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ATIVIDADE ANTIMICROBIANA E PROSPECÇÃO FITOQUÍMICA DE NOZ-MOSCADA (*MYRISTICA FRAGRANS*) CONTRA *ESCHERICHIA COLI* E *CANDIDA ALBICANS IN VITRO*

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF NUTMEG (*MYRISTICA FRAGRANS*) AGAINST *ESCHERICHIA COLI* AND *CANDIDA ALBICANS IN VITRO*

ACTIVIDAD ANTIMICROBIANA Y ANÁLISIS FITOQUÍMICO DE LA NUEZ MOSCADA (*MYRISTICA FRAGRANS)* FRENTE A *ESCHERICHIA COLI* Y *CANDIDA ALBICANS IN VITRO*

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Resumo
A resistência antimicrobiana constitui um importante problema de saúde pública e tem impulsionado a investigação de produtos naturais como fontes de substâncias com potencial antimicrobiano. Nesse contexto, *Myristica fragrans* (noz-moscada) apresenta interesse em razão da diversidade de metabólitos secundários descritos para a espécie. Este estudo teve como objetivo avaliar a atividade antimicrobiana in vitro de uma preparação hidroalcoólica de *M. fragrans* frente às cepas de referência Escherichia coli ATCC 25922 e *Candida a*lbicans ATCC 10231, bem como realizar a triagem fitoquímica qualitativa de classes de metabólitos secundários. A preparação foi obtida por maceração do material vegetal pulverizado em etanol 70% (v/v), na razão inicial planta/solvente de 200 mg·mL⁻¹. A atividade antimicrobiana foi avaliada por microdiluição em caldo, com determinação da Concentração Inibitória Mínima (CIM), seguida da investigação da Concentração Bactericida Mínima (CBM) e da Concentração Fungicida Mínima (CFM). Foram observadas CIM ≤ 0,195 mg·mL⁻¹ para E. coli e CIM de 1,56 mg·mL⁻¹ para *C. albicans*, expressas como equivalentes da massa inicial de material vegetal por volume da preparação. Não foi demonstrado endpoint bactericida ou fungicida até a maior concentração avaliada. O controle de solvente apresentou inibição apenas em concentrações de etanol superiores aos residuais presentes nas diluições correspondentes às CIMs da preparação. Na triagem fitoquímica, foram observadas reações compatíveis com alcaloides e resultados sugestivos para flavonoides e taninos, estes últimos com discordância entre os testes qualitativos empregados. Os achados demonstram atividade antimicrobiana in vitro da preparação hidroalcoólica de *M. fragrans* frente às cepas de referência avaliadas e sustentam investigações adicionais para caracterização de seus constituintes e avaliação frente a isolados clínicos com diferentes perfis de suscetibilidade aos antimicrobianos.
Palavras-chave: Resistência Antimicrobiana; Atividade Antimicrobiana; Myristica fragrans.

Abstract
Antimicrobial resistance poses a major public health threat, driving the search for natural products as potential sources of novel antimicrobial agents. In this context, *Myristica fragrans* (nutmeg) is of particular interest due to the diversity of secondary metabolites reported for the species. This study evaluated the in vitro antimicrobial activity of a hydroalcoholic preparation of *M. fragrans* against the reference strains *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231, as well as its qualitative phytochemical profile. The extract was prepared by macerating pulverized plant material in 70% (v/v) ethanol at an initial plant-to-solvent ratio of 200 mg·mL⁻¹. Antimicrobial activity was assessed by broth microdilution to determine the minimum inhibitory concentration (MIC), followed by the evaluation of the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). The preparation exhibited an MIC ≤ 0.195 mg·mL⁻¹ against *E. coli* and an MIC of 1.56 mg·mL⁻¹ against *C. albicans*, expressed as initial plant mass equivalents per volume of preparation. No bactericidal or fungicidal endpoints were detected within the tested concentration range. The vehicle control showed growth inhibition only at ethanol concentrations exceeding the residual levels present in the active MIC dilutions. Phytochemical screening revealed positive reactions for alkaloids and suggestive results for flavonoids and tannins—the latter showing discrepancies between the qualitative assays employed. Overall, *M. fragrans* hydroalcoholic preparation against the evaluated reference strains, supporting further investigation into its active constituents and potential activity against clinical isolates with diverse antimicrobial susceptibility profiles.
Keywords: *Myristica fragrans*; Antimicrobial activity; Antimicrobial resistance.

Resumen
La resistencia a los antimicrobianos constituye un importante problema de salud pública y ha impulsado la investigación de productos naturales como fuentes de sustancias con potencial

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antimicrobiano. En este contexto, *Myristica fragrans* (nuez moscada) resulta de interés debido a la diversidad de metabolitos secundarios descritos para esta especie. El objetivo de este estudio fue evaluar la actividad antimicrobiana *in vitro* de una preparación hidroalcohólica de *M. fragrans* frente a las cepas de referencia *Escherichia coli* ATCC 25922 y *Candida albicans* ATCC 10231, así como realizar el tamizaje fitoquímico cualitativo de clases de metabolitos secundarios. La preparación se obtuvo mediante maceración del material vegetal pulverizado en etanol al 70% (v/v), con una proporción inicial planta/solvente de 200 mg·mL^{-1}. La actividad antimicrobiana se evaluó mediante microdilución en caldo para determinar la Concentración Mínima Inhibitoria (CMI), seguida de la determinación de la Concentración Mínima Bactericida (CMB) y la Concentración Mínima Fungicida (CMF). Se observaron valores de CMI ≤ 0,195 mg·mL^{-1} para *E. coli* y de 1,56 mg·mL^{-1} para *C. albicans*, expresados como equivalentes de la masa inicial de material vegetal por volumen de la preparación. No se demostró un punto final bactericida o fungicida hasta la mayor concentración evaluada. El control del solvente mostró inhibición únicamente a concentraciones de etanol superiores a las concentraciones residuales presentes en las diluciones correspondientes a las CMI de la preparación. El tamizaje fitoquímico mostró reacciones compatibles con alcaloides y resultados sugestivos para flavonoides y taninos, estos últimos con discordancia entre las pruebas cualitativas empleadas. Los hallazgos demuestran actividad antimicrobiana *in vitro* de la preparación hidroalcohólica de *M. fragrans* frente a las cepas de referencia evaluadas y respaldan la realización de investigaciones adicionales para caracterizar sus constituyentes y evaluar su actividad frente a aislados clínicos con diferentes perfiles de susceptibilidad a los antimicrobianos.
Palabras clave: *Myristica fragrans*; actividad antimicrobiana; resistencia a los antimicrobianos.

1. Introduction

Antimicrobial resistance (AMR) occurs when microorganisms become able to survive exposure to therapeutic agents that were previously effective. This resistance can manifest as either intrinsic, representing a natural characteristic of a given species, or acquired, arising from the horizontal transfer of resistance genes (Sinha *et al*., 2025).

Although AMR is a naturally occurring phenomenon, anthropogenic factors have markedly accelerated this process—predominantly through the indiscriminate use of antimicrobial agents, which exerts selective pressure and drives the emergence and clonal expansion of resistant strains (Ndaki *et al*., 2025). Within this landscape, global forecasts suggest that AMR could be responsible for up to 10 million deaths annually by 2050, further establishing it as a critical global public health priority (GBD 2021 Antimicrobial Resistance Collaborators, 2024).

Among the pathogens of critical concern regarding antimicrobial resistance, *Escherichia coli* stands out as a major Gram-negative bacterium. Although a commensal member of the intestinal microbiota, it is implicated in a

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diverse range of clinical conditions, including diarrhea, colitis, urinary tract infections, and waterborne and foodborne illness (Geurtsen, 2022; Seixas & Muttoni, 2022). Numerous studies have reported resistance in *E. coli* to commonly used first-line antibiotics, notably penicillins, cephalosporins, quinolones, and sulfonamides (El Aila & El Aish, 2025; Tano *et al*., 2026).

Another clinically relevant microorganism is *Candida albicans*, a yeast species that commonly resides as a commensal organism in the human oral, genital, and gastrointestinal microbiota. However, *C. albicans* also acts as an opportunistic pathogen, shifting toward a pathogenic state under conditions such as dysbiosis, pH alterations, immunosuppression, prolonged antibiotic therapy, and hormonal fluctuations (Lopes & Lionakis, 2022). Furthermore, this pathogen possesses well-established virulence factors and molecular mechanisms that contribute to reduced antifungal susceptibility and the development of resistance to diverse antifungal classes (Vieira & Santos, 2025).

Given this scenario, effective therapeutic options are increasingly constrained, as the discovery of novel antimicrobials has failed to keep pace with the accelerating emergence of AMR (Sartori *et al*., 2026). Brüssow (2024) argues that the development pipeline for novel antimicrobials is hindered by pharmaceutical industry priorities that favor other therapeutic classes, as well as by persistent financial constraints faced by academic institutions in advancing translational research.

Consequently, the discovery and development of new therapeutic alternatives are increasingly necessary. A promising candidate is *Myristica fragrans*, commonly known as nutmeg—a widely commercialized culinary spice valued for its organoleptic properties, including its flavor and color. Beyond its dietary applications, *M. fragrans* demonstrates considerable therapeutic potential across diverse physiological systems, particularly due to its well-documented antimicrobial activity (Al-Rawi *et al*., 2024; Barman *et al*., 2021; Qi *et al*., 2025).

Accordingly, this study aimed to evaluate the in vitro antimicrobial activity of a hydroalcoholic extract of *Myristica fragrans* against the reference strains *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231, and to

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qualitatively screen for the major classes of secondary metabolites present in the preparation.

2. METHODS

Extract preparation

Nutmeg (*Myristica fragrans*) was obtained as a finely ground powder from a local retail market in Castanhal, Pará, Brazil (1°17′41.4″ S, 47°55′36.1″ W). The raw material corresponded to a locally sourced artisanal commercial product without industrial packaging or traceability information regarding the manufacturer, batch number, or expiration date. Because the plant material was acquired in a pre-pulverized form, botanical authentication could not be performed, representing a limitation regarding the traceability and taxonomic verification of the raw material.

To prepare the extractive solution, 6 g of pulverized nutmeg was suspended in 30 mL of 70% (v/v) ethanol, corresponding to an initial plant-to-solvent ratio of 200 mg of crude plant material per milliliter of solvent. The mixture was protected from light and heat and subjected to static maceration for seven days. Subsequently, the extract was filtered and stored under controlled conditions until use in the subsequent experimental assays.

Ethanol was not removed following extraction, and therefore remained in the final preparation used in the microbiological assays. Because solvent evaporation followed by gravimetric quantification of the dry extract yield was not performed, the concentration of 200 $mg \cdot mL^{-1}$ represents the initial ratio of crude plant material to solvent volume rather than the true dry extract concentration. Consequently, the extraction yield could not be determined.

Inoculum preparation

The reference strains *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) were resuspended in 5 mL of brain heart infusion (BHI) broth and incubated at 37 °C for 24 h. Following incubation, an aliquot of each culture was subcultured onto MacConkey agar (selective for Gram-negative

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bacilli) and Sabouraud dextrose agar (selective for fungi), respectively, and incubated under the same conditions to obtain isolated colonies.

Isolated colonies were suspended in sterile 0,85% physiological saline and adjusted to a turbidity equivalent to 0.5 McFarland, corresponding to approximately 2,0 × 10^6 CFU·mL^{-1} under the experimental conditions employed. Subsequently, a 10 µL aliquot of this standardized suspension was inoculated into each well, yielding an estimated final inoculum concentration of approximately 9,5 × 10^4 CFU·mL^{-1}

Minimum Inhibitory Concentration

To determine the minimum inhibitory concentration (MIC), defined as the lowest concentration that completely inhibits visible microbial growth, the entire assay was performed in two independent biological experiments conducted on separate days. In each experiment, two 96-well microplates were used, with each experimental condition evaluated in technical duplicate per plate.

Initially, 100 µL of BHI broth was dispensed into each well, followed by the addition of 100 µL of the nutmeg extract for twofold serial dilution. Brain heart infusion broth was selected as the growth medium for the microdilution assay because of its nutrient-rich composition and established ability to support robust growth of both the bacterial and yeast reference strains under the experimental conditions used.

Concentrations across the dilution series were expressed as initial plant biomass equivalents per volume of preparation, ranging from 100 to 0.195 mg·mL^{-1}. Due to the retention of ethanol in the hydroalcoholic preparation, its final concentration decreased progressively throughout the serial dilution, ranging from approximately 33.3% (v/v) in the first well to 0.065% (v/v) in the final well following inoculum addition. Subsequently, 10 µL of each standardized microbial inoculum was added to the respective wells.

For sterility control, uninoculated BHI broth alone was used, whereas growth (viability) control wells received 10 µL of the respective microbial suspension in BHI broth. For the vehicle (solvent) control, 70% (v/v) ethanol was

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subjected to the same twofold serial dilution scheme used for the *M. fragrans* preparation, with equivalent volumes and under identical experimental conditions, followed by the inoculation of 10 µL of the respective microbial suspensions.

Streptomycin and fluconazole were employed as positive reference controls for *E. coli* ATCC 25922 and *C. albicans* ATCC 10231, respectively. In addition, an uninoculated extract control, comprising the *M. fragrans* extract in BHI broth without microbial inoculation, was included and subjected to the same experimental conditions. Specific resazurin controls were also evaluated to confirm dye stability under the assay conditions and to exclude potential colorimetric interference during the reading.

The microplates were subsequently incubated at 37 °C for 24 h. To assess microbial viability and antimicrobial activity, 20 µL of resazurin solution was added to each well, followed by a 20-min incubation period for color development. Metabolically active (viable) microbial cells reduce the blue, non-fluorescent resazurin to pink, highly fluorescent resorufin; thus, a persistent blue coloration indicated growth inhibition, whereas a color transition to pink indicated microbial growth.

The broth microdilution assay was performed for both *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 following the identical extract dilution protocol. Species-specific cultivation and inoculum preparation parameters were maintained as described above.

Minimum Bactericidal and Fungicidal Concentrations

To determine the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), 100-µL aliquots were collected from the three highest concentrations in the dilution series (100, 50, and 25 $mg \cdot mL^{-1}$). For *E. coli*, aliquots were plated onto BHI agar, whereas Sabouraud dextrose agar was used for *C. albicans*. The aliquots were spread evenly across the agar surface using a sterile Drigalski spatula, and the plates were incubated at 37 °C for 24 h. Following incubation, plates were examined for the presence or absence of

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colony growth. The complete absence of microbial growth was defined as the criterion for bactericidal or fungicidal activity, depending on the tested organism.

Qualitative phytochemical screening

Finally, qualitative phytochemical assays were conducted on the nutmeg extract to screen for the major classes of secondary metabolites present in M. fragrans. The extract was pre-diluted to 10 $mg \cdot mL^{-1}$ to standardize the reaction conditions and minimize potential analytical interference.

All phytochemical tests were performed alongside appropriate controls, including reagent blanks and extract blanks, to ensure reliable interpretation of color changes, precipitate formation, and fluorescence alterations observed during the reactions.

Table 1: Qualitative phytochemical screening assays performed on *M. fragrans*.

Target metabolite	Assay	Reagents	Expected result	Reference
Alkaloids	Mayer's test	HCl 5% + Mayer's reagent	White precipitate or turbidity	Souza (2020); Henriques e Almeida (2013)
	Wagner's test	HCl 5% + Wagner's reagent	Orange-brown precipitate	
Flavonoids	Pew's test (Zinc reduction)	Methanol + Metallic zinc + Concentrated HCl	Red coloration	Silva *et al*. (2018); Sofiati (2009)
	Ferric chloride test	$FeCl_3$ (2%)	Green, yellow, or violet coloration	
	Sodium hydroxide test	NaOH (20%)	Yellow coloration	

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Tanins	Gelatin test	HCl 5% + 2,5% Gelatin	Precipitate formation	Souza (2020); Sofiati (2009)
	Ferric salts test	$FeCl_3$ (10%)	Blue-black (hydrolyzable) or Green-brown (condensed)	
	Lead acetate test	10% Acetic acid + 10% Lead acetate	Off-white precipitate (hydrolyzable tannins)	
Phenols	Colorimetric $FeCl_3$ test	Distilled water + 1% $FeCl_3$	Color change or precipitation	Souza (2020); Henriques e Almeida (2013)
Saponins	Froth (Foam) index test	Distilled water + Vigorous vortex agitation	Persistent foam column (>15 min)	Henriques e Almeida (2013)
Coumarins	UV fluorescence test	10% NaOH + Filter paper + UV light (365 nm)	Blue or green fluorescence	Costa (2011)
Terpenoids	Salkowski's test	Chloroform + Concentrated H_2SO_4	Red or reddish-brown coloration	Sasidharan *et al*. (2011)

RESULTS AND DISCUSSION

The *Myristica fragrans* hydroalcoholic preparation exhibited in vitro antimicrobial activity against the reference strains *Escherichia coli* ATCC 25922

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and *Candida albicans* ATCC 10231, as demonstrated by visible growth inhibition within the tested concentration ranges (Table 2).

Table 2: Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) determinations of the *M. fragrans* hydroalcoholic preparation.

Strain	MIC	MBC/MFC
E. coli	≤ 0,195 mg·mL^{-1}	MBC > 100 mg·mL^{-1}
C. albicans	1,56 mg·mL^{-1}	MFC > 100 mg·mL^{-1}

* Note: Concentrations are expressed as initial plant biomass equivalents per volume of preparation (mg·mL^{-1}), as dry extract mass was not determined. MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration. Values > 100 mg·mL^{-1} indicate that no bactericidal or fungicidal endpoint was reached within the maximum concentration evaluated.

Although the antimicrobial properties of *M. fragrans* are widely documented in the literature (Kaur *et al*., 2025; Rezende *et al*., 2021), there is a notable scarcity of studies evaluating crude plant extracts, as most published reports focus exclusively on essential oils. Consequently, the present study contributes to addressing this knowledge gap by evaluating the antimicrobial activity of a crude *M. fragrans* extract, thereby broadening the pharmacological understanding of non-volatile and whole-extract fractions from this species.

Investigating the antimicrobial activity of alcoholic and hydroalcoholic extracts represents a viable and efficient strategy for extracting plant secondary metabolites, consistent with the approach adopted in this study. In this regard, Bodoira & Maestri (2020) highlighted the critical role of alcoholic solvent systems in enhancing the extraction efficiency of bioactive compounds from nuts and other botanical matrices. Furthermore, Anjum *et al.* (2024) demonstrated that ethanolic extraction substantially increased both the chemical diversity and overall yield of secondary metabolites compared to aqueous extracts of *M. fragrans*.

In the vehicle control assay, growth inhibition of *E. coli* was observed at ethanol concentrations of 8.3% (v/v) and above, whereas for *C. albicans*, inhibition occurred at 33.3% (v/v). In contrast, the MICs of the *M. fragrans* preparation corresponded to substantially lower residual ethanol concentrations, approximately 0.065% (v/v) for *E. coli* and 0.52% (v/v) for *C. albicans*. These

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findings indicate that, at the concentrations corresponding to the preparation's MICs, ethanol alone was insufficient to produce the observed antimicrobial inhibition.

Under the evaluated experimental conditions, the *M. fragrans* preparation inhibited the growth of both *E. coli* and *C. albicans*, with no bactericidal or fungicidal endpoint reached at the maximum concentration tested.

The antimicrobial activity of *M. fragrans* has also been well documented in studies evaluating its essential oils. For instance, Al-Mariri & Safi (2024) and Rezende *et al*. (2022) reported activity against *E. coli*, whereas Özkan *et al*. (2018) demonstrated antifungal activity against *C. albicans*. These findings support the broad antimicrobial potential of different preparations derived from *M. fragrans*. Nevertheless, direct comparisons between MIC values must be made with caution, as hydroalcoholic extracts and essential oils differ substantially in their phytochemical composition and physicochemical properties. Furthermore, variations in reference strains, culture media, inoculum sizes, and specific assay parameters across different studies inherently preclude direct quantitative equivalence.

This finding is of clinical relevance because *E. coli* represents a major etiologic agent of gastrointestinal, urinary tract, and healthcare-associated infections, with its clinical impact further compounded by its propensity to develop multidrug resistance to commonly used antimicrobial agents (Grome *et al.*, 2026). Although the present investigation was conducted using a standard reference strain of *E. coli*, this result supports the need for future studies involving clinical isolates, particularly those harboring multidrug-resistant phenotypes.

The relevance of this finding extends to *C. albicans*, particularly relevant given that the World Health Organization (WHO, 2022) classifies *C. albicans* as a critical fungal threat to global public health. This opportunistic pathogen causes a wide spectrum of infections ranging from superficial mucosal candidiasis to life-threatening invasive candidemia associated with substantial mortality (Cornely *et al*., 2025). Furthermore, *C. albicans* possesses diverse resistance mechanisms against commercially available antifungal agents, significantly constraining

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current clinical treatment options and complicating infection management (Vieira & Santos, 2025).

In this context, the observed antimicrobial activity represents a preliminary finding that warrants further investigation of *M. fragrans* preparations. However, because this study evaluated standard reference strains, these outcomes cannot be directly extrapolated to antimicrobial-resistant microorganisms. Subsequent studies using clinical isolates with well-characterized resistance phenotypes are needed to determine whether this activity is retained against resistant strains and to further assess its therapeutic relevance.

The results of the qualitative phytochemical screening of the nutmeg extract are presented below:

Table 3: Qualitative phytochemical screening of the *M. fragrans* hydroalcoholic extract.

Metabolite	Assay	Result
Alkaloids	Mayer's test	+
	Wagner's test	+
	Pew's test	-
Flavonoids	Ferric chloride test	+
	Sodium hydroxide test	+
Tannins	Gelatin's test	-
	Ferric salts test	-
	Lead acetate test	+
Phenols	Colorimetric $FeCl_3$ test	+
Saponins	Froth (Foam) index test	-
Coumarins	UV fluorescence test	-
Terpenoids	Salkowski's test	-

* Note: (+), positive reaction in the qualitative assay; (−), negative reaction in the qualitative assay. The determination of each secondary metabolite class was based on the collective interpretation of all corresponding assays performed.

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Phytochemical screening revealed a positive reaction for alkaloids and suggestive evidence of flavonoids, as indicated by positive responses in both the ferric chloride and sodium hydroxide assays alongside a negative Pew's test. These findings are consistent with previous reports describing the presence of bioactive constituents in nutmeg (Barman *et al*., 2021; Wong *et al*., 2025; Luo *et al*., 2026).

Regarding tannins, suggestive evidence of their presence was observed, marked by a positive reaction in the lead acetate assay, despite negative outcomes in the gelatin and ferric chloride tests.

Alkaloids are nitrogenous secondary metabolites that may exhibit substantial antimicrobial activity through multiple mechanisms targeting bacterial and fungal cellular pathways; this broad-spectrum activity has been documented across multiple plant species (Zielińska *et al*., 2019; Fan *et al*., 2023; Qi *et al*., 2024).

The qualitative phytochemical profiling conducted in this study confirmed the presence of alkaloids in *M. fragrans*. The literature describes several mechanisms by which this class of metabolites may exert antimicrobial activity, including the inhibition of nucleic acid and protein synthesis, disruption of cell membrane permeability, and interference with bacterial cell division (Liang *et al*., 2022; Boberek *et al*., 2010; Silva *et al*., 2016; Thawabteh *et al*., 2024).

Furthermore, alkaloids have been reported to exhibit antifungal activity through mechanisms involving interference with cell wall biosynthesis and inhibition of spore, germ tube, and biofilm formation, thereby potentially attenuating fungal virulence and promoting cell lysis (Ding *et al.,* 2024).

The phytochemical screening also yielded suggestive evidence for the presence of flavonoids and tannins. Both metabolite classes have well-documented biological activities, including antimicrobial effects; however, their individual contributions to the antimicrobial activity observed in this study cannot be conclusively established based solely on these qualitative assays.

The suggestive evidence for flavonoids is consistent with previous reports characterizing this metabolite class in *M. fragrans*. Specifically, Trifan *et al*. (2023)

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and Moirangthem *et al*. (2024) reported the presence of flavonoids in the phytochemical analyses of *M. fragrans* extracts.

This metabolite class may exert antimicrobial effects through diverse mechanisms, disrupting bacterial metabolism, inhibiting key enzymes, alternation of outer membrane porin permeability, and interference with processes essential for bacterial survival (Tan *et al*., 2022; Xie *et al*., 2015). Furthermore, flavonoids may interfere with DNA and RNA biosynthesis, thereby suppressing bacterial replication (Yan *et al*., 2024).

Wang *et al*. (2023) demonstrated that tannins downregulate key proteins involved in peptidoglycan biosynthesis and outer membrane porin assembly, thereby compromising cell wall integrity and impairing the transport of essential nutrients. Furthermore, tannins have been shown to induce substantial perturbations in the bacterial metabolome, disrupting metabolic pathways critical for cellular survival (Virtanen *et al*., 2023).

In addition, Long *et al*. (2024) and Moreira *et al*. (2024) indicated that tannins may inhibit biofilm formation in both bacterial and fungal cells. This described mechanism is particularly relevant to our findings, as *C. albicans* exhibits a high capacity for biofilm production, which can limit antifungal penetration and contribute to increased recalcitrant to therapy (Fan *et al*., 2022).

Thus, the suggestive evidence for tannins indicates that this class warrants consideration in subsequent phytochemical and bioactivity studies, without establishing a direct causal contribution to the antimicrobial activity observed in the present study.

Although these metabolite classes have well-described antimicrobial activity in the literature, our findings do not permit the establishment of a direct causal relationship between the specific constituents detected by the qualitative screening and the observed antimicrobial effects, as these individual compounds were neither isolated nor evaluated separately.

A key limitation of this study is the lack of quantitative phytochemical profiling and chromatographic fractionation to isolate and characterize individual bioactive constituents, which precluded the assessment of isolated compounds

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and their potential synergistic interactions. Regarding future perspectives, subsequent investigations should evaluate the efficacy of *Myristica fragrans* extracts against multidrug-resistant clinical and field isolates. Ultimately, we anticipate that this study will contribute to the ongoing bioprospecting of plant-derived therapeutic alternatives to mitigate AMR.

Additionally, the use of commercially sourced, pre-pulverized plant material without lot traceability, documented geographical origin, or independent botanical authentication limits the chemical reproducibility and botanical traceability of the evaluated preparation. Furthermore, the absence of dry weight determination and extraction yield quantification constrains the quantitative standardization of the preparation, precluding direct concentration-dependent comparisons with studies reporting antimicrobial activity in terms of dry extract mass.

A further limitation of this study was the lack of in vitro cytotoxicity assays using mammalian cell lines, which prevented the calculation of the selectivity index (SI) and limited definitive assessment of the safety profile and therapeutic selectivity of the preparation at the antimicrobial concentrations evaluated.

4. Conclusion

The present study demonstrated that the hydroalcoholic preparation of *Myristica fragrans* exhibited in vitro antimicrobial activity against the reference strains *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231, as evidenced by growth inhibition under the evaluated conditions, with no bactericidal or fungicidal endpoint observed up to the highest concentration tested. Qualitative phytochemical screening revealed the presence of alkaloids and suggestive reactions for flavonoids and tannins, highlighting secondary metabolite classes of interest for subsequent investigations. These findings support further research on *M. fragrans* preparations, particularly focusing on detailed chemical characterization, cytotoxicity profiling, and bioactivity assays against clinical isolates with diverse antimicrobial susceptibility profiles.

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Received: 13/08/2026 - **Accepted:** 12/09/2026
Vol: 13.19
DOI: 10.66104/mph2hq73
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5. References

AL-RAWI, S. S. *et al.* Therapeutic and pharmacological prospects of nutmeg seed: A comprehensive review for novel drug potential insights. **Saudi Pharmaceutical Journal**, v. 32, n. 6, p. 102067, jun. 2024. DOI: https://doi.org/10.1016/j.jsps.2024.102067.

BARMAN, R. *et al.* Nutmegs and wild nutmegs: An update on ethnomedicines, phytochemicals, pharmacology, and toxicity of the Myristicaceae species. **Phytotherapy Research**, v. 35, n. 9, p. 4632–4659, set. 2021. DOI: https://doi.org/10.1002/ptr.7098.

BHATTACHARYA, S.; SOBEL, J. D.; WHITE, T. C. A Combination Fluorescence Assay Demonstrates Increased Efflux Pump Activity as a Resistance Mechanism in Azole-Resistant Vaginal Candida albicans Isolates. **Antimicrobial Agents and Chemotherapy**, v. 60, n. 10, p. 5858-5866, set. 2016. DOI: https://doi.org/10.1128/AAC.01252-16.

BOBEREK, J. M.; STACH, J.; GOOD, L. Genetic evidence for inhibition of bacterial division protein FtsZ by berberine. **PLoS ONE**, v. 5, n. 10, e13745, out. 2010. DOI: https://doi.org/10.1371/journal.pone.0013745.

BODOIRA, R.; MAESTRI, D. Phenolic compounds from nuts: extraction, chemical profiles, and bioactivity. **Journal of Agricultural and Food Chemistry**, v. 68, n. 4, p. 927–942, 2020. DOI: https://doi.org/10.1021/acs.jafc.9b07160.

BROWN, GORDON D. *et al.* The pathobiology of human fungal infections. **Nature Reviews Microbiology**, Londres, v. 22, p. 687–704, 2024. DOI: https://doi.org/10.1038/s41579-024-01062-w.

BRÜSSOW, H. The antibiotic resistance crisis and the development of new antibiotics. **Microbial Biotechnology**, v. 17, n. 7, e14510, jul. 2024. DOI: https://doi.org/10.1111/1751-7915.14510.

CORNELY, O. A. *et al.* Global guideline for the diagnosis and management of candidiasis: an initiative of the ECMM in cooperation with ISHAM and ASM. **The Lancet Infectious Diseases**, v. 25, n. 5, p. e280–e293, 2025. DOI: https://doi.org/10.1016/S1473-3099(24)00749-7.

COSTA, T. A. de C. **Perfil fitoquímico de materiais biológicos usados em dessalinizador caseiro de água salobra**. 2011. 116 f. Dissertação (Mestrado em Química) - Instituto de Química, Universidade Federal da Bahia, Salvador,

CC BY

REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 12/09/2026
Vol: 13.19
DOI: 10.66104/mph2hq73
Pages: 1-21

2011. Disponível em: https://repositorio.ufba.br/bitstream/ri/10676/1/Disserta%C3%A7%C3%A3o%20Tadeu%20Ant%C3%B4nio%20de%20Campos%20Costa.pdf. Acesso em: 17 jul. 2026.

DING, J. *et al.* Inhibitory effects of berberine on fungal growth, biofilm formation, virulence, and drug resistance as an antifungal drug and adjuvant with prospects for future applications. **World Journal of Microbiology and Biotechnology**, v. 41, n. 1, art. 5, dez. 2024. DOI: https://doi.org/10.1007/s11274-024-04223-4.

EL AILA, N. A.; EL AISH, K. I. A. Six-year antimicrobial resistance patterns of Escherichia coli isolates from different hospitals in Gaza, Palestine. **BMC Microbiology**, v. 25, n. 1, p. 559, ago. 2025. DOI: https://doi.org/10.1186/s12866-025-04335-3.

FAN, F. *et al.* Candida albicans biofilms: antifungal resistance, immune evasion, and emerging therapeutic strategies. **International Journal of Antimicrobial Agents**, v. 60, n. 5-6, p. 106673, 2022. DOI: https://doi.org/10.1016/j.ijantimicag.2022.106673.

FAN, K. *et al.* Antimicrobial indole alkaloids from Tabernaemontana corymbosa. **Chinese Journal of Natural Medicines**, v. 21, n. 2, p. 146-153, 2023. DOI: https://doi.org/10.1016/S1875-5364(23)60393-0.
GBD 2021 ANTIMICROBIAL RESISTANCE COLLABORATORS. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. **The Lancet**, v. 404, n. 10459, p. 1199-1226, set. 2024. DOI: https://doi.org/10.1016/S0140-6736(24)01867-1.

GEURTSEN, JEROEN *et al.* Genômica e patótipos das múltiplas faces da Escherichia coli. **FEMS Microbiology Reviews**, v. 46, n. 6, fuac031, nov. 2022. DOI: https://doi.org/10.1093/femsre/fuac031.

GONBADI, MOHAMMAD AMIN *et al.* Integrons e resistência a antibióticos mediada por bomba de efluxo em Escherichia coli uropatogênica multirresistente (MDR). **BioMed Research International**, v. 2026, art. 2158306, p. 1-8, 2026. DOI: https://doi.org/10.1155/bmri/2158306.

GROME, H. N. *et al.* Infecções invasivas extraintestinais por Escherichia coli nos EUA. **JAMA Network Open**, v. 9, n. 2, e2557201, 2026. DOI: https://doi.org/10.1001/jamanetworkopen.2025.57201.

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REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 12/09/2026
Vol: 13.19
DOI: 10.66104/mph2hq73
Pages: 1-21

HENRIQUES, S. V. C.; ALMEIDA, S. S. M. da S. de. Identificação do caráter medicinal da espécie Curatella americana por meio das folhas. **Estação Científica (UNIFAP)**, Macapá, v. 3, n. 2, p. 89-97, 2013. Disponível em: http://periodicos.unifap.br/index.php/estacao.

ISHAK, A. *et al.* Bactericidal versus bacteriostatic antibacterials: clinical significance, differences and synergistic potential in clinical practice. **Journal of Antimicrobial Chemotherapy**, v. 80, n. 1, p. 1–17, 2025. DOI: https://doi.org/10.1093/jac/dkae380.

KAUR, V. *et al.* Antimicrobial, synergistic and antibiofilm activities of Myristica fragrans Houtt. bioactive compounds and their derivatives. **Scientific Reports**, v. 15, n. 1, p. 33041, 2025. DOI: https://doi.org/10.1038/s41598-025-03152-y.

KEAWCHAI, K. *et al.* Synergistic effect of ampicillin and dihydrobenzofuran neolignans (myticaganal C) identified from the seeds of Myristica fragrans Houtt. against Escherichia coli. **Journal of Advanced Pharmaceutical Technology & Research**, v. 12, n. 1, p. 79-83, 2021. DOI: https://doi.org/10.4103/japtr.JAPTR_85_20.

LIANG, JINGRU; HUANG, XUAN; MA, GUO. Antimicrobial activities and mechanisms of extract and components of herbs in East Asia. **RSC Advances**, v. 12, n. 45, p. 29197-29213, out. 2022. DOI: https://doi.org/10.1039/d2ra02389j.

LONG, J. *et al.* Tannic acid inhibits Escherichia coli biofilm formation and underlying molecular mechanisms: biofilm regulator CsgD. **Biomedicine & Pharmacotherapy**, v. 175, p. 116716, 2024. DOI: https://doi.org/10.1016/j.biopha.2024.116716.

LOPES, J. P.; LIONAKIS, M. S. Pathogenesis and virulence of Candida albicans. **Virulence**, v. 13, n. 1, p. 89–121, 2022. DOI: https://doi.org/10.1080/21505594.2021.2019950.

MOIRANGTHEM, S. *et al.* Effect of nutmeg (Myristica fragrans) and tea tree (Melaleuca alternifolia) essential oils on the oxidative and microbial stability of chicken fillets during refrigerated storage. **Foods**, v. 13, n. 24, p. 4139, 2024. DOI: https://doi.org/10.3390/foods13244139.

MOREIRA, L. E. A. *et al.* Antifungal activity of tannic acid against Candida spp. and its mechanism of action. **Brazilian Journal of Microbiology**, v. 55, n. 4, p. 3679–3690, 2024. DOI: https://doi.org/10.1007/s42770-024-01477-w.

CC BY

REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 12/09/2026
Vol: 13.19
DOI: 10.66104/mph2hq73
Pages: 1-21

NDAKI, Pendo M. *et al.* Fatores que levam ao uso inadequado de antibióticos entre membros da comunidade em países de baixa e média renda: uma revisão sistemática de estudos qualitativos. **BMC Saúde Pública**, v. 25, n. 1, 705, fev. 2025. DOI: https://doi.org/10.1186/s12889-025-21553-6.

ÖZKAN, Osman Emre *et al.* Chemical composition and antimicrobial activity of Myristica fragrans & Elettaria cardamomum essential oil. **Kastamonu University, Journal of Forestry Faculty**, Kastamonu, v. 18, n. 2, p. 225-229, 2018. DOI: https://doi.org/10.17475/kastofod.446830.

QI, J. *et al.* Evaluation of the in vitro and in vivo antimicrobial activity of alkaloids prepared from Chelidonium majus L. using MRSA-infected Caenorhabditis elegans as a model host. **Fitoterapia**, v. 175, p. 105944, 2024. DOI: https://doi.org/10.1016/j.fitote.2024.105944.

QI, JUNNI *et al.* Nutmeg: A Review on Basic Source, Traditional Use, Chemical Components, Pharmacological Activities, Mechanism, and Quality Control. **Planta Medica**, 2025. DOI: https://doi.org/10.1055/a-2623-1165.

REZENDE, D. A. D. C. S. *et al.* Bactericidal and antioxidant effects of essential oils from Satureja montana L., Myristica fragrans H. and Cymbopogon flexuosus. **Letters in Applied Microbiology**, v. 74, n. 5, p. 741–751, 2022. DOI: https://doi.org/10.1111/lam.13659.

SARTORI, M.; TOPPO, S.; LAVEZZO, E. Molecular resistance mechanisms to newly approved antibiotics (2017–2025) in WHO priority pathogens. **Frontiers in Microbiology**, v. 16, e1719798, 2026. DOI: https://doi.org/10.3389/fmicb.2025.1719798.

SASIDHARAN, A. *et al.* Extraction, isolation and characterization of bioactive compounds from plants' extracts. **African Journal of Traditional, Complementary and Alternative Medicines**, v. 8, n. 1, p. 1-10, 2011. DOI: https://doi.org/10.4314/ajtcam.v8i1.60483.

SEIXAS, P.; MUTTONI, S. M. P. Doenças transmitidas por alimentos, aspectos gerais e principais agentes bacterianos envolvidos em surtos: uma revisão. **Nutrivisa Revista de Nutrição e Vigilância em Saúde**, Fortaleza, v. 7, n. 1, p. 23–30, 2022. DOI: https://doi.org/10.17648/nutrivisa-v7-14227.

SILVA, A. R. da *et al.* Berberine Antifungal Activity in Fluconazole-Resistant Pathogenic Yeasts: Action Mechanism Evaluated by Flow Cytometry and Biofilm Growth Inhibition in Candida spp. **Antimicrobial Agents and**

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REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 12/09/2026
Vol: 13.19
DOI: 10.66104/mph2hq73
Pages: 1-21

Chemotherapy, v. 60, n. 6, p. 3551-3557, maio 2016. DOI: https://doi.org/10.1128/AAC.01846-15.

SILVA, F. A.; BIZERRA, A. M. C.; FERNANDES, P. R. D. Testes fitoquímicos em extratos orgânicos de Bixa orellana L. (Urucum). **Holos**, Natal, v. 2, p. 484-498, 2018. DOI: https://doi.org/10.15628/holos.2018.6929.

SINHA, SAKSHI; UPADHYAY, LATA SHEO BACHAN. Compreendendo os mecanismos de resistência antimicrobiana (RAM) e os avanços no diagnóstico da RAM. **Microbiologia Diagnóstica e Doenças Infecciosas**, v. 113, n. 2, 116949, out. 2025. DOI: https://doi.org/10.1016/j.diagmicrobio.2025.116949.

SOFIATI, F. T. **Estudo fitoquímico e atividades biológicas preliminares de extratos de Polygonum acre (polygonaceae) h.b.k. e Synadenium carinatum (euphorbiaceae) boiss**. 2009. 100 f. Dissertação (Mestrado em Ciências Farmacêuticas) - Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Araraquara, 2009. Disponível em: https://repositorio.unesp.br/entities/publication/1c518146-f040-4fca-ba9f-64585a0894cc. Acesso em: 17 jul. 2026.

SOUZA, A. de O. **Prospecção fitoquímica e atividades biológicas de extratos das folhas da Spiranthera odoratissima A. St. - Hill (Rutaceae)**. 2020. 52 f. Dissertação (Mestrado em Agroquímica) - Instituto Federal de Educação, Ciência e Tecnologia Goiano, Rio Verde, 2020. Disponível em: https://sistemas.ifgoiano.edu.br/sgcursos/uploads/anexos_12/2020-09-11-12-03-37Dissertação%20Amanda%20de%20Oliveira%20Souza%20-%20Final.pdf. Acesso em: 17 jul. 2026.

TAN, Z. *et al.* The antibacterial activity of natural-derived flavonoids. **Current Topics in Medicinal Chemistry**, v. 22, n. 12, p. 1009–1019, 2022. DOI: https://doi.org/10.2174/1568026622666220221110506.

TANO, Z. N. *et al.* Fluoroquinolone resistant Escherichia coli in community: molecular analyses of resistance genes, virulence factors, and antimicrobial susceptibility test. **Frontiers in Microbiology**, v. 17, e1730332, jan. 2026. DOI: https://doi.org/10.3389/fmicb.2026.1730332.

THAWABTEH, A. M. *et al.* Antibacterial Activity and Antifungal Activity of Monomeric Alkaloids. **Toxins**, Basileia, v. 16, n. 11, art. 489, nov. 2024. DOI: https://doi.org/10.3390/toxins16110489.

CC BY

REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 12/09/2026
Vol: 13.19
DOI: 10.66104/mph2hq73
Pages: 1-21

TRIFAN, A. *et al.* Essential oils and sustainability: in vitro bioactivity screening of Myristica fragrans Houtt. post-distillation by-products. **Plants**, v. 12, n. 9, p. 1741, 2023. DOI: https://doi.org/10.3390/plants12091741.
VIEIRA, A. J. H.; SANTOS, J. I. dos. Mecanismos de resistência de Candida albicans aos antifúngicos anfotericina B, fluconazol e caspofungina. **RBAC - Revista Brasileira de Análises Clínicas**, Rio de Janeiro, v. 57, n. 3, 2025. DOI: https://doi.org/10.21877/2448-3877.201600407.

VIRTANEN, V. *et al.* NMR metabolomics and DNA sequencing of Escherichia coli and Staphylococcus aureus cultures treated with hydrolyzable tannins. **Metabolites**, v. 13, n. 3, p. 320, 2023. DOI: https://doi.org/10.3390/metabo13030320.

WORLD HEALTH ORGANIZATION (WHO). **WHO fungal priority pathogens list to guide research, development and public health action**. Geneva: World Health Organization, 2022. Disponível em: https://www.who.int/publications/i/item/9789240060241.

XIE, Y. *et al.* Antibacterial activities of flavonoids: structure-activity relationship and mechanism. **Current Medicinal Chemistry**, v. 22, n. 1, p. 132–149, 2015. DOI: https://doi.org/10.2174/0929867321666140916113443.

YAN, Y. *et al.* Antibacterial activity and mechanisms of plant flavonoids against Gram-negative bacteria based on the antibacterial statistical model. **Pharmaceuticals**, Basel, v. 17, n. 3, p. 292, 2024. DOI: https://doi.org/10.3390/ph17030292.

ZIELIŃSKA, S. *et al.* The activity of isoquinoline alkaloids and extracts from Chelidonium majus against pathogenic bacteria and Candida sp. **Toxins**, v. 11, n. 7, p. 406, 2019. DOI: https://doi.org/10.3390/toxins11070406.

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