In Vitro and In Silico Biological Evaluation of the Essential Oil from *Syzygium cumini* Leaves as a Source of Novel Antifungal and Trichomonacidal Agents

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Abstract: As microbes develop resistance to various drugs, the treatment of infections becomes increasingly challenging, leading to prolonged illness, heightened severity of infections, elevated mortality rates, and increased healthcare costs. Essential oils are lipophilic and volatile mixtures of compounds that have gained attention in research for novel antimicrobial agents. Therefore, the present study evaluated the essential oil of *Syzygium cumini* leaves (EOSC) in order to prospect its antifungal and trichomonacidal activities. The essential oil from the leaves was extracted by steam distillation and analyzed by GC-MS. Antifungal activity was evaluated using the serial microdilution method. Additionally, the potential of the EOSC as an enhancer of fluconazole (FCZ) action was tested at subinhibitory concentrations. To assess anti-*Trichomonas vaginalis* activity, concentrations ranging from 15.6 to 500 µg/mL of EOSC were tested. Finally, the SwissADME platform was employed to analyze the physicochemical and pharmacokinetic characteristics of the major component of EOSC. The GC-MS analysis identified 94.24% of the components of EOSC, with *α*-pinene (51.11%) and nerol (8.25%) as major constituents. EOSC exhibited low antifungal activity against the evaluated *Candida* strains. However, the combination of EOSC and FCZ reduced the IC50 against *Candida krusei* from 45.29 to 0.30 µg/mL. EOSC also demonstrated significant activity against *T. vaginalis* (IC50 = 88.2 µg/mL). In silico prediction with α-pinene showed low toxic action and important physicochemical aspects for drug production. The essential oil of *Syzygium cumini* emerges as a promising candidate for the discovery of molecules with potential antifungal and anti-*Trichomonas vaginalis* applications.

Keywords: *Syzygium cumini; Candida; Trichomonas vaginalis; pathogenic fungi; antimicrobial resistance
1. Introduction

At least 300 out of the 1.5–5 million existing fungal species are associated with human diseases. Among them, Candida genus leads morbidity and mortality rates. Several species are highlighted in invasive infections, with the predominant cases being attributed to C. albicans. However, they may also include C. glabrata, C. krusei, C. parapsilosis, and C. tropicalis [1]. This fungal diversity plays a crucial role in the dynamics of antimicrobial resistance (AMR), which represents a serious threat to global health. Alarming predictions indicate up to 10 million deaths per year by 2050 due to AMR, negatively impacting both public health and the economy [2].

As cases of AMR continue to rise, there is a need to prospect new therapeutic targets and drugs that can assist in the treatment of these infections. Unfortunately, the development of antifungal medications does not meet clinical needs, especially in light of the rapid development of resistance by clinically relevant fungi [3]. Therefore, fungal diseases are of great importance to public health, particularly when associated with AMR, which is also valid for drug-resistant parasitic and neglected diseases [4].

The neglected tropical diseases (NTDs) are a group of pathological conditions that predominate in tropical and subtropical regions. These diseases have a strong association with communities inhabiting low-income areas, especially in Africa, Asia, and Latin America [5]. They represent significant global causes of illness and death, contributing to stigma and discrimination among affected populations. Another infection often neglected by public health authorities and requiring new drugs for therapy is trichomoniasis. Trichomoniasis is the most common non-viral sexually transmitted infection (STI) worldwide, caused by the flagellated parasite Trichomonas vaginalis [6].

The parasite colonization in host cells poses complications to female reproductive health, in addition to risks for the predisposition of cervical and prostate cancer. Additionally, T. vaginalis infection can increase HIV (human immunodeficiency virus) transmission and acquisition [7]. Despite being curable, the excessive reliance on a single class of antimicrobials increases vulnerability if clinical resistance spreads. It is believed that market forces alone will not be sufficient to drive the development of new treatments for trichomoniasis [8]. The treatment of trichomoniasis has relied on the use of 5-nitroimidazoles for over 50 years, and T. vaginalis-resistant isolates have already been reported [9].

In recent years, there has been a significant increase in demand for medicinal and aromatic plants. This growth is driven by consumers’ preference for pharmaceuticals and natural foods. Essential oils and their constituents thus play a significant role due to their accessibility, low cost, and variety of biological activities. Furthermore, finding effective, safe, and economical antifungal agents to control the growth and production of mycotoxins by fungi is crucial from both sanitary and economic perspectives [10]. Several studies have been conducted to investigate the antifungal, antimicrobial, and anti-inflammatory properties of essential oils [10–13].

The Myrtaceae family comprises approximately 140 genera and 3,500 species. Among the members of this family, the genus Syzygium consists of fruit-bearing species primarily found in tropical and subtropical regions worldwide [14]. The species Syzygium cumini (L.) Skeels (synonym: Eugenia jambolana), popularly known as “ameixa-preta”, “azeitona-roxa”, or “jambolão” [15], is used in traditional medicine for the treatment of diabetes, colic, and digestive disorders [16,17]. Extracts and natural products obtained from the plant organs of S. cumini exhibit several biological activities, including hepatoprotective, antimicrobial, anti-inflammatory, antidiabetic, and hypolipidemic effects [18,19].

Studies have demonstrated that the essential oils obtained from S. cumini showed antioxidant, antimicrobial, antiparasitic, and anti-inflammatory properties [20–23]. A-Pinene is among the main compounds identified in the essential oil of this plant and it is suggested that this monoterpene is synergistically related to possible antiprotozoal and antimicrobial activities, potentiating such actions. These findings further prove the therapeutic potential of these essential oils and stimulate ongoing research into their benefits for human health, with promising implications for ethnopharmacology [20,23].
The computational methods known as in silico models are used to optimize molecules with potential for drug development, allowing for the assessment of crucial physicochemical properties for drug efficacy, as well as pharmacokinetic characteristics such as absorption, distribution, metabolism, and excretion (ADME) [24]. The SwissADME web tool simplifies the calculation of physicochemical and pharmacokinetic parameters of molecules, being useful for both specialists and non-specialists. Additionally, it includes access to BOILED-Egg, which predicts the gastrointestinal absorption and brain access of molecules, facilitating the evaluation of potential drugs [25].

In this study, our main objective is to evaluate the chemical composition of the essential oil of *S. cumini* leaves (EOSC), as well as to investigate its pharmacological effects, with special emphasis on its antifungal activity, its potential as a fluconazole modifier, and its anti-*Trichomonas vaginalis* activity. Additionally, we aim to predict the physicochemical, pharmacokinetic, and toxicological properties of the major compound using in silico computational tools, focusing on ADME activities.

2. Materials and Methods

2.1. License and Plant Material Collection

The collection of plant material was conducted with authorization from SISBIO (Sistema de Autorização e Informação em Biodiversidade), number 64011-1, and from SISGEN (Sistema Nacional de Gestão do Patrimônio Genético e Conhecimentos Tradicionais Associados), registration number A7AEBD7. The leaves of *S. cumini* were collected in the municipality of Jardim, Ceará, Brazil (7°33’’ W, 39°18’’ S), and specimens were deposited at the Herbário Caririense Dárdao de Andrade Lima (HCDAL), under the voucher number 13.593.

2.2. Extraction of Essential Oil

After collection, the leaves were dehydrated at room temperature and crushed manually. Subsequently, the crushed material (200 g) was mixed with 2 L of distilled water in a 5 L round-bottom flask. The essential oil was extracted using the hydrodistillation method in a Clevenger apparatus for a period of 2 h. At the end of extraction, a final yield of 0.159% was obtained. The essential oil of *S. cumini* was stored in amber bottles and kept refrigerated at −4 °C [23] (Figure 1).

![Figure 1. Essential oil extraction process from Syzigium cumini leaves.](image-url)
2.3. Gas Chromatography–Mass Spectrometry (GC-MS)

The EOSC underwent phytochemical analysis through GC-MS, using the Agilent Technologies AutoSystem XL GC-MS system (Agilent Technologies, Santa Clara, CA, USA). The process was conducted in EI mode at 70 eV, employing two distinct capillary columns: an HP 5MS (30 m × 0.35 mm, with a film thickness of 0.50 µm) and an HP Innowax (30 m × 0.32 mm, with a film thickness of 0.50 µm). A split/splitless injector (220 °C) connected to an FID detector was used. The thermal programming ranged from 60 °C (1 min) to 180 °C at a rate of 3 °C/min, with the detector temperature set to 220 °C.

Helium was used as the carrier gas with a flow rate of 1.0 mL/min. A volume of 1 µL of EOSC, diluted in chloroform at a ratio of 1:10, was injected into the system. Each sample was analyzed in duplicate, and the relative concentrations of the components were calculated based on the peak areas of GC, determined by the Flame Ionization Detector (FID) response, without the use of correction factors [26]. The identification of compounds was performed using retention index (RI) evaluation with the use of a set of standard n-alkanes (C7 to C30) under the same experimental conditions. Subsequently, this identification was compared with mass spectrometry information from the NIST and Wiley libraries, as well as with mass spectra reported in the literature [26].

2.4. Antifungal Activity

2.4.1. Fungal Strains, Culture Media, and Drugs

Standard strains of Candida albicans INCQS 40006 (isolated from a man with bronchomycosis), Candida krusei INCQS 40095 (clinical isolation), and Candida tropicalis INCQS 40042 (isolated from a man with bronchomycosis) were obtained from the Laboratório de Micologia Aplicada (LMAC) of the Coleção de Culturas Oswaldo Cruz do Instituto Nacional de Controle de Qualidade em Saúde (INCQS). The culture media used for fungal growth were Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB). The preparation of the media followed the manufacturer’s instructions and was sterilized by autoclaving at 121 °C for 15 min. The reference antifungal drug used for synergistic evaluation was Fluconazole (FCZ/FLUCOMED), diluted in the same manner as the EOSC.

2.4.2. Cultivation and Matrix Preparation

The growth of Candida strains was conducted on Petri dishes containing SDA medium at 37 °C for 24 h. Following growth, fungal suspensions were prepared in tubes containing 4 mL of sterile NaCl solution (0.9%), which were shaken and assessed for turbidity using the McFarland scale (reference of 0.5). The EOSC was weighed (0.0191 g) and dissolved in 1 mL of DMSO, the same way as FCZ. Subsequently, this solution was diluted in 9 mL of SDB culture medium to obtain a concentration of 1024 µg/mL, ensuring that the presence of DMSO does not interfere with the pharmacological effects [27].

2.4.3. Half-Maximal Inhibitory Concentration (IC₅₀)

The antifungal activity of EOSC was evaluated following the methodology described in Morais-Braga et al. [28]. The broth microdilution technique was employed to determine the IC₅₀. The EOSC and FCZ were separately diluted to concentrations ranging from 1024 to 2 µg/mL. The experiment was conducted in quadruplicate, with one well reserved for growth control and another for sterility control. After incubation at 37 °C for 24 h, concentrations were adjusted as necessary. Dilution and sterility controls were performed, and absorbance was measured at 630 nm using an ELISA reader (Termoplate® Kasuaki, Beijing, China).

2.5. Assessment of the Potentiation of Fluconazole Activity

To investigate the interaction between EOSC and FCZ, the compound was evaluated using the subinhibitory matrix concentration (CM/8) [29]. Fluconazole was tested at concentrations ranging from 2 to 1024 µg/mL. The plates used in the broth microdilution
technique and serial dilution were incubated at 37 °C for 24 h, and readings were taken using an ELISA spectrophotometer (Termoplate® Kasuaki, China).

2.6. Anti-Trichomonas Vaginalis Activity

The assays were conducted with the T. vaginalis ATCC 30236 (JH 31 A #4) metronidazole-sensitive clinical isolate (MIC: 3.1 µM; IC50:0.5 µM). Trophozoites were maintained in tryptase-yeast extract-maltose (TYM) medium, supplemented with heat-inactivated bovine serum (10%, v/v) and penicillin/streptomycin at 37 °C [30]. Trichomonads in the logarithmic growth phase exhibiting > 95% of normal motility and morphology were inoculated in fresh TYM for assays. The anti-T. vaginalis activity of EOSC was evaluated in vitro at concentrations ranging from 500 to 15.6 µg/mL, as described by Menezes et al. [31]. The serial diluted EOSC (50 µL) was added to 96-well microplates with 2.0 × 10^5 trophozoites/mL suspensions (150 µL). The plates were incubated at 37 °C, for 24 h, at 5% CO2. Trophozoite viability was assessed by comparisons with untreated parasites counted in a hemocytometer using trypan blue dye (0.2%). Two controls were used: negative control with trophozoites only in a supplemented TYM medium and vehicle control with 0.6% DMSO. All tests were performed in triplicate with three independent cultures (n = 3).

2.7. ADME Prediction In Silico

To analyze the physicochemical and pharmacokinetic characteristics of the major component (>20%) found in the essential oil of S. cumini, the SwissADME platform provided by the Swiss Institute of Bioinformatics (SIB) was used, focusing on toxic parameters, BOILED-egg, and the bioavailability radar [25].

2.8. Statistical Analysis

Statistical analyses were performed using GraphPad Prism software version 6 (GraphPad Software Inc., San Diego, CA, USA). The IC50 was calculated using non-linear regression. One-way Analysis of Variance (ANOVA) followed by Tukey’s test was applied. Antifungal and anti-Trichomonas vaginalis activity data were expressed as mean ± standard deviation (SD).

3. Results

3.1. Chemical Composition of EOSC

Following chromatographic analysis (GC-MS), it was possible to identify 94.24% of the compounds in EOSC, comprising a total of 16 chemical components, as illustrated in Table 1. Major components such as α-pinene (51.11%, monoterpene) and nerol (8.25%, monoterpene) were observed, along with trace elements such as nerolidol (6.56%), linalool (5.82%), nonalol (4.56%), caryophyllene (3.52%), and others.

Table 1. Chemical composition of essential oil of Syzygium cumini leaves.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RI a</th>
<th>RI b</th>
<th>Essential Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-pinene</td>
<td>937</td>
<td>939</td>
<td>51.11</td>
</tr>
<tr>
<td>β-pinene</td>
<td>979</td>
<td>981</td>
<td>2.98</td>
</tr>
<tr>
<td>β-myrcene</td>
<td>995</td>
<td>991</td>
<td>0.77</td>
</tr>
<tr>
<td>Limonene</td>
<td>1029</td>
<td>1031</td>
<td>1.42</td>
</tr>
<tr>
<td>Nonalol</td>
<td>1105</td>
<td>1103</td>
<td>4.56</td>
</tr>
<tr>
<td>Linalool</td>
<td>1099</td>
<td>1098</td>
<td>5.82</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>1187</td>
<td>1189</td>
<td>1.81</td>
</tr>
<tr>
<td>Nerol</td>
<td>1228</td>
<td>1228</td>
<td>8.25</td>
</tr>
</tbody>
</table>
3.2. Antifungal Effect

The assessment of the antifungal efficacy of EOSC is demonstrated in Table 2. A significant activity against *C. albicans* (541.4 µg/mL) and *C. krusei* (502.3 µg/mL) strains is observed, indicating its effectiveness as an antifungal agent in clinical contexts. However, no relevance was observed in the activity against the *C. tropicalis* strain, as the action was higher than the highest tested concentration (1024 µg/mL), suggesting a possible selective action against the previously mentioned Candida species.

### Table 2. Antifungal and modifying activity of essential oil from *Syzygium cumini* against *Candida* strains.

<table>
<thead>
<tr>
<th></th>
<th>IC₅₀ µg/mL</th>
<th>C. albicans</th>
<th>C. krusei</th>
<th>C. tropicalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOSC</td>
<td>541.4 ± 1.09</td>
<td>502.3 ± 2.93</td>
<td>&gt;1024 ± 2.75</td>
<td></td>
</tr>
<tr>
<td>FCZ</td>
<td>1.59 ± 0.91</td>
<td>45.29 ± 3.52</td>
<td>0.01 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>FCZ + EOSC</td>
<td>2.17 ± 0.08</td>
<td>0.30 ± 0.01</td>
<td>0.01 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

FCZ: Fluconazole, EOSC: essential oil of *Syzygium cumini* leaves.

3.3. Fluconazole Potentiating Action

The pharmacological potential of the EOSC as a FCZ enhancer is evident, as indicated in Table 2 and Figure 2. Remarkably, its modifying action was particularly highlighted concerning *C. krusei*, resulting in a significant reduction in the fluconazole IC₅₀ to 0.30 µg/mL. However, regarding the other strains, no significant alterations were observed; there was no substantial impact on *C. albicans* and *C. tropicalis*, demonstrating an antagonistic and indifferent action, respectively, in relation to the combination with FCZ.
Figure 2. Modifying activity of essential oil from Syzygium cumini combined with fluconazole. FCZ: Fluconazole, EOSC: essential oil of Syzygium cumini leaves. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

3.4. Anti-Trichomonas Vaginalis Activity

The evaluation of antiparasitic activity is demonstrated in Figure 3. The EOSC was demonstrated to be active against T. vaginalis with an IC50 of 88.2 μg/mL. At 500 μg/mL of EOSC, the trophozoite's viability was 0.16 ± 0.08, while at 250 μg/mL, it was 14.3 ± 1.98. No significant reduction in trophozoite viability was observed at the concentration of 15.6 μg/mL (Figure 3).
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![Figure 3. Activity of EOSC against *Trichomonas vaginalis* ATCC30236 isolate at 500, 250, 125, 62.5, 31.2, and 15.6 µg/mL. Control: non-treated trophozoites. VC: vehicle control, 0.6% DMSO. Data are presented as the mean ± standard deviation compared to the control (considering the viability of 100% of trophozoites). Results are representative of at least three independent experiments in triplicate. (*) Statistically significant difference (*p* < 0.05) when compared to the control by Student’s *t*-test.](image)

3.5. In Silico Tests (ADME)

The oral bioavailability graph (Figure 4) illustrates the pharmacokinetic characteristics of α-pinene, the main compound from EOSC, based on its ADME activity (absorption, distribution, metabolism, and excretion). The colored area represents the standards by which molecules exhibit better similarity to drugs, taking into account lipophilicity, saturation, size, flexibility, polarity, and solubility. It is notable that the molecule stands out from promising drug molecules due to its low flexibility (lack of rotational bonding), size (MW: 136.23 g/mol, reference range between 150 and 500 g/mol), and low polarity (TPSA: 0.00 Å²).

![Figure 4. ADME properties of α-pinene (major constituent of the essential oil of *Syzygium cumini*). LIPO: lipophilicity; SIZE: molecular size; POLAR: polarity; INSOLU: insolubility; INSATU: unsaturation; FLEX: flexibility.](image)
It is still possible to conclude that the parameters of lipophilicity (XLOGP3: +4.48, within the reference range between −0.7 and +5.0), saturation (fraction C sp.3: 0.80), and solubility (log S (ESOL): −3.51, indicating adequate solubility) comply with the predefined criteria for the production of suitable drugs. The cutaneous permeability coefficient (Log Kp: −3.95 cm/s, Table 3) is also considered adequate, as there are no obstacles to permeability; the similarity parameter is reinforced by fitting correctly into the rules of Verber, Egan, and Lipinski, having violated the rule of the lipophilic characteristic.

Table 3. Toxicity and ADME analysis of α-pinene (major constituent of the essential oil of Syzygium cumini).

<table>
<thead>
<tr>
<th>Pharmacokinetics</th>
<th>α-pinene</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI absorption</td>
<td>Low</td>
</tr>
<tr>
<td>BBB permeant</td>
<td>Yes</td>
</tr>
<tr>
<td>P-gp substrate</td>
<td>No</td>
</tr>
<tr>
<td>CYP1A2 inhibitor</td>
<td>No</td>
</tr>
<tr>
<td>CYP2C19 inhibitor</td>
<td>No</td>
</tr>
<tr>
<td>CYP2C9 inhibitor</td>
<td>Yes</td>
</tr>
<tr>
<td>CYP2D6 inhibitor</td>
<td>No</td>
</tr>
<tr>
<td>CYP3A4 inhibitor</td>
<td>No</td>
</tr>
<tr>
<td>Log Kp (skin permeation)</td>
<td>−3.95 cm/s</td>
</tr>
</tbody>
</table>

Drug-likeness

| Lipinski                  | Yes; 1 violation: MLOGP > 4.15   |
| Ghose                     | No; 1 violation: MW < 160        |
| Veber                     | Yes                               |
| Egan                      | Yes                               |
| Muegge                    | No; 2 violations: MW < 200, Heteroatoms < 2 |
| Bioavailability Score     | 0.55                              |

GI: gastrointestinal; BBB: blood–brain barrier; MW: molecular weight; CYP1A2: Cytochrome P450 1A2; CYP2C19: Cytochrome P450 2C19; CYP2C9: Cytochrome P450 2C9; CYP2D6: Cytochrome P450 2D6; CYP3A4: Cytochrome P450 3A4.

Additionally, Table 3 also lists potential toxic effects of α-pinene, with emphasis on the inhibition of the CYP2C9 isoenzyme. No significant changes were observed compared to other isoenzymes. The BOILED-egg graph (Figure 5, Table 3) provides data directly related to the distribution of the α-pinene molecule, simulating its behavior in the human body. The yellow region indicates the compound’s ability to cross the blood–brain barrier (BBB) and undergo passive gastrointestinal absorption (Human Intestinal Absorption, HIA). It is observed that α-pinene has partial penetration into the BBB and low HIA absorption, in addition to not being subject to active efflux (PGP−, permeability glycoprotein).
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4. Discussion

Syzygium cumini, popularly known as “amora-preta” or “jambolão”, stands out for its applications in traditional folk medicine, indicating its pharmaceutical potential [16,19]. Several pharmacological activities are attributed to S. cumini, including antioxidant, antidiabetic, antidiarrheal, antiparasitic, and anti-inflammatory properties [32]. Antimicrobial activity has been reported in the essential oil of S. cumini leaves, tested against clinically relevant bacterial strains such as Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. These factors highlight the importance of S. cumini in medicine and pharmaceutical research as a product with potential to provide therapeutic benefits [33].

Antifungal effects were observed in extracts from S. cumini leaves against different Candida spp. strains, demonstrating the ability to inhibit fungal growth with minimum inhibitory concentrations (MICs) ranging from 31.25 to 125 μg/mL [34,35]. Antifungal activity was also observed in studies conducted with essential oil of S. cumini leaves (EOSC) against strains of Aspergillus flavus (MIC: 0.083 mg/mL) and Rhizopus solani (MIC: 0.127 mg/mL). These results were corroborated by Hanif et al. [22], who also highlighted the composition of EOSC, evidencing the significant presence of hydrocarbon monoterpenes (27.0%), oxygenated monoterpenes (26.27%), hydrocarbon sesquiterpenes (20.95%), and oxygenated sesquiterpenes (18.13%).

Some recent studies have investigated the potential effects of compounds present in the EOSC, among which α-pinene, myrcene, and limonene stand out. These compounds have been associated with a wide range of in vitro biological activities [16,20,23]. However, other relevant compounds were also detected, suggesting the presence of significant chemical diversity in this essential oil. It is important to note that there is a disparity in the results found in the literature, with different major compounds identified in EOSC. In addition to those previously mentioned, τ-cadinol (21.44%) [19], 5-methyl-1,3,6-heptatriene (4.90%) [22], iso-caryophyllene (18.01%) [36], cis-β-ocimene (27.98%) [17], caryophyllene oxide (17.24%) [37], β-caryophyllene (37.65%) [38], and α-pinene (21.09%) [39] are also reported.

This variety in results can be attributed to multiple factors that affect the production of phytochemicals in plant organs. These factors can be directly influenced by soil characteristics, local climate, genetic variations of the plants [19], temperature and humidity, as well as

Figure 5. BOILED-Egg from α-pinene (major constituent of the essential oil of Syzygium cumini).
interactions with pollinators, predators, and rainfall [38]. Additionally, variations in light intensity over time, associated with different seasons of the year [40], play an important role in influencing the production of chemical compounds in plants. It has been observed that harvesting and extracting essential oil at different times of the year results in variations in the quantity of present chemical compounds, accompanied by changes in non-living elements of the environment [41].

Based on the literature analysis, it is evident that the compound α-pinene exhibits fungicidal efficacy against yeasts of the genus Candida, indicating a possible relationship with the inhibition of the antimicrobial efflux pump of fungi, among other mechanisms of activity, the fungicidal action present in the genus Rhizopus [42,43]. Thus, suggesting a possible mechanism of action of the EOSC, since there is no research that addresses this aspect, according to Nóbrega et al. [44], it was observed that this monoterpane inhibited virulence, inhibiting pseudohyphae and the growth of the pathogens C. albicans and C. parapsilosis at concentrations ranging from 64 to 128 µg/mL, effectively reducing blastoconidia. Additionally, they highlight the fungicidal activity, as well as its ability to inhibit and disintegrate fungal biofilms, especially the virulence mechanisms of C. albicans [45].

The proven effectiveness of the compound α-pinene as an antifungal agent is widely attributed to its mode of action targeting fungal yeasts. Studies have indicated that this compound interacts significantly with the cell membrane, resulting in its rapid destabilization and subsequent rupture, leading to the leakage of intracellular content [46]. This phenomenon has been observed in fungal species of both Candida spp. and Venturia inaequalis, in addition to demonstrating antibacterial activity [47]. Additionally, a theory suggests a specific interaction between α-pinene and the ergosterol present in the cytoplasmic membrane of Candida yeasts, directly influencing the production and inhibition of fungal hyphae and pseudohyphae [45].

Research on nerol as a single compound to prospect its antifungal potential has revealed cell damage effects in the cell membrane of Saccharomyces cerevisiae fungi, resulting in the inhibition of cell budding and the alteration of the metabolic profile [48]. Regarding Aspergillus flavus, it was observed that at concentrations of 0.8 µL/mL, nerol completely inhibited growth, suggesting a negative impact on mycelia development and spore germination [49]. When tested against C. albicans, nerol demonstrated a MIC of 0.77 µL/mL, inducing apoptosis by damaging the cell membrane structure and increasing its permeability [50]. When directed to antiparasitic activity, there are certain gaps in this activity; however, Geraniol (cis isomer of nerol) presented an IC₅₀ of 171.48 µg/ml against T. vaginalis [51].

However, substantial evidence points to the potential of caryophyllene, isolated from the essential oil of Syzygium species leaves, as a highly effective antifungal agent, specifically targeting the fungal cell wall of Aspergillus fumigatus [52]. Additionally, other varieties of the genus Syzygium, such as S. aromaticum, have also demonstrated antifungal capacity, significantly inhibiting the growth of fungi and biofilm formation in strains of C. albicans, C. glabrata, and C. tropicalis, with efficacy comparable to fluconazole, a standard antifungal [47]. In research conducted with Rhizoctonia solani and Helminthosporium oryzae, caryophyllene exhibited more promising activity, with concentrations of 450 and 510 µg/ml, respectively, for the species [53].

The anti-T. vaginalis activity from EOSC has not been described yet, but our results were shown to be promising. Although scarce, studies aiming to evaluate the anti-T. vaginalis potential of essential oils have been conducted, and such compounds are promising for prospecting new drugs [51,54]. Natural products obtained from S. cumini exhibit activity against other medically important protozoa, such as fruit extracts that recently showed potential against Plasmodium falciparum (IC₅₀ < 10µg/ml) [55]. Additionally, α-pinene, a major component of EOSC, demonstrates potential against intracellular amastigote forms (IC₅₀: 15.6µg/mL) and promastigote forms (IC₅₀: 19.7 µg/mL) of Leishmania amazonensis [56].

Regarding the antiparasitic potential, these findings demonstrate the biotechnological
potential of *S. cumini* for applications in the pharmaceutical industry, especially to produce α-pinene.

A remarkable characteristic of essential oils is their hydrophobic nature. The lipophilic properties of EOs enable them to traverse cell membranes to interact with intracellular components, thus compromising cellular functions and inducing cell death by enhancing cytoplasmic permeability. Despite being poorly understood, the mechanisms by which EOs act against *T. vaginalis* are mainly related to membrane damage [57]. Indeed, exposure of the *T. vaginalis* Tv2 isolate to EO from *Amomum tsao-ko* and its major component (geraniol) resulted in damage to the plasma membrane and cytoplasmic leakage, as well as dilation of the endoplasmic reticulum and disintegration of other organelles [51]. During the experiments with EOSC, we did not observe trophozoites stained with trypan blue dye, suggesting a possible rupture of the parasite membrane. However, these findings require further investigation.

The infection caused by the parasite *T. vaginalis* is associated with severe clinical complications, including infertility, cervical and prostate cancer, gestational disorders, and increased HIV/AIDS acquisition [58]. The World Health Organization estimates 156 million new cases of trichomoniasis per year [6]. Trichomoniasis treatment is mainly based on the use of metronidazole, but adverse side effects are frequent. Additionally, resistant isolates of *T. vaginalis* have been documented worldwide, with metronidazole resistance estimated at around 10%, implying a number of 15 million people without therapeutic options [59]. Thus, new drugs for the treatment of trichomoniasis are highly needed, and natural products have gained prominence as therapeutic alternatives. Previous studies have shown that essential oils from Myrtaceae species have potential against *T. vaginalis* [31].

Our results corroborate with these studies and point to EOSC as promising in combating *T. vaginalis* (IC$_{50}$: 88.2 µg/mL), highlighting the need for studies using α-pinene against metronidazole-sensitive isolates.

In silico tests aid in the search for promising molecules in pharmaceutical production. It is possible to use tools like SwissADME Web to calculate fundamental parameters, both physicochemical and pharmacokinetic, pertaining to drugs, for one or multiple molecules, with statistically significant predictions, utilizing models such as BOILED-egg and the bioavailability radar [25]. Regarding the major compound of EOSC, α-pinene, the inactivation of enzymes such as CYP2C9 can lead to potential drug interactions, resulting in toxic action [60]. However, research indicates the absence of cytotoxic activities [45]. Additionally, based on molecular docking in silico testing, there is a hypothesis suggesting its potential for breast cancer treatment [61].

The preference for treatments using *S. cumini* is acceptable given its proven low toxicity, as evidenced in the studies by Everton et al. [36] and Everton et al. [33]. These studies found that EOSC does not exhibit significant toxicity towards the model organism *Artemia salina*. It shows activity even when tested at extremely high concentrations [62]. It is also noteworthy that α-pinene is highly promising for the treatment of human diseases as it has not demonstrated adverse toxicological effects, being considered a molecule of minimal toxicity, establishing itself as a safe option for therapeutic use [45,63].

5. Conclusions

We can conclude that the use of *Syzygium cumini* in traditional medicine to treat fungal infections caused by *Candida* is supported by scientific studies. Our results demonstrate that the EOSC contains α-pinene and nerol as its main phytochemical components. Moreover, there is promising evidence of its efficacy in combating infections caused by microorganisms and its ability to enhance the effect of fluconazole, indicating its usefulness as a complementary therapy. In addition, the anti- *Trichomonas vaginalis* activity of EOSC was reported for the first time in this study. It was observed that this product was effective against *T. vaginalis* in in vitro tests.

Additionally, α-pinene exhibited low toxicological actions in in silico predictive tests. Despite being limited, our study emphasizes the need for further research to evaluate the
in vivo cytotoxicity in mammalian cells, possible mechanisms of action, and synergistic properties of EOSC phytoconstituents.

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