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Phytochemical screening and antibacterial activity of *Nigella sativa* and *Cinnamomum zeylanicum* against clinical isolates

Abstract

The escalating threat of antimicrobial resistance (AMR) underscores the urgent need for alternative therapeutic agents, particularly from natural sources. Nigella sativa (black seed) and *Cinnamomum zeylanicum* (Ceylon cinnamon) are two botanicals widely recognized in traditional medicine for their broad-spectrum antimicrobial properties. This study evaluated the antibacterial activity of extracts from these plants, prepared via maceration, against clinical isolates of both Gram-positive (*Staphylococcus aureus and Streptococcus pyogenes*) and Gram-negative (*Pseudomonas aeruginosa and Escherichia coli*) bacteria. Extracts demonstrated inhibitory effects across tested pathogens, with notable activity observed in *C. zeylanicum* extract at 1 g/mL, which produced zones of inhibition of 28 ± 1 mm against *S. aureus*, 15 ± 1 mm against *E. coli*, and 7 ± 1 mm against *P. aeruginosa*. The combined extract displayed enhanced inhibitory effects compared to individual extracts, suggesting possible synergistic interactions. These findings support continued investigation of *N. sativa* and *C. zeylanicum* as potential sources of antimicrobial agents for addressing resistant bacterial infections.[1,2]

Keywords: phytochemicals, antimicrobial resistance, *Nigella sativa*, *Cinnamomum zeylanicum*, clinical isolates, antibacterial activity

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Ewaoche Sunday Itodo, Andrew Yashim-Nuhu, Maryam Idris

Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Medicine and Health Sciences, Baze University, Nigeria

Correspondence: Ewaoche Sunday Itodo, Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Medicine and Health Sciences, Baze University, Nigeria, Tel +2348029869531

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Introduction

Phytochemicals are bioactive secondary metabolites synthesized by plants and have gained increasing attention as potential sources of novel antimicrobial agents. This interest is largely driven by the growing global burden of antimicrobial resistance (AMR), which has significantly reduced the effectiveness of conventional antibiotics and increased the need for alternative and less toxic therapeutic options.

In many African countries, including Nigeria, traditional medicine remains central to healthcare delivery, particularly in the management of infectious diseases. Medicinal plants represent a major component of indigenous healthcare systems and serve as reservoirs of biologically active compounds with therapeutic potential.

Two plants widely recognized for antimicrobial activity are *Nigella sativa L.* (black seed) and *Cinnamomum zeylanicum* Blume (*Ceylon cinnamon*). *N. sativa* contains several pharmacologically active compounds, notably thymoquinone, which has been extensively documented for antioxidant, anti-inflammatory, and antimicrobial properties.[3] Likewise, *C. zeylanicum* is rich in cinnamaldehyde and other phenolic compounds, which exert antibacterial effects by disrupting microbial membranes, inhibiting biofilm formation, and interfering with bacterial virulence pathways.[4] Evidence from systematic reviews has supported the antibacterial potential of true cinnamon against several clinically relevant bacterial pathogens.[2]

Modern validation of medicinal plants relies on standardized phytochemical screening and antimicrobial susceptibility assays. Manual qualitative phytochemical screening remains widely applied for identifying classes of secondary metabolites.[5] For compound-specific identification of volatile and semi-volatile phytochemicals, gas chromatography-mass spectrometry (GC-MS) remains a reliable analytical tool.[6,7]

The antimicrobial activity of plant extracts is commonly assessed using agar diffusion and broth dilution methods, with MIC and MBC values determined standardized protocols.[8,9] The present study investigated the phytochemical profile and antibacterial activity of *N. sativa* and *C. zeylanicum* extracts, individually and in combination, against selected pathogenic clinical isolates.

Materials and methods

Study area

The study was conducted in Abuja, Federal Capital Territory, Nigeria (9.0765° N, 7.3986° E). Sample analysis was performed at the Department of Medical Laboratory Science, Baze University, Abuja (9.0397° N, 7.5184° E).

Collection and identification of plant materials

Dried seeds of *Nigella sativa* and bark of *Cinnamomum zeylanicum* were obtained from an open herbal market in Kano, Nigeria. Botanical identification was confirmed using standard taxonomic references.[10,11]

Preparation and extraction of plant materials

The plant materials were air-dried, chopped or pounded, and ground into fine powder. Samples were stored in airtight containers and used individually and in a 1:1 combined ratio.

Extraction was carried out using the maceration method.[12] Briefly, 200 g of each powdered sample (or 100 g each for the mixture) was soaked in 1 L of ethyl acetate at room temperature for 48 hours with intermittent agitation. The extracts were filtered and oven-dried. Extraction yields were 10.24 g for *N. sativa*, 12.20 g for *C. zeylanicum*, and 12.20 g for the combined extract. Ethyl acetate was selected due to its efficiency in extracting semi-polar antimicrobial bioactive compounds.[13,14]

Preparation of stock extract solution

A stock extract solution was prepared by weighing 2 g (2000 mg) of dried extract and dissolving it in 10 mL dimethyl sulfoxide (DMSO) to obtain a concentration of 200 mg/mL. The mixture was vortexed to ensure complete dissolution and stored at 4°C until use.

Collection and confirmation of clinical isolates

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* were obtained from the National Hospital, Abuja. Identification was based on colony morphology, Gram staining, and biochemical tests, including catalase, coagulase, citrate, indole, KIA, oxidase, and urease tests.

Phytochemical screening

Extracts were screened for alkaloids, flavonoids, tannins, glycosides, saponins, coumarins, terpenoids, and phenolic compounds using manual test-tube screening procedures.[5] Thymoquinone and *cinnamaldehyde* were confirmed using GC-MS analysis based on established compound identification protocols.[7,15]

Preparation of 0.5 McFarland turbidity standard

The 0.5 McFarland turbidity standard was prepared for bacterial inoculum standardization. A 1% sulfuric acid solution was prepared by adding 1 mL of concentrated H_2SO_4 to 99 mL of distilled water. A 1% barium chloride solution was prepared by dissolving 0.5 g of barium chloride in 50 mL of distilled water. Thereafter, 0.6 mL of barium chloride solution was added to 99.4 mL of sulfuric acid with agitation to produce turbidity equivalent to 0.5 McFarland standard (≈ 1.5×10^8 CFU/mL).[8]

Antibacterial susceptibility testing (Agar well diffusion method)

The antibacterial activity of extracts was evaluated using the agar well diffusion method. Test isolates were standardized to 0.5 McFarland and inoculated onto Mueller-Hinton agar plates. Wells of 8 mm diameter were bored and filled with 100 µL of extract at concentrations of 1.0, 0.5, and 0.25 g/mL. Plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters. Ciprofloxacin (250 mg) served as a positive control. Sterility and viability controls were included.[16,17]

Determination of minimum inhibitory concentration (MIC)

The MIC was determined using the broth dilution method. A stock extract solution of 10 mg/mL was prepared by dissolving 500 mg crude extract in 5 mL ethyl acetate. Two-fold serial dilutions were prepared in nutrient broth to obtain concentrations of 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, and 0.078125 mg/mL. Each tube was inoculated with 0.5 mL standardized bacterial suspension and incubated at 37°C for 18 hours. Streptomycin was used as the reference drug. MIC was recorded as the lowest concentration showing no visible turbidity.[8,9]

Determination of minimum bactericidal concentration (MBC)

Aliquots from tubes showing no visible growth were subcultured onto Mueller-Hinton agar and incubated at 37°C for 24 hours. The MBC was recorded as the lowest extract concentration that produced no colony growth.[8,9]

Results and discussion

Gram staining and biochemical test reactions

The Gram reaction and biochemical test results for the bacterial isolates are presented in (Table 1**)**.

Table 1 Gram staining and biochemical tests of pathogenic isolates

Test	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Streptococcus pyogenes*
Gram Reaction	Gram-positive cocci	Gram-negative rods	Gram-negative rods	Gram-positive cocci
Catalase	+	+	+	–
Coagulase	+	–	–	–
Citrate	–	–	+	–
Indole	–	+	–	–
KIA (Acid/Alkali)	A/A	A/A	K/K	—
KIA (Gas)	–	+	–	—
KIA (H_2S)	–	–	–	—
Oxidase	–	–	+	–
Urease	+/variable	–	–	–

Phytochemical composition and bioactive potential

Phytochemical screening (Table 2) revealed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, and phenolic compounds in both plant extracts. *Cinnamaldehyde* was detected exclusively in *C. zeylanicum*, while thymoquinone was uniquely identified in *N. sativa*. These bioactive constituents have been widely reported as key antimicrobial agents in cinnamon and black seed, respectively.[3,4]

Flavonoids are known to exert antibacterial activity by inhibiting enzymes, chelating essential metal ions, and disrupting bacterial membranes.[18] Tannins precipitate microbial proteins and interfere with membrane functions,[19] while saponins promote membrane destabilization leading to leakage and lysis.[20] Terpenoids and volatile compounds in essential oils act primarily by membrane disruption and interference with respiration.[6] Phenolic compounds further contribute through oxidative stress generation and enzyme inhibition (Table 2).[7]

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Table 2 Phytochemical compounds of the plant extracts (Note: + = Present; – = Not present)

Phytochemical Compound	*C. zeylanicum*	*N. sativa*	Common in combination
Alkaloids	+	+	++
Flavonoids	+	+	++
Tannins	+	+	++
Glycosides	+	+	++
Saponins	+	+	++
Coumarins	+	–	+
Terpenoids	+	+	++
Phenolic compounds	+ (cinnamaldehyde, eugenol)	+ (thymoquinone, others)	+
Thymoquinone	–	+	+
Cinnamaldehyde	+	–	+

Antibacterial activity of extracts against clinical isolates

The antibacterial activity of extracts against the test organisms is presented in Table 3. The results showed concentration-dependent inhibition patterns. *C. zeylanicum* demonstrated notable activity against *S. aureus*, while *N. sativa* showed moderate activity. The combined extract demonstrated superior activity against *S. aureus* and *S. pyogenes*, suggesting potential synergistic interactions between cinnamaldehyde and thymoquinone (Table 3).[3,4]

Table 3 Antimicrobial activity of the extracts against selected pathogenic bacteria (Diameter of zone of inhibition (mm) ± S.E.).

Concentration / Extract	*S. aureus*	*S. pyogenes*	*E. coli*	*P. aeruginosa*
Cinnamon 1 g/mL	28 ± 1	—	15 ± 1	7 ± 1
Cinnamon 0.5 g/mL	0	—	0	—
Cinnamon 0.25 g/mL	0	0	0	0
Black seed 1 g/mL	15 ± 1	7 ± 1	0	5 ± 1
Black seed 0.5 g/mL	—	0	0	0
Black seed 0.25 g/mL	0	0	0	0
Cinnamon + Black seed 1 g/mL	25 ± 1	30 ± 1	7 ± 1	7 ± 1
Cinnamon + Black seed 0.5 g/mL	15 ± 1	7 ± 1	7 ± 1	5 ± 1
Cinnamon + Black seed 0.25 g/mL	15 ± 1	5 ± 1	5 ± 1	5 ± 1

MIC and MBC of extracts

The MIC and MBC results are shown in Table 4. The combined extract demonstrated enhanced inhibitory effects compared to individual extracts, consistent with evidence that combined phytochemicals may exert additive or synergistic antimicrobial activity. MIC determination was carried out in accordance with CLSI-recommended dilution protocols (Table 4).[8,9]

Table 4 MIC and MBC of plant extracts against selected isolates (Key: – = No growth; + = Growth. Streptomycin concentration = 200 mg/mL).

Extract	Organism	5	2.5	1.25	0.125	0.0625	0.03125	0.015625	0.0078125	*Streptomycin*
Cinnamon	*S. aureus*	+	+	+	+	+	+	–	–	–
Cinnamon	*E. coli*	+	+	+	+	+	+	+	–	–
Black seed	*S. aureus*	–	–	–	–	–	–	–	+	–
Black seed	*E. coli*	–	–	–	–	–	+	+	+	–
Cinnamon + Black seed	*S. aureus*	–	–	–	–	–	–	+	+	–
Cinnamon + Black seed	*E. coli*	–	–	–	–	+	+	+	+	–

Mechanistic interpretation and gram-specific susceptibility

Gram-positive bacteria demonstrated greater susceptibility compared to Gram-negative isolates. This trend may be attributed to the presence of an outer lipopolysaccharide barrier in Gram-negative bacteria which restricts penetration of hydrophobic bioactive compounds, thereby reducing susceptibility.[7,21] In contrast, Gram-positive organisms lack this barrier, allowing easier diffusion of phytochemicals such as cinnamaldehyde and thymoquinone.

The antibacterial effects observed in this study are likely attributable to synergistic phytochemical action through membrane disruption, enzyme inhibition, and oxidative stress generation.[4,6]

Implications for therapeutic development

The findings of this study underscore the potential of *C. zeylanicum and N. sativa* as sources of plant-derived antibacterial agents. The combined extract demonstrated enhanced efficacy, supporting the importance of herbal synergy. Furthermore, the selection of ethyl acetate as an extraction solvent emphasizes the need for solvent optimization to improve yield and antimicrobial activity.[13,14]

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Conclusion

This study demonstrates that *Nigella sativa* and *Cinnamomum zeylanicum*, particularly when combined and extracted with ethyl acetate, possess notable antibacterial activity against selected Gram-positive and Gram-negative clinical isolates. The synergy between key bioactive constituents such as thymoquinone and cinnamaldehyde likely contributes to their enhanced antimicrobial activity. These findings support further research into the development of plant-based antimicrobial alternatives for addressing antimicrobial resistance.[1,2]

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Conflict of interest

The authors declared that there are no conflicts of interest.

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