An Acad Bras Cienc (2024) 96(3): e20230309 DOI 10.1590/0001-3765202420230309
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

Antifungal, molecular docking and cytotoxic effect of the essential oil of *Cymbopogon citratus* (DC) Stapf. and *Cymbopogon nardus* (L.) Rendle against *Candida albicans*

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Abstract: Brazil is renowned for its extensive plant biodiversity, with emphasis on *Cymbopogon*, *C. citratus* and *C. nardus*, with broad antimicrobial potential. Candidemias caused by *Candida albicans* are highly prevalent in immunosuppressed individuals and are associated with infections by biofilms on medical devices. The aim of this study was to evaluate the antimicrobial potential of essential oils *C. citratus* and *C. nardus* against *C. albicans* in planktonic and biofilm forms. Essential oils were obtained by hydrodistillation and chemical composition evaluated by GC-FID and GC-MS. The minimum inhibitory concentration was determined by the broth microdilution method and the synergy effect of essential oils and amphotericin B were evaluated by the checkerboard test. Biofilm activity was determined by the XTT assay. Cytotoxicity assays performed with VERO cells and molecular docking were performed to predict the effect of oil interaction on the SAP-5 enzyme site. The results showed activity of essential oils against planktonic cells and biofilm of *C. albicans*. Furthermore, the oils had a synergistic effect, and low cytotoxicity. Molecular docking showed interaction between Cadinene, Caryophyllen oxide, Germacrene D with SAP-5. The results indicate that *Cymbopogon* spp. studied are anti-Candida, with potential for further application in therapy against infections caused by *C. albicans*.

Key words: Biofilm, *Cymbopogon* spp., essential oil, molecular docking, Synergism.

INTRODUCTION

Countries with tropical ecosystems, such as Brazil, have great potential for bioprospecting of native and cultivated plants. Among these plants, species of the *Cymbopogon* genus, belonging to the Poaceae family, have approximately 144 known varieties (Avoseh et al. 2015, Aguilar et al. 2019). This genus is characterized by having aromatic monocots, whose main representatives are *C. citratus* (DC.) Stapf and *C. nardus* (L.) Rendle, in that order, popularly named Capim-Santo and Citronela. These species are used by the community mainly for it's anxiolytic and insect repellent potential, respectively (Avoseh et al. 2015, Carreiro et al. 2020). Being therapeutic potential of their metabolism products of these plants, through secondary mevalonic acid, experimental studies support the possibility of using phytocomplexes, such as essential oils, for antimicrobial activity against viruses, bacteria, protozoa and fungi. Thus, the use of *Cymbogopon* species as medicinal plant and

its pharmacological products are promissors as alternative therapeutic to clinically invasive infections and with high antimicrobial resistance (Sousa et al. 2020, Kaur et al. 2021, Prado et al. 2022).

The use of medicinal plants for integrative and complementary health practices (ICHP) is a therapeutical alternative, alone or combined with conventional drugs, for the treatment of invasive infections (Borges & Sales 2018). This is particularly the case of treatment protocols that use drugs with high toxicity, such as antifungals of the azole and polyene classes, which reportedly are hepatotoxic and nephrotoxic, respectively (Bassetti et al. 2018).

Among the etiological agents that can cause opportunistic infections are species of the genus *Candida*. These yeasts are responsible for 400,00 cases of invasive candidiasis per year, resulting in 46 to 75% mortality in the world in 2022, being the fourth leading cause of sepsis in global health services, and the seventh in Brazil (Brown et al. 2012, Pappas et al. 2018, Machado et al. 2021). They can be grouped into *Candida albicans* and non-*albicans Candida*, the most prevalent being *C. albicans*. It is the species found in clinical isolates, causing 90% to 100% of mucosal infections and 40% to 70% of blood infections (Boucherit-Otmani et al. 2021, Sousa et al. 2020). Risk groups for invasive candidiasis are patients with acquired immunodeficiency syndromes (AIDS), diabetes and with immunosuppressive medication (Tong & Tang 2017, Pappas et al. 2018).

C. albicans stands out for its ability to establish, colonize and cause disease by overcoming host defenses, characteristics that are directly linked to the virulence of these microorganisms. The best-known virulence mechanisms are adherence, polymorphism, phenotypic variability, and production of extracellular enzymes and toxins. All these factors are directly linked to biofilm formation capacity (Tong & Tang 2017, Wall et al. 2019).

Biofilms are complex microbiological communities that have, among their many characteristics, the ability to adhere, multiply and form extracellular matrices and disperse, along with polymorphism. These mechanisms enable biofilms to develop on the most diverse surfaces, including medical devices such as prostheses, probes and catheters. Biofilm formation on these devices particularly affects immunocompromised patients, especially those in intensive care units (Lohse et al. 2018).

Thus, the aim of this study was to evaluate the cytotoxicity and antifungal activity of essential oils (EOs) of *C. citratus* and *C. nardus,* against clinical isolates and wild-type strains of *C. albicans* in planktonic and sessile forms. In addition, we evaluated the synergistic potential of the EOs with amphotericin B as pilot study to reduce doses of the standard drug, with subsequent determination of the interaction of the major EOs compounds with SAP5 adhesion protein by molecular docking.

MATERIALS AND METHODS

Plant material

The leaves of *C. citratus* and *C. nardus* were collected in the morning, from plants cultivated in the municipality of Sobral, Ceará (Brazil), located at coordinates 3°42'07"S 40°21'53"W. Voucher specimens were identified and deposited with the Professor Francisco de Abreu Matos Herbarium (HUVA) at Vale do Acaraú State University, registered as exsiccate number 18614 and 20807, respectively.

Extraction and chemical characterization of essential oil

Fresh leaves of *C. citratus* and *C. nardus* were macerated and subjected to hydrodistillation

for 2 h in a modified Clevenger apparatus. After extraction, the yield and relative density of essential oils were determined.

The chemical analysis of essential oils (EOs) was carried out according to Arantes et al. (2019), by gas chromatography. GC-FID analyses were performed *with a* Shimadzu Nexis GC-2030 gas chromatograph and flame ionization detector *(*GC-FID*)* equipped with an AOC-20i plus autoinjector (HERCULES Lab, Univ Évora, Portugal), with dimensions of 30 m x 0.25 mm i.d. and film thickness of 0.50 µm, and a Zebron ZB-5HT Inferno™ fused-silica non-polar capillary column (Phenomenex, USA), using the LabSolutions software version 5.92 (Shimadzu Corporation). GC-MS analyses were performed with a GC-MS-QP2010 Series (Shimadzu) gas chromatograph, fitted with Zebron ZB-5HT Inferno™ non-polar fused-silica (30 m × 0.25 mm i.d., film thickness 0.50 µm), interfaced with a detector model Polaris Q (E. I. quadrupole). Compounds were identified by their retention indices (RI) and their mass spectra of the NIST11 (National Institute of Standards and Technology) library. Retention indices were determined by interpolation relative to the C8–C22 n-alkanes retention times and compared with those of authentic samples, from the laboratory database and with literature data (Babushok et al. 2011, Videira et al. 2013, Arantes et al. 2019, Pandur et al. 2022).

For biological assays, the *C. citratus* essential oil (EOCC) and *C. nardus* essential oil (EOCN) were solubilized in RPMI-1640 medium, supplemented with L-glutamine (Sigma-Aldrich, St. Louis, MO, USA) and Tween® 80 at 0.1% (Nascimento et al. 2007).

Yeast strains

The standard *C. albicans* strain was obtained from the *American Type Culture Collection* (ATCC90028). Clinical isolates of *C. albicans* (LABMIC 0102, LABMIC 0104, LABMIC 0125 and LABMIC 0127) (Table II) were were provided by the Santa Casa de Misericordia Hospital, Sobral (Ceará, Brazil) and Norte Regional Hospital (Ceará, Brazil). Strains phenotypic and molecular identifications were performed by CHROMagar (CHROMagar *Candida*, France), a Vitek 2 system (BioMerieux Vitek, Hazelwood, France) and PCR (Cellco Biotech, Brazil), as published by Bastos, Alves, neves, Vasconcelos, Brito & Fontenelle (2023). This study was authorized by the Research Ethics Committee of Acaraú Valley State University under reference number 4.0633.262.

Inoculum preparation for antifungal susceptibility tests

The inoculum was prepared from cultures maintained in the laboratory, on Sabouraud dextrose agar (SDA) (Difco, Detroit, MI). Cells were cultured for 24 h at 35±2 ºC. Yeast colonies were transferred to tubes containing sterile PBS to obtain suspensions with turbidity equivalent to 0.5 on the McFarland scale (c. 10^6 CFU per ml). These suspensions were then diluted 1:2,000 with RPMI-1640 medium supplemented with L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), to obtain concentration $2x10^2$ CFU per ml, in agreement with the directions of the Clinical and Laboratory Standards Institute (CLSI) M27-A3 standard (CLSI 2008).

Broth microdilution method

The minimum inhibitory concentration (MIC) of yeast growth was determined by broth microdilution with 96-well plates, in accordance with the CLSI M27-A3 (CLSI 2008). The EOCC and EOCN was tested in the concentration range of 2.44-2,500 µg/ml. Then, 100 µL of inoculum was added to 100 µL of test solution. Amphotericin B (AMB) and fluconazole (FLC) were used as standard drug controls in the

ranges of 16 – 0.015µg/ml and 64 - 0.0625 µg/ml, respectively. The MIC was defined as the lowest oil concentration that caused 100% inhibition of visible fungal growth, according Fontenelle et al. (2007, 2008).

Checkerboard assay

To determine the modulatory effect, we used the two clinical isolates that showed the lowest MIC in the broth microdilution tests (LABMIC 0102 and LABMIC 0105), as well as the ATCC90028 strain. For this analysis, the antifungal drug of choice was amphotericin B. Despite being effective, this drug has a strong nephrotoxic effect, so the use of lower concentrations would also reduce this effect.

Initially, 50 µL of RPMI-1640 was added to each will of a 96-well plate and then 50 µL of EOCC and EOCN was added. Serial dilutions of the EOCC end EOCN were performed in the concentration range from MIC to MIC/10. Subsequently, 50 µL of different concentrations of AMB was added to each of the lines. Cells treated only with EOCC individually or AMB alone, at their respective MIC values (Table II), along with untreated fungal suspensions were used as controls. The plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration at which no visual growth (absence of turbidity) was observed. The FICI was calculated by the sum of FICo + FICa, where O represents EOCC and amphotericin B. In turn, FICo was calculated as MICo combined/MICo alone, while FICa was calculated as MICa combined/MICa alone. Synergism was defined as FICI ≤ 0.5, while no interaction was recorded when 0.5 < FICI ≤ 4.0, and antagonism when FICI >4.0 (White et al. 1996, Rosato et al. 2008). FIC values were plotted on an isobologram, allowing graphical representation of the resulting interactions in various associations. To analyze the results, lines were drawn between the FIC indices, visually discriminating interactions with synergistic activity (<0.5); additivity (between 0.5 and 1.0).

Biofilm assay

The susceptibility of *Candida* sp. biofilm by *C. citratus* and *C. nardus* was performed according to Gonçalves et al. (2017). Briefly, 100 µL of inoculum was transferred to each well of a 96-well microplate. Then, 100 µL of RPMI-140 supplemented with *C. citratus* or *C. nardus* essential oil were added twice the MIC value for each microorganism. The microplate was then incubated for 48h at 37°C. Wells containing only culture medium without inoculum or with inoculum and unsupplemented RPMI-1640 were used as controls and wells with RPMI-1640 supplemented with Amphoterician B 2 µg/mL were used as a positive control. After incubation, the biofilm activity was by tetrazole salt (XTT).

Determination of metabolism by XTT/Menadione

After the incubation period, the biofilm was washed three times with saline solution (0.85%; pH=7.00) to remove planktonic cells. To determine the metabolic activity, 100 µL of XTT-menadione solution (1 µL 1mM menadione in 10 mL of 0.5g/L XTT) was added to the wells of each plate and incubated in the dark for 2h at 37ºC. After this period, the supernatant solution was transferred to a new plate and read with optical density measured at 490nm (Gonçalves et al. 2017).

Cytotoxicity assay

The evaluation of cytotoxic activity the EOCC and EOCN was performed using the viability method of 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-tetrazolium bromide (MTT), described by Mosmann (1983) with modifications. The objective was to analyze the mitochondrial

activity of viable cells (Nery et al. 2014). Mammalian Vero cells (epithelial cells from the kidneys of African green monkeys) from the Rio de Janeiro (Brazil) Cell Bank (no. 0245) were used. The cells ($2x10^5$ cells/ml) were cultured in Leibovitz medium (Cultilab, SP, Brazil) supplemented with 10% fetal bovine serum and solution content of streptomycin (20 mg/mL), penicillin (10,000 U/ml) and AMB (1mg/ml). The EOCC and EOCN was tested in the concentration range of 1,000 to 31.25 µg/ml.

After formation of the monolayer cells, 48 h post-incubation, the medium was removed and the essential oils diluted in the predefined concentrations (31.25, 62.5, 125, 250, 500 and 1,000 µg/ml) were added. The plates were incubated for 7 days, after which the supernatant was removed from the wells, followed by addition of 100 µL of L-15 medium supplemented with 2% fetal bovine serum + 10 µL of the MTT solution. The experiments were performed in triplicates. After 4 h, the supernatant was discarded and 100 µL of DMSO was added, and the absorbance was read in a spectrophotometer at 540 nm (Mosmann 1983).

Molecular docking

Preparation of ligands

The PubChem repository (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the tridimensional structures of the ligands Citronellal (7794), Citronellol (8842), Geraniol (637566), Elemol (92138), α-cadinene (10398656), Caryophyllene oxide (1742210), eugenol (3314), γ-cadinene (15094), Geranial (638011), Geranyl acetate (1549026), Germacrene D (5317570), Isopulegol (170833), Limonene (22311), Linalol (6549), linalyl acetate (8294), terpinyl acetate (538936), β-myrcene (348293176), neral (643779), neryl acetate (1549025), as well the control ligands: amphotericin B (5280965), and fluconazole (3365). The low energy conformers were optimized with MMFF94 (Merck Molecular Force Field 94) and the steepest descent algorithm with cycles of five interactions through MarvinSketch™ (https://chemaxon.com/products/marvin) (Csizmadia, 2019; Chemaxon, 2019) and Avogadro ™ (http://avogadro.cc/) codes (Hanwell et al. 2012).

Anchoring procedure

The Protein Data Bank repository was utilized to obtain the target macromolecule denominated "Secreted aspartic proteinase (Sap) 5 from *Candida albicans*" (PDB ID: 2QZX), confirmed by X-ray diffraction (R-Value Free: 0.275 and R-Value Work: 0.224), deposited with a resolution of 2.50 Å and classified as a hydrolase enzyme. The target preparation removed water molecules, followed by addition of polar hydrogens and Gasteiger charges (Yan et al. 2014) through AutoDock Tools (Huey et al. 2012).

With a grid box configured at the centers x, y and z equal to 19,613 Å, 19.76 Å and 44,435 Å, respectively, fitted with Cartesian dimensions x = 64 Å, y = 58 Å and z = 108 Å, involving the entire surface of the protein, the AutoDock Vina software (Trott & Olson 2010) was used to perform molecular docking simulations with fifty independent simulations for each ligand.

To choose the best ligand, two criteria were used. The first was the root mean square deviation (RMSD), a validation criterion of simulations realized with ideal parameters until 2.0 Å (Yusuf et al. 2010). The second criterion utilized was the free energy of binding (ΔG_{bind}), which is considered ideal when values are lower than or equal to -6.0 kcal/mol (Shityakov & Förster 2014). The parameters proposed by Imberty et al. (1991) were used with the distances between the donor atoms with protein to evaluate the strength of the hydrogen bonds. The distances between hydrogen bonds of 2.5 Å to 3.1 Å, 3.1 Å to

3.55 Å, and greater than 3.55 Å, are classified as strong, moderate and weak, respectively.

Statistical analysis

The biofilm assay data were submitted to one-way analysis of variance (ANOVA), followed by the Tukey multiple comparison test using GraphPad® Prism version 8.0 (GraphPad Software, San Diego, California, USA). Statistical significance was set as $p < 0.05$. Cytotoxicity was assessed by calculating the IC50 values. Initially the logarithmic transformation was performed and subsequent normalization of data percentage, where negative controls (non-treated cells) optical density were stated as 100% of viability. The IC_{50} values obtained after treatment of cells with essential oils were calculated using non-linear regression curve using the GraphPad Prism program with confidence interval of 95%.

RESULTS AND DISCUSSION

Characterization of essential oils

The oil extracted from the leaves of *C. citratus* was hyaline and yellowish, with a citrus aroma, with a relative density of 0.832 ±0.038 g/ml and a yield of 0.399 ±0.103%. Obtained yield extraction was different from that found by Santos et al. (2009) and Domingues & Paiva (2021), who obtained yields of 0.66% to 1.15%. The essential oil of *C. nardus* was clear, colorless and with a citronella aroma. Yield extration and relative density were 0.71% and 0.88 g/mL, respectively. The yield obtained was lower than that found by Sawadogo et al. (2022) who reported values of 1.37% for EOCN. These differences in productivity may be related eith to the age of plants and environmental factors such as soil type, moisture content, seasonality and season of the year in which the plant was collected (Santos et al. 2009, Kaur et al. 2021).

The chemical composition of essential oils is shown in Table I. There were identified 22 compounds for *C. citratos* essential oil (98 %) and 14 compounds for *C. nardus* essential oil (97 %). Chemical profile of both essential oils showed a high oxygenated monoterpene content with 95 % for the EOCC and 91 % for the oil for EOCN, respectively. The major constituents were neral (36.13 %) and geranial (48.47 %) for the essential oil of *C. citratos* and citronellal (44.44 %), citronellol (16.97 %) and geraniol (26.55 %) for the oil of *C. nardus*.

Studies carried out with essential oils of *Cymbopogon* species have shown that these oils have unique chemical properties rich in oxygenated monoterpenes with significant inter/intra-species differences (Jin et al. 2022). The outcomes for the essential oil of *C. citratus* agree with the bibliography that suggest that this essential oil is rich in neral (31–45%) and geranial (27-55%), with a predominance of geranial in the Brazilian species (Barbosa et al. 2008, Silva et al. 2020, Jin et al. 2022, Paiva et al. 2022, Sawadogo et al. 2022, Sharma & Kaur 2022). They were also found in the essential oil of *C. citratus* collected and studied by Saboia et al. (2022) on the Maranhão State (Brazil) and correlated with the therapeutic potential of this plant. Among these compounds, citral and your isomers (Oxygenated monoterpenes) were present in the highest percentages in the EOCC.

The chemical profile of *C. nardus* is according to the other Brazilian *C. nardus* essential oils described in bibliography, with some seasonal and geographical differences , in different periods and locations in Brazil, were citronellal (28 - 50%), geraniol (17 - 34%) and citronellol (11 - 25%) (Aguiar et al. 2014, Andrade et al. 2012, Castro et al. 2010, da Silva et al. 2020, Gaspar de Toledo et al. 2020, Guandalini Cunha et al. 2020, Kaur 2021, Pontes et al. 2019, Trindade 2015). Guandalini Cunha et al. (2020) and da

Table I. Chemical composition of the essential oil of *C. citratus* and *C. nardus* identified by GC-MS.

Class	Compound	*RI $_{Exp.}$	RI $_{bib.}$	*C. citratus*	*C. nardus*
				Area (%)	Area (%)
				Mean $_{±SD}$	Mean $_{±SD}$
HM	β-Myrcene	990	990 [a]	2.17 ± 0.05	__
HM	Limonene	1024	1026 [a]	__	1.82 ± 0.02
HM	α-Terpinolene	1078	1076 [b]	0.23 ± 0.01	__
OM	Linalool	1096	1095 [a]	0.71 ± 0.01	0.53 ± 0.01
OM	*trans*-Pinocarveol	1134	1134 [a]	0.16 ± 0.01	__
OM	Isopulegol	1144	1144 [a]	0.12 ± 0.02	0.92 ± 0.01
OM	Menthone	1151	1150 [a]	0.12 ± 0.01	__
OM	Citronellal	1155	1153 [d]	__	**44.44 ± 0.15**
OM	Borneol	1163	1162 [a]	0.46 ± 0.01	__
OM	4-Terpineol	1175	1174 [a]	0.11 ± 0.01	__
OM	α-Terpineol	1181	1190 [d]	0.72 ± 0.03	__
OM	Myrtenal	1191	1192 [d]	0.14 ± 0.01	__
OM	Citronellol	1230	1228 [d]	__	**16.97 ± 0.09**
OM	Neral (Citral-b)	1241	1242 [c]	**36.13 ± 0.18**	__
OM	Geraniol	1257	1255 [d]	2.11 ± 0.03	**26.55 ± 0.12**
OM	Geranial (Citral-a)	1273	1274 [c]	**48.47 ± 0.19**	0.35 ± 0.01
OM	Thymol	1293	1294 [a]	0.12 ± 0.01	__
OM	Myrtenyl acetate	1330	1329 [d]	0.31 ± 0.01	__
OM	Terpinyl acetate	1338	1347 [d]	1.86 ± 0.01	0.19 ± 0.01
OM	Eugenol	1363	1358 [d]	0.78 ± 0.03	0.85 ± 0.02
HS	α-Copaene	1366	1369 [b]	0.43 ± 0.07	__
OM	Neryl acetate	1374	1363 [d]	2.40 ± 0.02	__
OM	Geranyl acetate	1382	1380 [d]	0.29 ± 0.01	0.41 ± 0.02
HS	β-Caryophyllene	1427	1417 [a]	0.17 ± 0.01	__
HS	D-Germacrene	1478	1481 [d]	__	0.33 ± 0.01
HS	γ-Cadinene	1511	1515 [c]	__	0.10 ± 0.01
OS	Elemol	1548	1548 [d]	__	3.25 ± 0.03
OS	Caryophyllene oxide	1582	1582 [a]	0.11 ± 0.01	__
OS	α-Cadinol	1653	1652 [d]	__	0.63 ± 0.01
Total identified				98.12 ± 0.74	97.47 ± 0.53

***RI $_{Exp.}$ - Retention index relative to C8–C22 *n*-alkanes on the Zebron ZB5HT Inferno™ apolar column.**
[a] Retention index reported by Arantes et al. (2019).
[b] Retention indices reported by Videira et al. (2013).
[c] Retention indices reported by Pandur et al. (2022).
[d] Retention indices reported by Babushok et al. (2011).

Silva et al. (2020) also detected the presence of nerol (>10%) in the essential oil of *C. nardus* from Araçatuba, São Paulo, and Seropédia, Rio de Janeiro (Brazil), respectively, while Gaspar de Toledo et al. (2020) observed the presence of geranial (13%) and neral (10%) in the essential oil of *C. nardus* from Araraquara, São Paulo (Brazil). In the analysed essential oil of *C. nardus* it was observed the presence of geranial (0.4%) but that neral and nerol were absent. These findings show that the phytochemical profile of EOCN and EOCC is stable in major components, with variation in the concentration of their major and minor components regardless of the period and place where the plant was collected (Avoseh et al. 2015).

Minimum inhibitory concentration

For EOCC and EOCN, the antifungal potential was observed by the broth microdilution method, with MIC values in the range between 156.25 and 78.12 µg/ml and 625 to 312.5 µg/mL, respectively, against *C. albicans*, type strain and clinical isolates (Table II). *Cymbopogon* essential ois slhowed antifungal activity against *C. albicans*, with EOCC showing the highest anti-*Candida* activity.

The results of the antifungal activity observed by the broth microdilution method indicate that essential oils have components with antimicrobial activity, corroborating the antimicrobial activity against *C. albicans* for monoterpenes components reported for other plants, such as *Mentha arvensis* L., *Mentha pulegium* L., *Ocimum basilicum* L. (Zabka et al. 2014, Rhimi et al. 2022). Other studies with *Cymbopogon* species reported antifungal activity against species *Candida*, with a MIC of 1.25 to 562 µg/mL (Trindade et al. 2015, Kandimalla et al. 2016, Toledo et al. 2016, Domingues & Paiva 2021, Rhimi et al. 2022).

Paiva et al. (2022), in a study carried out on the antimicrobial action of the essential oil of *C. citratus* (84.53% citral and 13.76% myrcene), observed that both showed antifungal activity against 193 strains of *Candida albicans* isolated from the oral cavity. In another study, Boukhatem et al. (2014) observed that the essential oil of *C. citratus* from Algeria (42.2% geranial, 31.5% neral, and 7.5% β-myrcene) showed antifungal activity (disk diffusion assay) against 15 isolates of yeast and filamentous fungi strains, with zone of growth inhibition of 15-90mm of diameter (40 µL of essential oil) for the 8 strains of *Candida* spp. Previous studies carried out with citral (*cis*-isomer geranial and *trans*-isomer neral) - the major component of the essential oil of *C. citratus* - concluded that this compound has significant antifungal activity against *Candida*

Table II. Antifungal activity of essential oil of *C. citratus* and *C. nardus* against *C. albicans*.

C. albicans* strains	**Source**	***Cymbopogon citratus***	***Cymbopogon nardus***
		MIC (µg/mL)	**MIC (µg/mL)**
LABMIC 0102	Hemoculture	78.12	312.50
LABMIC 0104	Tracheal aspirate	78.12	312.50
LABMIC 0105	Hemoculture	78.12	312.50
LABMIC 0125	Diabetic foot	156.25	625.00
LABMIC 0127	Diabetic foot	78.12	625.00
ATCC 90028	ATCC, EUA	156.25	625.00

***The values of MICs of controls AMB and FLU were 1 µg/mL and 0.5 - 0.25 µg/mL, respectively.**

spp (Leite et al. 2016, Silva et al. 2008, Zore et al. 2011).

Toledo et al. (2020), in another study using essential oil of *C. nardus* (27.34 percent citronellal, 23.21 percent geraniol, 13.37 percent geranial, 12.49 percent citronellol, and 10.31 percent neral), observed the sensitivity of two *C. albicans* strains, with MIC values ≥ 500 µg/mL, suggesting that the antifungal activity of the essential oil of *C. nardus* is greater the higher its citronellal content. Additionally, Singh et al. (2016) and Saibabu et al. (2017) observed the strong antifungal activity of citronellal against *C. albicans* strains, attributing its anticandidal mechanism: i) interference in membrane homeostasis, increasing fungal hypersensitivity to membrane disturbing agents, reducing ergosterol levels and decreasing glucose-induced H+ extrusion; ii) the induction of oxidative and genotoxic stress through an increase in the production of reactive oxygen species; iii) inhibition of the virulent attributes of the transition from yeast to hyphae and biofilm formation; as well as iv) the reduction of cell adherence to the polystyrene surface and to human oral epithelial cells.

The differences in minimal inhibitory concentration between studies are associated with the seasonal variation in the composition of volatile oils. EUCAST establishes cut-off points, compliance ranges for MICs that indicate resistance or susceptibility. According to these indices, the strains studied here are sensitive to both amphotericin B and fluconazole, while the essential oil showed variation of only two concentrations between strains.

All clinical isolates were sensitive to amphotericin B and fluconazole. For natural products, there are no cutoff values that indicate resistance or sensitivity of the isolates, however, concentrations in which the natural compound has a MIC lower than or equal to 1,000µg/mL indicate sensitivity of the microorganism to the product of plant origin (Toledo et al. 2016).

This antifungal potential presented by these essential oils of the genus *Cympopogon*, rich in oxygenated monoterpenes, has the potential to interfere with replication, fixation, production of hyphae or fluidity of fungal cell walls, possibly interfering through the interference of these compounds with the fluidity of the *Candida albicans* membrane (Almeida et al. 2020, Shaban et al. 2020).

Checkerboard assay

Antifungals play an important role in the therapy of infectious diseases, especially in the treatment of invasive fungal infections. However, cases of resistance and drug toxicity make treatments difficult. Currently amphotericin B is the most effective antifungal in the treatment of these infections, however, its use is limited due to its nephrotoxic effects. In view of this, the search for combined therapy is an alternative for a more effective treatment where different mechanisms of action of two or more drugs can make the treatment more effective against resistant strains, in addition to being safer by reducing toxic concentrations of the antifungal.

The results obtained from the checkerboard test to determine the effects of the combination of amphotericin B and essential oils are shown in Table III, with fractional inhibitory concentration (FICI) indices between 0.16 and 0.31 for samples tested with EOCC and reduction of the MIC of the EOCC together with AMB by up to 8 times, indicating a synergistic effect according to the standards proposed by Rosato et al. (2008). As for the combination between EOCN and amphotericin B, synergistic activity was observed, presenting FICI between 0.50 and 0.37, reducing the concentration with inhibitory activity of amphotericin B by up to 4 times and

Table III. Evaluation of the synergistic effect of EOCC or OECN and AMB against *C. albicans*.

Strains	*Cymbopogon citratus*		Amphotericin B		FICI	Effect
	MIC µg/mL (Individual)	MIC µg/mL (Combined)	MIC µg/mL (Individual)	MIC µg/mL (Combined)		
LABMIC0102	78.12	9.77	1	0.125	0.25	Synergistic
LABMIC0105	78.12	2.44	1	0.125	0.16	Synergistic
ATCC 90028	156.25	9.77	1	0.250	0.31	Synergistic
	Cymbopogon nardus		*Amphotericin* B			
	MIC µg/mL (Individual)	MIC µg/mL (Combined)	MIC µg/mL (Individual)	MIC µg/mL (Combined)		
LABMIC0102	312.5	78.125	1	0.25	0.5	Synergistic
LABMIC0105	312.5	39.0625	1	0.25	0.375	Synergistic
ATCC 90028	625	78.125	1	0.25	0.375	Synergistic

FICI: Fractional Inhibitory Concentration Index Synergism was defined as FICI ≤ 0.5, while no interaction was annotated when 0.5 < FICI ≤ 4.0, and antagonism when FICI >4.

of the essential oil by up to 8 times the value of the MIC of the compound.

The isobolograms show the oil combinations with AmB that showed some synergistic or additive effect. EOCC and AmB presented two to three synergistic combinations for the clinical isolates tested (Fig. 1a-b) and four synergistic combinations against the ATCC 90028 strain (Fig. 1c). EOCN showed one synergistic combination with amphotericin B for clinical isolate 0102 (Fig. 1d). For the other two strains, EOCN showed the same synergism pattern, presenting two synergistic combinations with the same compound concentrations against both *C. albicans* strains (Fig. 1e-f).

This study is promising, considering that in the literature we found few studies experimenting with EOCC or EOCN. Citronellal, one of the compounds from EOCN, showed indifferent effect when associated with amphotericin B against ATCC76615 and a clinical isolate of *Candida albicans* (Silva et al. 2019). In contrast, other compounds present in *Cymbopogon* spp. such as citronellol and geraniol showed a synergistic effect when associated with amphotericin B against *C. albicans* (Silva et al. 2020, Khan et al. 2012).

Amphotericin B is a polyene that acts on the yeast cell membrane, causing changes in its permeability and causing the extravasation of intracellular contents. Whereas, secondary metabolites from plants are known to act as ergosterol synthesis inhibitors, proton pump inhibition, genetic material damage and ATPase inhibition in yeast mitochondria (Tian et al. 2012, Zhou et al. 2017). Therefore, the FICI indices and representation of isobolograms indicating synergism of the oils with amphotericin B can be result of distinct mechanisms exerted between the compounds, contributing to the increase of the antimicrobial activity against *C. albicans*.

Biofilm assays (XTT/Menadione)

In the XTT assay, it evaluates the metabolic activity of reducing the dehydrogenase enzymes presents in the mitochondrial electron transport system, consequently evaluating the metabolism of the sessile cells that make up the biofilm (Silva et al. 2008).

Thus, after carrying out the test with EOCC, a metabolic reduction of 58 to 81% of biofilm (Fig.

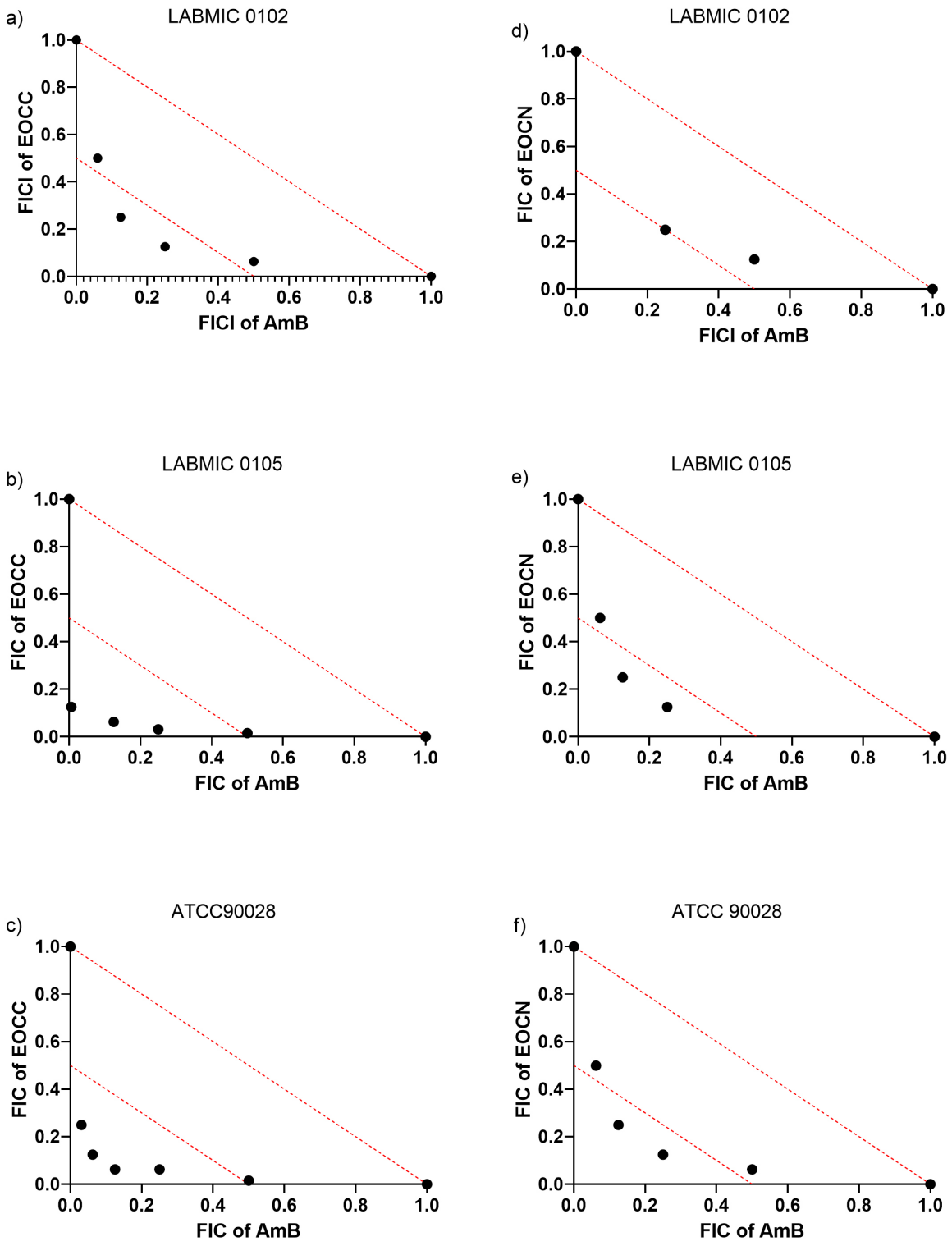

Figure 1. Isobolograms of the associations of *C. citratus* essential oil (EOCC) [a-c] and *C. nardus* essential oil (EOCN) [d-e] with amphotericin B against *C. albicans*. A red line was drawn between the FIC indices, visually discriminating interactions with synergistic (≤ 0.5) and non-synergistic (> 0.5) activity.

2), while for the EOCN it reduced the metabolic activity of the biofilm by 83.66 to 93.4% (Figure 1), results that demonstrate a significant reduction in metabolism obtained with concentrations of 2 times the MIC for a clinical isolate (LABMIC 0102) and ATCC 90028, a result close to those found by Khan et al. (2012) and Da Silva et al. (2020) who observed anti-biofilm activity at concentrations of 360 to 125 µg/mL with *Cymbopogon* sp. This reduction is correlated to the phytocomponents of the EOCN, which according to Singh et al. (2016) can act on membrane homeostasis and consequently leaving it more vulnerable and inhibiting aspects related to virulence such as the transition from yeast to hypha.

Cytotoxicity assay

The MTT assay with EOCC and EOCN showed an IC_{50} of 329.4 and 8.535 µg/mL, respectively (Fig. 3). These results show that *C. nardus* oil has a cytotoxicity value up to 25 times lower than *C. citratus* oil. Therefore, although both oils present cytotoxic concentrations below the MIC values, EOCN appears to be safer. This assay is based on the metabolization of MTT salt (yellow color) by mitochondrial dehydrogenase enzymes present in viable cells. When metabolized, the salt is converted into insoluble formazan crystals (purple color), allowing indirect quantification of the percentage of cell viability (Mosmann 1983).

In assays with HaCaT cells, Chinese hamster ovary (CHO) cells, EOCC killed 47% and 55% of cells at concentrations of 150 and 200 µl/mL, respectively, presenting IC50 in this concentration range. While EOCN showed lower cytotoxicity with IC_{50} of 450 µl/mL (Koba et al. 2009). In Chinese hamster ovary (CHO) cells and non-cancerous human fibroblast cell line (WI38) EOCC showed IC_{50} of 10 and 39.77 µg/mL, respectively while EOCN showed IC_{50} >50 µg/mL for both cell lines (Kpoviessi et al. 2014).

In human erythrocytes, EOCC tested diluted in saline and water, with and without neutralized pH, showed a variation between 0 and 12.6% of hemolysis at concentrations of up to 1,000 µg/mL (Silva et al. 2020). Another cytotoxicity study with EOCN showed IC_{50} of the oil of 96.6 and 33.1 µg/mL for MRC-5 (fibroblast) and HepG-2 (liver) cells, respectively (Toledo et al. 2016), showing high toxicity for these lineages compared to the HeLa cells used in our study. EOCC tested against MRC-5 (fibroblast) showed an IC_{50} of 19.63 µg/mL (Chaure et al. 2023). These findings show that although toxicity values vary greatly depending on the cell lineage and methodology used, EOCC is always more cytotoxic in relation to EOCN.

Such toxicity of essential oils is related to terpenic compounds and the ability of these substances to interfere with the fluidity of membranes, with toxicity depending on the

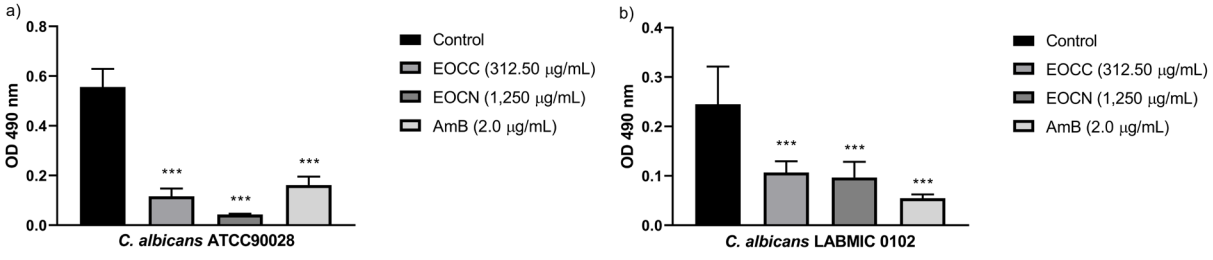

Figure 2. Effect of essential oil from *C. citratus* and *C. nardus* on mature biofilms of wild type (a) and clinical isolate (b) of *C. albicans*. Data are expressed as mean and standard deviation of XTT absorbance values normalized with those of the drug-free growth control (p<0.05). ***Significant reduction of biofilm biomass compared to negative control.

dose. Therefore, these natural compounds can be used for therapy with careful consideration of the level of toxicity by monitoring and supervision (Ortega-Cuadros, Tofiño-Rivera, Merini, Martínez-Pabón 2018, Popova et al. 2019).

Molecular docking

The molecular docking simulations were performed to understand the possible mechanism action of essential oils components against *Candida albicans*. SAP5 is a protease from the SAPS family (Secreted Aspartic Proteases) that has been associated with virulence characteristics in *C. albicans*. The main function of these enzymes is to degrade proteins, but they play an important role in biofilm formation. It is recognized that *C. albicans* biofilms secrete more SAPs than planktonic cells. Therefore, here we analyze the interaction of this protein with the EOCC and EOCN compounds (Min et al. 2013).

The results of ΔG (free energy of binding) and RMSD values of ligands with the SAP5 protein can be observed between figure 4 for EOCN constituents and EOCC components, while the types of interactions are listed in table IV.

Among the major ligands identified in the chromatogram, it was possible to observe that ligands linalyl acetate, geranial, β-myrcene, Neral, terpinyl acetate, neryl acetate, Citronelal, Elemol and Geraniol occupied the comparative binding site to Fluconazole. However, the ligands showed free energy values outside the ideality spectrum, that is, -6.0 kcal/mol (Shityakov & Förster 2014), in relation to Fluconazole itself (-6.1 kcal/mol).

The ligands showed interactions in common with the Lys 257 residues, with calculated

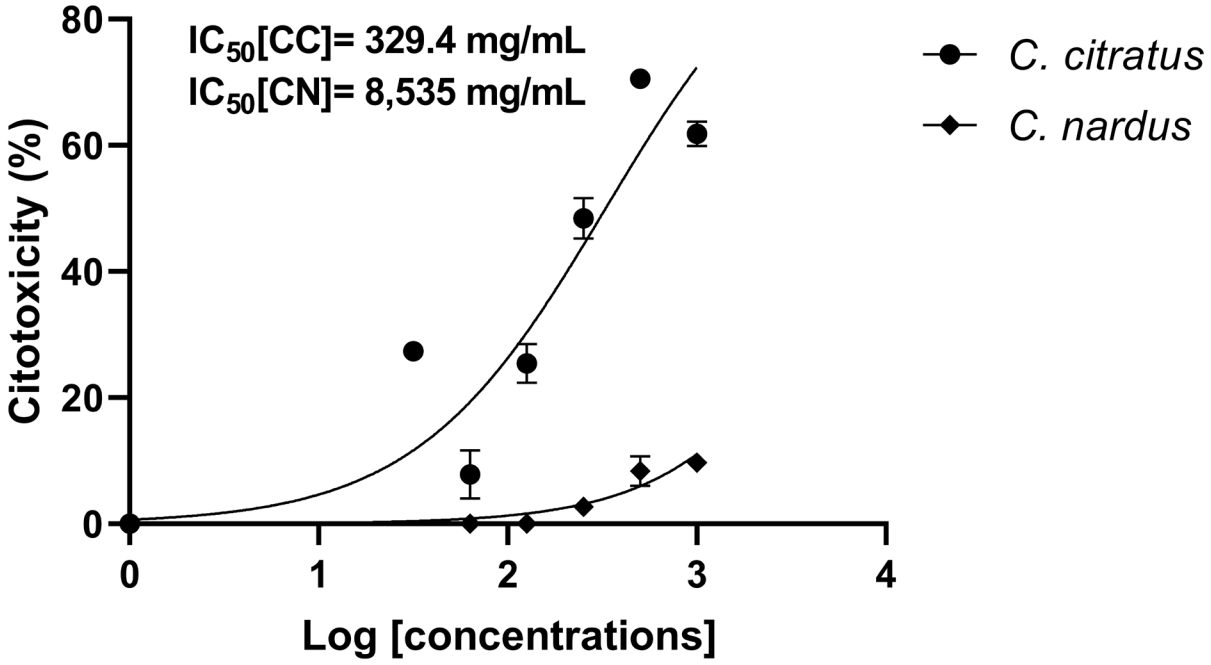

Figure 3. Citotoxicity effect of *C. citratus* [CC] and *C. nardus* [CN] IC_{50} values are indicated at the top of the graph. The 95% confidence intervals for the oils were [CC]: 246.6 – 460.7 and [CN]: 4,398 – 26,017. Results are given in percentages related to untreated control cells. Data are shown as the mean±standard deviation values of three replicates measurements.

distances of 3.52 Å, 3.71 Å and 3.47 Å for Citronelal, Elemol and Geraniol ligands (respectively), which characterize interactions of moderate strength (Imberty et al. 1991), as well as occurs with the comparative Fluconazole (3.51 Å), where the interactions are predominantly hydrophobic. The linalyl acetate ligand exhibited four hydrophobic interactions, with residues Ala 162 A (3.95 Å), Thr 222 A (3.73 Å), Phe 281 A (3.51 Å and 3.49 Å), Lys 598 B (3.64 Å) and a bridge salt with the residue Arg 297 A (5.14 Å). The terpinyl acetate molecule registered a strong hydrogen bond and two hydrophobic interactions with the residue Arg 312 A (2.46 Å) and Lys 598 B (3.57 Å and 3.48 Å), respectively. The ligands geranial and β-myrcene registered only hydrophobic interactions with residues Ala 162 A (3.99 Å), Phe 281 A (3.73 Å), Arg 297 A (3.53 Å) and Lys 598 B (3. 65 Å) and Thr 222 A (3.81 Å), Ile 223 A (3.91 Å), Arg 297 A (3.79 Å), Asn 309 A (3.82 Å), Lys 598 B (3, 82 Å and 3.71 Å), respectively. Four hydrophobic interactions and one strong hydrogen bond were observed for the Neral ligand with Pro 329 A (3.85 Å and 3.73 Å), Val 330 A (3.85 Å), Thr 602 B (3.86 Å) and Gly 601 B (2.73 Å) residues, respectively. The amphotericin B ligand recorded five hydrophobic interactions and a moderate hydrogen bond with Lys 289 A (3.78 Å), Trp 393 B (3.46 Å), Phe 622 B (3.64 Å and 3.47 Å), Thr 627 B (3.52 Å) and Tyr Residues 625 B (3.10 Å), respectively. Furthermore, seven strong hydrogen bonds were observed for the same ligand, with Arg 394 B (2.08 Å), Tyr 566 B (2.67 Å, 2.27 Å and 2.50 Å), Asp 586 B (2.20 Å), Ser 587 B (2.96 Å Å), Ala 588 B (2.71 Å) and Thr 627 B (2.87 Å). Regarding the fluconazole ligand, this molecule exhibited a hydrophobic interaction and a halogen bond with Val 671 B (3.44 Å) and Glu 619 B (3.23 Å), respectively. In addition, five

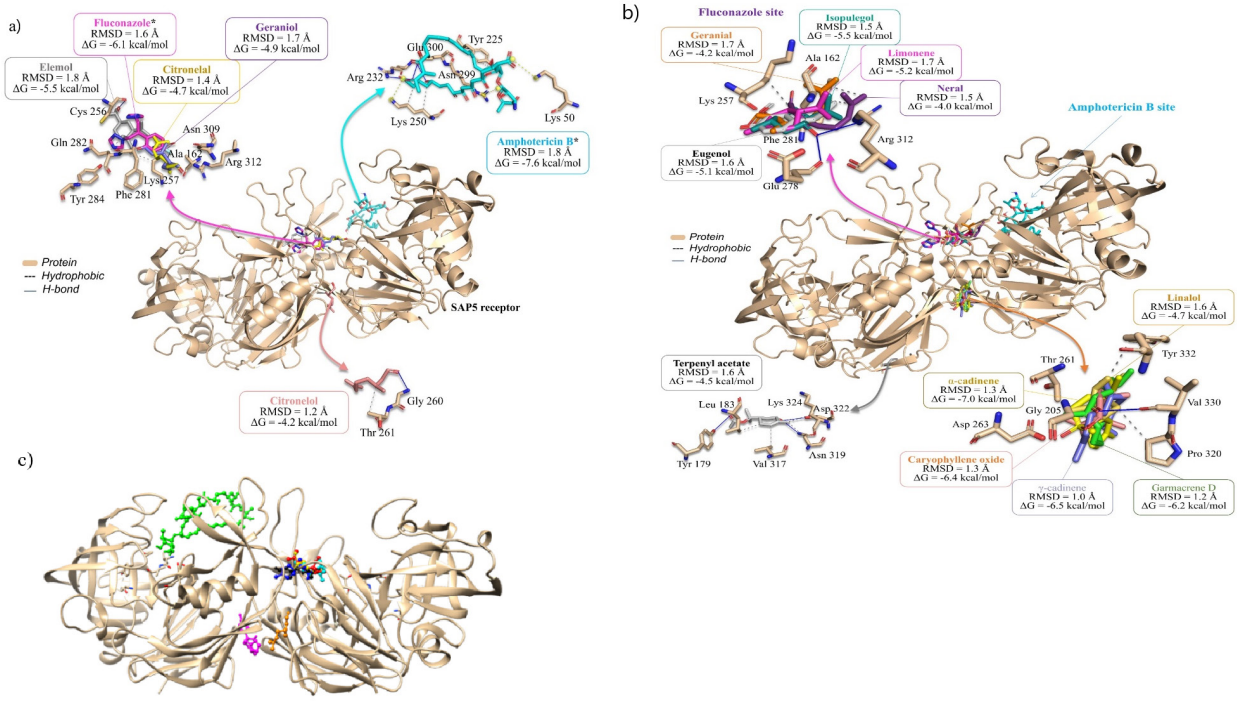

Figure 4. The binding sites between Citronelal, Elemol and Geraniol ligands (a); The binding sites between α-cadinene, caryophyllene oxide, eugenol, γ-cadinene, geraniol, germacrene D, isopulegol, limonene, linalool, Neral, terpenyl acetate (minor ligands) (b); and binding sites between, linalyl acetate (red), terpenyl acetate (blue), geranial (black), β-myrcene (yellow), neral (orange) and neryl acetate (cyan) (c). All represent binding with the SAP5 protein and with the control ligands Amphotericin B and Fluconazole*.

hydrogen bonds were recorded (four strong and one moderate), with residues Asn 202 A (2.57 Å), Ser 522 B (2.40 Å), Thr 261 A (3.02 Å and 2 .93 Å) and Gly 205 A (3.20 Å), respectively. Therefore, the interactions observed between the analyzed ligands with the SAP5 protein explain the ΔGbind values observed in Table IV.

The strong hydrogen bonds observed in terpinyl acetate-SAP5 and fluconazole-SAP5 complexes may contribute about 2.63 to 14.33 kcal/mol of free binding energy (Steed & Atwood 2022) because the intermolecular force is the strongest and most influential in molecular recognition (Dong & Davis 2020). Furthermore, Figure 4 shows the binding site between the ligands analyzed with the SAP5 protein. On the other hand, none of the phytochemicals showed affinity for the catalytic site of the ligand Amphotericin B, which showed excellent affinity with the receptor in these tests (-7.6 kcal/mol). In addition, RMSD values lower than 2.0 Å infer that the ligands have a free energy within the reliability standard, but that they have low affinity with the SAP5 receptor.

In relation to simulations for the minor ligands identified in the chromatogram, it was possible to observe that the ligands Eugenol, Isopulegol and Limonene showed low affinity with the SAP5 receptor and occupy the same catalytic site of the comparative ligand Fluconazole, where the free energy values are higher to -6.0 kcal/mol, although they are within the mean squared deviation range, ie RMSD < 2.0 Å. Furthermore, the compounds showed interactions in common with the Phe 281 residue (except for Limonene), with distances in the order of 3.79 Å and 3.75 Å for the ligands Eugenol

Table IV, Interactions with distances (Å) between the analyzed EOCC and EOCN binding constituents and SAP5 protein amino acid residues.

Compound	Residue	AA	Distance (Å)	Interaction
Caryophyllene oxide	205B	Gly	2.80	Hydrogen bonds
Caryophyllene oxide	261B	Thr	3.30	Hydrophobic
Caryophyllene oxide	330A	Val	3.72	Hydrophobic
Citronelal	162A	Ala	3.78	Hydrophobic
Citronelal	257B	Lys	3.52	Hydrophobic
Citronelal	281A	Phe	3.70	Hydrophobic
Citronelal	312A	Arg	2.77	Hydrogen bonds
Citronelol	260B	Gly	2.78	Hydrogen bonds
Citronelol	261B	Thr	3.82	Hydrophobic
Elemol	256A	Cys	2.83	Hydrogen bonds
Elemol	257A	Lys	3.71	Hydrophobic
Elemol	282A	Gln	3.36	Hydrophobic
Eugenol	257B	Lys	3.75	Hydrophobic
Eugenol	281A	Phe	3.79	Hydrophobic
Geranial	281A	Phe	3.71	Hydrophobic
Geranial	162 A	Ala	3.99	Hydrophobic
Geranial	281 A	Phe	3.73	Hydrophobic

Table IV. Continuation.

Geranial	297 A	Arg	3.53	Hydrophobic
Geranial	598 B	Lys	3.65	Hydrophobic
Geraniol	257B	Lys	4.00	Hydrophobic
Geraniol	281A	Phe	3.74	Hydrophobic
Geraniol	282A	Gln	2.38	Hydrogen bonds
Geraniol	309A	Asn	3.74	Hydrophobic
Germacrene D	261B	Thr	3.11	Hydrophobic
Germacrene D	330A	Val	3.77	Hydrophobic
Isopulegol	257B	Lys	3.79	Hydrophobic
Isopulegol	278A	Glu	1.84	Hydrogen bonds
Isopulegol	281A	Phe	3.75	Hydrophobic
Isopulegol	312A	Arg	2.53	Hydrogen bonds
Limonene	162A	Ala	3.72	Hydrophobic
Limonene	257B	Lys	3.56	Hydrophobic
Linalol	329A	Pro	3.77	Hydrophobic
Linalol	330A	Val	1.95	Hydrogen bonds
Linalol	332A	Tyr	3.58	Hydrophobic
Linalyl acetate	162 A	Ala	3.95	Hydrophobic
Linalyl acetate	222 A	Thr	3.73	Hydrophobic
Linalyl acetate	281A	Phe	3.51	Hydrophobic
Linalyl acetate	598B	Lys	3.64	Hydrophobic
Linalyl acetate	297A	Arg	5.14	Salt bridges
Neral	162A	Ala	3.68	Hydrophobic
Neral	257B	Lys	3.64	Hydrophobic
Neral	281A	Phe	3.85	Hydrophobic
Neral	329A	Pro	3.85	Hydrophobic
Neral	330A	Val	3.85	Hydrophobic
Neral	602B	Thr	3.86	Hydrophobic
Neral	601B	Gly	2.73	Hydrogen bonds
Terpenyl acetate	179B	Tyr	3.06	Hydrogen bonds
Terpenyl acetate	183B	Leu	3.90	Hydrophobic
Terpenyl acetate	317B	Val	3.73	Hydrophobic
Terpenyl acetate	319B	Asn	2.85	Hydrogen bonds
Terpenyl acetate	322B	Asp	3.17	Hydrogen bonds
Terpenyl acetate	324B	Lys	3.87	Hydrophobic
Terpenyl acetate	598B	Lys	3.57	Hydrophobic
Terpenyl acetate	312A	Arg	2.46	Hydrogen bonds

Table IV. Continuation.

α-cadinene	261B	Thr	3.47	Hydrophobic
α-cadinene	329A	Pro	3.57	Hydrophobic
α-cadinene	330A	Val	3.61	Hydrophobic
α-cadinene	332A	Tyr	3.77	Hydrophobic
β-Myrcene	222A	Thr	3.81	Hydrophobic
β-Myrcene	223A	Ile	3.91	Hydrophobic
β-Myrcene	297A	Arg	3.79	Hydrophobic
β-Myrcene	309A	Asn	3.82	Hydrophobic
β-Myrcene	598B	Lys	3.82	Hydrophobic
γ-cadinene	329A	Pro	3.66	Hydrophobic
Amphotericin B*	289A	Lys	3.78	Hydrophobic
Amphotericin B*	393B	Trp	3.46	Hydrophobic
Amphotericin B*	622B	Phe	3.64	Hydrophobic
Amphotericin B*	627B	Thr	3.52	Hydrophobic
Amphotericin B*	394B	Arg	2.08	Hydrogen bonds
Amphotericin B*	566B	Tyr	2.50	Hydrogen bonds
Amphotericin B*	586B	Asp	2.20	Hydrogen bonds
Amphotericin B*	587B	Ser	2.96	Hydrogen bonds
Amphotericin B*	588B	Ala	2.71	Hydrogen bonds
Amphotericin B*	625B	Tyr	3.10	Hydrogen bonds
Amphotericin B*	627B	Thr	2.87	Hydrogen bonds
Amphotericin B*	50A	Lys	4.57	Salt bridges
Amphotericin B*	225A	Tyr	3.61	Hydrophobic
Amphotericin B*	250A	Lys	3.84	Hydrophobic
Amphotericin B*	250A	Lys	4.27	Salt bridges
Amphotericin B*	232A	Arg	3.20	Hydrogen bonds
Amphotericin B*	299A	Arg	5.22	Salt bridges
Amphotericin B*	300A	Glu	2.23	Hydrogen bonds
Fluconazole*	671B	Val	3.44	Hydrophobic
Fluconazole*	202A	Asn	2.57	Hydrogen bonds
Fluconazole*	205A	Gly	3.20	Hydrogen bonds
Fluconazole*	261A	Thr	2.93	Hydrogen bonds
Fluconazole*	522B	Ser	2.40	Hydrogen bonds
Fluconazole*	619B	Glu	3.23	Halogen bonds
Fluconazole*	257B	Lys	3.93	Hydrophobic
Fluconazole*	284A	Tyr	3.03	Hydrogen bonds

***Comparative ligands used in molecular docking testing.**

and Isopulegol (respectively), which characterize interactions of moderate strength (Table IV).

Furthermore, it is possible to point out that the α-cadinene, Caryophyllene oxide, and Germacrene D ligands occupy an allosteric site with hydrophobic interactions in common with the residues of Thr 261B, where distances > 3.11 Å characterize interactions of moderate strength, and with the residue of Val 330A, where distances > 3.55 Å characterize weak interactions (Table IV), including the possibility of an H-bond interaction between the ligand Caryophyllene oxido, by the presence of an epoxide group, and the residue of Gly 205B, where the distance on the order of 2.80 Å characterizes a strong hydrogen interaction (Table IV). It is noteworthy that the compounds showed, at the same time, excellent affinity energy with the SAP5 receptor, that is, with ΔG values lower than -6.0 kcal/mol, as well as RMSD values that guarantee the statistical reliability of the test (RMSD < 2.0 Å), indicating that they can act as SAP5 modulators by synergism associated with the ligand Fluconazole (Figure 4).

CONCLUSION

Essential oils of *Cymbopogon citratus* and *Cymbopogon nardus* are rich in oxygenated monoterpenes with a higher content of geranial and neral for EOCC and citronellal, geraniol and citronellol for EOCN. Phytochemical substances which are associated with the antifungal properties of these oils from *Cymbopogon* spp. Both essential oils have antimicrobial against *Candida albicans* strains, in planktonic and biofilm form, in non-toxic concentrations. In addition to presenting synergistic potential when combined with amphotericin B, thus raising the possibility of formulations that use both substances together to a more promising therapy, with less toxicity to the patient.

Regarding to the study of molecular docking, we observed a low interaction between the major constituents of both essential oils with the SAP-5 enzyme, however the minor components of EOCN, such as α-Cadinene, Caryophyllen oxide and Germacrene D, approved a better front to the SAP5, it showed interaction in different binding sites of the standard drugs, thus supporting a synergistic effect.

Thus, *C. citratus* and *C. nardus* have potential against *C. albicans* in planktonic form, carefully or together with amphotericin B, and at concentrations of 2x the MIC, they have inhibitory potential to the *Candida* biofilm. Nevertheless, there is a need for tests to determine molecular dynamics, verifying the stability of the formation of complexes between these ligands and the SAP5 receptor, and further studies are needed to understand the mechanism of action of this essential oil, through preclinical and clinical trials for greater safety and measurement of the effectiveness of possible therapies and subsequent production of pharmaceutical formulations.

Acknowledgments

The authors thank to Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico, from the state government, for financial support.

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How to cite

PRADO GM ET AL. 2024. Antifungal, molecular docking and cytotoxic effect of the essential oil of *Cymbopogon citratus* (DC) Stapf. and *Cymbopogon nardus* (L.) Rendle against *Candida albicans*. An Acad Bras Cienc 96: e20230309. DOI 10.1590/0001-3765202420230309.

Manuscript received on April 26, 2023;
accepted for publication on October 27, 2023

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