

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITIES OF AQUEOUS AND ETHANOLIC LEAF AND STEM EXTRACTS OF *EUPHORBIA HIRTA* ON SOME CLINICAL ISOLATES

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ABSTRACT

Medicinal plants have been widely used for centuries as sources of therapeutic agents, with *Euphorbia hirta* traditionally used to treat respiratory, gastrointestinal, and skin infections. The increasing prevalence of multidrug-resistant bacterial strains has intensified the need for alternative antimicrobial agents derived from natural sources. This study aims to evaluate the phytochemical constituents and antibacterial activities of ethanolic and aqueous leaf and stem extracts of *E. hirta* against clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. The plant materials were collected, identified, and extracted by maceration in ethanol and distilled water. Phytochemical screening was performed to detect the presence of polyphenols, tannins, flavonoids, saponins, and alkaloids. Antimicrobial activity was assessed using the agar well diffusion method, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Results revealed that the ethanol extracts contained higher concentrations of polyphenols, flavonoids, and alkaloids compared to the aqueous extracts, whereas tannins were more abundant in the aqueous extracts. The ethanol leaf extract exhibited the highest antimicrobial activity, particularly against *P. aeruginosa* and *S. aureus*, with inhibition zones of 23.5 mm and 19.5 mm, respectively, at 500 mg/ml. The aqueous stem extract showed notable activity against *S. pneumoniae* (16.5 mm at 500 mg/ml). The MIC values indicated that the ethanol stem extract was the most effective against *P. aeruginosa* (62.5 mg/mL), whereas aqueous extracts required higher concentrations (500 mg/mL). MBC results suggested that all extracts were more bacteriostatic than bactericidal. These findings support the traditional medicinal use of *E. hirta* and highlight its potential as a natural antimicrobial agent. The ethanol extracts, particularly from the leaves, demonstrated the strongest antibacterial effects, suggesting that ethanol is an optimal solvent for extracting bioactive antimicrobial compounds. Further research is recommended to isolate and characterize specific compounds and explore their mechanisms of action for potential pharmaceutical applications.

Keywords: Convective Flow, Dusty Viscous Fluid, MHD, Viscous Dissipation.

INTRODUCTION

Medicinal plants have played a pivotal role in human culture and healthcare systems for thousands of years, serving as foundational elements of traditional medicine. These plants have been used to treat a wide array of health conditions, prevent diseases, and even preserve food, showcasing their versatility and importance in

human life (Abdallah *et al.*, 2023). In recent years, the alarming rise of multidrug-resistant pathogens has necessitated a renewed focus on exploring alternative antimicrobial agents, particularly those derived from natural sources (Ahmad *et al.*, 2017). Among these, *Euphorbia hirta*, commonly known as asthma weed, has gained significant attention for its extensive use in traditional medicine to treat respiratory tract infections, gastrointestinal disorders, and skin conditions (Al-Snafi, 2017). Its potential as a source of novel antimicrobial compounds makes it a promising candidate for further scientific investigation.

Infectious diseases remain one of the leading causes of death globally, a problem exacerbated by the rapid emergence and spread of multidrug-resistant bacterial strains (Bloom & Cadarette, 2019). Antibiotic resistance, a phenomenon where bacteria evolve mechanisms to survive exposure to drugs designed to kill them, has become a critical public health challenge. The overuse, misuse, and inappropriate prescription of antibiotics in human medicine, agriculture, and livestock have accelerated the development of resistant strains, rendering many conventional antibiotics ineffective (Sofowora *et al.*, 2013; Caneschi *et al.*, 2023). This resistance not only complicates the treatment of common infections but also increases the risk of severe complications, prolonged hospital stays, and higher healthcare costs. In some cases, it has led to the emergence of "superbugs," which are resistant to nearly all available antibiotics, posing a dire threat to global health security.

The growing prevalence of antibiotic resistance underscores the urgent need for innovative solutions. There is a critical demand for new antibacterial agents that are effective, affordable, and associated with minimal side effects. Plant-based antimicrobials represent a vast and largely untapped resource for discovering such agents. Plants produce a diverse array of bioactive compounds, such as alkaloids, flavonoids, tannins, and terpenoids, which exhibit significant antimicrobial activity (Kubmarawa *et al.*, 2008). These natural compounds often exhibit unique mechanisms of action, making them less likely to induce resistance compared to synthetic antibiotics. Furthermore, the use of medicinal plants aligns with the principles of sustainability and environmental conservation, as they are renewable resources that can be cultivated with minimal ecological impact.

Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* are widely used in antimicrobial studies due to their clinical relevance and rising antibiotic resistance. These pathogens serve as important model organisms for evaluating the efficacy of novel antimicrobial agents,

understanding resistance mechanisms, and developing effective therapeutic strategies against multidrug-resistant infections. Specifically, the study focuses on the ethanolic and aqueous extracts of *Euphorbia hirta* leaves and stems and assesses their potential antibacterial effects against clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Clinical isolates, which are bacterial strains obtained directly from patients, are particularly relevant to this research, as they represent real-world pathogens with established resistance mechanisms (Kumari & Pandey, 2017). Studying these isolates ensures that the findings are directly applicable to practical healthcare scenarios, where antibiotic resistance poses a significant threat. This study aims to evaluate the phytochemical constituents and antibacterial activities of ethanolic and aqueous leaf and stem extracts of *E. hirta* against clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*.

MATERIALS AND METHODS

Study Area

The study was conducted at the Department of Microbiology and Biochemistry, Faculty of Science, Kaduna State University, Kaduna, Nigeria.

Collection, Identification, and Authentication of Plant Material

Fresh samples of *Euphorbia hirta* (leaves and stems) were collected from a local farm in the Rigasa area of Kaduna. The plant material was identified and authenticated by a botanist at the Department of Biological Sciences, Nigerian Defence Academy, Kaduna. A voucher number NDA/BIOH/2023/23 was issued and deposited in the Departmental Herbarium Unit for future reference. The leaves and stems were washed and dried in the shade. The dried samples were then ground into powder in the laboratory using a mortar and pestle. The powdered sample was kept in a closed plastic container for further analysis.

Collection of Clinical Isolates

The clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* used in this study were collected from the Department of Microbiology Laboratory, Kaduna State University. All samples were collected on nutrient agar slants, labelled, placed in a cold box, and transported to the postgraduate laboratory of the Biochemistry Department at Kaduna State University. Samples were incubated at 37 °C for 24 h.

Plant Extraction

Exactly 200 grams of each plant sample (leaves and stems) were cold-extracted separately by maceration in 1.5 litres of distilled water and 600 mL of ethanol, and then allowed to stand for 48 hours with intermittent shaking. The extracts were filtered using Whatman No. 1 paper. The extract filtrates were then evaporated in a hot-air oven at 40 °C for 72 hours before being lyophilized under vacuum at -80 °C and a pressure of 0.06 mbar. The dried/powdered extracts were weighed, the percentage yields were

calculated, and the extracts were stored at -20 °C for further analysis (Kong et al., 2014).

Phytochemical Screening

Qualitative and quantitative phytochemical analyses were conducted to detect the presence of polyphenols, tannins, flavonoids, saponins, and alkaloids using standard methods (Trease & Evans, 2002).

Antimicrobial Activity Testing

The agar well diffusion method was used to determine the antimicrobial activity of the extracts against the clinical isolates. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using standard protocols (Doughari et al., 2017).

Data Analysis

Data were analyzed using one-way ANOVA, and Duncan's multiple range test was used for post hoc analysis. Statistical significance was set at $p \leq 0.05$.

RESULTS

Plant Extraction

The percentage yield of *Euphorbia hirta* extracts varied based on the extraction solvent and plant part used. The ethanol extraction of the leaves yielded 15.0%, while the ethanol extract of the stem yielded 12.0%. In contrast, the aqueous extraction produced higher yields: the leaf extract 20.0%, and the stem extract 16.0%.

Phytochemical Contents

Table 1: Qualitative Phytochemical Screening of *Euphorbia hirta* Extracts

Phytochemical Compound	Ethanol Leaf Extract	Ethanol Stem Extract	Aqueous Leaf Extract	Aqueous Stem Extract
Carbohydrates	+	+	+	+
Polyphenols	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+

Key: (+) = Present, (-) = Absent

The qualitative phytochemical screening of the ethanol and aqueous extracts of the leaves and stem of *Euphorbia hirta* revealed the presence of all the tested phytochemical constituents, including carbohydrates, polyphenols, tannins, flavonoids, saponins, and alkaloids in all the extracts (Table 1).

Table 2: Concentration of polyphenol, tannins, and flavonoids in the aqueous and ethanol extracts of the leaves and stem of *Euphorbia hirta*

Extracts	Polyphenols (mg/g)	Tannins (mg/g)	Flavonoids (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)
Stem (EtOH)	0.024±0.008 ^a	9.83±4.17 ^a	0.112±0.012 ^a	2.18±0.16 ^b	1.39±0.03 ^b
Leaves (EtOH)	0.061±0.041 ^b	8.62±2.10 ^a	0.215±0.023 ^b	53.6±0.82 ^d	16.9±0.97 ^d
Stem (Aq)	0.019±0.008 ^a	13.0±1.55 ^b	0.123±0.229 ^a	1.05±0.08 ^a	0.19±0.04 ^a
Leaves (Aq)	0.022±0.009 ^a	28.9±3.84 ^c	0.108±0.011 ^a	12.3±0.21 ^c	8.36±0.26 ^c
p value	< 0.001	0.001	0.001	0.048	0.018

Values are given as mean ± standard deviation; in each column, mean values with different superscripts have a statistically significant difference ($p < 0.05$). Stem (EtOH) = ethanol extract of the stem of *Euphorbia hirta*; Leaves (EtOH) = ethanol extract of the leaves of *Euphorbia hirta*; Stem (Aq) = aqueous extract of the stem of *Euphorbia hirta*; Leaves (Aq) = aqueous extract of the leaves of *Euphorbia hirta*

The quantitative phytochemical analysis of the aqueous and ethanol extracts of the leaves and the stem revealed significant variations in the concentrations of polyphenols, tannins, flavonoids, saponins, and alkaloids among the different extracts. The ethanol leaf extract recorded the highest concentration of polyphenols (0.061±0.041 mg/g) and flavonoids (0.215±0.023 mg/g), indicating that ethanol was more effective in extracting these phytochemicals from the leaves compared to the aqueous solvent. In contrast, the aqueous leaf extract showed the highest tannin content (28.9±3.84 mg/g), suggesting that water favored the extraction of tannins from the leaves. Saponins were markedly abundant in the ethanol leaf extract (53.6±0.82 mg/g), which was significantly higher than those observed in the stem extracts and aqueous leaf extract. In contrast,

the stem aqueous extract had the lowest saponin concentration (1.05±0.08 mg/g). Similarly, alkaloid content was highest in the ethanol leaf extract (16.9±0.97 mg/g), followed by the aqueous leaf extract (8.36±0.26 mg/g). In contrast, the aqueous stem extract recorded the lowest alkaloid concentration (0.19±0.04 mg/g). Generally, leaf extracts exhibited higher concentrations of most phytochemicals than stem extracts, and ethanol extraction yielded greater amounts of polyphenols, flavonoids, saponins, and alkaloids, while aqueous extraction favored tannin extraction. The observed differences among the extracts were statistically significant as indicated by the different superscripts within each column and the corresponding p-values ($p < 0.05$).

Antimicrobial Activities

Table 3: Zones of Inhibition (mm) of *Euphorbia hirta* Extracts Against *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*

Organism	Conc. (mg/ml)	ALE	ASE	ELE	ESE	Control
<i>Pseudomonas aeruginosa</i>	500	13.5 ± 0.04 ^a	15.5 ± 0.07 ^b	23.5 ± 0.28 ^c	20.0 ± 0.83 ^d	63.5 ± 0.42 ^e
	250	11.5 ± 0.20 ^a	14.5 ± 0.32 ^b	20.5 ± 0.27 ^c	17.5 ± 0.22 ^d	63.5 ± 0.42 ^e
	125	NI	12.5 ± 0.13 ^a	18.5 ± 0.23 ^b	16.5 ± 0.57 ^c	63.5 ± 0.42 ^d
	62.5	NI	10.5 ± 0.45 ^a	17.5 ± 0.28 ^b	14.5 ± 0.23 ^c	63.5 ± 0.42 ^d
<i>Streptococcus pneumoniae</i>	500	NI	16.5 ± 0.24 ^a	19.5 ± 0.15 ^b	NI	58.5 ± 0.28 ^c
	250	NI	13.5 ± 0.29 ^a	15.5 ± 0.47 ^b	NI	58.5 ± 0.28 ^c
	125	NI	NI	NI	NI	58.5 ± 0.28 ^a
	62.5	NI	NI	NI	NI	58.5 ± 0.28 ^a
<i>Staphylococcus aureus</i>	500	16.5 ± 0.28 ^a	14.5 ± 0.29 ^b	19.5 ± 0.25 ^c	NI	57.5 ± 0.54 ^d
	250	NI	12.5 ± 0.37 ^a	18.0 ± 0.58 ^b	NI	57.5 ± 0.54 ^c
	125	NI	11.0 ± 0.48 ^a	NI	NI	57.5 ± 0.54 ^b
	62.5	NI	NI	NI	NI	57.5 ± 0.54 ^a

Key: ALE= Aqueous Leaf Extract, ASE= Aqueous Stem Extract, ELE= Ethanol Leaf Extract, ESE= Ethanol Stem Extract, NI= No inhibition, Control= Ciprofloxacin, Superscripts (a, b, c, d, e): Indicate statistically significant differences at $p < 0.05$ within each row (microorganism and concentration). Values with different superscripts are significantly different.

The ethanol leaf extract exhibited the highest antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with inhibition zones of 23.5 ± 0.28 mm and 19.5 ± 0.25 mm, respectively, at 500 mg/ml. The aqueous stem extract showed

notable activity against *Streptococcus pneumoniae*, with an inhibition zone of 16.5 ± 0.24 mm at 500 mg/ml. The results are summarized in Table 3 above.

Minimum Inhibitory and Bactericidal Concentrations

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Euphorbia hirta* Extracts

Microorganisms	Extract	MIC (mg/mL)	MBC (mg/mL)
<i>Pseudomonas aeruginosa</i>	Aqueous stem	500	ATC
	Aqueous leaf	500	ATC
	Ethanol stem	62.5	ATC
	Ethanol leaf	500	ATC
<i>Streptococcus pneumonia</i>	Aqueous stem	500	ATC
	Aqueous leaf	ATC	ATC
	Ethanol stem	500	ATC
	Ethanol leaf	Neg	ATC
<i>Staphylococcus aureus</i>	Aqueous stem	500	ATC
	Aqueous leaf	500	ATC
	Ethanol stem	ATC	ATC
	Ethanol leaf	500	ATC

Key: ATC = Above tested concentrations

The MIC values for the ethanol stem extract against *P. aeruginosa* were the lowest (62.5 mg/mL), while the MIC values for the aqueous extracts were higher (500 mg/mL). The MBC values for all extracts were above the tested concentrations, indicating bacteriostatic rather than bactericidal activity. The results are summarized in Table 4.

DISCUSSION

The study confirms the presence of significant bioactive compounds in *Euphorbia hirta*, including polyphenols, tannins, flavonoids, saponins, and alkaloids. These findings align with previous studies reporting similar phytochemical profiles in *E. hirta* and other medicinal plants (Al-Snafi, 2017; Ahmad et al., 2017). Ethanolic extracts, particularly from the leaves, exhibited the most potent antimicrobial activity, indicating ethanol's effectiveness as a solvent for extracting antimicrobial compounds. This is consistent with studies on other medicinal plants, such as *Azadirachta indica* and *Adansonia digitata*, in which ethanol extracts outperformed aqueous extracts in antimicrobial assays (Sharma et al., 2020). The ethanol leaf extract exhibited the highest concentrations of polyphenols (0.061 ± 0.041 mg/g) and flavonoids (0.215 ± 0.023 mg/g), known for their antioxidant and anti-inflammatory properties (Dias et al., 2021). These results are consistent with Asha et al. (2016), who reported high levels of polyphenols and flavonoids in *E. hirta* leaf extracts. Similarly, the aqueous leaf extract had the highest tannin content (28.9 ± 3.84 mg/g), consistent with Patel et al. (2024), who noted that tannins are more readily extracted with polar solvents such as water due to their hydrophilic nature. Tannins disrupt microbial cell walls and inhibit enzyme activities, contributing to their antimicrobial properties (Kováč et al., 2022).

The ethanol leaf extract showed the highest antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with inhibition zones of 23.5 ± 0.28 mm and 19.5 ± 0.25 mm, respectively, at 500 mg/ml. These results are comparable to those of Subramani et al. (2022), who found that ethanol extracts of *E. hirta* exhibited significant antimicrobial activity against oral pathogens. The aqueous stem extract showed notable activity against *Streptococcus pneumoniae*, with an inhibition zone of 16.5 ± 0.24 mm at 500 mg/mL, consistent with Kumar et al. (2017), who reported that aqueous extracts of *Hibiscus rosa-sinensis* were effective against Gram-positive bacteria, including *S. pneumoniae*.

The antimicrobial activity of *E. hirta* observed in this study is comparable to that of other medicinal plants. For example, Tran et al. (2020) reported that ethanol extracts of *E. hirta* exhibited significant antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*. Similarly, Waheed et al. (2020) found that ethanol extracts of *Euphorbia helioscopia* showed potent antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These findings suggest that *Euphorbia* species possess significant antimicrobial properties, attributed to their rich phytochemical composition.

The MIC values for the ethanol stem extract against *P. aeruginosa* were the lowest (62.5 mg/mL), indicating high effectiveness at lower concentrations. This is consistent with Doughari et al. (2017), who reported low MIC values for ethanol extracts of *Balanites aegyptiaca* against *Salmonella Typhi*. The MBC values for all extracts were above the tested concentrations, suggesting bacteriostatic rather than bactericidal activity. This aligns with Gonçalves et al. (2020), who found that plant extracts often inhibit bacterial growth without directly killing the organisms. The findings provide a scientific basis for the traditional use of *Euphorbia hirta* in treating respiratory infections and other ailments. Ethanolic extracts, particularly from the leaves, exhibited the most potent antimicrobial activity, suggesting that ethanol is the best solvent for extracting effective antimicrobial compounds. These results are consistent with Sharma et al. (2020), who reported that ethanol extracts of medicinal plants generally exhibit higher antimicrobial efficacy than aqueous extracts.

While this study provides valuable insights into the phytochemical and antimicrobial properties of *E. hirta*, there are limitations. The study was conducted *in vitro*, and further research is needed to evaluate the efficacy and safety of the extracts *in vivo*. Additionally, the specific compounds responsible for the observed antimicrobial activities have not been isolated and characterized. Future studies should focus on isolating these compounds and evaluating their mechanisms of action. Furthermore, the potential synergistic effects of *E. hirta* extracts with conventional antibiotics should be explored, as this could lead to the development of more effective combination therapies.

Conclusion

This study demonstrates that *Euphorbia hirta* contains significant bioactive compounds with antimicrobial properties. The ethanol extracts, particularly those from the leaves, exhibited the most

potent antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. These findings provide a scientific basis for the traditional use of *E. hirta* and suggest its potential as a source of natural antimicrobial agents. Further research is recommended to isolate and characterize specific compounds and evaluate their efficacy in clinical settings.

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Conflict of Interest:

The authors declare no conflict of interest.

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