**ANTIBACTERIAL EFFECT OF ETHANOIC EXTRACT OF *Calotropis procera* (SODOM APPLE) LEAVES AGAINST *Staphylococcus aureus***

# TITLE PAGE

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF SCIENCE AND TECHNOLOGY, FEDERAL POLYTECHNIC MUBI, ADAMAWA STATE.**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF NATIONAL DIPLOMA (ND) IN SCIENCE LABORATORY TECHNOLOGY**

**AUGUST, 2025**

# DECLARATION

We hereby declare that the work in this project titled **“Antibacterial Effect of Ethanoic Extract of *Calotropis procera* (Sodom apple) Leaves against *Staphylococcus aureus*”** was performed by us under the supervision of Mrs. Ummi M. Nuhu. The information derived from literatures has been duly acknowledged in the text and a list of references provided. The work embodied in this project is original and had not been submitted in part or in full for any other diploma or certificate of this or any other institution.

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# APPROVAL PAGE

This project titled **“Antibacterial Effect of Ethanoic Extract of *Calotropis procera* (Sodom apple) Leaves against *Staphylococcus aureus*”** meets the regulations governing the award of National Diploma (ND) in Science Laboratory Technology, Federal Polytechnic Mubi, Adamawa State

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(External Examiner) Sign/Date

# DEDICATION

This project work is dedicated to Almighty God for his enabling strength he bestowed on us during the course of this project work. Also, our gratitude goes to our lovely parents for their never-ending support and encouragement during the course of this research work.

# ACKNOWLEDGEMENTS

We want to acknowledge Almighty God for his infinite mercy and protection throughout our academic activities. And for the understanding in achieving our academic success.

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# ABSTRACT

*This study investigates the presence of phytochemicals and heavy metals in two commonly consumed leafy vegetables Bitter leaf (Vernonia amygdalina) and Water leaf (Talinum triangulare) sourced from Mubi, Nigeria. Qualitative phytochemical screening revealed the presence of essential bioactive compounds such as saponins, phenols, flavonoids, terpenoids, glycosides, tannins, anthraquinones, and alkaloids in both plant samples, while steroids were present only in Water leaf. These compounds are known for their therapeutic and antioxidant properties, which may contribute to the health benefits associated with the consumption of these vegetables. Heavy metal analysis was conducted using standard atomic absorption spectrophotometry methods. The results showed that lead (Pb) and chromium (Cr) were not detected in either plant, indicating a relatively low risk of toxicity from these elements. However, cadmium (Cd) was detected at 0.08 mg/kg in Bitter leaf and 0.15 mg/kg in Water leaf. Copper (Cu) concentrations were 0.44 mg/kg and 0.51 mg/kg, while zinc (Zn) levels were 0.33 mg/kg and 1.18 mg/kg in Bitter leaf and Water leaf, respectively. Although these values are within the permissible limits set by WHO/FAO, continued monitoring is recommended to prevent potential health hazards. The study concludes that both Bitter leaf and Water leaf possess valuable medicinal properties due to their rich phytochemical content and are generally safe for consumption concerning heavy metal contamination. However, the presence of cadmium, though low, warrants attention to environmental and agricultural practices in the area. This research provides essential baseline data for public health safety and further pharmacological studies.*

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**CHAPTER ONE**

**INTRODUCTION**

**1.1 Background of the Study**

*Calotropis procera*, commonly known as Sodom apple, is a medicinal shrub native to tropical and subtropical regions of Africa, Asia, and the Middle East. It is widely distributed in the northern parts of Nigeria, where it grows along roadsides, farmlands, and arid areas. The plant is traditionally used for treating a variety of ailments including skin infections, wounds, fever, and respiratory disorders (Usman *et al.,* 2022). Among the different parts of the plant, the leaves are known to contain significant phytochemical compounds such as alkaloids, flavonoids, tannins, and saponins, which contribute to its biological activities including antibacterial, antifungal, and anti-inflammatory properties (Aliyu *et al.,* 2021).

One of the most common bacterial pathogens affecting public health is *Staphylococcus aureus*, a Gram-positive bacterium responsible for a wide range of infections including boils, skin abscesses, pneumonia, and food poisoning. The growing resistance of *S. aureus* to commonly used antibiotics, including methicillin, has become a serious global concern (Oluwafemi & Onifade, 2023). This has triggered an increased interest in exploring plant-based alternatives with potent antibacterial effects.

Extracts of *Calotropis procera* have shown promising antimicrobial activity in various studies. Ethanoic (ethanol-based) extraction, in particular, has been proven to be effective in isolating active compounds from plant materials due to its polarity and ability to dissolve a wide range of phytoconstituents. Ethanoic extracts of *C. procera* leaves have demonstrated significant activity against bacterial strains including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in in-vitro studies (Bashir *et al.,* 2023).

In northern Nigeria, where both *C. procera* and infectious diseases are prevalent, scientific exploration of the antimicrobial potential of local plants is essential. There is, however, a scarcity of recent localized data on the antibacterial efficacy of ethanoic extracts of *C. procera* against resistant strains of *S. aureus*. By evaluating this plant’s antibacterial activity using standardized methods, this study aims to provide scientific validation for its traditional use and promote the development of natural therapeutic agents.

Moreover, considering the socio-economic constraints and limited access to conventional healthcare in rural communities, the exploration of cost-effective, plant-based antibacterial agents is timely and necessary. If found effective, such plant extracts could reduce dependency on synthetic antibiotics, help combat antimicrobial resistance, and promote the integration of traditional medicine with modern healthcare systems (Adamu *et al.,* 2022).

## 1.2 Plant Description

*Calotropis procera* is a fast-growing, perennial shrub commonly found in arid and semi-arid environments. It thrives along roadsides, farmlands, and dry wastelands. The plant is characterized by its thick, waxy leaves, milky latex, and white to purplish flowers. It is highly resilient and can grow in nutrient-poor soils. Traditionally, different parts of the plant (leaves, bark, roots, and latex) are used to treat ailments such as fever, skin infections, wounds, respiratory disorders, and digestive issues.

## 1.3 Scientific Classification

**Kingdom:** Plantae

**Division:** Angiosperms

**Class:** Eudicots

**Order:** Gentianales

**Family:** Apocynaceae

**Genus:** *Calotropis*

**Species:** *Calotropis procera*

**1.4 Statement of the Problem**

The increasing resistance of *Staphylococcus aureus* to existing antibiotics poses a significant threat to public health in Nigeria and beyond. Despite the traditional use of *Calotropis procera* for treating infections, there is limited scientific documentation on the efficacy of its ethanoic leaf extract against *S. aureus*, particularly in the northern region of Nigeria. This study seeks to fill this gap by evaluating the antibacterial potential of the ethanoic extract of *C. procera* leaves against *Staphylococcus aureus* isolates. The findings could support the development of natural antimicrobial agents and enhance local healthcare practices.

## ****1.5 Justification for the Study****

Given the increasing threat of antibiotic resistance and the socio-economic limitations in accessing conventional medicine in rural communities, the investigation of accessible, affordable, and effective plant-based alternatives is both timely and necessary. Calotropis procera is abundantly available in the study area and is traditionally known for its medicinal properties. By scientifically validating its antibacterial activity against Staphylococcus aureus, this study aims to bridge the gap between traditional knowledge and scientific research. The outcome could contribute to the development of natural antibacterial agents, support local healthcare practices, and offer a sustainable approach to managing resistant infections.

**1.6 Aim and Objectives of the Study**

**1.6.1 Aim**

The aim of the research is to study the antibacterial effect of ethanoic extract of *Calotropis procera* leaves against *Staphylococcus aureus*.

**1.6.2 Objectives**

1. To determine the phytochemicals, present in ethanolic extract of *Calotropis procera* leaves.
2. To measure the antibiotic susceptibility using various antibiotics against *Staphylococcus aureus* using standard microbiological techniques.
3. To identify the bioactive components in ethanolic extract of *Calotropis procera* leaves.

# CHAPTER TWO

## LITERATURE REVIEW

### 2.1 Literature Review

This chapter reviews existing literature relevant to the study on the antibacterial properties of Calotropis procera leaf extracts against Staphylococcus aureus. It highlights previous research on the phytochemical composition of C. procera, its traditional uses, extraction techniques, and antibacterial activity, particularly in comparison to standard antibiotics.

Calotropis procera (Aiton) W.T. Aiton, belonging to the family **Apocynaceae**, is a perennial, soft-wooded, xerophytic shrub or small tree that typically grows between 1–5 meters in height, though it may reach up to 6 meters under favorable conditions. It is commonly referred to as **Sodom apple, giant milkweed**, or **"bom bom"** in various local Nigerian dialects. The plant is adapted to arid and semi-arid environments and is widely distributed across tropical and subtropical regions of Africa, the Middle East, and South Asia (Ibrahim *et al.,* 2023).

The leaves of C. procera are opposite, broadly ovate to obovate in shape, thick, leathery, and covered with a waxy coating that helps minimize water loss. The plant is easily recognized by its **milky white latex,** which exudes from any injured part of the plant and contains a variety of biologically active compounds such as calotropin and uscharin (Adeoye *et al.,* 2022).

The flowers of C. procera are bisexual, five-petaled, and range in color from purple to pale lavender with white centers. The plant produces **follicular fruits** (pods) that contain numerous seeds attached to silky hairs, aiding wind dispersal. It is commonly found on roadsides, farmlands, fallow lands, and waste areas, especially in the **savannah regions of Nigeria** such as Borno, Adamawa, and Yobe States (Yakubu & Abdullahi, 2022).

The deep-rooted system and tough stem structure of the plant allow it to survive in extreme drought conditions, and it is often considered a weed in some regions due to its aggressive growth and ability to colonize degraded lands (Chinedu *et al.,* 2023). Despite this, its importance in traditional medicine and ethnobotany has led to increased interest in its conservation and pharmacological evaluation.

Recent botanical surveys and morphological studies have also highlighted the plant's potential role in soil stabilization and ecological restoration of degraded drylands in northern Nigeria, further underscoring its ecological and medicinal significance (Salam *et al.,* 2023).

### 2.2 Traditional Medical Uses of C. procera

*Calotropis procera* has been widely employed in traditional medicine systems such as Ayurveda, Unani, and African ethnomedicine for centuries. Its diverse pharmacological applications are rooted in the rich composition of bioactive compounds found in its leaves, latex, roots, and bark. The plant is often referred to as a “miracle shrub” in rural communities due to its therapeutic versatility (Nwachukwu *et al.,* 2023).

In Africa, particularly in the Sahelian regions and among rural communities in northern Nigeria, various parts of the plant are used for treating common health problems such as fever, leprosy, asthma, toothache, abdominal pain, dysentery, and diarrhea. The leaves are commonly heated and applied as a poultice for wounds, ulcers, and swollen joints to relieve inflammation and promote healing (Yusuf & Bala, 2023).

The milky latex of *C. procera* is one of its most notable components and is traditionally used to treat ringworm, eczema, scabies, and warts. It is often applied directly to affected skin areas or mixed with other herbal preparations. In some local practices, the latex is also used to relieve toothaches and earaches, though caution is advised due to its strong irritant properties (Adebayo et al., 2022).

The bark and root extracts are used for their purgative, antihelminthic, and analgesic effects. Decoctions from the root are consumed to alleviate constipation, malaria, and stomach disorders (Okonkwo & Ibrahim, 2023). In Sudan and India, powdered flowers are used as a tonic or stimulant and have been reported in ethnobotanical surveys as remedies for bronchial asthma and coughs (Rahman *et al.,* 2023).

Despite its toxicity in high doses, local practitioners have developed techniques for detoxifying certain parts of the plant through boiling, drying, or combining with other herbs. This knowledge has been passed down through generations and remains relevant in contemporary herbal practices (Emeka *et al.,* 2022).

The increasing interest in ethnopharmacology has led to scientific validation of many of these traditional uses, with studies confirming the antimicrobial, anti-inflammatory, wound-healing, antipyretic, and antidiarrheal properties of various *C. procera* extracts (Ajao *et al.,* 2023).

### 2.3 ****Pharmacological Activities of**** Calotropis procera

Calotropis procera has garnered considerable scientific attention for its pharmacological versatility, as it possesses a wide range of bioactivities that support its traditional medicinal applications. The plant is rich in secondary metabolites such as alkaloids, flavonoids, cardenolides, saponins, terpenoids, and tannins, which contribute to its therapeutic potential. These compounds function individually or synergistically to exhibit biological activities that are beneficial for managing infections, inflammation, oxidative stress, cancer, and parasitic diseases (Salam *et al.,* 2023).

The pharmacological value of C. procera has been affirmed through both in vitro and in vivo studies. The various plant parts—leaves, latex, flowers, and root bark—have been explored for their medicinal properties, with notable results in the areas of antimicrobial, anti-inflammatory, antioxidant, and anticancer research. Moreover, C. procera is considered a valuable natural resource for developing plant-based therapeutic agents, especially in resource-limited regions where access to conventional drugs may be restricted (Salam *et al.,* 2023).

Its pharmacological properties are especially important in the context of rising antibiotic resistance and the growing need for alternative, plant-based treatments. With the increasing interest in ethnopharmacology, Calotropis procera continues to offer promising avenues for drug discovery and development.

### ****2.3.1 Antibacterial Activity****

The antibacterial potential of Calotropis procera has been widely documented. Methanol and ethanol extracts from the leaves and latex exhibit significant zones of inhibition against both Gram-positive and Gram-negative bacteria. Notably, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae are among the pathogens effectively inhibited by these extracts (Bashir et al., 2023). This suggests the plant’s potential in managing skin infections, respiratory tract infections, and gastrointestinal diseases caused by these bacteria.

Mechanistically, the bioactive compounds in C. procera, such as flavonoids, phenolics, and cardenolides, are believed to compromise bacterial cell membrane integrity, disrupt protein synthesis, and inhibit nucleic acid replication. Scanning electron microscopy (SEM) studies on bacterial morphology post-treatment with the extract have shown cell wall damage and leakage of intracellular contents. These effects are more pronounced in crude latex and polar extracts, indicating that the polarity of solvents plays a significant role in extracting effective antibacterial agents.

In light of the growing challenge of antibiotic resistance, C. procera provides a strong basis for developing alternative antibacterial agents. Combinations of C. procera extracts with conventional antibiotics have also shown synergistic effects in recent studies, suggesting that it can enhance the efficacy of existing drugs. This combination therapy could help reduce the dosage of antibiotics needed and mitigate side effects or resistance development.

### ****2.3.2 Antifungal Activity****

In addition to antibacterial effects, C. procera has demonstrated potent antifungal activity. Methanol and aqueous extracts of the leaves and latex have shown inhibitory effects on fungal pathogens such as Candida albicans, Aspergillus niger, Trichophyton mentagrophytes, and Microsporum gypseum (Ahmed et al., 2022). These pathogens are associated with dermatophytic infections, candidiasis, and systemic fungal infections, especially in immunocompromised individuals.

The antifungal mechanism of C. procera appears to involve the disruption of fungal cell membranes and inhibition of ergosterol synthesis, a key component of fungal membranes. Flavonoids and saponins present in the plant increase membrane permeability and induce apoptosis in fungal cells. Studies using electron microscopy have confirmed cellular deformation and leakage of cytoplasmic materials in fungi exposed to the extracts.

Given the limitations of current antifungal therapies—such as toxicity and resistance—C. procera could serve as a source of safer, plant-derived antifungal agents. Incorporation of its extracts into topical formulations, creams, and antifungal soaps has been suggested for managing skin and mucosal infections, especially in rural healthcare systems where pharmaceutical antifungals may not be readily available.

### ****2.3.3 Anti-inflammatory and Analgesic Effects****

Calotropis procera has been shown to exert significant anti-inflammatory activity in preclinical models. Latex and leaf extracts have been tested in carrageenan-induced rat paw edema models, revealing substantial suppression of inflammation. The effect is comparable to that of standard non-steroidal anti-inflammatory drugs (NSAIDs), as demonstrated by Adekunle et al. (2023), who reported a dose-dependent decrease in paw swelling and leukocyte infiltration.

The anti-inflammatory action is largely attributed to inhibition of pro-inflammatory mediators such as histamines, prostaglandins, TNF-α, and interleukins. Flavonoids and triterpenoids in the plant inhibit the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, reducing the synthesis of these inflammatory mediators. Furthermore, antioxidant compounds in the extract help neutralize reactive oxygen species that exacerbate inflammation, contributing to its therapeutic effect.

In addition to anti-inflammatory properties, C. procera also exhibits strong analgesic (pain-relieving) effects. This has been demonstrated in the tail-flick and hot-plate tests in rats, where the extract significantly delayed pain responses. The dual action of anti-inflammation and analgesia makes C. procera particularly suitable for treating inflammatory conditions such as arthritis, muscle pain, and chronic wounds.

### ****2.3.4 Antioxidant Properties****

The antioxidant capacity of C. procera plays a crucial role in its therapeutic effects. Oxidative stress is a contributing factor to many chronic diseases, including diabetes, neurodegeneration, cancer, and cardiovascular disorders. The leaf and latex extracts of C. procera have shown strong antioxidant activities in DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, FRAP (ferric reducing antioxidant power), and ABTS assays (Okonkwo et al., 2022).

Phytochemical analysis reveals that phenolic acids and flavonoids—particularly quercetin and kaempferol—are responsible for this antioxidant activity. These compounds donate electrons or hydrogen atoms to neutralize free radicals and break the chain reaction of lipid peroxidation. The ability of C. procera to reduce malondialdehyde (MDA) levels and increase superoxide dismutase (SOD) and catalase activities in animal models further supports its antioxidant potential.

Due to its antioxidant properties, C. procera may offer protective effects in disease models involving oxidative tissue damage. Its use in herbal formulations for managing aging, metabolic syndrome, and cardiovascular diseases is gaining popularity. Current research is exploring its potential as a supplement to reduce oxidative stress markers in diabetic and hypertensive patients.

### ****2.3.5 Anticancer Activity****

Recent studies have demonstrated that C. procera possesses significant anticancer activity. Its latex and leaf extracts exhibit cytotoxicity against multiple human cancer cell lines including breast (MCF-7), colon (HT-29), and cervical (HeLa) cells (Singh & Rajan, 2023). This activity is largely attributed to the presence of cardenolides and flavonoids, which interfere with cancer cell proliferation.

One of the key mechanisms behind this anticancer effect is the induction of apoptosis. Extracts of C. procera have been found to activate caspase enzymes, increase the Bax/Bcl-2 ratio, and cause DNA fragmentation—all hallmarks of programmed cell death. Moreover, these extracts also inhibit angiogenesis (the formation of new blood vessels), thereby depriving cancer cells of nutrients and oxygen.

Importantly, C. procera shows selectivity towards cancer cells while having limited cytotoxicity against normal cells in vitro, making it a promising candidate for drug development. Ongoing in vivo studies are evaluating its safety profile and potential use as an adjuvant in chemotherapy. If further validated, C. procera-based extracts or compounds could form the basis of plant-derived anticancer drugs.

### ****2.3.6 Anthelmintic and Antimalarial Activity****

The anthelmintic (anti-worm) and antimalarial activities of Calotropis procera are well supported by traditional knowledge and modern pharmacological research. Gana et al. (2021) demonstrated that the methanol extract of C. procera was highly effective in expelling intestinal worms in rodent models. It exhibited dose-dependent paralysis and death of Pheretima posthuma, a model for human intestinal helminths.

The plant’s anthelmintic effect is believed to stem from the presence of proteolytic enzymes, alkaloids, and saponins that disrupt the cuticle and internal metabolism of parasites. These compounds interfere with the parasites' neuromuscular function, leading to paralysis and expulsion from the host. The anthelmintic action is particularly useful in rural communities where parasitic infections are prevalent and synthetic drugs may be unaffordable or unavailable.

Additionally, C. procera has shown inhibitory activity against Plasmodium falciparum, the parasite responsible for the most severe form of malaria. The plant’s bioactive compounds interfere with the parasite’s lifecycle, possibly by targeting its mitochondrial membrane or protein synthesis. These properties suggest that C. procera may have value as a supplementary or alternative treatment in malaria-endemic regions.

### ****2.3.7 Wound Healing and Dermatological Applications****

Calotropis procera has long been used in traditional medicine for treating wounds, ulcers, and skin infections. Recent studies have confirmed that its latex and leaf extracts significantly accelerate the wound healing process. Mohammed et al. (2022) demonstrated that rats treated with topical C. procera extracts exhibited faster wound contraction, epithelialization, and granulation tissue formation compared to controls.

The wound healing effect is attributed to multiple actions, including antibacterial activity, anti-inflammatory properties, and promotion of collagen synthesis. Flavonoids and triterpenoids stimulate fibroblast proliferation and angiogenesis, which are essential for tissue regeneration. Furthermore, antioxidant activity reduces oxidative stress at the wound site, facilitating healing. In dermatological applications, C. procera has been used to treat eczema, ringworm, and acne. Its extracts are being tested in herbal ointments, creams, and antiseptic gels. With growing interest in natural skincare, C. procera offers a sustainable and effective option for treating various skin conditions, particularly in settings where access to conventional dermatological treatments is limited.

### 2.3.8 ****Hepatoprotective Activity****

*Calotropis procera* has demonstrated hepatoprotective effects in various experimental models. Ethanolic and aqueous extracts of its leaves and latex have shown significant protection against chemically induced liver damage, particularly in models using hepatotoxins such as carbon tetrachloride (CCl₄) and paracetamol. In treated animal groups, markers such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were significantly reduced compared to untreated controls, suggesting hepatic recovery and membrane stabilization (Kumar et al., 2022).

The hepatoprotective effect is mainly attributed to the presence of flavonoids, saponins, and polyphenolic compounds that exhibit antioxidant properties. These phytochemicals neutralize free radicals and reduce lipid peroxidation in liver cells, thereby restoring normal hepatic histology and function (Abdel-Azeem et al., 2021).

Given its potent bioactive components, C. procera is a promising candidate for managing liver-related disorders, including drug-induced hepatotoxicity, fatty liver disease, and viral hepatitis. Its inclusion in herbal formulations could offer a cost-effective liver tonic in traditional and integrative medicine.

### 2.3.9 ****Antidiabetic Activity****

Calotropis procera has also shown promising antidiabetic potential. Studies using alloxan and streptozotocin-induced diabetic rats revealed that administration of methanolic and aqueous leaf extracts significantly lowered fasting blood glucose levels, improved oral glucose tolerance, and enhanced insulin secretion (Sani et al., 2022).

The mechanism of action appears to involve several pathways: inhibition of intestinal glucose absorption, improvement of peripheral glucose uptake, and regeneration of pancreatic β-cells. Alkaloids, flavonoids, and terpenoids in the extract are believed to contribute to the modulation of carbohydrate metabolism and insulin signaling pathways (Ahmed et al., 2021).

Considering the global burden of diabetes, especially in low-resource communities, C. procera may serve as a low-cost, plant-based alternative or adjunct in diabetes management, aligning with ethnomedical practices in sub-Saharan Africa and South Asia.

### 2.3.10 ****Immunomodulatory Activity****

Emerging research indicates that Calotropis procera possesses significant immunomodulatory effects. Its extracts have been shown to stimulate both innate and adaptive immune responses in murine models. In one study, administration of latex and leaf extracts resulted in enhanced phagocytic activity, elevated total leukocyte counts, and increased antibody titers, suggesting both cellular and humoral immune stimulation (Chaudhary et al., 2023).

Bioactive compounds such as flavonoids and cardenolides are believed to play a key role in modulating immune cell proliferation and cytokine release, particularly interleukin-2 (IL-2) and interferon-gamma (IFN-γ), which are essential for immune defense (Rahman et al., 2022).

These findings support the potential application of C. procera in managing conditions associated with immune suppression or immune dysregulation, such as chronic infections, cancer, or autoimmune diseases.

### 2.4 Phytochemical Composition of C. procera Leaves

Phytochemical investigations of *Calotropis procera* leaves have consistently confirmed the presence of diverse bioactive compounds that contribute to the plant’s wide range of therapeutic effects. These include alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, anthraquinones, and cardiac glycosides (Bashir *et al.,* 2023; Umar *et al.*, 2024).

**2.4.1 Alkaloids**

Alkaloids are nitrogen-containing compounds that have been widely studied for their pharmacological effects, including antimicrobial, anti-inflammatory, and antidiabetic properties. In *Calotropis procera*, several alkaloids have been identified, with studies highlighting the presence of compounds such as calotropine and uscharine. These alkaloids contribute to the plant’s toxic properties but also exhibit bioactive effects, such as antimicrobial and analgesic activities. Research by Mounira et al. (2023) confirmed that alkaloid-rich extracts of *C. procera* demonstrate strong antibacterial activity against a wide range of pathogens, including *Staphylococcus aureus* and *Escherichia coli*.

**2.4.2 Flavonoids**

Flavonoids are a class of polyphenolic compounds with known antioxidant, anti-inflammatory, and anticancer activities. The presence of flavonoids like quercetin, kaempferol, and rutin in *C. procera* has been well documented. A study by Okonkwo et al. (2022) confirmed that the leaf and flower extracts of *C. procera* contain significant levels of flavonoids, which contribute to its free radical-scavenging properties. These compounds help reduce oxidative stress, which plays a major role in the development of several chronic diseases such as cancer, diabetes, and cardiovascular diseases. The antioxidant properties of flavonoids also support the plant's use in treating inflammatory conditions and improving immune function.

**2.4.3 Cardenolides**

Cardenolides, a subclass of steroids, are found abundantly in the latex of *C. procera*. These compounds have demonstrated a variety of biological activities, including antimicrobial, anticancer, and anti-inflammatory effects. *Calotropis procera* is particularly rich in cardenolides, such as calotropin and procera. A study by Singh and Rajan (2023) revealed that the cardenolide content in the plant is responsible for its cytotoxic effects against cancer cells, inducing apoptosis in breast cancer cell lines (MCF-7). Cardenolides act by inhibiting the Na+/K+ ATPase pump, which disrupts cellular homeostasis, leading to the death of malignant cells. These compounds also play a significant role in managing heart conditions due to their effects on the cardiovascular system.

**2.4.4 Saponins**

Saponins are glycoside compounds known for their surfactant properties and have been shown to possess antifungal, antimicrobial, and antidiabetic activities. In *C. procera*, saponins are found in both the leaves and latex. These compounds have been associated with the modulation of immune responses and the inhibition of lipid accumulation, which may contribute to their hypolipidemic and antidiabetic properties (Akinmoladun et al., 2021). Saponins are also known to aid in the absorption of other active phytochemicals, enhancing the overall therapeutic effect of the plant.

**2.4.5 Tannins and Phenolic Compounds**

Tannins and phenolic compounds are well-known for their antioxidant and anti-inflammatory properties. In *C. procera*, these compounds contribute to its ability to neutralize free radicals and reduce oxidative stress. Recent studies have shown that the plant’s leaf extracts are rich in tannins such as catechins and epicatechins, as well as other polyphenolic compounds like phenolic acids. According to a study by Mohammed et al. (2022), these phytochemicals are responsible for the plant's antimicrobial, wound-healing, and anti-inflammatory effects. The presence of phenolics and tannins also supports the plant’s traditional use in treating wounds and ulcers, as these compounds accelerate tissue regeneration and fight infection.

**2.4.6 Terpenoids**

Terpenoids are another group of bioactive compounds found in *C. procera*. These compounds, including essential oils and steroids, contribute to the plant’s anti-inflammatory, antimicrobial, and anticancer properties. Studies have demonstrated that terpenoid-rich extracts of *C. procera* possess strong anti-inflammatory activity, with some compounds acting as natural COX-2 inhibitors, similar to non-steroidal anti-inflammatory drugs (NSAIDs) (Saidu et al., 2021). Terpenoids also possess antidiabetic and hepatoprotective effects, further broadening the plant's potential therapeutic applications.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Materials

The materials used in this study included: fresh leaves of *Calotropis procera*, mortar and pestle, 2500 ml conical flasks, ethanol (95%), distilled water, rotary evaporator (R110), test tubes, water bath, Fehling’s solution, dimethylsulphoxide (DMSO), acetic anhydride, concentrated sulphuric acid, ferric chloride solution (5%), chloroform, magnesium metal, concentrated hydrochloric acid, Dragendorff’s and Mayer’s reagents, nutrient agar (Oxoid), clinical isolates of *Escherichia coli,* *Staphylococcus* *aureus*, *Salmonella* spp., and *Pseudomonas* spp., Bijou bottles, pipettes, and incubators set at 37±1°C.

## 3.2 Collection and identification of the plant material

The leaves actively-growing *C. procera* plants were randomly and aseptically collected from around Federal Polytechnic, Mubi, Adamawa State, Northern Nigeria. The plant was first identified at the field using standard keys and descriptions.

## 3.3 Preparation of Samples

This was carried out in accordance with the method of Kareem *et al* (2013). Here, the leaves of *C. procera* plant was obtained as exudates by hand plucking of fresh leaves of actively growing plant using aseptic techniques. The leaves were obtained by hand-plucking. They were thoroughly washed under running tap water, rinsed with distilled water and finally air dried. The dried leaves were made into powder form using mortar and pestle as described by Fatope *et al* (2013). The content was then stored in air-dried containers until required for use.

## 3.4 Extraction protocols

This was carried out according to the method of Fatope *et al* (2013) using soxhlet extraction technique. A quantity (100 g) of the fine powder of the leaves was weighed and suspended into a 2500ml-capacity conical flask. This was percolated with 1000 ml of 95% ethanol while another (100g) was suspended into separate conical flask of 800 ml of distilled water. Each was allowed to stand for two weeks with constant shaking at regular intervals under room temperature. The percolates were then filtered and the solvents (ethanol and water) were evaporated using rotar evaporator (R110) to obtain the ethanolic and aqueous extracts of the leaves and latex respectively. These served as the stock solutions, which were stored in a refrigerator at 4ºC until needed for analysis.

## 3.5 Phytochemical screening

**Test for reducing sugars**

One gram of the aqueous extract was weighed and placed into a test tube. This was diluted using 10 ml of de-ionised distilled water. This was followed by the addition of Fehling’s solution. The mixture warmed to 40ºC in water bath. Development of brick-red precipitate at the bottom of the test tube was indicative of the presence of a reducing sugar. Same procedure was repeated using dimethylsulphoroxide (DMSO) as the diluent for the ethanolic extract (Brain & Turner, 2015).

**Test for resins**

Two grams of the ethanolic extract was dissolved in 10ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple colour, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material (Cuilel, 2014).

**Test for tannins**

Two grams of the aqueous extract was weighed and placed in a test tube. Two drops of 5% ferric chloride solution was then added. The appearance of a darkgreen color was indicative of the presence of tannins. The same procedure was repeated using the ethanolic extract (Cuilel, 2014).

**Test for steroid glycosides**

One gram of the ethanolic extract was weighed and placed in a test tube. This was dissolved in 2 ml of acetic anhydride, followed by the addition of 4 drops of chloroform. Two drops of concentrated sulphuric acid were then added by means of a pipette at the side of the test tube. The development of a brownish ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer were indicative of the presence of steroid glycosides. Same procedure was repeated using the aqueous extract (Cuilel, 2014).

**Test for flavonoids**

Two grams of the ethanolic extract was weighed, placed in a test tube, followed by the addition of 10 ml of DMSO. The mixture was heated, followed by the addition of magnesium metal and 6 drops of concentrated hydrochloric acid. The appearance of red colour was indicative of the presence of flavonoids. Same procedure was repeated using aqueous extract (Sofowora, 2013).

**Test for alkaloids**

One gram each of the ethanolic extract was weighed and placed into two separate test tubes. To the first test tube, 2-3 drops of Dragendoff’s reagent was added while 2-3 drops of Meyer’s reagent were added to the second test tube. The development of an orange-red precipitate (turbidity) in the first test tube (with Dragendoff’s reagent) or white precipitate (turbidity) in the second test tube (with Meyer’s reagent) was indicative of the presence of alkaloids. Same procedure was repeated using aqueous extract (Cuilel, 2014).

**Test for saponins**

Five grams of the aqueous extract was weighed and placed in a test tube. This was followed by the addition of 5 ml de-ionised distilled water. The content was vigorously shaken. The appearance of a persistent froth that lasted for 15 minutes was indicative of the presence of saponins. Same procedure was repeated using DMSO for the ethanolic extract (Brain and Turner, 1975).

## 3.6 Test organisms

Clinical isolates of bacteria were used for the bioassay studies. The isolates included *Escherichia* *coli*, *Staphylococcus aureus* and the species of *Salmonella* and *Pseudomonas*. The isolates were obtained from the Department of Biochemical Science Technology, Federal Polytechnic, Mubi, Adamawa State, Nigeria. They were further confirmed using standard biochemical tests (citrate utilization, coagulase, oxidase and catalase) as described by Cheesbrough (2022). The isolates were maintained on freshly-prepared nutrient agar (oxoid) slants and kept in a refrigerator at 4ºC until required for use.

## 3.7 Preparation of extract concentrations

This was carried out using standard method (Cheesbrough, 2022). Stock solution of the ethanolic extract was prepared by weighing 10 mg of it and dissolved in 1ml of dimethylsulphoroxide (DMSO) in Bijou bottle. This gave an extract concentration of 10,000 µg/ml (stock solution). Three varied extract concentrations (1000 μg/ml, 2000 μg/ml and 5000 μg/ml) were prepared from the stock solution (10,000 µg/ml) using 10-fold serial dilution. The same procedure was repeated using de-ionised distilled water for the aqueous extract.

## 3.8 Determination of minimum inhibitory and bactericidal concentrations of the extracts

The MIC and MBC were determined in accordance with the method of Cheesbrough (2022). Varied extract concentrations were prepared to arrive at 2000, 1000, 500 and 250 µg/ml. A quantity (0.1 ml) of the suspension of the test bacterium (standardised inoculum) was inoculated onto fresh nutrient agar (oxoid) plates at the different extract concentrations. The plates were incubated at 37±1ºC for 18 hours. The lowest concentration of the extract that inhibited the growth of the test bacterium was noted and recorded as the MIC while the MBC was determined by taking a loopful from each negative (no growth) tube in the MIC assay and inoculated onto fresh nutrient agar (oxoid). The plates were incubated at 37±1ºC for 24 hours after which they were observed for growth or otherwise of the test organism.

# CHAPTER FOUR

# RESULT AND DISCUSSION

## 4.1 Results

## 4.2 Phytochemical Analysis

**Table 4.1: Result of qualitative analysis of phytochemicals in *Calotropis Procera* leaves**

|  |  |  |
| --- | --- | --- |
| **Test** | **Aqueous** | **Methanol** |
| Alkaloid | **+** | **+** |
| Flavonoid | **+** | **+** |
| Phenolic | **-** | **-** |
| Steroids | **+** | **-** |
| Glycosides | **+** | **+** |
| Tannins | **++** | **+** |
| Saponins | **+** | **+** |
| Terpenoids | **++** | **+** |

**Key:**

++ = indicate presence (deep in colour)

+ = Presence (lighter in colour)

– = Absent (undetected)

## 4.3 Results of the antibacterial effect of ethanolic extract of *Calotropis procera* leaves acting against the selected bacterial isolates (*E. coli*, *Salmonella*, *Pseudomonas*, Staphylococcus aureus) using different concentration (200, 400, 600).

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate** | **200 (mg/ml)** | **400 (mg/ml)** | **600 (mg/ml)** |
| *Escherichia coli* | - | - | - |
| *Pseudomonas Aeruginosa* | - | 8 | 10 |
| Salmonella | - | - | - |
| Staphylococcus aureus | - | - | - |

The result indicated that the extract had no antimicrobial activity against the selected bacterias buut showed minimal inhibitory activity against *Pseudomonas Aeruginosa* which is a potential urinary tract pathogen, meaning that ethanolic extract of the plant (*Calotropis procera* leaves) should be useful for the treatment of urinary tract infections.

## 4.3 Discussion

In this study, ethanolic leaf extract of *Calotropis procera* exhibited no detectable antibacterial activity against *Escherichia coli*, *Salmonella* spp., or *Staphylococcus aureus* at concentrations up to 600 mg/mL, and showed only minimal inhibition against *Pseudomonas aeruginosa* (8 mm at 400 mg/mL; 10 mm at 600 mg/mL). These results contrast sharply with the findings of Saddiq *et al.* (2022), who reported significant zones of inhibition approximately 18.7 mm for *S. aureus*, 21.3 mm for *Klebsiella pneumoniae*, and 21.9 mm for *E. coli* with MICs ranging from 0.60–1.50 mg/mL. Similarly, Kareem, Ojo, and colleagues (2024) found that ethanolic latex and leaf extracts produced strong inhibition of *E. coli* (~14.1 mm) and *S. aureus* using agar well diffusion methods. The disparity between these studies and the current findings may stem from differences in geographic origin of the plant material, extraction protocols, and specific microbial strains tested.

Adding nuance to the present findings, Umaru *et al.* (2024) demonstrated that ethanol‐based stem extracts of *C. procera* completely eradicated *P. aeruginosa* and *E. coli* within 20 hours, while corresponding leaf extracts were significantly less effective. Their GC–MS profiling linked superior activity to bioactives such as lupenyl acetate and phytol. This aligns with the minimal activity observed in the current study using only leaf material, suggesting that plant part selection and phytochemical content are key determinants of antibacterial efficacy.

Phytochemical screening in the current investigation confirmed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids (in aqueous extract), and terpenoids (in methanol extract), with phenolics notably absent. This matches patterns reported by Molecules investigators (Saddiq *et al.,* 2022) who identified α‑amyrin (≈39 %), lupeol acetate (≈18 %), phytol (≈13 %), linolenic acid, and other compounds in ethanolic leaf extracts exhibiting notable antimicrobial activity. Yet, the lack of phenolic compounds which are often associated with strong broad‑spectrum inhibition may partly explain the absence of activity in this study, particularly against *S. aureus*. Collectively, these results underscore the influence of extract origin, phytochemical composition, and microbial target on the observed antibacterial potential of *C. procera* leaf extracts.

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

The findings of this study indicate that ethanolic leaf extract of *Calotropis procera* possesses limited antibacterial potential against the tested pathogens, with only minimal activity observed against *Pseudomonas aeruginosa*. The absence of phenolic compounds often associated with strong antimicrobial effects may partly account for the lack of broad-spectrum activity, especially against *Staphylococcus aureus*. In line with previous studies that highlight stronger antibacterial effects from stem or latex extracts, the present results suggest that the antimicrobial efficacy of *C. procera* is highly dependent on the plant part utilized and the phytochemical profile of the extract. While *C. procera* remains a plant of pharmacological interest, the ethanolic leaf extract, at the tested concentrations, may not be suitable for therapeutic application against the bacterial strains examined in this study without further optimization.

## 5.2 Recommendations

Based on the findings, the following recommendations are made:

1. Future studies should evaluate the antibacterial activity of stem and latex extracts of *C. procera*, as they have been reported to possess higher antimicrobial potency.
2. Employing different solvents (e.g., methanol, chloroform, acetone) and advanced extraction techniques (e.g., Soxhlet extraction, ultrasonic-assisted extraction) may improve the yield and potency of bioactive compounds.
3. Targeted isolation and characterization of specific bioactive molecules such as α‑amyrin, lupeol acetate, and phytol should be prioritized to determine their individual and synergistic antibacterial effects.
4. Testing should be extended to include multidrug-resistant strains, especially methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing bacteria.