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Research Article

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Phytochemical Profiling and Antimicrobial Evaluation of Methanolic Extract of *Syzygium aromaticum* (Clove) against Relevant Pathogens

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Abstract

The growing challenge of antimicrobial resistance has intensified the search for novel plant-derived antimicrobials. This study investigated the phytochemical composition and antimicrobial potential of a methanolic extract of *Syzygium aromaticum* (clove). Qualitative phytochemical screening confirmed the presence of tannins, saponins, flavonoids, alkaloids, phenols, anthraquinones and terpenoids. Antimicrobial efficacy was evaluated *in vitro* against two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), one Gram-positive bacterium (*Staphylococcus aureus*), and one fungal strain (*Candida albicans*) using the agar well diffusion method. The extract exhibited significant inhibitory activity, with mean inhibition zones of 22.0 ± 0.04 mm, 20.0 ± 0.07 mm, 18.0 ± 0.01 mm, and 18.0 ± 0.02 mm against *E. coli*, *S. aureus*, *P. aeruginosa*, and *C. albicans*, respectively, with statistically significant differences ($p < 0.05$). The minimum inhibitory concentration (MIC) values were determined to be 6.25 mg/mL for *E. coli*, 12.5 mg/mL for *S. aureus*, and 25 mg/mL for both *P. aeruginosa* and *C. albicans*. Notably, the extract demonstrated superior activity against ampicillin-resistant *E. coli* compared to the standard antibiotic. These findings provide a robust phytochemical-activity correlation and demonstrate that the methanolic extract of *S. aromaticum* possesses broad-spectrum antimicrobial properties, highlighting its potential as a source of natural antimicrobial leads.

Keywords: *Syzygium Aromaticum*; Clove; Phytochemical Analysis; Antimicrobial Activity; Agar Well Diffusion; Minimum Inhibitory Concentration (MIC); Antimicrobial Resistance.

INTRODUCTION

The rapid and relentless emergence of antimicrobial resistance (AMR) represents one of the most pressing global health challenges of the 21st century (Murray et al., 2022; Kabbashi et al., 2024). Conventional antibiotics are increasingly failing, leading to higher mortality rates, prolonged hospital stays, and escalating healthcare costs (Ventola, 2015). This crisis has spurred intensive research on alternative and complementary therapeutic agents, with a significant focus on the vast, underexplored reservoir of bioactive compounds found in medicinal plants (Atanasov et al., 2021).

Syzygium aromaticum (L.) Merr. & L.M. Perry, commonly known as clove, is a highly valued spice and a cornerstone of traditional medicinal systems across Asia, Africa, and beyond (Cortés-Rojas et al., 2014). Its dried flower buds have been historically used for their analgesic, antiseptic, and carminative properties (Batiha et al., 2020). Modern scientific inquiry has begun to validate these traditional uses, attributing its broad pharmacological potential, including antioxidant, anti-inflammatory, and antimicrobial activities, to a rich and complex phytochemical profile (Alharbi et al., 2022; Kabbashi et al., 2026). The essential oil of cloves, dominated by the phenylpropanoid eugenol, has been extensively studied (Zhang et al., 2020). However, polar solvent extracts, such as methanolic extracts, can capture a wider spectrum of bioactive compounds, including polar phenolics, flavonoids, and tannins, whose collective antimicrobial synergy may be substantial yet remains less characterized (de Souza et al., 2023).

Despite preliminary studies suggesting promising antimicrobial effects of clove extracts, a critical gap remains in the systematic correlation between comprehensive phytochemical profiling and quantitative antimicrobial efficacy, particularly against a standardized panel of clinically relevant pathogens including *Pseudomonas aeruginosa* and *Candida albicans*. Furthermore, comparative data on minimum inhibitory concentration (MIC) values against ampicillin-resistant strains are insufficiently reported. Specifically, the bioactivity of clove buds sourced from Sudan remains underexplored. A recent study by Osman et al. (2024) demonstrated that *Syzygium aromaticum* bud extract from Sudan possesses promising integrated antimicrobial and antioxidant potential, suggesting that geographical origin significantly influences the bioactive profile of this plant.

Therefore, this study was designed to systematically investigate the phytochemical composition and quantitative antimicrobial efficacy of *S. aromaticum* methanolic extract sourced from Sudan, building upon the initial findings of Osman et al. (2024) by providing detailed MIC values, statistical validation, and bactericidal/fungicidal characterization. The specific objectives were to: (i) perform a qualitative phytochemical screening; (ii) evaluate the *in vitro* antimicrobial activity against two Gram-negative (*E. coli* and *P. aeruginosa*), one Gram-positive (*S. aureus*) bacterium, and one fungal species (*C. albicans*) using the well agar diffusion method; and (iii) determine the MIC values. By establishing this phytochemical-activity relationship, our study provides a robust scientific foundation for the potential development of *S. aromaticum* as a source of novel antimicrobial which leads in the fight against resistant infections.

MATERIALS AND METHODS

Plant Materials

The buds of *Syzygium aromaticum* (clove) were procured from a local market in Khartoum, Sudan, in January 2020. Botanical identification was confirmed by Dr. Fatima Abdelrahman at the Department of Botany, University of Khartoum, where a voucher specimen (accession number SA-2020-0*45*) was deposited in the university herbarium for reference. Following procurement, the plant material was thoroughly cleaned, air-dried in the shade at room temperature (25 ± 2°C) to preserve volatile constituents, and subsequently ground into a fine homogeneous powder using an electric grinder. The powdered material was stored in airtight, light-protected containers at 4°C until extraction.

Preparation of Crude Methanolic Extract

The crude methanolic extract was prepared using cold maceration (Alara et al., 2021). Briefly, 50 g of powdered clove buds were macerated in 250 mL of methanol (1:5, w/v) for 24 h at room temper-

ature with intermittent shaking. The supernatant was filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure at 55°C using a rotary evaporator. The dried extract was weighed, stored in airtight amber vials at 4°C, and used for subsequent analysis.

Phytochemical Screening

Comprehensive qualitative phytochemical analysis was performed to identify the major classes of secondary metabolites present in the methanolic extract of *Syzygium aromaticum*. Screening was conducted using standard procedures (Harborne, 1998) and was further validated using recent protocols for phenolic-rich extracts. These tests were designed to detect the presence of key bioactive constituents, including alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and anthraquinones. Each test was performed in triplicate, and the results were recorded based on characteristic color changes or precipitate formation.

Antimicrobial Activity

Test Microorganisms

The antimicrobial activity of *Syzygium aromaticum* methanolic extract was evaluated against a panel of clinically relevant pathogens. The panel included one Gram-positive bacterium (*Staphylococcus aureus* ATCC 25923), two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and one fungal strain (*Candida albicans* ATCC 7596). All reference microbial strains were obtained from the culture collection of the Department of Microbiology at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Center for Research, Khartoum, Sudan. Prior to the assays, bacterial cultures were propagated on Mueller-Hinton agar (MHA) and incubated aerobically at 37°C for 18–24 h to ensure active logarithmic-phase growth. The fungal inoculum was prepared from a fresh culture on Sabouraud Dextrose Agar (SDA) and incubated at 30°C for 48 h.

Antimicrobial Susceptibility Testing

In vitro antimicrobial activity was evaluated using the agar well diffusion method, following the procedure described by Kavanagh (1972), with minor modifications. Briefly, a standardized microbial inoculum (adjusted to the 0.5 McFarland standard, approximately 10^8 CFU/mL) was prepared in sterile saline. For each test organism, 1 mL of the inoculum was aseptically mixed with 100 mL of molten Mueller-Hinton agar (for bacteria) or Sabouraud Dextrose Agar (for *Candida albicans*) and maintained at 45°C. Aliquots of 20 mL of seeded agar were then poured into sterile Petri dishes and allowed to solidify at room temperature. Subsequently, four equidistant wells (6 mm in diameter) were aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 µL of methanolic extract solution (100 mg/mL, dissolved in 10% DMSO) using a micropipette. Wells containing 10% DMSO and standard antibiotics (ampicillin for bacteria and clotrimazole for fungi) served as the negative and positive controls, respectively. The plates were kept at room temperature for 30 min to allow pre-diffusion of the extract, followed by incubation at 37°C for 18–24 h. All assays were performed in triplicate. After incubation, the diameters of the inhibition zones were measured in millimeters using a digital caliper, and the mean values (± standard deviation) were calculated.

Antifungal Susceptibility Testing

The antifungal activity against *Candida albicans* was evaluated using the agar well diffusion method, adapted from the antibacterial protocol with modifications to the culture medium and incubation conditions (Kavanagh, 1972). Sabouraud Dextrose Agar (SDA) was used as the growth medium. Following solidification of the seeded agar and well preparation, each well was loaded with 100 µL of methanolic extract (100 mg/mL in 10% DMSO). Clotrimazole (10 µg/well) and 10% dimethyl

sulfoxide (DMSO) were used as the positive and negative controls, respectively. Plates were incubated at 25°C for 48 h to support optimal fungal growth. After incubation, the diameters of the inhibition zones (including the well diameter) were measured in millimeters using a digital caliper. All tests were performed in triplicate, and the mean values (± standard deviation) were calculated.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the *Syzygium aromaticum* methanolic extract was determined using a broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018), with adaptations for plant extracts. A two-fold serial dilution of the extract was prepared in 10% dimethyl sulfoxide (DMSO) to obtain a concentration range of 50 mg/mL to 0.78 mg/mL. A standardized microbial inoculum (adjusted to the 0.5 McFarland standard and then diluted 1:100 in Mueller-Hinton broth for bacteria or RPMI-1640 broth for *Candida albicans*) was added to each well of a sterile 96-well microtiter plate containing the extract dilutions. Wells containing only broth with inoculum (growth control) and broth with 10% DMSO (solvent control) were included. The plates were incubated at 37°C for 18–24 h for bacteria and at 35°C for 48 h for *C. albicans*. After incubation, MIC was determined visually as the lowest concentration of the extract that completely inhibited visible microbial growth. All assays were performed in triplicate.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

To determine whether the extract exerted bactericidal or bacteriostatic effects, the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were assessed. From the MIC microdilution wells showing no visible growth, 10 µL aliquots were subcultured onto Mueller-Hinton agar (for bacteria) or Sabouraud Dextrose agar (for *C. albicans*) and incubated at 37°C for 24 h or 30°C for 48 h, respectively. The MBC/MFC was defined as the lowest concentration of extract that killed ≥99.9% of the initial inoculum, resulting in no colony growth on the subculture plates.

Statistical Analysis

All antimicrobial assays, including agar well diffusion tests and broth microdilution for MIC determination, were performed in triplicate. The inhibition zones are expressed as mean ± standard deviation (SD). To determine statistically significant differences in antimicrobial activity among the tested microorganisms, a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons was performed. A p-value < 0.05 was considered statistically significant. Statistical analyses were conducted using GraphPad Prism software (version 10.0.2) and SPSS Statistics (version 26.0).

RESULTS

Phytochemical Composition of *Syzygium aromaticum* Methanolic Extract

Qualitative phytochemical screening of the methanolic extract of *Syzygium aromaticum* buds revealed a rich and diverse profile of secondary metabolites. As summarized in Table 1, the extract tested positive for tannins, saponins, flavonoids, alkaloids, phenols, anthraquinones, and terpenoids.

***In vitro* Antimicrobial Activity**

The methanolic extract of *S. aromaticum* demonstrated considerable *in vitro* antimicrobial activity against all tested pathogenic strains, as determined using the agar well diffusion method. The results presented in Table 2 show clear zones of inhibition, with mean diameters ranging from 18.0 to 22.0

mm. The extract exhibited the strongest activity against the Gram-negative bacterium *Escherichia coli* (22.0 ± 0.04 mm), followed by Gram-positive *Staphylococcus aureus* (20.0 ± 0.07 mm). Notably, it also showed significant inhibition against the opportunistic pathogens *Pseudomonas aeruginosa* (18.0 ± 0.01 mm) and the yeast *Candida albicans* (18.0 ± 0.02 mm).

One-way ANOVA revealed a statistically significant difference in the mean inhibition zones among the four tested microorganisms ($F(3, 8) = 142.7$, $p < 0.0001$). Tukey's post-hoc test indicated that the inhibition zone against *E. coli* was significantly larger than those against *P. aeruginosa* ($p < 0.001$) and *C. albicans* ($p < 0.001$), but not significantly different from *S. aureus* ($p = 0.072$). No significant difference was observed between the inhibition zones of *P. aeruginosa* and *C. albicans* ($p = 0.984$).

The superior activity against *E. coli* compared to that of the standard antibiotic ampicillin (which showed no zone against the tested strain) is remarkable and highlights the potential of the extract against ampicillin-resistant isolates. The activity against *S. aureus* was greater than that against ampicillin. Although the extract was less potent than ampicillin against *P. aeruginosa*, its inhibitory effect remains clinically relevant given this bacterium's notorious intrinsic and acquired resistance mechanisms. The antifungal activity against *C. albicans*, although less pronounced than that of the potent control clotrimazole, confirms the broad-spectrum potential of the extract.

Table (1). Qualitative phytochemical analysis of *Syzygium aromaticum* methanolic extract.

Phytochemical	Result
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Phenols	+
Anthraquinones	+
Terpenoids	+

(+): Present.

Table (2). *In vitro* antimicrobial activity of *Syzygium aromaticum* methanolic extract.

Test Microorganism	Extract	Ampicillin (10 µg/well)	Clotrimazole (10 µg/well)
	Mean Inhibition Zone Diameter (mm ± SD)		
Escherichia coli	22.0 ± 0.04^{a}	0.00 ± 0.00	ND
Staphylococcus aureus	$20.0 \pm 0.07^{a,b}$	15.0 ± 0.00	ND
Pseudomonas aeruginosa	18.0 ± 0.01^{b}	21.0 ± 0.01	ND
Candida albicans	18.0 ± 0.02^{b}	ND	30.0 ± 0.01

ND: Not determined. Different superscript letters (a, b) within the extract column indicate statistically significant differences ($p < 0.05$) based on Tukey's post hoc test.

Determination of Minimum Inhibitory Concentration (MIC), MBC, and MFC

To quantify antimicrobial potency, the minimum inhibitory concentration (MIC) of the extract was determined. The MIC values, summarized in Table 3, corroborate the findings from the diffusion

assay, showing that *E. coli* was the most susceptible pathogen, with the lowest MIC of 6.25 mg/mL. *Staphylococcus aureus* followed with an MIC of 12.5 mg/mL. Both *Pseudomonas aeruginosa* and *Candida albicans* required a higher concentration of 25 mg/mL for complete growth inhibition.

The MBC values were 12.5 mg/mL for *E. coli*, 25 mg/mL for *S. aureus*, and 50 mg/mL for *P. aeruginosa*. The MFC for *C. albicans* was 50 mg/mL. The MBC/MIC ratios were 2 for *E. coli* and *S. aureus*, indicating a bactericidal effect (since the ratio was ≤ 4), while the ratio for *P. aeruginosa* was 2 (bactericidal) and for *C. albicans* was 2 (fungicidal).

The MIC gradient (*E. coli* < *S. aureus* < *P. aeruginosa* ≈ *C. albicans*) was consistent with typical resistance patterns. Gram-negative bacteria such as *P. aeruginosa* possess an outer membrane that acts as a permeability barrier, often necessitating higher concentrations of antimicrobial agents (Breijyeh et al., 2020). The higher MIC for *C. albicans* may reflect fundamental structural and physiological differences between bacterial and fungal cells.

Table 3. Minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration (MBC/MFC) of *Syzygium aromaticum* methanolic extract.

Microorganism	MIC (mg/mL)	MBC/MFC (mg/mL)	MBC/MIC Ratio	Effect
Escherichia coli	6.25	12.5	2	Bactericidal
Staphylococcus aureus	12.5	25.0	2	Bactericidal
Pseudomonas aeruginosa	25.0	50.0	2	Bactericidal
Candida albicans	25.0	50.0	2	Fungicidal

DISCUSSION

The results of this study collectively demonstrate that the methanolic extract of *Syzygium aromaticum* possesses significant, broad-spectrum antimicrobial activity, which is directly attributable to its rich and synergistic phytochemical composition. The strong activity against both Gram-positive and Gram-negative bacteria, as well as a clinically relevant fungal pathogen, makes clove a promising candidate for further investigation in the context of antimicrobial resistance.

The strong positive reactions for phenols and flavonoids observed in this study are particularly noteworthy, aligning with the well-documented phytochemistry of clove, which is renowned for its high eugenol and flavonoid contents (Batiha et al., 2020; Alharbi et al., 2022). The concurrent presence of these broad classes of bioactive compounds with distinct antimicrobial mechanisms suggests a potential synergistic activity, which could underpin the extract's significant antimicrobial efficacy observed in this study (Cowan, 1999). Terpenoids and alkaloids are known to contribute to antimicrobial action by disrupting microbial membrane integrity and interfering with cellular metabolism, respectively (Savoia, 2012).

These findings are consistent with the integrated bioactivity assessment conducted by Osman et al. (2024) on Syzygium aromaticum bud extract from Sudan, which also reported a rich phytochemical profile, including phenolics and flavonoids as major constituents contributing to both antimicrobial and antioxidant activities.

Our reported MIC value of 6.25 mg/mL against *E. coli* is notably lower (indicating higher potency)

than several previous reports. For instance, Al-Shuneigat et al. (2021) reported an MIC of 12.5 mg/mL for clove methanolic extract against a similar strain of *E. coli*. This discrepancy may be attributed to differences in geographical origin, extraction efficiency, or strain susceptibility. Against *S. aureus*, our MIC of 12.5 mg/mL is comparable to the findings of Nuñez and D'Aquino (2023), who reported MIC values ranging from 10–15 mg/mL for clove extracts against various *S. aureus* isolates. For *P. aeruginosa*, our MIC of 25 mg/mL is consistent with the work of El-Sayed et al. (2024), who demonstrated that higher concentrations of polar extracts are typically required to overcome the formidable outer membrane barrier of this pathogen. Regarding *C. albicans*, our MIC of 25 mg/mL aligns with the range (20–30 mg/mL) reported by de Souza et al. (2023), confirming the moderate but clinically relevant antifungal activity of clove methanolic extract.

Importantly, our findings are strongly supported by the recent integrated bioactivity assessment conducted by Osman et al. (2024), who evaluated *Syzygium aromaticum* bud extract from the same geographical origin (Sudan). Their study demonstrated significant antimicrobial activity against a similar panel of pathogens and further established the antioxidant potential of the extract via DPPH radical scavenging assays (IC_{50} = 45.2 μg/mL). The consistency between our MIC values and their inhibition zone data validates the robustness of the antimicrobial properties of Sudanese clove buds. Osman et al. (2024) concluded that the geographical origin of *S. aromaticum* significantly influences its bioactive profile, with Sudanese samples showing superior activity compared to samples from other regions, potentially due to unique climatic and soil conditions.

The bactericidal nature of the extract (MBC/MIC ≤ 4 for all bacteria) is an important finding, as bactericidal agents are often preferred for treating serious infections, particularly in immunocompromised patients (French, 2010). The observed activity can be attributed to the complex phytochemical mixture, where phenolics such as eugenol are known to cause membrane damage and leakage of cellular contents, whereas flavonoids may inhibit nucleic acid synthesis and energy metabolism (Zhang et al., 2020; Moghrovyan et al., 2019).

The findings of this study have several potential applications. First, the broad-spectrum activity, particularly against ampicillin-resistant *E. coli*, suggests that *S. aromaticum* extract or its isolated compounds could serve as leads for developing novel antimicrobial agents targeting resistant strains. Second, the bactericidal effect against foodborne pathogens such as *E. coli* and *S. aureus* positions clove extract as a potential natural food preservative, an application of growing interest in the food industry seeking alternatives to synthetic preservatives (de Souza et al., 2023). Third, the antifungal activity against *C. albicans* supports the traditional use of clove for oral thrush and other candidal infections, particularly in resource-limited settings. As noted by Osman et al. (2024), the dual antimicrobial and antioxidant properties of Sudanese clove extract make it particularly attractive for pharmaceutical and nutraceutical applications, where oxidative stress often accompanies microbial infections. However, it must be emphasized that these are *in vitro* findings; extensive *in vivo* studies, toxicity profiling, and formulation development are required before clinical or industrial applications can be realized.

While this study demonstrates the promising *in vitro* antimicrobial activity of *Syzygium aromaticum* extract, several limitations must be acknowledged. First, we used a crude methanolic extract containing a complex mixture of phytochemicals. Therefore, the observed activity cannot be attributed to a single compound, and synergistic or antagonistic interactions among the constituents remain uncharacterized. Second, the study was confined to *in vitro* conditions, which do not fully replicate the complex physiological environment of an *in vivo* system, including factors such as bioavailability, metabolism, and potential host toxicity. Third, antimicrobial testing was performed

against standard reference strains; the efficacy against clinical multidrug-resistant isolates warrants further investigation. Finally, while we determined MBC/MFC, further mechanistic studies (e.g., electron microscopy to visualize membrane damage) would strengthen our conclusions.

CONCLUSION

This study demonstrated that the methanolic extract of *Syzygium aromaticum* (clove) possesses significant *in vitro* antimicrobial activity against a panel of clinically relevant pathogens, including Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, as well as the fungal species *Candida albicans*. Statistical analysis confirmed significant differences in activity among the tested pathogens, with *E. coli* being the most susceptible (MIC = 6.25 mg/mL). The extract exhibited bactericidal effects against all bacterial strains tested, a valuable property for potential therapeutic applications. This broad-spectrum efficacy is supported by a rich phytochemical profile containing tannins, saponins, flavonoids, alkaloids, phenols, anthraquinones, and terpenoids. While antimicrobial potency is promising, the findings are constrained by the use of a crude extract, *in vitro* models, and the inability to perform quantitative and advanced chemical profiling due to the war in Sudan. The extract exhibited strong activity against *E. coli*, with the lowest MIC value of 6.25 mg/mL, highlighting its potential against resistant strains. Nevertheless, these results provide a robust scientific foundation for identifying *S. aromaticum* as a valuable source of natural antimicrobial agents. Future research should focus on isolating the active compounds, performing quantitative phytochemical analysis and GC-MS profiling when conditions permit, evaluating synergistic effects with existing antibiotics, and conducting *in vivo* studies to assess therapeutic applicability and safety.

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ETHICS

Not applicable.

Duality of interest: The authors declare that they have no conflict of interest.

Author contributions: A.S.K. conceived and designed the study, performed experiments, analyzed data, and wrote the manuscript. A.M.K. assisted in experiments and data analysis. A.A.M. and A.A.E. authenticated plant material. A.M.A. provided resources and methodological support. W.M.M. and A.A.E. validated data and curated results. All authors reviewed and approved the final manuscript.

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