AJBCPS African Journal of Biological, Chemical and Physical Sciences

Vol 4, Issue 1, 2025

ISSN: 2790-9522 (Print) | 2790-9530 (Online)
Website: https://journals.jozacpublishers.com/ajbcps/index
https://doi.org/10.5281/zenodo.17055452

OPEN ACCESS

Unveiling the intriguing properties: A Study on the powerful Phytochemicals and Biological Marvels of the *Calotropis procera* plant

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Received: July 24, 2024 | **Accepted:** August 14, 2025 | **Published:** September 04, 2025

Abstract

Calotropis procera, known as giant milkweed, is notable for its rich array of phytochemicals that showcase various biological activities. This study aimed to analyze the plant's phytochemical constituents and assess its potential antimicrobial properties. Key phytochemicals identified include flavonoids (with antioxidant properties), tannins (providing astringent qualities), saponins (possibly boosting immunity), steroids (known for anti-inflammatory effects), glycosides (involved in physiological functions), alkaloids (medicinal properties), and cardiac glycosides (influencing heart function). For antimicrobial testing, specific clinical isolates were evaluated in vitro, including *Salmonella typhi, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, along with *Candida albicans* for opportunistic infections. Results showed that both ethyl acetate and aqueous leaf fractions were highly effective against *Salmonella typhi, Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*, indicating the plant's antimicrobial potential. Meanwhile, the aqueous and methanol root fractions effectively inhibited *Escherichia coli* and *Staphylococcus aureus* as well. Notably, hexane fractions were active only against *Candida species*, indicating selective anti-fungal properties.

Keywords: Calotropis procera, Phytochemicals, MIC and MBC

1. Introduction

Secondary metabolites are vital for plants' adaptation to environmental stress, serving as chemical defenses against diseases, insects, and herbivores. These bioactive compounds are abundant in medicinal plants and are recognized for their strong pharmacological activities, such as antioxidant, anti-inflammatory, and analgesic effects (Gupta et al., 2002; Alzahrani, 2017; Punetha et al., 2022). *Calotropis procera,* a perennial evergreen species from the Apocynaceae family, stands out for its medicinal value. Known by various names including "Apple of Sodom," "Calotrope," "giant milkweed," "*ewe bomubomu*" in South-West Nigeria, and *"tumafafiya*" in Northern Nigeria it is widely used in traditional medicine to address diverse health conditions (Alikhan & Khanum, 2005; Murti et al., 2010; Qasim et al., 2011; Singh & Dubey, 2012). This study aims to scientifically assess the

biological and pharmacological properties of *Calotropis procera,* providing validation for its traditional uses.

2. Literature review

Calotropis procera has been extensively documented for its therapeutic uses in traditional medicine systems. The plant's various parts, particularly its leaves and latex, are employed in numerous medicinal applications. For instance, the leaves are traditionally used as a coagulant in milk-based drinks such as "warankachi" and "nono" by the Hausa and Fulani communities (Alikhan & Khanum, 2005). The latex extracts of *Calotropis procera* are well-known for their anti-inflammatory, antioxidant, wound-healing, cough-suppressing, muscle-contracting, and neuromuscular blocking properties (Dewan et al., 2000; Murti et al., 2010). Phytochemical analyses have revealed that the plant contains a diverse array of secondary metabolites contributing to its pharmacological effects. The leaves and latex are rich in compounds such as glycosides, triterpenoids, cardenolides, flavonoids, proteins, and various enzymes (Alencar et al., 2006; Khanzada et al., 2008; Bezerra et al., 2017). Additionally, the plant contains saponins, saturated and unsaturated fatty acids, and hydrocarbons (Doshi et al., 2012). These constituents are believed to underpin the plant's therapeutic properties, making it a valuable candidate for further scientific investigation to validate its traditional uses and explore its potential in modern pharmacology.

Plate 1: *Calotropis procer* plant

3. Research methodology

3.1. Plant material

Calotropis procera plants were collected at New Site FHA Lugbe, Abuja-FCT, Nigeria. The identification and authentication of the plant specimens were confirmed at the Herbarium Department of the National Institute for Pharmaceutical Research and Development, Idu, Abuja, FCT, Nigeria, and assigned the identification number NIPRD/H/7259.

3.2. Collection of plant material

The leaf and root bark of *Calotropis procera* were carefully hand-picked, thoroughly washed, and air-dried at room temperature. The dried plant materials were ground into a near-powder form. A total of 120 g of each plant part was subjected to sequential extraction using a Soxhlet apparatus.

3.3. Extraction of plant

The leaf and root bark of the plant specimens were carefully hand picked, thoroughly washed and air dried at room temperature. They were ground to near powder and 120 g each was extracted with 1000 ml each of 95% n-hexane for 6–8 h in Soxhlet apparatus. Sequential extraction process was repeated in Ethyl acetate, Methanol, and Distilled water in a respective manner. The extract thus obtained was cooled, filtered and concentrated to remove excess solvent in a rotary evaporator.

% Yield = weight of the obtained extract (g)/weight of plant material (g) × 100.

3.4. Preliminary phytochemical studies

The crude extract was examined for the presence of different classes of natural chemicals using established methods (Harbone, 1998).

3.5. Antimicrobial screening

The experiment was conducted using a sterile 96-well plate format, where leaf and root extracts of *Calotropis procera* were tested in duplicate. Each well received 50 µL of Middle Brook 7H9 broth, followed by a serial dilution from wells 1 to 9. Control wells were designated as follows: well 10 for extract sterility, well 11 for media sterility, and well 12 for organism viability. Test microorganisms were inoculated into each well and appropriately labeled. A 24-hour incubation at 37°C was conducted after the plates were sealed. Following this period, 25 µL of p-iodonitrotetrazolium chloride (INT) was added to indicate bacterial growth. After an additional 48 hours, 25 µL of tetrazolium was added to the control wells to evaluate microbial growth. A lack of color change indicated inhibition, while a color change suggested growth. The highest dilution that effectively inhibited microbial growth was recorded as the Minimum Inhibitory Concentration (MIC) of the plant extract (Caviedes et al., 2002; Ncube et al., 2008).

3.6. Minimum Inhibitory concentrations (MIC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits microbial growth, serving as a key indicator of susceptibility (Turnidge & Paterson, 2007). To ascertain the MIC, serial dilutions of the centrifuged crude extract were prepared and inoculated with a sterile, standardized concentration of the target microorganism. The resulting MIC represents the threshold concentration needed to inhibit growth, measured in micrograms per milliliter (µg/ml) (Jorgensen et al., 2009).

3.7. Minimum bacteriocidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined based on MIC results, specifically selecting wells with no visible growth for further analysis (Rodríguez-Melcón et al., 2021). To establish the MBC, 100 µL of fresh sterile Muller Hinton Agar (MHA) was inoculated and incubated for 24 hours at 37°C, followed by colony counting. If the growth rate was below 0.1%, the MBC was calculated (Rakholiya et al., 2015).

4. Results

Table 1: Percentage Yield of Crude Extracts of leaf and root of *Calotropis procera*

Solvents	Leaves (%)	Roots (%)
n-Hexane	1.40	1.56
Ethylacetate	7.20	7.26
Methanol	7.90	9.37
Aqueous	9.30	9.92

Table 2: Phytochemical analysis of *Calotropis procera* leaf

Phytochemicals	n-hexane	Ethylacetate	Methanol	Aqueous
Alkaloids	-	+	+	+
saponin	+	-	-	+
Terpenes	-	+	+	+
Flavonoids	+	+	+	-
Tannin	_	+	+	+
Steroids	-	+	+	+
Glycosides	-	-	-	+
Terpenoids	-	-	+	+
Carbohydrates	+	+	+	+
Phenols	-	-	+	+

Table 3: Phytochemical Analysis of *Calotropis procera* root extracts

Phytochemicals	n-hexane	Ethylacetate	Methanol	Aqueous
Alkaloid	-	+	-	+
saponin	+	+	+	_
Terpenes	-	+	+	+
Flavonoids	+	-	-	_
Tannin	-	-	-	+
Steroids	-	+	+	+
Glycosides	-	-	+	+
Terpenoids	-	-	+	+
Carbohydrates	+	+	+	+
Phenols	-	-	+	+

Keys = Positive (+) Negative (-)

Table 4: Antimicrobial screening of *Calotropis procera* activity using 96 micro web plate method Minimun Inhibition Concentration (MIC) of the leaf and root extracts and fractions

	Leaf (mg/L)				Root (mg/L)			
Test organisms	HCPL	ECPL	MCPL	ACPL	HCPR	ECPR	MCPR	ACPR
S. typhi	0.00	12.50	0.00	3.13	0.00	3.13	0.00	12.50
S.aureus	3.13	12.50	0.00	0.00	3.13	12.50	3.13	0.00

E. Coli	0.00	12.50	0.00	6.25	0.00	0.00	12.50	12.50
P.aeruginosa	0.00	12.50	12.50	12.50	0.00	12.50	3.13	12.50
Bacillius spp	0.00	0.00	6.25	6.25	0.00	0.00	3.13	12.50
C.albican	1.56	0.00	0.00	0.00	0.00	1.56	0.00	0.00

Keys: 0.00 represents no activity**;** HCPL: n-Hexane leaf extract, ECPL: Ethyl acetate leaf extract, MCPL: Methanol leaf extract, ACPL: Aqueous leaf extract, HCPR: n-Hexane root extract, ECPR: Ethyl acetate root extract, MCPR: Methanol root extract, ACPR: Aqueous root extract

Table 5: Minimum Bacteriocidal Concentration (MBC) of leaf and root extracts and fractions of *Calotropis procera*

	Leaf (mg/L)				**Root (mg/L)**			
Test organisms	HCPL	ECPL	MCPL	ACPL	HCPR	ECPR	MCPR	ACPR
S. typhi	0.00	25.00	0.00	6.25	0.00	6.25	0.00	25.00
S.*aureus*	6.25	25.00	0.00	0.00	6.25	25.00	6.25	0.00
E.*coli*	0.00	25.00	0.00	12.50	0.00	0.00	25.00	25.00
P.*aeruginosa*	0.00	25.00	25.00	25.00	0.00	25.00	6.25	25.00
Bacillius spp	0.00	0.00	12.50	12.50	0.00	0.00	6.25	25.00
C.albican	3.13	0.00	0.00	0.00	0.00	3.13	0.00	0.00

Keys: 0.00 represents no activity**;** HCPL: n-Hexane leaf extract, ECPL: Ethyl acetate leaf extract, MCPL: Methanol leaf extract, ACPL: Aqueous leaf extract, HCPR: n-Hexane root extract, ECPR: Ethyl acetate root extract, MCPR: Methanol root extract, ACPR: Aqueous root extract

HCP: n-hexane extract

5. Discussion

The bioassay-guided protocol (Table 1) detailed the percentage yield of crude extracts and fractions. It employeds sequential solvent extraction using hexane, ethyl acetate, methanol, and water, each capturing different soluble compounds. Soluble fractions were collected and weighed at each stage before extracting the residues. Phytochemicals are found to have great potential to prevent chronic and degenerative diseases (Wang et.al., 2013). In this study *Calotropis procera* plant was examined for its phytochemical composition, which included alkaloids saponins, terpenes, flavonoids, tannins, steroids, phenols, glycosides and carbohydrates (Table 2 and 3). Methanol and aqueous fractions of the leaf and roots mostly contains the main bio-active compounds whereas hexane and ethyl acetate fractions reveal few compounds. Saponins are triterpenoid and steroid glycosides that have biological activities such as regulating plasma cholesterol and nutrient absorption (Moses et al., 2014). Flavonoids and tannins, which are components of lignans, have all been shown to have pharmacological potential as well as significant antioxidant, anticancer, antioxidant, anti-inflammatory, and antiviral effects (Mendki et al., 2005; Ullah et al., *2020*).

In this study the plant crude leaf and root bark extracts fractions against the six microbial strains (Table4), the antimicrobial screening results demonstrate that *Salmonella typhi* showed the highest sensitivity to the ethyl acetate fraction of the leaf (ECP, 12.50 mg/L) and the aqueous fraction of the root (ACPR, 12.50 mg/L), with no activity noted in the hexane fractions. Conversely, *Staphylococcus*

aureus was notably susceptible to the hexane fractions from both the leaf (HCPL, 3.13 mg/L) and the root (HCPR, 3.13 mg/L), while methanol fractions exhibited no inhibitory effect. For *Escherichia coli*, significant activity was observed with the aqueous fraction of the leaf (ACPL, 6.25 mg/L) and the methanol fraction of the root (MCPR, 12.50 mg/L). *Pseudomonas aeruginosa* was inhibited by multiple fractions, with the lowest minimum inhibitory concentration (MIC) in the methanol fraction of the leaf (MCPL, 1.56 mg/L), while root fractions generally required higher concentrations (12.50 mg/L). Bacillus spp. were susceptible to the methanol fraction of the leaf (6.25 mg/L) and the aqueous fraction of the root (12.50 mg/L).

Candida albicans was inhibited only by the hexane fraction of the leaf (HCPL, 1.56 mg/L) and the ethyl acetate fraction of the root (ECPR, 1.56 mg/L). the findings suggest that hexane fractions showed strong antibacterial and antifungal activity against C. albicans and S. aureus. Methanol fractions were less effective overall, with exceptions noted for specific bacteria such as *Bacillus spp.* and *P. aeruginosa.* Ethyl acetate fractions demonstrated moderate activity against Gram-negative bacteria like S. typhi and E. coli, while aqueous fractions exhibited moderate inhibition, typically requiring higher MIC values. Notably, the ethyl acetate fractions of *Calotropis procera* leaf was more effective against all bacteria than the root fractions which significantly agreed with previous reports (Yesmin *et al.,* 2008; Mainasara *et al.,* 2012).The minimum bacteriocidal concentration (MBC) in (Table 5) indicated distinct sensitivities among bacterial strains to different extracts. *Salmonella typhi* was notably sensitive to the ethyl acetate root extract (ECPR) and aqueous leaf extract (ACPL) at 6.25 mg/L, while hexane fractions (HCPL, HCPR) showed no activity.

ECPL and aqueous root extract (ACPR) required higher concentrations of 25 mg/L for inhibition.*Staphylococcus aureus* was highly susceptible to hexane extracts, particularly HCPL and HCPR at 6.25 mg/L, with the methanol root extract (MCPR) showing moderate inhibition. ECPL and ECPR needed 25 mg/L for effectiveness, and aqueous extracts (ACPL, ACPR) were inactive.*Escherichia coli* responded to ACPL at 12.50 mg/L and MCPR at 25 mg/L, whereas hexane fractions exhibited no activity. ECPL and ACPR showed reduced efficacy at 25 mg/L.*Pseudomonas aeruginosa* was primarily inhibited at 25 mg/L across most extracts, with MCPR demonstrating stronger inhibition at 6.25 mg/L; hexane fractions were ineffective.For *Bacillus* spp. methanol extracts (MCPL and MCPR) effectively inhibited at 12.50 mg/L and 6.25 mg/L, respectively, while ACPL showed moderate activity. Hexane and ethyl acetate fractions were inactive. Regarding *Candida albicans,* only HCPL and ECPR demonstrated antifungal activity at 3.13 mg/L, with all other fractions inactive.In summary, hexane extracts were potent against *Staphylococcus aureus* and *Candida albicans,* while methanol extracts were more effective against Bacillus spp. and *Pseudomonas aeruginosa.* Ethyl acetate extracts exhibited activity but generally required higher concentrations.

Aqueous extracts provided moderate effects, typically at elevated concentrations. Primarily, *S. aureus, S,typhi* and *E. coli* has been linked to hospital-acquired infections and has increasingly become a common cause of community-based infections (Bachir & Abouni, 2015). The strains were suppressed by *C procera solven*t extracts implying that it could be a source of novel antibacterial compounds. The minimum inhibitory concentrations (MICs) and the minimum bacteriocidal concentration (MBC) of the extract and fractions were found to be in the range of μg/ml.

6. Implication of the study

To fully leverage the therapeutic potential of *Calotropis procera,* further research and development are crucial. Focus should be directed toward drug discovery by investigating its antimicrobial and antioxidant compounds for innovative treatment options. Clinical studies are needed to validate the plant's traditional uses, ensuring safety and efficacy for human use. Additionally, identifying and characterizing specific bioactive compounds and their mechanisms will enhance understanding and improve therapeutic outcomes. Lastly, developing targeted formulations to address resistant microbial strains, especially those linked to hospital-acquired infections, may position *Calotropis procera* as a valuable asset in modern healthcare.

7. Recommendations

Calotropis procera appears to possess significant medicinal potential, as several bioactive compounds have antimicrobial properties. The methanol and aqueous fractions, rich in flavonoids, tannins, and saponins, effectively targeted pathogens like Staphylococcus aureus and Escherichia coli. The n-hexane fraction showed antifungal activity against *Candida albicans* (HCP: 6.25 μg/ml). These results validate the plant's traditional medicinal uses and highlight its promise for developing new antibacterial and antifungal agents. Moreover, prior studies have indicated that these compounds possess antioxidant and anti-inflammatory properties that may be beneficial for treating chronic diseases, so further research is warranted (Sangraula et al., 2001; Kumar et al., 2013; Wang et al., 2013; Ullah et al., 2020).

8. Conclusion

This study enhanced our knowledge of *Calotropis procera*'s phytochemical properties, demonstrated the importance of exploring traditional medicinal plants for novel bioactive compounds that can be deployed to enhance healthcare, as well as laid the foundation for future research into its application potential. Nevertheless, further research is required to determine its precise mode of action and identify its active ingredients responsible for its specific antimicrobial properties.

9. Acknowledgement

The authors express their gratitude to the management and staff of the Department of Chemistry at the University of Abuja and the Sheda Science and Technology Complex (SHESTCO) for providing access to the laboratories and supplying some of the reagents used in this study.

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