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Phytochemical screening and antimicrobial studies on the aqueous ethanol extract of the leaves of *Walteria indica.* Linn (Sterculiaceae) and aerial parts of *Euphorbia hirta* Linn (Euphorbiaceae)

Umar Faruk Shehu[*1], Given Vihityo Toma[1], Abdulrahman Adamu[1], Ibrahim Muazzam Aliyu[2]

[1]*Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria;*
***Email:** *ufshehu@abu.edu.ng*

[2]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria;*

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ABSTRACT

Background & Aim: *Waltheria indica* and *Euphorbia hirta* are traditionally employed in African ethnomedicine to manage microbial infections. This study aims to scientifically validate these uses through phytochemical analysis and antimicrobial evaluation of their aqueous ethanol extracts.

Experimental: The aerial parts of *E. hirta* and leaves of *W. indica* were extracted using 75% ethanol. Phytochemical screening was conducted using standard qualitative methods. Antimicrobial activities were assessed via agar well diffusion against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Aspergillus niger*. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) were determined using serial dilution and sub-culturing methods based on Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds in both extracts. Antimicrobial tests indicated that both plant extracts had notable inhibitory effects on Gram-positive and Gram-negative bacteria, but not on *A. niger*. Notably, *W. indica* exhibited a lower MIC (12.5 mg/mL) and MBC (50 mg/mL) against *S. aureus* compared to *E. hirta*. The combination of both extracts showed enhanced zones of inhibition, especially against *E. coli* (22 mm), *S. aureus* (20 mm), and *B. subtilis* (22 mm), suggesting potential synergistic interactions. However, antifungal activity was absent across all samples.

Recommended applications/industries: These findings support the development of plant-based antimicrobial agents from *W. indica* and *E. hirta* for the treatment of bacterial infections. Further studies should isolate and characterize the active constituents, explore mechanisms of action, and assess toxicity and efficacy *in vivo* to support pharmaceutical applications.

1. Introduction

Plant extracts have long been used in the treatment of infectious diseases, predating the development of synthetic drugs, particularly in regions where traditional medicine remains integral to healthcare (Sofowora *et al.*, 2013; Yuan *et al.*, 2016). In developing nations, medicinal plants continue to play a crucial role in primary healthcare due to their accessibility, affordability, and cultural acceptance (WHO, 2019; Ekiert *et al.*, 2020). The increasing ineffectiveness of conventional antibiotics, driven by the global rise in antimicrobial resistance (AMR), has renewed interest in plant-derived antimicrobial agents

as potential alternatives or adjuvants to existing therapies (Ventola, 2015; Atanasov *et al.*, 2021). The World Health Organization (WHO) has also emphasized the importance of integrating traditional medicine into modern healthcare systems, particularly in combating drug-resistant infections (WHO, 2023).

Waltheria indica has been traditionally utilized for its purported anti-inflammatory, antimicrobial, and detoxifying properties (Mukherjee *et al.*, 2017; Rather *et al.*, 2021). In Ayurvedic and African traditional medicine, it has been employed to treat skin infections, wounds, and microbial diseases due to its bioactive constituents (Chaudhary *et al.*, 2020). Recent studies have demonstrated its antimicrobial activity against various pathogens, supporting its ethnomedicinal use (Al-Snafi, 2020). Similarly, *Euphorbia hirta* L. has been widely documented in ethnobotanical literature for its therapeutic effects against respiratory infections, gastrointestinal disorders, and skin ailments (Tona *et al.*, 2001; Sharma and Kumar, 2021). Pharmacological studies have confirmed its antibacterial, antifungal, and anti-inflammatory properties, aligning with its traditional applications (Ahmad *et al.*, 2019; Pandey and Singh, 2022).

Despite their extensive use in traditional medicine, there remains a need for comprehensive scientific validation of the antimicrobial efficacy of *E. hirta* and *W. indica* through rigorous phytochemical and microbiological analyses. This study aims to bridge this gap by evaluating their bioactive compounds and antimicrobial potential against clinically relevant pathogens, thereby contributing to the growing body of evidence supporting plant-based antimicrobial therapies.

2. Materials and Methods

2.1 Plant collection and preparation

The aerial parts of *E.hirta* and the leaves of *W. indica* were collected from the surroundings of Main campus of Ahmadu Bello University Zaria, Kaduna State, Nigeria (11.0855° N 7.7190° E). The collections were made in July 2023. The taxonomical identification of the plants was confirmed by Mr. Namadi Sanusi of the Department of Botany Faculty of Life Sciences Ahmadu Bello University Zaria, Nigeria and assigned specimens Voucher number -ABU0918 for *E. hirta* and ABU0337for *W. indica* for future reference.

The plant parts collected were rinsed with tap water and air dried under the shade for 14days to arrest enzymatic degradation or hydrolysis of the active constituents. The dried parts were then reduced to coarse powder using mortar and pestle. The powders were then packed into polythene bag, labeled and kept dried as much as possible.

2.2 Extraction of plant material

Fifty grams each of the plant materials were macerated separately in 500 mL of 75% ethanol in a clean, sterilized glass bottle. The bottle was sealed tightly with a rubber cap and intermittently shaken throughout the extraction process. The mixture was allowed to macerate at room temperature for 72 hours (3 days), with occasional shaking to enhance extraction. After maceration, the mixture was first filtered through clean muslin cloth and then through Whatman No. 1 filter paper to obtain a clear filtrate. The resulting filtrate was evaporated to dryness using a water bath maintained at approximately 65 °C for about 7 hours. The dried extracts were subsequently weighed and stored in tightly sealed containers for further analysis.

2.3 Phytochemical screening

Phytochemical screening of the aqueous ethanol extract of each of the plants were carried out using the methods described by Evans (2009) and Sofowora (2008).

2.4 Antimicrobial studies

2.4.1Preparation of culture media and microorganism inoculation

Sabouraud Dextrose Agar (SDA), Nutrient Agar (NA), and Nutrient Broth (NB) were prepared in both single and double strengths according to standard protocols. Appropriate quantities of each medium were dissolved in distilled water, boiled with constant stirring, *dispensed* into sterile bottles (20 mL for agar, 10 mL for broth), and autoclaved at 121 °C for 15 minutes. A 0.9% sodium chloride solution was also prepared and sterilized for use as a diluent.

Test microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Aspergillus niger*) were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, ABU Zaria. These

organisms were sub-cultured into prepared media and incubated (bacteria at 37 °C for 18 hours; fungi at 30 °C for 24 hours). Cultures were stored at 4 °C for further use. For antimicrobial assays, overnight bacterial cultures were standardized using sterile saline dilutions to achieve approximately 10^6 CFU/mL: 1:5000 for Gram-negative and 1:1000 for Gram-positive bacteria. All procedures were conducted under aseptic conditions.

2.4.2. Antimicrobial susceptibility testing (Agar well diffusion method)

Standardized overnight cultures of test organisms were prepared by adjusting the turbidity to match the 0.5 McFarland standard (approximately 10^6 CFU/mL for bacteria and 10^3 CFU/mL for fungi) using 0.9% sterile normal saline. Sterile nutrient agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) were poured into petri dishes (20 mL per plate) and allowed to solidify. Each plate was flooded with 10 mL of standardized inoculum, and excess was decanted.

Four equidistant wells were bored into each agar plate using a sterile No. 6 cork borer. A drop of molten agar was added to seal the base of each well to prevent leakage. Wells were filled with 150 µL of plant extract at concentrations of 100, 50, 25, and 12.5 mg/mL. Plates were left at room temperature for one hour to allow pre-diffusion of the extracts before incubation. Bacterial plates were incubated at 37 °C for 18 hours, and fungal plates at room temperature for 24 hours. Zones of inhibition were measured in millimeters. Control plates were included and processed under the same conditions (CLSI, 2014). The same procedure was carried out for each of plant extracts and the mixture of the extracts in ratio 1:1.

2.4.3. Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extracts and mixture of the extracts (1:1) were determined using the agar dilution method. A two-fold serial dilution of the extracts was prepared in sterilized nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) to yield final concentrations ranging from 100 mg/mL to 0.195 mg/mL. This was achieved by mixing 10 mL of double-strength nutrient agar with 10 mL of extract solution, followed by serial dilution into bottles containing 10 mL of single-strength agar. The same procedure was followed for SDA in the case of fungal testing. The molten media were poured into sterile petri dishes and allowed to solidify.

Standardized overnight cultures of the test organisms (10^6 CFU/mL for bacteria, 10^3 CFU/mL for fungi) were inoculated onto the agar surfaces and allowed to diffuse for 30 minutes before incubation. Plates were incubated at 37 °C for 18 hours (bacteria) and at 30 °C for 24 hours (fungi). The lowest concentration of extract that inhibited visible microbial growth was recorded as the MIC (CLSI, 2014).

2.7. Determination of Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC)

To determine MBC/MFC, samples from MIC tubes or plates showing no visible growth were aseptically transferred into fresh nutrient broth or SDA supplemented with 3% Tween 80 (as inactivator). These were incubated at 37 °C for 18 hours (bacteria) and 30 °C for 24 hours (fungi). The lowest concentration of extract that showed no visible growth after sub-culturing was recorded as the MBC or MFC (CLSI, 2014).

3. Results and discussion

3.1. Phytochemical screening

Phytochemical analysis of *Waltheria indica* and *Euphorbia hirta* revealed the presence of bioactive compounds, including flavonoids, alkaloids, tannins, saponins, triterpenes, and steroids (Table 1).

Table 1. Phytochemical screening of the ethanol extracts of *Walteria indica* and *Euphorbia hirta.*

S/no	Phytochemical class	Test	Inference	
			A	B
1	Flavonoids	Ferric chloride test	Positive	Positive
		Shinoda test	Positive	Positive
		Alkaline reagent test	Positive	Positive
2	Saponins	Frothing test	Positive	Positive
		Haemolysis test	Positive	Positive
3	Anthraquinones	Borntrager's test	Negative	Negative
4	Cardiac glycosides	Kella-Kiliani test	Negative	Negative
5	Tannins	Lead subacetate test	Positive	Positive
6	Alkaloids	Dragendorff`s test	Positive	Positive
		Mayer`s test	Positive	Positive
7	Steroids and Triterpenes	Liebermann Burckhard test	Positive	Positive

KEY: A-*Walteri aindica*, B-*Euphorbia hirta*

These phytoconstituents are known to exhibit antimicrobial properties through multiple mechanisms, such as protein precipitation (tannins), membrane disruption (saponins), enzyme inhibition (flavonoids), and interference with microbial DNA replication (alkaloids) (Dzotam *et al.*, 2018; Rather *et al.*, 2021). Recent studies have highlighted the role of flavonoids and tannins in bacterial cell wall destabilization (Kuete, 2017), while saponins have been shown to disrupt fungal membrane integrity (Sharma *et al.*, 2022).

3.2. Antimicrobial studies

The ethanolic extracts of *W. indica* and *E. hirta* demonstrated significant antimicrobial activity against all tested bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*) but showed no activity against the fungal strain *Aspergillus niger* (Table 2).

Table 2. The zone of inhibition of the ethanol extract of *Walteria indica*, *Euphorbia hirta a*nd combination of the extracts.

Test Organisms	Mean zone of inhibition (mm) ± SD												
	100mg/mL			50mg/mL			25mg/mL			12.5mg/mL			Control
	A	B	C	A	B	C	A	B	C	A	B	C	
Pseudomonas aeruginosa	17±0.001	15±0.00	16±0.001	15±0.003	13±0.001	14±0.00	13±0.001	12±0.00	13.5±0.001	11.5±0.00	0	10±0.00	24±0.001
Escherichia coli	18±0.001	16±0.00	22±0.00	15±0.00	14±0.001	18±0.00	13±0.00	10±0.001	15±0.001	0±0.000	0	13±0.00	30±0.00
Bacillus substilis	18±0.001	15±0.00	22±0.00	16±0.00	13±0.001	20±0.001	15±0.001	11.5±0.001	17±0.00	14±0.00	0	14±0.00	33±0.00
Staphylococcus aureus	19±0.00	19±0.00	20±0.00	15±0.00	14±0.00	18±0.00	12±0.00	12±0.00	17±0.00	0	0	12±0.00	27±0.00
Aspergillus niger	15±0.00	0	0	13±0.00	0	0	0	0	0	0	0	0	33±0.00

A: *Waltheria indica,* B: *Euphorbia hirta***,** C: Combination of the two extract, SD: Standard Deviation**.**

Notably, *W. indica* exhibited stronger antimicrobial effects, with lower minimum inhibitory concentration (MIC) values—particularly against *S. aureus* (12.5 mg/mL)—compared to *E. hirta*, which required higher concentrations (MIC up to 100 mg/mL) for inhibition (Al-Snafi, 2020; Pandey and Singh, 2022).

The combined extract of both plants produced slightly larger zones of inhibition compared to individual extracts, suggesting a possible synergistic interaction (Table 3).

Table 3. The Minimum Inhibitory Concentration (MIC) of the aqueous ethanol extract of *Walteria indica*, *Euporbia hirta* and combination of the extracts.

Test organism	Minimum Inhibitory Concentration (mg/mL)		
	A	B	C
Pseudomonas aeruginosa	100	100	100
Bacillus subtilis	50	100	100
Escherichia coli	50	100	100
Staphlococcus aureus	12.5	50	25
Aspergilus niger	0	0	0

A: *Waltheria indica,* B: *Euphorbia hirta*, C: Combination of the two extract

However, MIC determination revealed that *W. indica* alone was more potent at lower concentrations, indicating that while combination therapy may enhance initial bacterial inhibition, *W. indica* possesses superior intrinsic antimicrobial activity (Kuete *et al.*, 2019).

Minimum bactericidal concentration (MBC) assays (Table 4) further supported these findings.

Table 4. Minimum Bactericidal Concentration (MBC) of the ethanol extract of *Waltheria indica*, *Euphorbia hirta* and combination of the extracts.

Test Organism	Minimum Bactericidal Concentration(mg/mL)		
	A	B	C
Pseudomonas aeruginosa	—	—	100
Bacillus substilis	100	—	100
Escherichia coli	100	—	—
Staphlococcus aureus	50	100	50

A: *Waltheria indica,* B: *Euphorbia hirta***,** C: Combination of the two extract**.**

W. indica exhibited bactericidal effects at 50–100 mg/mL, whereas *E. hirta* only demonstrated MBC against *S. aureus* at 100 mg/mL. The combination extract achieved bactericidal activity against *P. aeruginosa*, *S. aureus*, and *B. subtilis*, suggesting broader-spectrum efficacy (Ntie-Kang *et al.*, 2020). None of the extracts showed fungicidal activity against *A. niger*, possibly due to the limited antifungal properties of the identified phytochemicals (Toukam *et al.*, 2021).

According to CLSI (2023) standards, the susceptibility profiles derived from zone of inhibition, MIC, and MBC values indicate that while the combined extracts improved overall antimicrobial coverage, *W. indica* remained superior in potency. These differences likely stem from variations in phytochemical composition, with specific compounds in *W. indica* (e.g., flavonoids and alkaloids) contributing more effectively to microbial growth inhibition (Rather *et al.*, 2021; Sharma *et al.*, 2022).

4. Conclusion

The study demonstrates that *Waltheria indica* and *Euphorbia hirta* possess significant antimicrobial properties, attributable to their rich phytochemical composition. *W. indica* showed superior antimicrobial potency, particularly against *Staphylococcus aureus*, as evidenced by lower MIC and MBC values. While the combination of both extracts exhibited slightly enhanced zones of inhibition against bacterial strains, it did not outperform *W. indica* in terms of minimum inhibitory concentration. The absence of antifungal activity against *Aspergillus niger* suggests that the extracts may be more effective against bacterial pathogens. These findings support the traditional use of these plants in managing microbial infections and provide a scientific basis for further investigation into their active compounds, mechanisms of action, and potential development as alternative antimicrobial agents.

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