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Phytochemical analysis and antimicrobial activity of *Cymbopogon citratus* essential oil and hydrosol against *Candida albicans*, *Escherichia coli*, and *Pseudomonas aeruginosa*

Análise fitoquímica e actividade antimicrobiana de óleo essencial e do hidrolato de *Cymbopogon citratus* frente a estirpes de *Candida albicans, Escherichia coli e Pseudomona aeruginosa*

Análisis fitoquímico y actividad antimicrobiana del óleo esencial e hidroloato de *Cymbopogon citratus* contra cepas de *Candida albicans, Escherichia coli* y *Pseudomona aeruginosa*

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Abstract
Background: Natural therapeutic agents, such as *Cymbopogon citratus* essential oil, have gained prominence for their potential antimicrobial properties. This study aimed to evaluate the antimicrobial activity of the essential oil and hydrolate of Cymbopogon against clinically relevant strains of Candida albicans, Escherichia coli, and Pseudomonas aeruginosa. Methods. Essential oil was extracted using the steam distillation method with a Clevenger apparatus, and prepared in three concentrations (50, 75 and 100%) using Dimethyl Sulfoxide. Component quantification was performed using gas chromatography coupled with mass spectrometry. Antimicrobial activity was evaluated using the Kirby-Bauer disk diffusion technique, employing discs impregnated with essential oil. Pure cultures of microorganisms, including Escherichia coli, Pseudomonas aeruginosa, and Candida albicans isolated from Leukorrhea samples, were utilized. The Welch Test was applied to analyze potential differences in inhibition zones of microorganisms in response to essential oil at different concentrations. Results: The essential oil exhibited a yield of 0.98%, with a yellow coloration, clear appearance, and pH of 5.65. Major compounds identified through gas chromatography coupled with mass spectrometry included Beta Myrcene, 6-Methyl-5-hepten-2-one, Ethanol, 2-(3,3-dimethylcyclohexylidene)-, (Z), (1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene, Isoneral, and Isogeranial. Antimicrobial activity was observed against all three tested species, where the complete inhibition of C. albicans growth was observed with an inhibitory concentration of 50%. However, the hydrosol exhibited modest antibacterial activity against the tested strains. Statistically significant differences in inhibition diameters were observed for the different studied concentrations ($p<0.05$). Conclusion: The *C. citratus* essential oil possesses effective antimicrobial activity, proposing a potential antimicrobial alternative. Therefore, the importance of future investigations to explore its therapeutic applicability is underscored.
Keywords: Antimicrobial activity; *Candida albicans*; *Cymbopogon citratus*; *Escherichia coli*; *Pseudomonas aeruginosa*.

Resumo

Introdução: Os agentes terapêuticos naturais, como o óleo essencial de Cymbopogon citratus, ganharam destaque pelas suas potenciais propriedades antimicrobianas. Este estudo teve como objetivo avaliar a atividade antimicrobiana do óleo essencial e do hidrolato de Cymbopogon contra estirpes clinicamente relevantes de Candida albicans, Escherichia coli e Pseudomonas aeruginosa. Métodos. O óleo essencial foi extraído utilizando o método de destilação a vapor com um aparelho Clevenger e preparado em três concentrações (50, 75 e 100%) utilizando dimetilsulfóxido. A quantificação dos componentes foi efectuada por cromatografia gasosa acoplada a espetrometria de massa. A atividade antimicrobiana foi avaliada utilizando a técnica de difusão em disco de Kirby-Bauer, empregando discos impregnados com óleo essencial. Foram utilizadas culturas puras de microorganismos, incluindo Escherichia coli, Pseudomonas aeruginosa e Candida albicans isoladas de amostras de leucorréia. O Teste de Welch foi aplicado para analisar potenciais diferenças nas zonas de inibição dos microrganismos em resposta ao óleo essencial em diferentes concentrações. Resultados: O óleo essencial apresentou um rendimento de 0,98%, com coloração amarela, aspeto límpido e pH de 5,65. Os principais compostos identificados através de cromatografia gasosa acoplada a espetrometria de massa incluíram Beta Mirceno, 6-Metil-5-hepten-2-ona, Etanol, 2-(3,3-dimetilciclohexilideno)-, (Z), (1R)-2,6,6-Trimetilbiciclo [3.1.1] hept-2-eno, Isoneral e Isogeranial. A atividade antimicrobiana foi observada contra as três espécies testadas, onde a inibição completa do crescimento de C. albicans foi observada com uma concentração inibitória de 50%. No entanto, o hidrossol exibiu uma atividade antibacteriana modesta contra as estirpes testadas. Foram observadas diferenças estatisticamente significativas nos diâmetros de inibição para as diferentes concentrações estudadas ($p<0,05$). Conclusão: O óleo essencial de C. citratus possui atividade antimicrobiana eficaz, propondo uma potencial alternativa antimicrobiana. Portanto, ressalta-se a importância de futuras investigações para explorar sua aplicabilidade terapêutica.

Palavras-chave: *Actividade antimicrobiana; Candida albicans; Cymbopogons citratus; Escherichia coli; Óleo essencial; Pseudomona aeruginosa.*

Resumen

Antecedentes: Los agentes terapéuticos naturales, como el aceite esencial de Cymbopogon citratus, han cobrado importancia por sus posibles propiedades antimicrobianas. El objetivo de este estudio era evaluar la actividad antimicrobiana del aceite esencial y el hidrolato de Cymbopogon contra cepas clínicamente relevantes de Candida albicans, Escherichia coli y Pseudomonas aeruginosa. Métodos. El aceite esencial se extrajo mediante el método de destilación al vapor con un aparato Clevenger, y se preparó en tres concentraciones (50, 75 y 100%) utilizando dimetilsulfóxido. La cuantificación de los componentes se realizó mediante cromatografía de gases acoplada a espectrometría de masas. La actividad antimicrobiana se evaluó mediante la técnica de difusión en disco de Kirby-Bauer, empleando discos impregnados con aceite esencial. Se utilizaron cultivos puros de microorganismos, incluidos Escherichia coli, Pseudomonas aeruginosa y Candida albicans aislados de muestras de leucorrea. Se aplicó la prueba de Welch para analizar las posibles diferencias en las zonas de inhibición de los microorganismos en respuesta al aceite esencial a distintas concentraciones. Resultados: El aceite esencial presentó un rendimiento del 0,98%, con una coloración amarilla, aspecto claro y pH de 5,65. Los principales compuestos identificados mediante cromatografía de gases acoplada a espectrometría de masas fueron: beta mirceno, 6-metil-5-hepten-2-ona, etanol, 2-(3,3-dimetilciclohexilideno)-, (Z), (1R)-2,6,6-trimetilbiciclo [3.1.1] hept-2-eno, isoneral e isogeranial. Se observó actividad antimicrobiana frente a las tres especies ensayadas, observándose la inhibición completa del crecimiento de C. albicans con una concentración inhibitoria del 50%. Sin embargo, el hidrosol mostró una modesta actividad antibacteriana contra las cepas ensayadas. Se observaron diferencias estadísticamente significativas en los diámetros de inhibición para las distintas concentraciones estudiadas ($p<0,05$). Conclusiones: El aceite esencial de C. citratus posee una eficaz actividad antimicrobiana, proponiendo una potencial alternativa antimicrobiana. Por lo tanto, se subraya la importancia de futuras investigaciones para explorar su aplicabilidad terapéutica.

Palabras clave: *Actividade antimicrobiana; Candida albicans; Cymbopogons citratus; Escherichia coli; Óleo essencial; Pseudomona aeruginosa.*

1. Introduction

Antimicrobial agents are crucial substances in the fight against various infectious diseases worldwide. However, due to the emergence of resistant strains of microorganisms, these agents are becoming increasingly ineffective or even powerless against a range of pathogens (Shin et al., 2018). Several factors contribute to the rise in antimicrobial resistance (AMR), with the primary one being the inappropriate and excessive use of antibiotics, which enables these pathogens to more easily develop resistance capabilities (Xavier et al., 2022). While antibiotics have helped prevent and treat infections, thereby increasing life expectancy globally, the rise of AMR now threatens the lives of many people worldwide (Moyo et al., 2023). Estimates suggest that approximately five million people died in 2019 from causes associated with AMR, with 1.3 million of these deaths

directly related to bacterial resistance globally. Sub-Saharan Africa (SSA), however, experienced the highest mortality rate, with around 1.1 million deaths linked to AMR. Approximately a quarter of a million deaths in this region were associated with AMR. The six main pathogens responsible for AMR-related deaths in SSA were Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Pseudomonas aureus, and Acinetobacter baumannii (Murray et al, 2022). It is also estimated that, each year, around 300 million people worldwide suffer from severe fungal infections (Denning, 2022). AMR poses a major problem for both health and the economy globally, especially in low- and middle-income countries (Naylor et al., 2018). The search for new antimicrobial agents is ongoing, and one promising option is antimicrobials derived from medicinal plants, whose mechanism of action differs from synthetic antibiotics and may be essential in treating diseases caused by resistant microbial strains (Abreu et al., 2012). Plants have been potential sources of new antibiotics and lead compounds in drug design and development, as they are less toxic and not associated with the side effects often observed in synthetic drugs (Masoko & Makgapeetja, 2015; Okigbo et al., 2009). The pursuit of solutions to address the challenge of multi-resistant bacterial infections has led to a growing interest in natural-source medicines (Subramaniam et al., 2020). In this context, Cymbopogon citratus, widely known as lemongrass or chablate, emerges as a promising alternative (Shah et al., 2011). Beyond its culinary uses, this plant has been the focus of studies due to its therapeutic properties, including antifungal, antidiarrheal, antibacterial, and anti-inflammatory activities, with its extract applicable to fresh and open wounds (Karkala Manvitha & Bhushan Bidya, 2014). The remarkable efficacy of lemongrass essential oil in inhibiting the growth of bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant Staphylococcus epidermidis (MRSE), and gram-negative bacteria underscores the potential of this substance as a promising weapon against antimicrobial resistance (Sharma et al., 2013). This study aimed to evaluate the antimicrobial activity of the essential oil and hydrolate of Cymbopogon against clinically relevant strains of Candida albicans, Escherichia coli, and Pseudomonas aeruginosa.

2. Methodology

In the essential oil extraction process, various methods can be used, such as hydrodistillation, maceration, solvent extraction, enfleurage, supercritical gases and microwaves. Among these, the most widely used is hydrodistillation, which is divided into two techniques - vapour drag. However, it is necessary to minimum knowledge of the theoretical approach of the distillation process and in the design of distillation equipment for the essential oil extraction, both on a laboratory and industrial scale(Santos et al., 2004).

Sample Collection and Botanical Description

The plant was cultivated in the Municipal District of Muatala, Nampula City, Nampula Province, Mozambique, in March 2022, with strict growth conditions and protection from physical damage. Sample collection took place in January 2023, in the early morning between 6 and 7 a.m.

Cymbopogon citratus, commonly known as lemongrass, is a versatile, tropical herb that grows in dense clusters, reaching up to 1.8 meters in height and about 1.2 meters in width, with a short rhizome. Its thin, long leaves make it one of the primary medicinal and aromatic plants cultivated in Algeria. Additionally, it is widely grown for its essential oil (EO) in tropical and subtropical regions of Asia, South America, and Africa (Boukhatem et al., 2014).

The botanical identification was conducted at the National Herbarium of Mozambique, part of the Agricultural Research Institute of Mozambique, where the plant's non-toxicity was also confirmed. This identification process, carried out in a specialized setting, ensures the accuracy and reliability of botanical and toxicological information associated with the studied plant.

Preparation of Plant Extract

Fresh *Cymbopogon citratus* plant material underwent initial sorting and was subsequently air-dried in shaded, ambient conditions for seven days. After drying, it was cut into small pieces. The essential oil extraction was conducted by steam distillation using a Clevenger apparatus in a 6000 mL volumetric flask. A total of 235.5 grams of dried plant material (equivalent to 244.6 grams of fresh material) was used, cut into small pieces. The distillation process lasted two hours. The obtained oil was filtered through anhydrous Na2SO4 crystals and stored in a sterile amber-colored tube, kept refrigerated at 4ºC in a light-protected, well-ventilated environment.

The extraction of the essential oil was conducted at the Ethnobotanical Research and Development Center Laboratories in Namaacha, Maputo City, Maputo Province, Mozambique.

Determination of Chemical Composition of Cymbopogon citratus Essential Oil

Analyses were conducted using a gas chromatograph (GC 7820A) coupled to a mass spectrometer (MSD 5977B) by Agilent Technologies. The components were separated on a 30-meter SH-Rtx-5MS capillary column with an internal diameter of 250 μm and a film thickness of 0.25 μm, operating within a temperature range of -60ºC to 330ºC. A 1 μL injection of the essential oil sample was made in splitless mode.

The carrier gas used was helium (99.9997%) at a flow rate of 35 ml/min and a pressure of 13.265 kPa. Analyses were conducted in scan mode (SCAN) with an ionization energy of 70 eV. The injection temperature was maintained at 250°C, and the oven temperature was initially set at 70ºC and held for 5 minutes. Subsequently, the oven temperature was increased to 190ºC at a rate of 30ºC/min and held for 20 minutes, then raised to 300ºC at 20ºC/min and held for 15 minutes. The total analysis runtime was 49.50 minutes.

Obtaining Strains and Antimicrobial Activity Assay

The strains used in this study were isolated from patients with different clinical conditions: Escherichia coli from patients with diarrhea, Pseudomonas aeruginosa from skin infections, and Candida albicans from cases of leucorrhea. Microorganism cultures were conducted at the Microbiology Laboratory of the Faculty of Medicine, Eduardo Mondlane University, Mozambique.

The antimicrobial activity evaluation of *Cymbopogon citratus* essential oil and hydrolate was conducted using the Kirby-Bauer disk diffusion technique (Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, 2009), following the M44-A2 document guidelines (Wayne, 2002). Pure cultures of microorganisms were tested, with bacterial growth observed between 18 and 24 hours and fungal growth between 24 and 48 hours. Mueller-Hinton Agar and Sabouraud Dextrose Agar were used as culture media. The activity assessment was performed at three different concentrations diluted in DMSO (dimethyl sulfoxide) (v/v): 50%, 75%, and undiluted (100%) for the essential oil, and a concentration expressed in g/L for the hydrolate.

Artificial antibiotic disks, such as Fluconazole 10 mcg, Nystatin 100 mcg, and Norfloxacin 10 mcg, were used as positive controls. DMSO 99.9% served as a negative control. The technique was performed in triplicate. The impregnated disks were placed on the surface of the medium where the test microorganism was incorporated. Plates were incubated at 37°C for 18-24 hours for bacteria and 24-48 hours for fungi. The inhibition zone diameters were subsequently measured using a calibrated caliper. The interpretation of inhibition zone diameters followed the criteria established by the Clinical and Laboratory Standards Institute (Wikler et al., 2008).

Statistical Analysis

Statistical analysis was conducted using one-way ANOVA (analysis of variance) and the Welch test for comparing means. Post hoc tests (Games-Howell) were applied for multiple mean comparisons. Data and residual normality were verified using the Ryan-Joiner test, similar to the Shapiro-Wilk test. Variances were assumed to be unequal. p-values below 0.05 were considered statistically significant. All statistical analyses were performed using Minitab software version 21.2.

3. Results

Essential Oil Analysis and Identification of Chemical Compounds by Mass Spectrometry

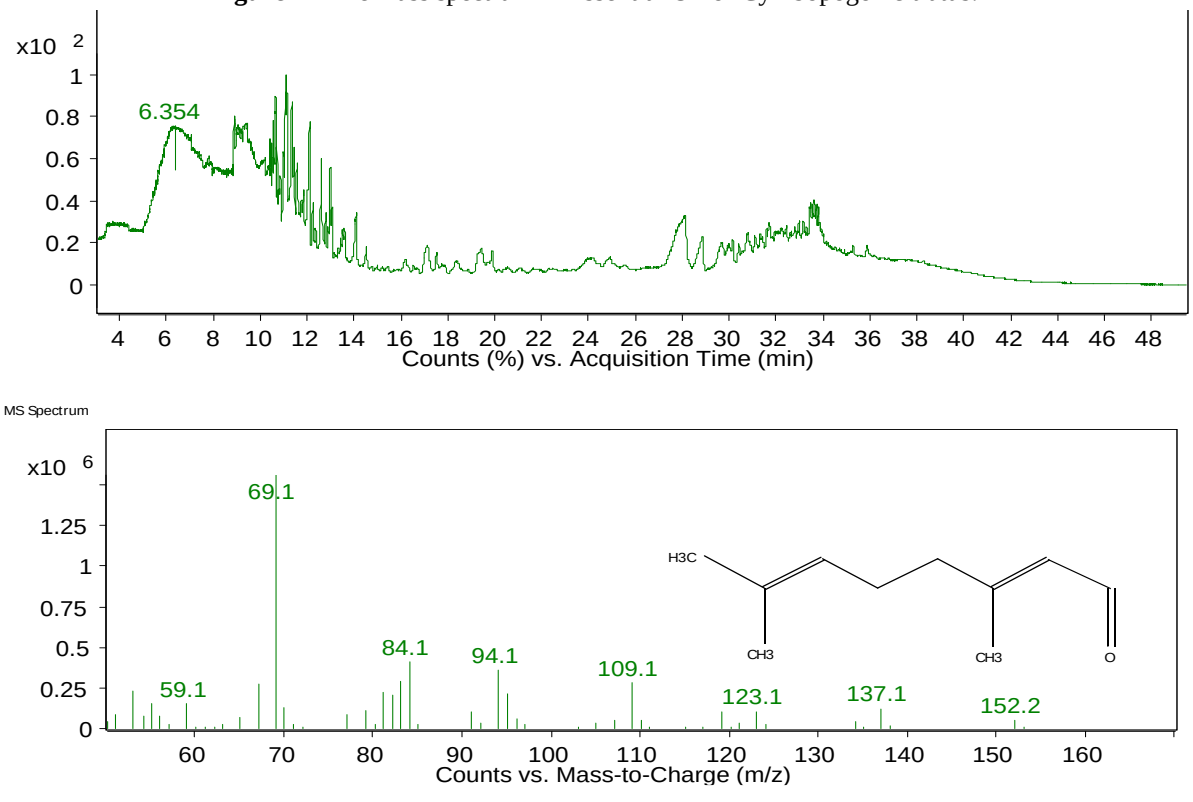
The essential oil obtained exhibited a yellowish color with a clear appearance and a characteristic odor. During the extraction process, a volume of 2.2991 mL was obtained, resulting in a yield of 0.98%, calculated based on dry weight. Compound identification was performed by comparing the spectra of their characteristic molecular ions (m/z) with those in the NIST 14 library, using an 80% z-score threshold. To ensure reliable identification, retention data were also considered, as shown in Table 1.

Table 1 - Major Compounds Identified in the Essential Oil of Cymbopogon citratus.

Compound	Formula	Retention Time (RT) (min)
Beta-Mircene	$C_{10}H_{16}$	12,61-13,33
6-Methyl-5-hepten-2-one	$C_8H_{14}O$	13,46-
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-eno	$C_{10}H_{16}$	14,13-15,22
Ethanol, 2-(3,3-dimethylcyclohexylidene)-, (Z)	$C_{10}H_{18}O$	18,54
Citral	$C_{10}H_{16}O$	18,80
Isogeranial	$C_{10}H_{16}O$	19,19

Source: NIST 14 library, adapted with Authors.

Figure 1 - The mass spectrum in Essential Oil of Cymbopogon citratus.



Source**:** NIST 14 library, adapted with Authors

The mass spectrum shows peaks with varying intensities, each corresponding to ions with a mass-to-charge ratio (m/z). Only peaks with relatively high intensities were considered.

Antimicrobial Activity Assay

As shown in Table 2, the essential oil at 100% concentration demonstrated an average antimicrobial activity against E. coli of 17.33 (± 5.8; 95% CI: 11.2, 23.4). However, this efficacy decreased at lower concentrations: 75% (16.5 ± 5.2) and 50% (14.8 ± 5.9). The hydrosol displayed lower activity (6.0 ± 3.0; 95% CI: 3.0, 9.1). Norfloxacin showed a mean activity of 23.0 ± 6.9 (95% CI: 15.8, 30.2). For P. aeruginosa, the essential oil at 100% concentration exhibited an average activity of 11.0 ± 4.1 (95% CI: 7.2, 14.8). At lower concentrations, the efficacy slightly decreased: at 75%, the average was 9.9 ± 3.4, and at 50%, it was 9.3 ± 3.5. The hydrosol showed minimal activity, with an average of 1.0 ± 2.7.

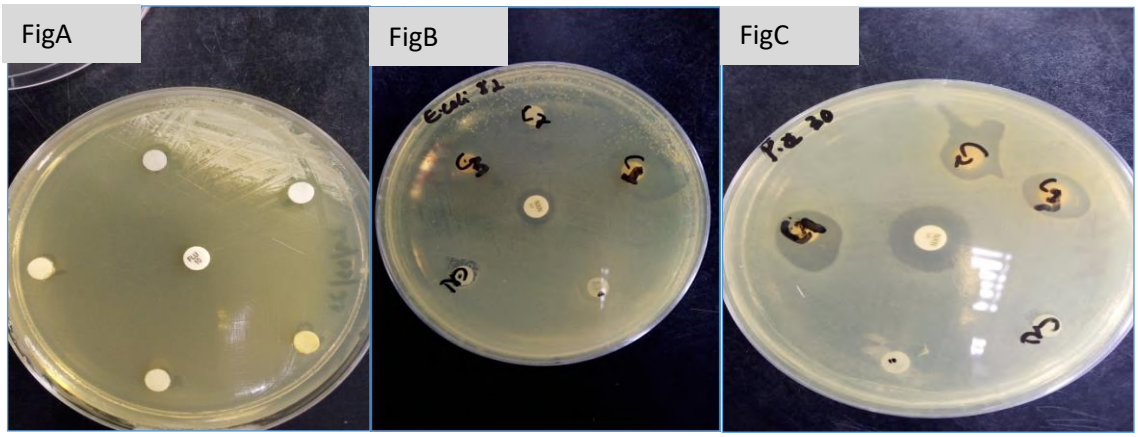
Table 2 - Antimicrobial Activity of Essential Oils against E. coli, P. aeruginosa, and Candida albicans.

Microorganisms			***E. coli***		***P. aeruginosa***	***Candida albicans***
Compounds		**N**	**x̄ ± SD (95% CI)**	**N**	**x̄ ± SD (95% CI)**	**x̄ ± SD (95% CI)**
Essential Oil	100%	6	17,33±5,8 (11,2; 23,4)	7	11,0 ± 4,1 (7,2; 14,8)	Full
	75%	6	16,5 ± 5,2 (11,0; 22,0)	7	9,9 ± 3,4 (6,7; 12,5)	Full
	50%	6	14,8 ± 5,9 (8,7; 21,0)	7	9,3 ± 3,5 (6,1; 12,5)	Full
Hydrosol		6	6,0 ± 3,0 (3,0; 9,1)	7	1,0 ± 2,7 (-1,5; 3,5)	Full
Dimethyl Sulfoxide		6	----------		----------	----------
Norfloxacin		6	23,0 ± 6,9 (15,8; 30,2)	7		Full

Note: Replace (N) with the actual values from the experimental data. This table summarizes the antimicrobial activity of essential oils at different concentrations (100%, 75%, and 50%) and hydrosol against E. coli, P. aeruginosa, and Candida albicans. The values include sample size (N), mean ± standard deviation (x̄ ± SD), and the 95% confidence interval (CI) for each measurement.

Source: Authors.

Figure 2 - Inhibition zones for Candida albicans (A), Escherichia coli (B), and Pseudomonas aeruginosa (C) in response to the essential oil and hydrosol of *C. citratus*.



Source: Authors.

In the minimum inhibitory concentration tests, the largest inhibition zone observed with both the essential oil and hydrosol was associated with the fungal species Candida albicans, as shown in Figure 2 (A). In comparison, the inhibition zones for Escherichia coli (disk B) were less pronounced, while those for Pseudomonas aeruginosa (disk C) were the smallest.

Comparative Analysis of Mean Concentrations

The assay evaluated the antimicrobial activity of different treatments (factor) at five levels: 100.00%, 75.00%, 50.00%, Hydrosol, and Norfloxacin. The Welch Test results indicated a statistically significant difference between groups (F-Value = 10.88, p-Value = 0.001). Based on the Games-Howell simultaneous tests for mean differences in colony-forming unit (CFU) counts (TSA) of E. coli across treatments, statistically significant differences were observed for Hydrosol vs. 100% essential oil (p-value = 0.020) and Hydrosol vs. 75% essential oil (p-value = 0.017). The hydrosol showed a significant reduction in CFU counts (95% CI = -20.72, -1.94; -19.02, -1.98). Norfloxacin had a significantly higher CFU count (5.98, 28.02), indicating a more pronounced antimicrobial effect compared to the hydrosol (p-value = 0.006).

Table 3 - Games-Howell Simultaneous Tests for Mean Differences in TSA for E. coli.

Level Comparison	**95% CI**	**t-value**	**p-value**
75% Essential Oil vs 100% Essential Oil	(-11,37; 9,70)	-0,26	0,999
50% Essential Oil vs 100% Essential Oil	(-13,57; 8,57)	-0,74	0,941
Hydrosol vs 100% Essential Oil	(-20,72; -1,94)	-4,25	0,020*
Norfloxacin vs 100% Essential Oil	(-6,50; 17,83)	1,54	0,562
50% Essential Oil vs 75% Essential Oil	(-12,23; 8,90)	-0,52	0,983
Hydrosol vs 75% Essential Oil	(-19,02; -1,98)	-4,27	0,017*
Norfloxacin vs 75% Essential Oil	(-5,28; 18,28)	1,84	0,406
Hydrosol vs 50% Essential Oil	(-18,26; 0,59)	-3,30	0,067
Norfloxacin vs 50% Essential Oil	(-4,02; 20,35)	2,22	0,250
Norfloxacin vs Hydrosol	(5,98; 28,02)	5,56	0,006*

Source: Authors.

The results of the Games-Howell simultaneous tests for mean differences in TSA for P. aeruginosa revealed several significant comparisons among the analyzed samples. First, the Hydrosol showed a statistically significant difference compared to the 100% concentration (CI: -15.11 to -4.89; $p<0.001$). Similarly, Norfloxacin also displayed a statistically significant difference compared to the 100% concentration (CI: 5.88 to 19.55; $p<0.001$), highlighting its efficacy.

When comparing the Hydrosol with the 75% concentration, a statistically significant difference was observed (CI: -13.27 to -4.43; $p<0.001$), reinforcing the distinct effect of the Hydrosol. Likewise, the comparison between Norfloxacin and the 75% concentration revealed a significant difference (CI: 7.37 to 20.36; $p<0.001$). A significant difference was also identified when comparing the Hydrosol with the 50% concentration (CI: -12.76 to -3.81; $p<0.001$), as well as in the comparison between Norfloxacin and the 50% concentration (CI: 7.91 to 20.94; $p<0.001$). Finally, the comparison between Norfloxacin and Hydrosol showed a highly significant difference (CI: 16.46 to 28.97; $p<0.001$), with Norfloxacin demonstrating a superior effect relative to the Hydrosol.

Table 4 - Games-Howell Simultaneous Tests for Mean Differences in TSA for P. aeruginosa.

Level Comparison	**95% CI**	**t-value**	**p-value**
75% Essential Oil vs 100% Essential Oil	(-6.62; 4.32)	-0,66	0,962
50% Essential Oil vs 100% Essential Oil	(-7.23; 3.80)	-0,97	0,862
Hydrosol vs 100% Essential Oil	(-15.11; -4.89)	-6,24	< 0,001*
Norfloxacin vs 100% Essential Oil	(5.88; 19.55)	5,84	< 0,001*
50% Essential Oil vs 75% Essential Oil	(-5.50; 4.37)	-0,36	0,996
Hydrosol vs 75% Essential Oil	(-13.27; 4.43)	-6,29	0,000*
Norfloxacin vs 75% Essential Oil	(7.37; 20.36)	6,81	< 0,001*
Hydrosol vs 50% Essential Oil	(-12.76; 3.81)	-5,82	< 0,001*
Norfloxacin vs 50% Essential Oil	(7.91; 20.94)	7,05	< 0,001*
Norfloxacin vs Hydrosol	(16.46; 28.97)	11,86	< 0,001*

Source: Authors.

4. Discussion

Previous studies have shown that gram-negative bacteria are generally less sensitive to the essential oil extract of *C. citratus* compared to gram-positive bacteria (Naik et al., 2010). However, in this study, the tested gram-negative strains showed susceptibility to *C. citratus* essential oil using the disk diffusion method. Antimicrobial activity was observed against all three tested species, with inhibition zones ranging from 15 mm to 17 mm for E. coli, 9 mm to 11 mm for P. aeruginosa, and complete inhibition of C. albicans growth, with a minimum inhibitory concentration of 50%. Inhibition zones between 8 mm and 13 mm are considered moderately active extracts, while zones larger than 14 mm are regarded as highly active (Mothana & Lindequist, 2005). Previous studies have demonstrated moderate antimicrobial effects of C. citratus extracts against E. coli (Boeira et al., 2020) and activity of the essential oil extract against P. aeruginosa (Subramaniam et al., 2020). One study also found relatively higher effects of the extract against C. albicans, with a moderate inhibition zone of 9 mm (Sherif et al., 2023).

The effects observed in this study may be attributed to the synergistic activity of the main component of *C. citratus*, pseudo-limonene, in combination with other antibacterial compounds present in the extract, such as D-limonene, ɣ-terpinene, citronellol, and hydrated sabinene (Han et al., 2019; Lopez-Romero et al., 2015; Ramos et al., 2011; Sonia et al., 2015; Sousa et al., 2022). Similarly, the antifungal activity of the essential oils may be attributed to the presence of D-limonene, ɣ-terpinene, and citronellol, as well as citral and carveol (Couto et al., 2015; Han et al., 2019; Lim et al., 2022). These effects are explained by interactions with bacterial cell structures, affecting their function (Saad et al., 2013), altering fatty acid composition (Di Pasqua et al., 2006), or changing surface physicochemical properties, such as hydrophobicity, electrical conductivity, soluble protein filtration, and reducing sugars (Li & Yu, 2015; Lopez-Romero et al., 2015). Additionally, modification of calcium and potassium ion channel activity can cause irreversible damage to cytoplasmic membranes, leading to microorganism death through ionic homeostasis imbalance. Membrane damage was identified in a study on the essential oil of C. citratus in E. coli (Saad et al., 2013), including cellular content coagulation (Siewe et al., 2015).

The activity against P. aeruginosa has been attributed to the presence of quercetin, which can increase the permeability of the microorganism's inner membrane and cause membrane potential loss (Jayaraman et al., 2010). Additionally, tannic acid has been reported to play a role in inhibiting acyl-homoserine lactones (AHLs) and pyocin production. Further studies are necessary to evaluate and confirm the efficacy of C. citratus essential oil against variable strains and its potential interactions with different antimicrobials.

5. Conclusion

This study demonstrated that the essential oil of *Cymbopogon citratus* is effective against various microbial strains, including gram-negative bacteria like E. coli and P. aeruginosa, as well as the fungus C. albicans. With inhibition zones ranging from moderately active to highly active, the essential oil shows promise as a natural antimicrobial agent. These findings suggest its potential to complement synthetic antibiotics and possibly offer alternatives in the context of increasing antimicrobial resistance. Further studies are necessary to evaluate and ensure the efficacy of *C. citratus* essential oil against variable strains and its potential interactions with different antimicrobials.

Declarations

Acknowledgments

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Authors' Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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Not applicable

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Informed Consent Statement

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