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Antimicrobial activity of ethanolic extract of *Hunteria umbellata* seeds on selected clinical isolates

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Abstract

Hunteria umbellata, a medicinal plant widely used in African ethnomedicine, is reputed for its broad pharmacological properties. This study investigated the antimicrobial activity of ethanolic extract of *Hunteria umbellata* seeds (EEHUS) against selected clinical isolates, including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, *Streptococcus* spp., *Enterobacter* spp., *Candida albicans,* and *Aspergillus niger*. The antimicrobial efficacy was determined using agar well diffusion assays, minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC) analyses. Results revealed notable antibacterial activity, with *Escherichia coli, Streptococcus* spp., *and Enterobacter spp*. exhibiting high susceptibility (MIC = 100 mg/mL), while *Salmonella* spp., *Pseudomonas aeruginosa, and Vibrio cholerae* showed reduced sensitivity (MIC = 200 mg/mL). EEHUS also demonstrated promising antifungal potential, particularly against *Candida albicans* (MIC = 100 mg/mL, MFC = 150 mg/mL) and *Aspergillus niger* (MIC = 200 mg/mL, MFC = 300 mg/mL), though *Mucor* spp. exhibited complete resistance. The findings support the antimicrobial potential of *Hunteria umbellata* seed extract and validates its traditional usage. However, its limited activity against resistant strains suggests the need for phytochemical optimization and further pharmacological evaluation.

Keywords: *Hunteria umbellata* seeds; Antimicrobial activity; Clinical isolates; Minimum Inhibitory Concentration (MIC); Zone of inhibition; Natural antimicrobial agents

1. Introduction

Antimicrobial resistance (AMR) has become a global public health crisis, compromising the treatment of infections and threatening essential procedures such as surgery, chemotherapy, and organ transplants (1). According to the World Health Organization (WHO), AMR was directly responsible for 1.27 million deaths and contributed to nearly 4.95 million deaths globally in 2019 (2). The misuse and overuse of antimicrobials in medicine, veterinary practice, and agriculture have accelerated the emergence of multidrug-resistant pathogens (3).

Economically, the burden of AMR is immense projected to cost up to US$1 trillion in healthcare expenses by 2050, with potential annual losses in global GDP ranging from US$1 trillion to US$3.4 trillion by 2030 (4). These projections underscore the urgency to explore novel, sustainable sources of antimicrobial agents. Infectious diseases continue to pose significant challenges, especially in low- and middle-income countries where poor sanitation, poverty, and inadequate healthcare infrastructure prevail (5). Medicinal plants are an accessible and culturally accepted option in such regions and have long been used in traditional, complementary, and alternative medicine systems (6). These plants are a rich source of natural antimicrobial agents such as tannins, flavonoids, alkaloids, and terpenoids, that exhibit various mechanisms of action with a lower risk of resistance (7). Traditional medicine, defined by WHO as knowledge,

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skills, and practices based on cultural beliefs and experiences used to maintain health and treat illness, plays a critical role in the primary healthcare systems of countries like Nigeria (8; 9;10).

Hunteria umbellata (K. Schum) Hallier f., of the *Apocynaceae* family, is widely distributed across West Africa and locally known by several names, including "madaci" (Hausa), "mkpokiri" (Igbo), "aberere" (Yoruba), and "Asewa" (Ibibio) (11; 12). Various parts of the plant—seeds, leaves, bark, and roots—have traditionally been used to treat fever, menstrual irregularities, intestinal worms, stomach disorders, and microbial infections (13; 14). Phytochemical analyses have shown that *Hunteria umbellata* is rich in alkaloids, flavonoids, tannins, and phenols, which may account for its antimicrobial properties (15). Despite its wide use in traditional medicine, limited scientific data exist on its efficacy against clinically significant microbial pathogens.

Therefore, this study aims to investigate the antimicrobial potential of the ethanolic extract of *Hunteria umbellata* seeds on selected clinical isolates, with the goal of validating its traditional use and assessing its potential as a plant-based antimicrobial agent.

2. Material and methods

2.1. Plant collection and identification

Mature fruits of *Hunteria umbellata* were collected from the Pharmacy Plantation at the University of Uyo, Uyo, Akwa Ibom State, Nigeria. Mrs. Emmanuella Udoma, a taxonomist in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy Herbarium, University of Uyo, Nigeria, identified and authenticated the plant samples. A voucher specimen UUPH 6(I) was deposited in the Departmental herbarium for future reference.

2.2. Preparation of Extract and Extraction of the Plant Material

Seeds were extracted manually from the fruits, dehusked, washed thoroughly with clean water, and air-dried at room temperature for three weeks. Dried seeds were ground into fine powder using a VTCL Solitaire mixer grinder. A total of 3324 g of powdered seeds was soaked in 7.5 L of absolute ethanol for 72 hours with intermittent shaking. The mixture was filtered with Whatman No. 1 filter paper, and the filtrate was concentrated in a water bath at 40 °C to yield the crude extract, which was then stored at 4 °C in a sealed container until use.

2.3. Preparation of Ethanolic Extract of *Hunteria umbellata* Seeds

The ethanolic extract of *Hunteria umbellata* seeds was prepared and subsequently dissolved in sterile water and dimethyl sulfoxide (DMSO) to obtain different concentrations. For antibacterial assays, concentrations of 100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL, and 500 mg/mL were used. For antifungal assays, concentrations of 50 mg/mL, 25 mg/mL, and 12.5 mg/mL were prepared.

2.4. Collection of Isolates

The clinical isolates used included: Gram-positive bacteria- *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus* spp*., Clostridium* spp., *Gram-negative bacteria- Salmonella* spp*., Serratia* spp., *Escherichia coli, Pseudomonas aeruginosa, Shigella* spp*., Enterobacter* spp*., Klebsiella* spp., *Proteus* spp*., Vibrio cholerae, and Vibrio haemolyticus.* Fungi- *Candida albicans, Aspergillus niger, and Mucor* spp.

Isolates were obtained from the Microbiology Stock Culture Unit, Department of Microbiology, University of Uyo. All isolates were sub-cultured on Nutrient Agar (bacteria) or Sabouraud Dextrose Agar (SDA) (fungi) and maintained under refrigeration until use.

2.5. Antibacterial Activity Assay

Bacterial strains were cultured in nutrient broth at 37 °C for 18 hours and standardized to 0.5 McFarland turbidity. 90 mm Petri dishes were prepared with Nutrient Agar and inoculated using sterile swabs. Wells (6 mm) were bored and filled with 0.1 mL of extract at various concentrations. Gentamicin (100–500 μg/mL) served as the positive control. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimeters.

2.6. Antifungal Activity Assay

Fungal isolates were cultured on SDA at 28 °C for 24 hours. Fungal spore suspensions were adjusted to 0.5 McFarland standard and used to inoculate SDA plates. Wells (6 mm) were filled with 0.2 mL of the extract. Nystatin (40 mg/mL) was used as the positive control. Plates were incubated at 28 °C for 5 days, and inhibition zones were measured.

2.7. Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined by serial dilution in Nutrient Broth (bacteria) or Sabouraud Dextrose Broth (fungi), followed by inoculation with standardized microbial suspensions. Incubation was carried out at 37 °C for 24 hours (bacteria) or 28 °C for 48–72 hours (fungi). MIC was recorded as the lowest concentration with no visible turbidity.

2.8. Determination of Minimum Bactericidal Concentration (MBC)

From MIC tubes with no visible growth, 0.1 mL aliquots were sub-cultured onto fresh Nutrient Agar and incubated at 37 °C for 24 hours. MBC was the lowest concentration with no colony growth.

2.9. Determination of Minimum Fungicidal Concentration (MFC)

Similarly, 0.1 mL aliquots from MIC tubes (fungi) were cultured on SDA plates and incubated at 28 °C for 48 hours. MFC was defined as the lowest extract concentration with no fungal colony growth. All assays were conducted in triplicate to ensure reproducibility.

3. Results and discussion

The ethanolic extract of *Hunteria umbellata* seeds (EEHUS) showed varying degrees of antimicrobial activity against a range of Gram-positive and Gram-negative bacteria, as well as pathogenic fungi. The ethanolic extract of *Hunteria umbellata* seeds demonstrated notable antibacterial activity against selected Gram-positive and Gram-negative bacteria in a concentration-dependent manner. Among the tested organisms, *Staphylococcus aureus* exhibited a progressive increase in susceptibility, with a maximum zone of inhibition diameter (ZID) of 20 mm at 500 mg/mL, matching the activity of gentamycin at 40 mg/mL. This suggests that the extract contains potent antimicrobial phytochemicals, such as flavonoids and alkaloids, known for disrupting microbial membranes and inhibiting protein synthesis (16;17).

Streptococcus species exhibited the highest susceptibility to the extract, with an inhibition zone of 21 mm at the highest concentration, significantly surpassing gentamycin, which recorded a 9 mm zone of inhibition. This remarkable antibacterial activity highlights the potential of EEHUS as an effective alternative treatment for streptococcal infections, particularly in light of rising antibiotic resistance. This finding is supported by 18, who also demonstrated the antimicrobial activity of plant extracts against *Streptococcus pneumoniae*.

Moderate antibacterial activity was observed against *Staphylococcus epidermidis* and *Bacillus subtilis*, particularly at concentrations of 300 mg/mL and above. The inhibition zone for *S. epidermidis* peaked at 15 mm, while *B. subtilis* showed a maximum of 10 mm. These results align with previous studies suggesting that the structural characteristics of bacterial cell walls and inherent resistance mechanisms influence susceptibility to plant-based antimicrobials (19).

In contrast, *Clostridium* sp. exhibited complete resistance to the extract across all tested concentrations, likely due to its robust cell wall structure, spore-forming ability, and efflux systems that counteract plant-derived antimicrobial agents (20). These findings support the traditional use of *Hunteria umbellata* in managing bacterial infections such as pneumonia and skin conditions. The extract's dose-dependent efficacy suggests enhanced antibacterial properties at higher concentrations, attributed to bioactive constituents including alkaloids, flavonoids, phenols, tannins, and saponins (21).

Enterobacter sp. and *Escherichia coli* exhibited the highest sensitivity to the extract, recording zones of inhibition diameters of 30 mm and 27 mm at 500 mg/mL, surpassing gentamycin's 20 mm and 22 mm, respectively. 19; 22 also reported similar findings, attributing antibacterial activity to the presence of bioactive phytochemicals such as flavonoids, alkaloids, saponins, and tannins, which are known for their ability to disrupt bacterial membranes and inhibit key enzymes critical for microbial survival.

The extract also demonstrated dose-dependent inhibition of *Shigella* sp. and *Salmonella* sp., with zones of inhibition diameters (ZIDs) of 22 mm and 16 mm, respectively, at 500 mg/mL, reinforcing the antimicrobial potential of the EEHUS. These results align with previous studies showing that plant-derived antimicrobials exhibit enhanced activity at higher concentrations, making them valuable in managing multidrug-resistant organisms (23; 24).

Moderate antibacterial activity was observed against *Proteus* spp., *Pseudomonas aeruginosa,* and *Serratia* spp., with zone of inhibition diameters ranging from 16 mm to 20 mm at the highest concentrations of 500 mg/mL. However, bacterial resistance mechanisms such as efflux pumps and biofilm formation may influence susceptibility, reducing the effectiveness of plant extracts. These findings are consistent with previous reports on the antimicrobial effects of plant phytochemicals against Gram-negative bacteria, including *Pseudomonas* and *Proteus* species (25; 26, 27). Conversely, *Klebsiella* spp. and *Vibrio haemolyticus* exhibited limited susceptibility, possibly due to biofilm formation, thick polysaccharide capsules, or reduced membrane permeability (28, 29, 30). These findings support the traditional use of plant extracts such as EEHUS in managing bacterial infections and affirm their broad-spectrum antibacterial potential, particularly against *Escherichia coli, Enterobacter* spp*.,* and *Shigella* spp*.* (23).

Table 1 Zones of Inhibition Diameter (mm) of Ethanolic Extract of *Hunteria umbellata* Seeds and Gentamycin Against Gram-Positive and Gram-Negative Bacteria

Test organisms	**Zone of Inhibition (mm) Concentration of extract (mg/mL)**					**Gentamycin (mg/mL)**
	100	**200**	**300**	**400**	**500**	**40**
Gram-Positive Bacteria						
Staphylococcus aureus	8	11	14	16	20	20
Bacillus subtilis	NA	NA	NA	7	10	6
Staphylococcus epidermidis	NA	NA	9	11	15	23
Streptococcus spp	9	12	15	18	21	9
Clostridium spp	NA	NA	NA	NA	NA	6
Gram-Negative Bacteria						
Salmonella spp	NA	8	11	14	16	18
Escherichia coli	14	17	20	24	27	22
Serratia spp	NA	8	11	15	18	12
Pseudomonas aeruginosa	NA	8	10	12	16	18
Shigella spp	8	11	14	17	22	19
Enterobacter spp	10	16	20	24	30	20
Klebsiella spp	NA	NA	NA	10	13	14
Proteus spp	7	9	11	16	20	13
Vibrio cholerae	NA	7	10	13	16	15
Vibrio haemolyticus	NA	NA	NA	NA	10	17

Species: spp, No activity: NA

The observed minimum inhibitory concentrations (MICs) of 100 mg/mL for *Staphylococcus aureus* and *Streptococcus* species indicated the highest susceptibility. This activity suggests the presence of bioactive phytochemicals such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds in the extract, such as EEHUS. These compounds are known to interfere with bacterial membrane integrity and protein synthesis (31; 32)

The elevated minimum inhibitory concentration (MIC) of 300 mg/mL observed for *Staphylococcus epidermidis* can be attributed to species-specific factors such as membrane composition, biofilm formation, and intrinsic resistance mechanisms. Studies have shown that *S. epidermidis* biofilms exhibit significantly higher antibiotic tolerance than planktonic cells, with resistance driven by the extracellular matrix acting as a physical barrier, reduced metabolic activity, and persister cells. Additionally, biofilms hinder antimicrobial diffusion, leading to subtherapeutic drug concentrations within the biofilm and contributing to infection persistence (29;33).

In contrast, *Bacillus subtilis* and *Clostridium* spp. exhibited no detectable inhibition (ND), indicating strong protective barriers such as thick peptidoglycan layers or spore-forming capabilities that limit the effectiveness of plant-derived antimicrobials. Studies have also shown that Bacillus and *Clostridium* species develop metabolically dormant

endospores, making them highly resistant to environmental stressors and antimicrobial treatments (34). Additionally, spore formation enhances bacterial survival by providing structural defenses that hinder antimicrobial penetration, further contributing to resistance (20). These findings support the selective antimicrobial action of EEHUS, which effectively targets certain Gram-positive bacteria while being ineffective against others due to structural resistance mechanisms.

The extract demonstrated strong inhibitory activity against *Serratia* sp., *Escherichia coli*, *Shigella* spp., *Enterobacter* spp., and *Proteus* spp., with minimum inhibitory concentration (MIC) values ranging from 20 mg/mL to 100 mg/mL, indicating high bacterial susceptibility (35). Studies have shown that medicinal plants contain bioactive compounds such as coumarins, flavonoids, phenolics, alkaloids, terpenoids, and tannins, which may contribute to bacterial membrane disruption and enzyme inhibition, ultimately impeding microbial growth (36).

Conversely, Salmonella spp., *Pseudomonas aeruginosa*, and *Vibrio cholerae* exhibited higher MIC values of 200 mg/mL, indicating reduced susceptibility. This diminished activity may be attributed to structural resistance features such as low outer membrane permeability and active efflux mechanisms (37), particularly in *P. aeruginosa*, which is inherently resistant to many antimicrobials due to its robust efflux systems and biofilm formation capacity (38). These resistance mechanisms limit the intracellular accumulation of antimicrobial agents, thereby reducing their efficacy (39).

Klebsiella spp. and *Vibrio haemolyticus* exhibited no detectable inhibition (ND) across all tested concentrations, suggesting either highly efficient resistance mechanisms or insufficient phytochemical concentrations for effective antibacterial action. Studies have indicated that *Klebsiella* species possess multiple resistance adaptations, including capsular structures that enhance immune evasion, production of β-lactamase enzymes that confer resistance to β-lactam antibiotics, and thick lipopolysaccharide layers that reduce membrane permeability (40). Similarly, *Vibrio haemolyticus* has been reported to exhibit resistance due to biofilm formation and efflux pump activity, further limiting the effectiveness of antimicrobial agents (41). These findings highlight the complexity of bacterial resistance mechanisms and the challenges in developing effective plant-derived antibacterial treatments.

Table 2 Minimum Inhibitory Concentration (MIC, mg/ml) of Ethanolic Extract of *Hunteria umbellata* Seeds against Gram-Positive and Gram-Negative Bacteria

Test organisms	**MIC (mg/ml)**
Gram-Positive Bacteria	
Staphylococcus aureus	100
Bacillus subtilis	ND
Staphylococcus epidermidis	300
Streptococcus spp	100
Clostridium spp	ND
Gram-Negative Bacteria	
Salmonella spp	200
Escherichia coli	100
Serratia spp	20
Pseudomonas aeruginosa	200
Shigella spp	100
Enterobacter spp	100
Klebsiella spp	ND
Proteus spp	100
Vibrio cholerae	200
Vibrio haemolyticus	ND

Species: spp, No activity: NA

Table 3 presents the Minimum Bactericidal Concentration (MBC) values of the ethanolic extract of *Hunteria umbellata* seeds (EEHUS) against selected Gram-positive and Gram-negative bacterial strains. The bactericidal efficacy of the extract varied considerably among the organisms, reflecting differences in bacterial structure, resistance mechanisms, and interactions with phytochemicals present in the extract.

The EEHUS extract exhibited moderate bactericidal activity against *Staphylococcus aureus* and *Streptococcus* species, with minimum bactericidal concentration (MBC) values of 150 mg/mL. This suggests the presence of potent bioactive compounds, such as flavonoids, alkaloids, tannins, and saponins, which are known to disrupt bacterial membranes, inhibit protein synthesis, and induce cell death (42; 43). The efficacy against these pathogens supports the traditional use of *Hunteria umbellata* in treating infections such as skin abscesses, pneumonia, and streptococcal pharyngitis (44).

In contrast, Staphylococcus epidermidis exhibited reduced susceptibility, requiring 400 mg/mL for bactericidal action. This higher MBC may be attributed to biofilm formation and other adaptive resistance mechanisms, which necessitate higher antimicrobial concentrations for effective bacterial eradication (15). While the extract retains bactericidal properties against S. epidermidis, its reduced efficacy at lower concentrations suggests that higher doses or synergistic combinations may be needed to overcome resistance and achieve therapeutic success (45).

Bacillus subtilis and *Clostridium* spp. showed no detectable bactericidal activity (ND) at all tested concentrations. This lack of effect highlights the strong protective barriers of these bacteria, such as thick peptidoglycan layers and spore-forming capabilities, which limit the penetration and efficacy of plant-derived antimicrobials *(*44, 15). These findings suggest that *Hunteria umbellata* exhibits selective bactericidal action, being effective against certain Gram-positive bacteria while ineffective against others, potentially due to differences in cell wall architecture and resistance mechanisms.

The EEHUS extract exhibited strong bactericidal activity against *Escherichia coli*, *Shigella* spp., *Enterobacter* spp., and *Proteus* spp., with minimum bactericidal concentration (MBC) values of 150 mg/mL, indicating high bacterial susceptibility to the bioactive compounds present in the extract. The presence of bioactive compounds such as flavonoids, tannins, and alkaloids likely contributed to bacterial membrane disruption, inhibition of nucleic acid synthesis, and cellular leakage, ultimately leading to bacterial cell death (19; 46; 44). Given their clinical relevance in gastrointestinal and urinary tract infections, this finding supports the potential therapeutic potential of EEHUS in managing infections (15).

Conversely, *Salmonella* spp., *Serratia* spp., *Pseudomonas aeruginosa*, and *Vibrio cholerae* exhibited higher MBC values of 300 mg/mL, suggesting reduced susceptibility. These bacteria are known for their complex resistance mechanisms, including robust efflux systems, biofilm production, and decreased outer membrane permeability, which collectively diminish antimicrobial efficacy (41). The partial susceptibility observed suggests that higher extract concentrations or combination therapies with other antimicrobials may be necessary to achieve effective bactericidal outcomes.

Klebsiella spp. and *Vibrio haemolyticus* were not inhibited (ND) at all tested concentrations, suggesting complete resistance or the need for higher extract doses beyond the tested range. This resistance could be attributed to protective adaptations such as capsule polysaccharide production, β-lactamase secretion, and thick lipopolysaccharide layers that impede antimicrobial components (47). These findings suggest that while *Hunteria umbellata* is effective against certain Gram-negative bacteria, its bactericidal action is limited against others.

Table 3 Minimum Bactericidal Concentration (MBC, mg/mL) of Ethanolic Extract of *Hunteria umbellata* Seed against Gram-Positive and Gram-Negative Bacteria

Test organisms	**MIC (mg/ml)**
Gram-Positive Bacteria	
Staphylococcus aureus	150
Bacillus subtilis	ND
Staphylococcus epidermidis	400
Streptococcus spp	150
Clostridium spp	ND

Gram-Negative Bacteria	
Salmonella spp	300
Escherichia coli	150
Serratia spp	300
Pseudomonas aeruginosa	300
Shigella spp	150
Enterobacter spp	150
Klebsiella spp	ND
Proteus spp	150
Vibrio cholerae	300
Vibrio haemolyticus	ND

Species: spp, No activity: NA

Table 4 presents the antifungal activity of the ethanolic extract of *Hunteria umbellata* seeds (EEHUS) against selected fungal pathogens like *Candida albicans*, *Aspergillus niger*, and *Mucor* spp. with Nystatin (10 mg/mL) as the reference drug. The extract demonstrated dose-dependent antifungal activity, with varying susceptibility among the test organisms.

Among the tested fungi, *Candida albicans* exhibited the highest sensitivity to the extract, with its zone of inhibition increasing from 7 mm at 100 mg/mL to 20 mm at 500 mg/mL. While Nystatin showed a larger inhibition zone (30 mm), EEHUS still demonstrated significant antifungal potential at higher concentrations. This suggests that the phytochemicals such as flavonoids, alkaloids, tannins, and saponins known to compromise fungal cell wall integrity, inhibit ergosterol synthesis, and interfere with membrane function (46;47;16;44;15). Given the pathogenicity of *C. albicans*, especially in immunocompromised patients, this result supports the extract's potential in treating candidiasis (48).

The extract also demonstrated moderate inhibition activity against *Aspergillus niger*, with inhibition zones increasing from 9 mm at 200 mg/mL to 17 mm at 500 mg/mL. This *dose-dependent inhibition* suggests that EEHUS may be effective in treating *Aspergillus*-related infections, particularly at higher concentrations, due to its active constituents' *disruption of fungal cell walls or inhibition of spore germination*. Previous studies have indicated that plant-derived antifungal agents can effectively target *Aspergillus* species, often disrupting fungal cell wall integrity and metabolic pathways (44).

Mucor spp. showed the least susceptibility among the tested fungi, with no inhibition (NA) at 100 and 200 mg/mL, and moderate zones of inhibition (7–14 mm) at concentrations of 300 mg/mL and above. However, the extract exhibited a slightly higher inhibition zone (14 mm at 500 mg/mL) compared to Nystatin (12 mm), indicating potential efficacy against mucormycosis-causing fungi at elevated concentrations. The reduced susceptibility at lower doses is likely due to the robust hyphal structure and rapid growth rate of *Mucor* species, which pose a barrier to the penetration of phytochemicals (49; 50).

Table 4 Zones of Inhibition (Diameter in mm) of Ethanolic Extract of *Hunteria umbellata* Seed and Nystatin Against Fungi

S/N	**Test Isolates**	**Concentration (mg/ml) and ZID (mm)**					**Nystatin (mg/mL)**
		100	**200**	**300**	**400**	**500**	**10**
1	*Candida albicans*	7	10	13	16	20	30
2	*Aspergillus niger*	NA	9	11	14	17	14
3	*Mucor* spp	NA	NA	7	10	14	12

No activity: NA, Zones of Inhibition Diameter (ZID) in mm

The antifungal potential of the ethanolic extract of *Hunteria umbellata* seeds (EEHUS) was evaluated using MIC determination against three clinically relevant fungal isolates: *Candida albicans*, *Aspergillus niger*, and *Mucor* species as presented in Table 5. EEHUS demonstrated variable degrees of antifungal activity, with *Candida albicans* exhibiting the highest susceptibility (MIC = 100 mg/mL), followed by *Aspergillus niger* (MIC = 200 mg/mL). No inhibitory activity was observed against *Mucor* species, as indicated by the 'not determined' (ND) MIC value. In contrast, the reference antifungal agent, Nystatin, was markedly more effective, with MICs ranging from 2.00 to 2.50 mg/mL across all tested fungi (44; 15).

Previous studies have attributed the antimicrobial activity of medicinal plant extracts to their rich phytochemical constituents, such as flavonoids, alkaloids, tannins, and saponins, which are known to disrupt fungal membrane integrity, interfere with ergosterol biosynthesis, and inhibit essential enzymatic processes (46;47;16). The strong inhibition of *C. albicans*, a common opportunistic pathogen, highlights the therapeutic relevance of *Hunteria umbellata* in treating fungal infections such as oral thrush and *vulvovaginal candidiasis* (48).

Aspergillus niger exhibited a MIC value of 200 mg/mL, indicating reduced sensitivity compared to *C. albicans*. This aligns with previous studies on *Aspergillus* resistance, where fungal spores and hyphal structures contribute to decreased permeability of antifungal agents (51). The extract's moderate activity suggests potential efficacy in managing *Aspergillus*-related infections, particularly in cases of pulmonary aspergillosis, though higher concentrations may be necessary for therapeutic applications. *Previous reports have also indicated that plant-based antifungals may act on Aspergillus species by disrupting cell wall biosynthesis or targeting energy metabolism pathways* (52, 53).

In contrast, *Mucor* species showed no detectable (ND) susceptibility to EEHUS at all the tested concentrations. This resistance is consistent with literature suggesting that zygomycetes, such as *Mucorales*, possess unique cell wall compositions and metabolic characteristics that confer innate resistance to many plant-based and conventional antifungal agents (54). Additionally, the thick hyphal walls and rapid growth of *Mucor* spp. may impede the penetration and efficacy of bioactive phytochemicals and many conventional antifungals (49).

Table 5 Minimum Inhibitory Concentration (MIC, mg/mL) of Ethanolic Extract of *Hunteria umbellata* Seed and Nystatin Against Fungi

S/N	Test isolates	Minimum Inhibitory Concentration (mg/mL)	Nystatin (Standard control) (mg/mL)
1	*Candida albicans*	100	2.00
2	*Aspergillus niger*	200	2.50
3	*Mucor* spp	ND	2.50

Not determined: ND

Table 6 presents the Minimum Fungicidal Concentration (MFC) values of ethanolic extract of *Hunteria umbellata* seeds (EEHUS) against selected fungal isolates, with Nystatin as the reference antifungal agent. The results demonstrate varying fungicidal potency across fungal species, highlighting a concentration-dependent effect. *The Minimum* Fungicidal Concentration (MFC) of the ethanolic extract of *Hunteria umbellata* seeds (EEHUS) was evaluated against three clinically important fungal isolates such as Candida albicans, *Aspergillus niger*, and *Mucor* species using Nystatin as the reference antifungal agent. Among the tested fungi, *Candida albicans* exhibited the highest sensitivity to EEHUS, with an MFC of 150 mg/mL. Although this value is significantly higher than that of Nystatin (4.00 mg/mL), it suggests the presence of bioactive compounds in the extract capable of compromising fungal viability (44; 15).

Phytochemicals such as flavonoids, alkaloids, tannins, and saponins have been reported to exert antifungal effects by disrupting membrane integrity, inhibiting ergosterol biosynthesis, and interfering with key metabolic pathways (19; 46; 47; 16). The fungicidal activity observed against *C. albicans*, a common opportunistic pathogen associated with candidiasis, supports the potential of *Hunteria umbellata* as a candidate for natural antifungal therapy.

In contrast, *A. niger* required a higher concentration (MFC = 300 mg/mL) to achieve Fungicidal activity, indicating reduced sensitivity. This is consistent with previous reports highlighting the inherent resistance mechanisms of *Aspergillus* species, which include robust cell wall architecture, active efflux pumps, and a propensity for rapid sporulation (55). Despite this relative resistance, the observed fungicidal effect suggests that EEHUS retains antifungal potential, particularly when optimized in formulation or used synergistically with conventional agents. Studies have also shown that natural antifungal compounds can inhibit *Aspergillus* by targeting β-glucan synthesis, chitin metabolism, and mitochondrial functions (52; 56).

No fungicidal activity was observed against *Mucor* species (ND), indicating resistance to EEHUS at the tested concentrations. This is consistent with existing literature highlighting the intrinsic resistance of *Mucorales* to many antifungal agents, particularly phytochemicals, due to their unique cell wall composition, rapid growth, and high chitosan content (54; 49). The resilience of *Mucor* species further underscores the difficulty in treating mucormycosis and the need for either more potent plant extracts or combination therapies

Table 6 Minimum Fungicidal Concentration (MFC, mg/ml) of Ethanolic Extract of *Hunteria umbellata* Seeds and Nystatin Against Fungi

S/N	Test isolates	Minimum Fungicidal Concentration (mg/mL)	Nystatin (Standard control) (mg/ml)
1	*Candida albicans*	150	4.00
2	*Aspergillus niger*	300	4.50
3	*Mucor* spp	ND	4.50

Not determined: ND

4. Conclusion

The ethanolic extract of *Hunteria umbellata* seeds (EEHUS) demonstrated promising antimicrobial activity against a range of clinically relevant pathogens, notably *Escherichia coli, Streptococcus* spp*., Enterobacter* spp*., and Candida albicans*. These findings validate its traditional medicinal use and highlight its potential as a source of natural antimicrobial agents.

However, the extract showed limited or no activity against more resistant organisms such as *Clostridium* spp*., Klebsiella* spp*., Vibrio haemolyticus, and Mucor* spp., suggesting that its efficacy is spectrum-specific.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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