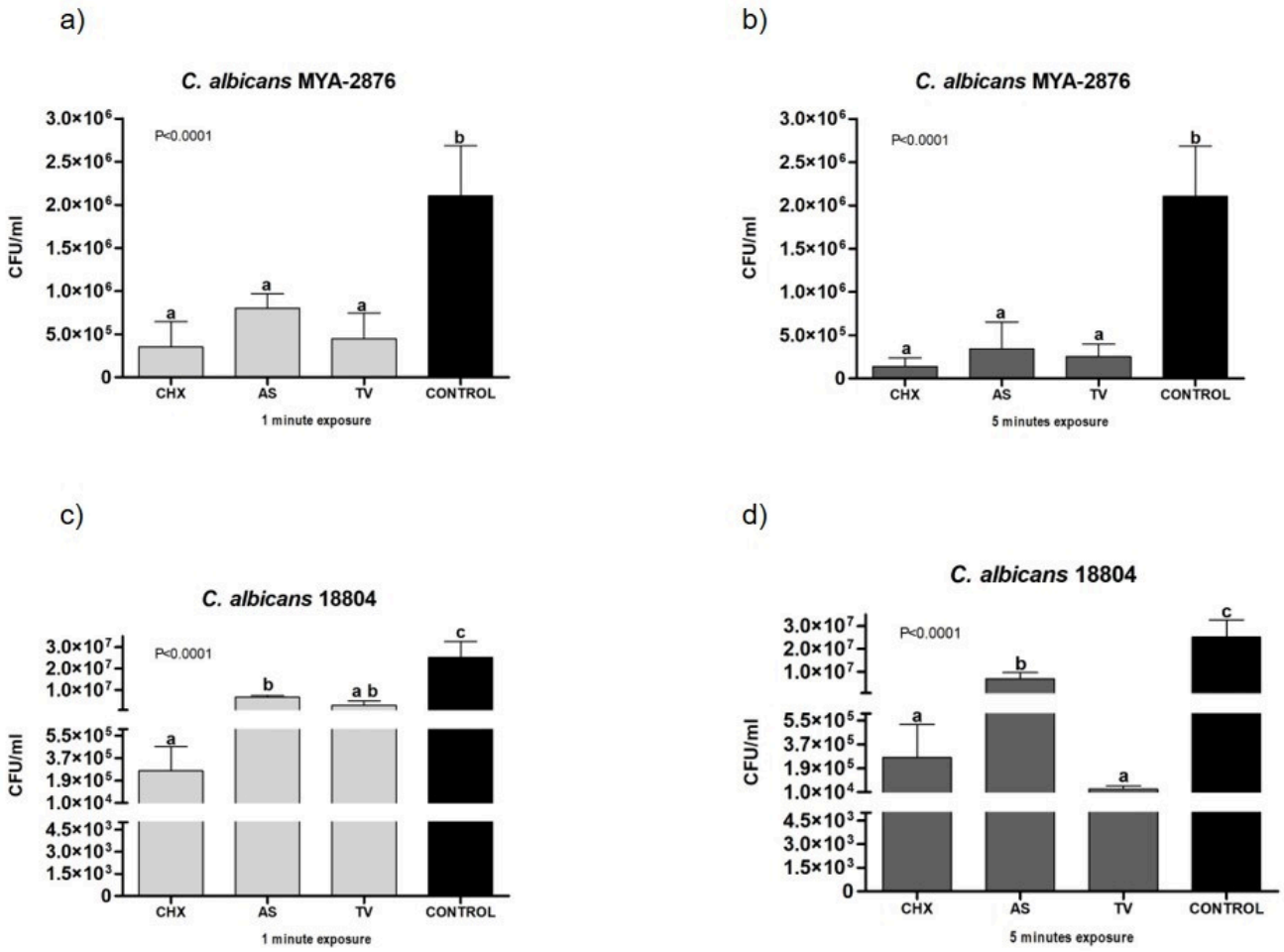

Figure 1

C. albicans MYA-2876 and ATCC 18804 biofilm viability after treatment for 1 or 5 min (CFU/mL; Mean (± sd). CHX: 0.12% chlorhexidine digluconate; AS: *A. sativum* essential oil (MYA-2876: 8.32 mg/mL, 18804: 1.664 mg/mL); TV: *T. vulgaris* essential oil (MYA-2876: 3.375 mg/mL, 18804: 1.687 mg/mL); CONTROL: 0.9% NaCl. n = 9, ANOVA, $p \leq 0.05$.

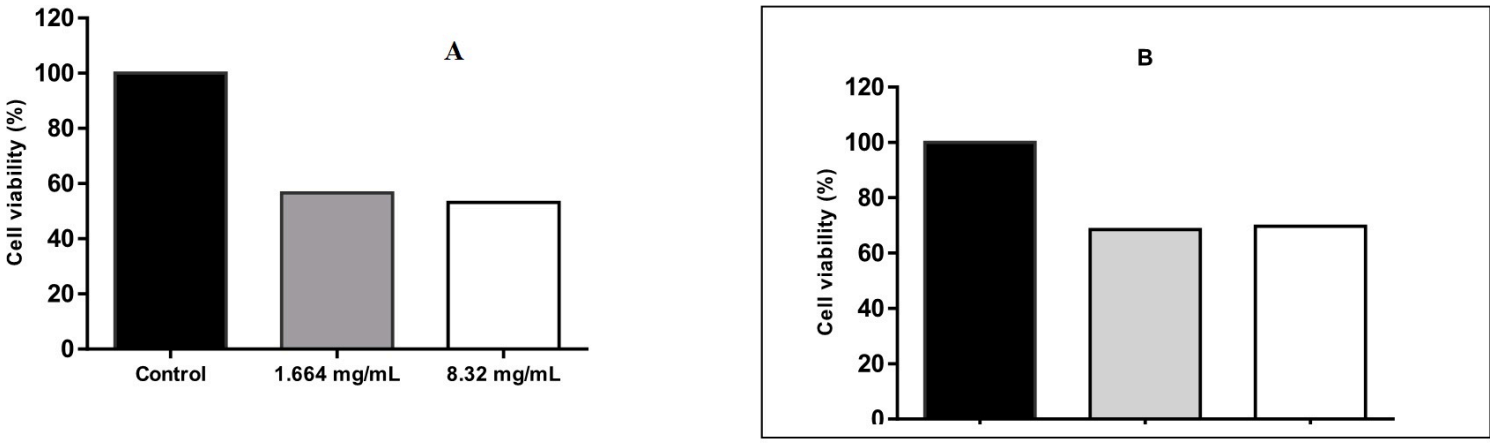

Figure 2

Viability of Vero cells (mean percentage) after exposure to (A) *Thymus vulgaris* essential oil at 1.664 and 8.32 mg/ml and (B) *Allium cepa*essential oil at 1.687 and 3.375 mg/ml.

Supplementary Files

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- Table1.docx