FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 24, December, 2026, pp 32 – 39
DOI: https://doi.org/10.33003/fjs-2026-1024-6497

In Vitro Antimicrobial Activity and *In Vivo* Wound-Healing Potential of Hydro-Ethanol Plant Extract of *Euphorbia Hirta* in Wistar Rats with *Staphylococcus Aureus*-Infected Wounds

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Received: 11 Nov. 2026
Accepted: 19 Nov. 2026
Published: 23 Nov. 2026

ABSTRACT
Euphorbia hirta is a medicinal-plant widely recognized in traditional-medicine and has demonstrated considerable potential in the treatment of infections and wounds. This study focused on evaluating the *in-vitro* antimicrobial-activity and wound-healing potential of a hydro-ethanol plant extract of *Euphorbia hirta* in *Staphylococcus aureus*-infected wounds in Wistar-rats. Preliminary-phytochemical screening of the extract was performed using standard-procedures. The acute-toxicity of the extract was also evaluated using Locke's-method. The antimicrobial-activity was assessed *in-vitro* against selected bacterial and fungal-isolates using the agar-well diffusion-technique. For the wound-healing experiment, 30 Wistar-rats were randomly assigned to five-groups of six-rats each. A wound measuring 5mm was created on the dorsal thoracic-region of each rat after disinfection. The wounds were inoculated with *Staphylococcus-aureus* (1×10^5CFU/mL) and left for 48hours to establish infection. The rats were then treated with graded oral-doses of *E. hirta* extract (150, 300 and 600mg/kg) with topical-formulations of 0.5, 1.0 and 1.5g (group 1-3), clindamycin (50mg/kg orally) with 0.1% w/w gentamicin topically (group 4) and vaseline as the topical-control (group 5). Wound-contraction was monitored from day-1 through day-21. Bacterial-samples were collected from the wound sites before treatment and on day-17 to determine bacterial-load. Phytochemical-screening revealed several biologically-active constituents. The acute-toxicity study produced an LD_{50} above 5000mg/kg. *E. hirta* demonstrated concentration-dependent antimicrobial-activity against the tested bacterial and fungal-isolates in the *in-vitro*-study. In the wound-healing study, treatment with the extract significantly reduced bacterial-load and enhanced wound-contraction. The findings indicate that *Euphorbia hirta* possesses significant antimicrobial and wound-healing properties and may have potential applications in the management of bacterial wound-infections.

Keywords: Wound healing, *Staphylococcus aureus*, Bacteria wound infection, *Euphorbia hirta*, Bacteria count

INTRODUCTION

Microorganisms are essential components of the human microbiome and ecosystem, contributing to physiological processes and maintenance of host health; however, pathogenic species remain important causes of infectious diseases. A diverse range of microorganisms including viruses, parasites, fungi and bacteria are implicated in human infections and this has imposed substantial burden on global health systems (Anju *et al.,* 2023).

Among these microbial pathogens, bacteria constitute a persistent public health challenge contributing significantly to morbidity and mortality worldwide, particularly in low and middle-income countries where healthcare infrastructure, diagnostic capacity and access to effective antimicrobial therapy may be limited (Murray *et al.,* 2019; Lewnard *et al.,* 2024). The clinical outcome of bacterial infection is influenced by the virulence of the infecting organism, bacterial burden, host immune status, infection site and the effectiveness of antimicrobial therapy (Soni *et al.,* 2024).

Skin and soft-tissue infections are among the common manifestations of bacterial disease and frequently arise following trauma, surgery, burns, ulcers or other disruptions of the skin barrier. Under physiological conditions, intact skin constitutes a highly effective first line of host defense, functioning not only as a physical barrier but also as an immunologically active interface that restricts microbial colonization and invasion; once the integrity of the skin is compromised, protective function of the skin will be affected and this will creates a favourable micro-environment for microbial adherence, proliferation, tissue invasion and subsequent wound infection. Persistent infection may prolong inflammation, promote tissue damage, impair re-epithelialization and ultimately delay wound closure (Bessa, 2023; Uberoi *et al.,* 2024; Prodinger *et al.,* 2025).

Several Gram-positive and Gram-negative bacteria have been implicated in wound infections, this include *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter spp*. Among these, *S. aureus* is of particular clinical importance because of its ability to colonize damaged tissue, invade underlying structures, evade host defense mechanisms and persist within infected wound micro-environment (Pieniążek *et al.,* 2024).

Although *S. aureus* commonly occurs as a component of the normal skin and nasal microbiota, disruption of the skin barrier can facilitate its transition from commensalism to pathogenicity. Its pathogenicity is mediated by multiple virulence factors involved in adhesion, tissue invasion, immune evasion and host tissue damage (Touaitia *et al.,* 2025).

Management of bacterial wound infections typically involves wound cleansing, debridement, microbiological assessment, susceptibility testing and appropriate antimicrobial therapy such as β-lactams (Malone *et al.,* 2022). However, the increasing prevalence of antimicrobial resistance has substantially compromised the effectiveness of conventional antibiotics. Approximately, one in six laboratory-confirmed bacterial infections globally is reportedly resistant to standard antibiotic treatment, highlighting the need for alternative or complementary therapeutic strategies (Monk *et al.,* 2024; WHO, 2024).

Medicinal plants are increasingly being investigated as potential sources of bioactive compounds with antimicrobial and wound-healing properties. *Euphorbia hirta* L. (*Euphorbiaceae*), commonly known as asthma weed or garden spurge, is a medicinal plant widely distributed in tropical and subtropical regions. It has traditionally been used in the management of gastrointestinal, respiratory, inflammatory and skin-related conditions (Salehi *et al.,* 2019). Phytochemical studies have identified compounds such as flavonoids, phenolic compounds, terpenoids, tannins, triterpenoids, phytosterols, polyphenols and other secondary metabolites in *E. hirta*, several of which possess reported antioxidant, anti-inflammatory and antimicrobial activities (Atanasov *et al.,* 2021; Goyal *et al.,* 2024; Kumari *et al.,* 2026). The detection of bioactive constituents such as quercetin and kaempferol derivatives provides a plausible phytochemical basis for some of the biological activities attributed to *E. hirta* (Kumari *et al.,* 2026).

Recent studies have demonstrated the phytochemical profiling, antimicrobial and wound-healing properties of *E. hirta* (Mwalongo *et al.,* 2025; Shuwa *et al.,* 2025; Cong *et al.,* 2026. However, its potential against *S. aureus*-infected wounds remains unexplored. Therefore, this study evaluated the *in vitro* antimicrobial activity and wound-healing potential of *Euphorbia hirta* in a *Staphylococcus aureus*-infected wound model.

MATERIALS AND METHODS

Drugs and Chemicals

Ciprofloxacin, Griseofulvin, Clindamycin and Gentamicin were purchased from Fidson Pharmaceutical Company, Nigeria. All other reagents used were obtained commercially and of analytical grade.

Plant Collection and Identification

Fresh plant of *Euphorbia hirta* was purchased from Ijebu-ode, Ogun state in March, 2025. The plant was identified and authenticated at the Department of Pharmacognosy, Olabisi Onabanjo University, Sagamu Campus and a voucher specimen was deposited in the herbarium section of the Department and voucher number PHS/OOU/515 were obtained for future reference.

Extract Preparation

The fresh plant of *Euphorbia hirta* were air dried under shade (at room temperature), weighed until a constant weight was obtained and then reduced to a coarse powder using a grinding machine. 250 g of powdered plant was soaked in 750 ml of 80% ethanol and left at room temperature for 3 days (72 hours) and then filtered. The resultant filtrate was concentrated using a rotator evaporator and then dried over a water bath at 40°C. The extract was stored in refrigerator in airtight container (universal bottle) until required (Aderinola *et al.,* 2025).

Phytochemical Screening

Preliminary qualitative phytochemical analysis of the plant extract was carried out to assess for the presence of anthraquinones, flavonoids, alkaloids, saponins, tannins, steroids, carbohydrate, triterpenes and cardiac glycosides using the method of Trease and Evans, (2002).

Acute Study in Mice

The oral median lethal dose (LD_{50}) of the plant extract of *Euphorbia hirta* was determined in mice according to the method described by Lorke (1983). The study was carried out in two phases with a total of 12 animals. In phase 1, three groups of 3 mice per group were orally administered with graded doses of 10, 100 and 1,000 mg/kg of the plant extract of *Euphorbia hirta* and observed for signs of toxicity and death in 24 hours. The second phase of the experiment based on the outcome of the first phase, was conducted using doses of 1,600, 2,900 and 5,000 mg/kg of the plant extract in 3 mice and again observed for 24 hours.

The square root of the smallest dose that caused mortality and the highest dose that did not was taken as the estimated median lethal dose (LD_{50}) for each of the hydro-ethanol plant extract of *Euphorbia hirta.*

LD_{50}=√highest non-lethal dose × lowest lethal dose.

Test Organisms

The pathogenic strains of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus*, *Bacillus substilis* and one fungus *Candida albicans* were used for the study. These strains were obtained from the Department of Medical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State.

Standardization of Inoculum

The test organisms were sub-cultured onto fresh plates of Mc-Conkey agar (Oxoid Laboratories, England) and Saboraud (4%) Dextrose agar (Oxoid Laboratories, England) for 24 hours at 37^0C for bacteria and fungus.

Colonies from these plates were suspended in Muller Hinton broth (Oxoid Laboratories, England) and adjusted to a turbidity matching 0.5 Mc farland standard.

Evaluation of Antmicrobial Activity

Agar Disc Diffusion Method

The anti-microbial activity of the plant extract was determined using agar-well diffusion method (Cup plate method) according to method described by Perez *et al.,* 1990 with slight modification. Mueller Hinton agar media was sterilized by autoclaving and cooled, with the aid of a sterile cotton swab mildly soaked with the individual isolated bacteria and fungus, the isolates were inoculated on the surface of Mŭeller Hinton agar by surface spreading and allowed to set, wells of approximately 6 mm in diameter, 2.5 mm deep and 2 cm apart were made on the surface of the solid medium using a sterile 6 mm cup-borer, the agar plugs were removed using sterile needles, the well were labeled 1 - 6. Well 1 - 4 was then filled with 0.1 ml of the reconstituted hydro-ethanol plant extract of *Euphorbia hirta* serially diluted to four different concentrations (100%, 50%, 25% and 12.5%) each with sterile distilled water. Sterile distilled water was used as negative control (well 5) while Ciprofloxacin

and/ or Griseofulvin was used as positive control (well 6). The inoculated plates were maintained at room temperature for 2 hours enabling the diffusion of the extracts into the medium. The plates were then incubated at 37°C for 24 hours. The antimicrobial activity tests were undertaken under aseptic conditions and in triplicates. After incubation at 37^0C for 24 hours, all plates were examined for any zones of growth inhibition. With the aid of a transparent ruler, the diameters of zones of inhibition around the wells were measured in millimeters (mm) and the average of the triplicate measurements recorded.

Animals

Wistar Albino rats ranging from 200 - 220 g were purchased from the animal house in Babcock University, Ilisan, Ogun State. All animals were housed and maintained in the animal house of department of Pharmacology and Therapeutics, Olabisi Onabanjo University, Sagamu Campus, Ogun state, Nigeria. The animals were housed, fed and treated in accordance with the in-house guidelines for animal care (Hedrich and Bullock, 2006). Animals were kept for two (2) weeks in order to acclimatize prior to the investigation. During this time, they were given Top feed (Premier Feed Mill Company) quality growers mash with water *ad libitum.* All experimental protocols for this study adhered to the guideline of National Institutes of Health (Mukerjee, 1997) and were executed in strict accordance with the Ethical Guidelines and Research Policy of Olabisi Onabanjo University, Ago-iwoye, Ogun state, Nigeria.

Wound Healing Activity and Wound Contraction Measurement

Thirty (30) Wistar albino rats were anesthetized intraperitoneally using a cocktail of ketamine (91 mg) at a dose of 1 mL/kg. The dorsal thoracic regions of the rats were shaved and antisepsis was performed with 70% alcohol and an impression of 5 x 5 mm was made. The skin of the impressed area was excised to full thickness. The excised skin of the rats were inoculated with *Staphylococcus aureus* bacteria (10^5organism cfu/ml) and left for 48 hours for establishment of bacterial infection. The rats were then allocated into five groups of 6 rats per group. Group 1, 2 and 3 received hydro-ethanol plant extract of *Euphorbia hirta* at 150, 300 and 600 mg/ kg (per oral) respectively, group 4 received clindamycin (50 mg/kg) while group 5 received distilled water p.o. control (infected non-treated), all lasted for 10 days.

The wounds of groups 1, 2 and 3 were topically treated with 0,5 g, 1.0 g and 1.5 g of *Euphorbia hirta* plant extract formulation once daily, group 4 were treated topically with gentamicin (0.1% w/w) while rats in group 5 were topically treated with the base (Vaseline). The topical and oral treatments started in the same day in all the groups. Wound area measurement was taken on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th and 21st day with the aid of transparent ruler.

Bacterial Count

After disinfection with 70% ethanol, the skin area corresponding to the infection site and the underlying tissue were removed from the rats on day 0 and day 17 and homogenized in 2 mls sterile water. Samples were diluted to 1:10 to quantify the bacteria numbers, then placed on Mac-Conkey agar plates and incubated for 24 hours at 37^0C.

Bacterial counts were expressed as numbers of *S. aureus* colony forming units per gram (cfu/g) of tissue (Godin *et al*., 2005).

Statistical Analysis

Statistical analysis of the data was done using Graph Prism version 9.00, utilizing One Way Analysis of Variance (ANOVA) and Dunnett's post-hoc test to evaluate differences between groups. Results gotten were expressed as mean ± standard error of mean (SEM), with $p \leq 0.05$ and $p \leq 0.01$ indicating statistical significance.

RESULTS AND DISCUSSION

The Phytochemical Profile of Hydro-Ethanol Plant Extract of *Euphorbia Hirta*

Qualitative phytochemical screening of the hydro-ethanol plant extract of *Euphorbia hirta* identified the presence of diverse secondary metabolites, comprising tannins, phenolic compounds, alkaloids, saponins, steroids, cardiac glycosides, terpenoids and flavonoids which are of beneficial pharmacological importance in the plant (Table 1).

Table 1: Phytochemical Profile of Hydro-Ethanol Plant Extract of *Euphorbia Hirta*

	Tanins	Alkaloids	Saponins	Steriods	Cardiac glycosides	Flavonoids	Phenols	Terpenoids
EH	+	+	+	+	+	+	+	+

+ (present) Key: EH: *Euphorbia hirta*

The Acute Toxicity Profile of Hydro-ethanol Plant Extract of *Euphorbia Hirta*

Evaluation of the acute oral toxicity of the hydro-ethanol plant extract of *Euphorbia Hirta*in mice demonstrated a favourable acute tolerability profile. No observable signs of toxicity, overt behavioural or clinical abnormalities, adverse reactions or mortality were recorded following oral administration at doses of up to 5,000 mg/kg body weight. These findings suggest that the median lethal dose (LD_{50}) of the extract is greater than 5,000 mg/kg under the experimental conditions employed, indicating a relatively wide acute safety margin and low acute oral toxicity potential.

***In-vitro* Antibacterial Activity of the Hydro-ethanol Plant Extract of *Euphorbia Hirta* against Selected Bacterial Isolates**

The *in-vitro* antibacterial susceptibility assessment of the hydro-ethanol plant extract of *Euphorbia hirta* demonstrated a pronounced concentration-dependent inhibitory effect against the selected bacterial isolates. At a concentration of 200 mg/mL, the extract exhibited varying degrees of antibacterial activity across the tested organisms, with zone of growth inhibition values of 19.50 mm for *Staphylococcus aureus*, 14.25 mm for *Escherichia coli*, 12.25 mm for *Pseudomonas aeruginosa* 14.50 mm for *Klebsiella pneumoniae* and 16.30 mm for *Bacillus substilis*, respectively.

Staphylococcus aureus exhibited the highest inhibition value (19.50 mm), suggesting greater susceptibility to the extract, whereas *Pseudomonas aeruginosa* demonstrated the lowest inhibitory response (12.25 mm), potentially reflecting differences in bacterial cell envelope characteristics and intrinsic resistance mechanisms. The comparatively higher inhibitory activities observed against *Klebsiella pneumoniae* and *Bacillus substilis* further suggest a broad spectrum of antibacterial potential (Table 2).

Table 2: ***In-Vitro*** **Antibacterial Activity of Hydro-Ethanol Plant Extract of** ***Euphorbia Hirta*** **against Selected Bacteria Isolates**

Treatment groups	25 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	Ciprofloxacin	Negative Control
Staphylococcus aureus	12.25±0.25	14.25±0.25	17.5±0.50	19.5±0.50*	25.50±0.50	0±0
Escherichia coli	9.5±0.25	10.25±0.25	13.5±0.25	14.25±0.25*	24.50±0.50	0±0
Pseudomonas aeruginosa	8.25±0.25	9.50±0.50	11.25±0.2	12.25±0.2*	22.00±1.00	0±0
Klebsiella pneumoniae	11.5±0.25	12.25±0.2	13.25±0.25	14.50±0.5*	21.00±1.00	0±0
Bacillus substilis	11.25±0.13	12.50±0.29	14.10±0.12	16.3±0.13*	24.00±0.58	0±0

n =3; Statistics: One Way ANOVA followed by Dunnett's Posthoc test at *p ≤0.05.

In-vitro **Antifungal Activity of the Hydro-Ethanol Plant Extract of** ***Euphorbia Hirta*** **against Fungal Isolate**

Evaluation of *in vitro* antifungal susceptibility of the hydro-ethanol plant extract of *Euphorbia hirta* showed a significant concentration-dependent inhibitory effect against the tested fungal isolate. The extract exhibited appreciable antifungal activity against *Candida albicans*, producing an inhibition value of 14.50 mm at a concentration of 200 mg/mL (Table 3).

Table 3: ***In-Vitro*** **Antifungal Activity of Hydro-ethanol Plant Extract of** ***Euphorbia hirta*** **against Fungi Isolate**

Treatment groups	25 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	Griseofulvin	Negative Control
Candida albicans	9.50±0.5	11.25±0.2	12.50±0.50	15.25±0.25*	22.50±0.50	0±0

n =3; Statistics: One Way ANOVA followed by Dunnett's Posthoc test at *p ≤0.05.

The Effect of the Hydro-ethanol Plant Extract of ***Euphorbia Hirta*** **on the Bacterial Load of** ***Staphylococcus aureus*****-Infected Wounds in Rats**

Administration of the hydro-ethanol plant extract of *Euphorbia hirta* demonstrated appreciable antibacterial activity in rats with experimentally induced *Staphylococcus aureus* wound infections, as evidenced by a significant reduction in bacterial burden in the extract-treated groups. The observed antibacterial response was concentration-dependent, with the highest administered extract concentration (600 mg/kg) producing a greater reduction in microbial load compared with the negative control group, which received no treatment. However, the standard reference antibacterial drug produced a more pronounced reduction in bacterial count than the plant extract. These findings suggest that the hydro-ethanol plant extract possesses *in-vivo* antibacterial potential against *S. aureus* although its efficacy was comparatively lower than that of the conventional reference drug (Table 4).

Table 4: Effect of hydro-Ethanol Plant Extract of ***Euphorbia Hirta*** **on Bacteria Count of** ***Staphylococcus Aureus*** **Infected Wound in Rats**

Treatment groups	Initial count	Final count
Distil. Water	2650±45.83	2796±14.00
150 mg/kg	2637±54.57	495.5±25.50
300 mg/kg	2613±63.33	361±59.00*
600 mg/kg	2633±26.03	146.5±36.50**
Clindamycin (150 mg/kg)	2627±79.65	95.50±15.50**

n =6; Statistics: One Way ANOVA followed by Dunnett's Posthoc test at *p ≤0.05 and **(p<0.01)

The Wound-Healing Efficacy of the Hydro-ethanol Plant Extract of ***Euphorbia Hirta*****in** ***Staphylococcus Aureus*****-Infected Rats**

Evaluation of the wound-healing potential of the hydro-ethanol plant extract of *Euphorbia hirta* in rats with experimentally induced *Staphylococcus aureus* wound infections revealed a significant improvement in wound contraction across the extract-treated groups. Oral administration of the extract at doses of 150, 300 and 600 mg/kg body weight together with topical extract formulation of 0.5 g, 1.0 g, and 1.5 g resulted in progressive wound contraction, with statistically significant reductions in wound size observed on days 11, 13, 15, 17, 19 and 21 compared with the initial wound dimensions and the untreated infected control group.

Furthermore, the standard treatment group exhibited marked wound contraction relative to the negative control group, indicating a pronounced wound-healing response following administration of the standard drug (Table 5).

Table 5: Wound Healing Ability of Hydro-ethanol Plant Extract of ***Euphoria Hirta*** **in** ***Staphylococcus Aureus*** **Infected Rats**

Treatment group	1	3	5	7	9	11	13	15	17	21
Distilled Water	5.04±0.17	5.11±0.28	5.70±0.14	5.24±0.35	5.31±0.26	5.33±0.15	5.37±0.14	5.42±0.11	5.46±0.18	5.49±0.23
150 mg/kg/ 0.5g EH	5.05±0.13	5.01±0.18	4.93±0.21	4.69±0.45	3.81±0.1	3.47±0.13	3.31±0.1	2.25±0.35*	1.93±0.26*	1.51±0.17*
300 mg/kg/ 1.0g EH	5.03 ±0.18	4.96±0.39	4.87±0.62	4.52±0.15	3.54±0.41	3.28±0.6	3.09±0.55	2.01±0.04*	1.40±0.50*	1.22±0.11*
600 mg/kg/ 1.5g EH	5.04±0.20	4.81±0.13	4.75±0.05	4.41±0.10	3.23±0.5	2.97±0.2*	2.51±0.04*	2.16±0.1*	1.25±0.02*	1.04±0.06**

Treatment group	1	3	5	7	9	11	13	15	17	21
Clindamycin (150 mg/kg)/Gentamicin (0.1% w/w)	5.02±0.31	4.77±0.28	4.64±0.06	3.92±0.05	2.84±0.1*	2.45±0.2*	2.28±0.1*	2.05±0.2*	1.19±0.1*	0.86±0.3**

n =6; Statistics: One Way ANOVA followed by Dunnett's Posthoc test at * p ≤0.05 and ** (p<0.01)
Key: EH: *Euphoria hirta*

Discussion

Bacterial pathogens are important causative agents of a wide range of human infections, including wound infections, and their increasing resistance to antimicrobial agents has become a major challenge to healthcare systems worldwide (WHO, 2024). The growing prevalence of antimicrobial resistance has consequently intensified the search for alternative and complementary therapeutic approaches, particularly those derived from natural sources.

Medicinal plants are recognized as valuable reservoirs of diverse phytochemical constituents with considerable pharmacological activities (Jangir *et al.,* 2026). *Euphorbia hirta* is one of such medicinal plants that has traditionally been employed in the management of inflammatory conditions, bacterial infections, and various skin disorders (Salehi *et al.,* 2019). The therapeutic relevance of this plant may be associated with its rich composition of biologically active phytochemicals.

In the present study, phytochemical screening of the *Euphorbia hirta* extract identified notable phytochemicals including flavonoids, tannins, alkaloids, saponins, phenolic compounds, and terpenoids. These classes of phytochemicals have been widely associated with antimicrobial, antioxidant and anti-inflammatory activities (Ajayi *et al.,* 2026). The phytochemical profile observed in this study is consistent with the findings of Shuwa *et al.,* (2026), who similarly reported the presence of these constituents in the leaf extract of *Euphorbia hirta*. The presence of these bioactive compounds may, therefore, contribute to the antimicrobial and wound-healing effects demonstrated by the extract in the present investigation.

The *in vitro* antibacterial findings demonstrated that the extract exhibited significant ($P < 0.05$) concentration-dependent inhibitory activity against all the bacterial isolates evaluated. The greatest antibacterial effect was recorded against *Staphylococcus aureus* with an inhibition zone of 19.50 mm, followed by *Bacillus subtilis* (16.30 mm), *Klebsiella pneumoniae* (14.50 mm), *Escherichia coli* (14.25 mm), and *Pseudomonas aeruginosa* (12.25 mm). The comparatively lower susceptibility observed among *E. coli, K. pneumoniae* and *P. aeruginosa* may be associated with the intrinsic structural and physiological characteristics of Gram-negative bacteria, which can confer greater resistance to antimicrobial compounds (Ashig *et al.,* 2025).

The comparatively greater susceptibility of Gram-positive bacteria observed in this study may be explained in relation to differences in bacterial cell-envelope architecture. Gram-negative bacteria possess an additional outer membrane enriched with lipopolysaccharide, which constitutes an important permeability barrier and may restrict the penetration of several antimicrobial compounds into the bacterial cell (Borges *et al.,* 2015; Ashig *et al.,* 2025; Arbad *et al.,* 2026). In contrast, Gram-positive bacteria lack this additional outer membrane and possess a relatively more accessible peptidoglycan-rich cell wall, potentially allowing greater interaction between antimicrobial agents, phytochemicals and cellular targets. Consequently, compounds such as flavonoids, tannins, alkaloids, saponins and phenolics may exert greater inhibitory effects against some Gram-positive organisms (Arbad *et al.,* 2026). The present findings are in line with those of Shuwa *et al.* (2026), who reported greater antibacterial activity of the ethanol leaf extract of *Euphorbia hirta* against Gram-positive organisms than against Gram-negative strains.

The antibacterial activity observed in this study may further reflect the combined or synergistic action of multiple phytochemical constituents present in the extract. Medicinal plants frequently contain complex mixtures of secondary metabolites capable of acting through different antimicrobial mechanisms. Flavonoids, phenolics, tannins, alkaloids and saponins, in particular, have been widely implicated in the antibacterial activity of medicinal plants and are recognized for their antimicrobial properties (Ajayi *et al.,* 2026). Flavonoids and phenolic compounds may compromise microbial membrane integrity, interfere with cellular energy metabolism and inhibit nucleic acid synthesis. Tannins may exert antibacterial effects through protein precipitation and interaction with microbial enzymes while alkaloids may interfere with DNA replication and other essential cellular processes. Saponins, on the other hand, may increase membrane permeability, thereby promoting cellular leakage and compromising microbial viability (Arbad *et al.,* 2026). The combined presence of these compounds may therefore account, at least partly, for the pronounced antibacterial activity demonstrated by the extract.

Although ciprofloxacin produced a larger inhibition zone than the *Euphorbia hirta* extract, the antibacterial activity demonstrated by the crude extract remains noteworthy. Unlike ciprofloxacin, which is a purified antimicrobial compound with a defined molecular target, plant extracts contain complex mixtures of bioactive constituents that may act through multiple mechanisms. Therefore, the observed inhibitory activity of the crude extract provides evidence of its antimicrobial potential, although the activity cannot be directly equated with that of a standardized antibiotic.

In addition to its antibacterial activity, the extract demonstrated significant ($P<0.05$), concentration-dependent antifungal activity against *Candida albicans,* with the highest inhibition zone of 15.25 mm recorded at 200 mg/mL. This finding indicates that the antimicrobial activity of *Euphorbia hirta* may extend beyond bacterial pathogens to include fungal organisms. The observed antifungal activity may be associated with phytochemicals such as flavonoids, tannins and saponins, which have been reported to interfere with fungal membrane integrity, inhibit essential fungal enzymes, and disrupt cellular metabolic processes (Al Aboody & Mickymaray, 2020; Azeem *et al.,* 2024). The antifungal effect observed in the present study may therefore reflect the combined contribution of these phytochemical constituents.

Although the antifungal activity of the extract was lower than that of griseofulvin, which produced an inhibition zone of 22.50 mm, the significant activity demonstrated by the extract indicates the presence of constituents with potential antifungal properties. This finding further supports the possibility that *Euphorbia hirta* possesses broad-spectrum antimicrobial activity. However, further studies involving

isolation and characterization of the active compounds would be necessary to establish the specific constituents responsible for the observed antifungal effect.

The *in vivo* antibacterial assessment further demonstrated that treatment with *Euphorbia hirta* extract resulted in a marked and dose-dependent reduction in bacterial counts in Staphylococcus aureus-infected wounds. The group treated with 600 mg/kg exhibited the greatest reduction in bacterial burden among the extract-treated groups, whereas the untreated control group showed an increase in bacterial count, indicating continued bacterial proliferation and persistence of the infection. Bacterial colonization and proliferation within wounds are important factors associated with prolonged inflammation, delayed formation of granulation tissue, impaired collagen deposition, and delayed epithelialization (Guo *et al.,* 2023). Consequently, effective reduction of microbial burden constitutes an important component of the wound-healing process.

The reduction in bacterial burden observed in the extract-treated rats may have contributed to the improved wound-healing response recorded in this study. The antimicrobial effect of the extract may have limited bacterial proliferation within the wound environment, thereby reducing microbial-associated tissue damage and creating more favorable conditions for tissue repair. This effect may be attributable to the antimicrobial phytochemicals identified in the extract, particularly flavonoids, phenolics, tannins, alkaloids and saponins.

Clindamycin produced a greater reduction in bacterial wound burden than the plant extract, which is consistent with its pharmacological role as a purified antibiotic specifically formulated for the treatment of susceptible bacterial infections. Nevertheless, the substantial reduction produced by the 600 mg/kg extract treatment indicates that *Euphorbia hirta* possesses meaningful antibacterial activity in infected wounds. The dose-dependent pattern further suggests that increasing extract concentration may enhance its ability to suppress bacterial proliferation under the experimental conditions employed.

The therapeutic potential of the hydro-ethanol extract was further demonstrated by its ability to enhance wound contraction and accelerate the wound-healing process. Rats treated with 300 and 600 mg/kg of the extract exhibited significantly greater wound contraction than the untreated control group, particularly between days 11 and 21. By day 21, the wound diameter in the 600 mg/kg group had markedly decreased, approaching near-complete wound closure and producing a response that was comparable to that observed in the clindamycin-treated group.

The wound-healing activity of *Euphorbia hirta* may be attributable to the combined actions of its pharmacologically active phytoconstituents. Flavonoids may promote tissue repair by attenuating oxidative stress and excessive inflammatory responses, thereby supporting fibroblast activity and collagen synthesis. Tannins may contribute through their astringent properties, which can facilitate tissue contraction and help protect damaged tissues while saponins have been associated with angiogenesis and extracellular matrix formation (Kumari *et al*., 2025). These activities, together with the antimicrobial effect of the extract, may collectively contribute to the enhanced wound contraction observed in the treated rats.

The findings indicate that the hydro-ethanol plant extract of *Euphorbia hirta* possesses both antimicrobial and wound-healing properties. The *in vitro* antibacterial and antifungal activities, together with the reduction in bacterial burden and accelerated wound contraction observed *in vivo*, suggest that the therapeutic effects of the plant may arise from multiple complementary pharmacological actions. The antimicrobial activity may reduce the microbial burden within infected wounds, while the antioxidant, anti-inflammatory, and tissue-repair properties of its phytochemical constituents may facilitate subsequent stages of wound healing.

CONCLUSION

The findings of this study demonstrate that the hydro-ethanol plant extract of *Euphorbia hirta* possesses significant antibacterial, antifungal, and wound-healing activities. The extract inhibited the growth of the tested bacterial and fungal organisms *in vitro* and produced a dose-dependent reduction in bacterial burden and enhancement of wound contraction in *Staphylococcus aureus*-infected wounds *in vivo*. These findings provide experimental support for the traditional use of *Euphorbia hirta* in the management of bacterial infections and wounds and indicate that the plant may represent a promising source of bioactive compounds for the development of complementary or adjunctive therapeutic approaches for infected wounds.

Further investigations are required to isolate and characterize the specific compounds responsible for the observed effects, elucidate their molecular mechanisms of action, establish appropriate therapeutic doses and comprehensively evaluate the toxicological and safety profiles of *Euphorbia hirta*.

REFERENCES

Aderinola, A. A., Ejiofor, J. I., Ogunjimi, L. O., Murtala, A. A., Oladapo, O. E. & Oladoja, F. A. (2025). *Lecaniodiscus cupanioides* leaf extract attenuates *Plasmodium berghei*-induced malaria infection in albino mice via modulation of serum biochemical and hematological markers. *Pharmacological Research-Natural Products*, 7, 100257. Doi: https://doi.org/10.1016/j.prenap.2025.100257.

Ajayi, I. A., Odejobi, B. S., Ifedi, E. N., Raji, A. A., & Ajayi, O. O. (2026). Phytochemical composition and wound healing activity of *Azadirachta indica* seed extract in wistar rats. *Journal of Chemical Society of Nigeria*, 51(2). https://doi.org/10.4314/jcsn.v51i2.13.

Al-Aboody, M. S., & Mickymaray, S. (2020). Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics,* 9(2), 45. https://doi.org/10.3390/antibiotics9020045.

Anju, V.T., Busi, S., Mohan, M.S.,& Dyavaiah, M. (2023). Bacterial infections: Types and pathophysiology. In Antibiotics-Therapeutic Spectrum and Limitations Chapter 2. Pp.21-38. Academic Press. ISBN 9780323953887. https://doi.org/10.1016/B978-0-323-95388-7.00004-8.

Arbad S., Ullah, H., Alzahrani, K. J., Alsharif, K. F., Alzahrani, F. M., Abuderman, A., & Zeng, Z. (2026). Methanolic plant extracts: emerging biotherapeutic alternatives for animal wound infection control. *Frontiers in Veterinary Science,* 13, 1812558. https://doi.org/10.3389/fvets.2026.1812558.

Ashiq, A., Das, S. C., Biswas, S., Akter, R., Masum, M. A., & Bedoura, S. (2025). Accelerating wound healing: Unveiling a synergistic neem-chitosan composite for rapid tissue regeneration. *Next Research,* 2(3), 100541. ISSN 3050-4759. https://doi.org/10.1016/j.nexres.2025.100541.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: advances

and opportunities. *Nature reviews Drug discovery,* 20(3), 200-216. https://doi.org/10.1038/s41573-020-00114-z.

Azeem, K., Irfan, I., Shakir, M., Rawat, D. S., & Abid, M. (2024). Therapeutic potential of phytochemicals and their derivatives as antifungal candidates: recent discovery and development. In: Manzoor, N. (eds) Advances in Antifungal Drug Development. Springer, Singapore. https://doi.org/10.1007/978-981-97-5165-5_19.

Bessa, G. (2023). Bacterial Infections. In: Rangel Bonamigo, R. (eds) Dermatology in Public Health Environments. Springer, Cham. Pp.183-202. https://doi.org/10.1007/978-3-031-13505-7_9.

Borges, A., Saavedra, M., & Simoes, M. (2015). Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents. *Current Medicinal Chemistry*, 22(21), 2590-2614. https://doi.org/10.2174/0929867322666150530210522

Cong, N. D., Van Thu, N., Thanh, D. V., Yen, NTH., Yen, TH., Ha, HTT., & Thao, NTP. (2026). Study of Antibacterial Activity of Extract of Large-Leaf Milky Grass (*Euphorbia hirta* L.). *TNU Journal of Science and Technology,* 231 (13), 484-490.

Godin, B., Touitou, E., Rubinstein, E., & Athamna, A. M. (2005). A New approach for treatment of deep skin infections by an Ethosomal antibiotic preparation: An *in-vivo* study. *Journal of Antimicrobial Chemotherapy,* 55: 989-994. https://doi.org/10.1093/jac/dki125

Goyal, M., Sharma, R., and Verma, P. (2024). Recent advances in pharmacological studies on *Euphorbiaceae* medicinal plants. *Industrial Crops and Products*, 210, 118102.

Guo, S. A., & DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of Dental Research,* 89(3), 219-229. https://doi.org/10.1177/0022034509359125

Hedrich H. H., & Bullock, G. (2006). The Laboratory Mouse: The Handbook of Experimental Animals. Elsevier Academic press, UK. pp. 463-492.

Jangir, R. K., Singh, J., & Haider, F. (2026). *In vitro* and *in vivo* Antimicrobial Activity of Azadirachta indica (Neem) Nanoformulations against Multidrug-Resistant Bacterial Strains Isolated from Indian Hospital Settings: Implications for Wound Healing in Tropical Climates. *CME Journal Geriatric Medicine*, 18(2), 202-207. https://doi.org/10.61336/cmejgm/2026-02-2.

Kumari, S., Sharma, T. K., & Kumar, V. B. (2026). Ethnobotanical, Phytochemical and Pharmacological Importance of Selected Members of *Euphorbiaceae*: A Review. *Journal of Scientific Research and Technology*, 4(5): 22-36. . ISSN: 2583-8660. www.jsrtjournal.com.

Lewnard, J. A., Charani, E., Gleason, A., Hsu, L. Y., Khan, W. A., Karkey, A., & Laxminarayan, R. (2024). Burden of bacterial antimicrobial resistance in low-income and middle-income countries avertible by existing interventions: an evidence review and modelling analysis. *The Lancet,* 403(10442), 2439-2454. http://doi.org/10.1016/s0140-6736%2824%2900862-6.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing, PMID: 6667118, *Arch. Toxicol.* 54 275–287. https://doi.org/10.1007/BF01234480.

Malone, M., & Schultz, G. (2022). Challenges in the diagnosis and management of wound infection. *British journal of dermatology*, 187(2), 159-166. https://doi.org/10.1111/bjd.21612

Monk, E. J., Jones, T. P., Bongomin, F., Kibone, W., Nsubuga, Y., Ssewante, N., & van Zandvoort, K. (2024). Antimicrobial resistance in bacterial wound, skin, soft tissue and surgical site infections in Central, Eastern, Southern and Western Africa: a systematic review and meta-analysis. *PLOS global public health*, 5(5), e0004608. https://doi.org/10.1371/journal.pgph.0004608

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., & Tasak, N. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet*, 399(10325), 629-655. https://doi.org/10.1016/s140-6736(21)02724-0.

Mukerjee, M. (1997). Trends in animal research. *Scientific American,* 276(2), 86-93. https://www.jstor.org/stable/24993615.

Mwalongo, O., Kisula, L., Credo, D., & Sauli, E. (2025). Phytochemical composition, toxicity, and *in vivo* antimicrobial properties of *Euphorbia hirta* and *Plectranthus barbatus* ethanolic extracts' polyherbal formulation. *Natural Product Communications*, 20(10), 1934578X251388330. https://doi.org/10.1177/1934578X251388330

Perez C., Paul M., & Bazerque P. (1990). An antibiotic assay by the agar-well diffusion method. *ActaBiol. Med. Exp*. 15: 113-115.

Pieniążek, J. M., Padkowska, A., Dobosz, M., Arciszewska, K., Buczkowski, J., Miazga, M., & Mataczyńska, A. (2024). Overview of the Main Bacterial Infections in Humans. *Quality in Sport*, 23, 54786-54786. https://doi.org/10.12775/QS.2024.23.54786.

Prodinger, C., Lechner, A., Hintersteininger, A., Kern, J. M., Meißnitzer, M., Meyersburg, D., & Laimer, M. (2025). Complicated and deep bacterial skin and soft tissue infections. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, 23(3), 357-375. https://doi.org/10.1111/ddg.15493

Salehi, B., Iriti, M., Vitalini, S., Antolak, H., Pawlikowska, E., Kręgiel, D., & Seca, A. M. (2019). *Euphorbia*-derived natural products with potential for use in health maintenance. *Biomolecules,* 9(8), 337. https://doi.org/10.3390/biom9080337

Shuwa, F. A. A., Yakubu, M., & Sani, A. M. (2026). Phytochemical analysis and antibacterial activities of aqueous and ethanolic leaf and stem extracts of *Euphorbia hirta* on some clinical isolates. *Science World Journal*, 21(2), 698-702. https://doi.org/10.4314/swj.v21i2.34.

Soni, J., Sinha, S., & Pandey, R. (2024). Understanding bacterial pathogenicity: a closer look at the journey of harmful microbes. *Frontiers in Microbiology,* 15, 1370818. https://doi.org/10.3389/fmicb.2024.1370818.

Touaitia, R., Mairi, A., Ibrahim, N. A., Basher, N. S., Idres, T., & Touati, A. (2025). *Staphylococcus aureus*: A review of the pathogenesis and virulence mechanisms. *Antibiotics*, 14(5): 470. https://doi.org/10.3390/antibiotics14050470.

Trease G.E & Evans W.C (1983). Pharmacognosy. 1st edition. Ahmadu Bello University press, Zaria, Nigeria. pp188-198.

Uberoi, A., McCready-Vangi, A., & Grice, E. A. (2024). The wound microbiota: microbial mechanisms of impaired wound healing and infection. *Nature Reviews Microbiology*, 22(8), 507-521. https://doi.org/10.1038/s41579-024-01035-z

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