



Investigating the Photochemical Composition and Antimicrobial Activity of *Carica papaya* Leaf Extract in Relation to Wound Healing.

BY

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**A RESEARCH PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF
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DECLARATION

I, GIBEDI BRIAN, declare that this research project is my original work and has not been submitted to any other institution for academic credit.

Signature: 

Date: 04/09/2026

APPROVAL

This research project has been submitted with the approval of the supervisor.

Mr. KATWESIGYE RICHARD

Signature: 

Date: 04/09/2026

DEDICATION

To my beloved parents, whose sacrifices and unwavering belief in the power of education have brought me this far. Your unconditional love, guidance, encouragement and selfless support have been the foundation of my academic journey and this achievement is a reflection of your enduring commitment to my success.

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ABSTRACT

Delayed wound healing and wound infections continue to pose major public health concerns, especially in low- and middle-income countries where antimicrobial resistance is rising. *Carica papaya* leaves are commonly used in traditional medicine for wound management, though scientific validation remains limited in many local settings. This study investigated the phytochemical composition and antimicrobial activity of ethanolic leaf extract of *C. papaya* against selected wound-associated bacterial pathogens; *S. aureus* and *E. coli*. Fresh leaves were collected from Nagongera Town Council, validated, air-dried, and powdered before extraction using 70 % ethanol by soaking for 72 hours. Qualitative phytochemical screening was performed using standard biochemical tests, while antimicrobial activity was evaluated using the agar well diffusion method at extract concentrations ranging from 30 to 100 mg/ml. Phytochemical analysis

confirmed the presence of flavonoids, steroids, saponins, alkaloids, and tannins. The extract exhibited concentration-dependent antibacterial activity against both test organisms. At the highest concentration (100 mg/ml), mean zones of inhibition were 5.83 mm against *S. aureus* and 6.50 mm against *E. coli*. The positive control, ciprofloxacin, produced larger inhibition zones, while the negative control showed no activity. These findings indicate that *C. papaya* leaf ethanolic extract contains bioactive compounds with measurable antibacterial properties, supporting its traditional use in wound care. Further studies involving quantitative phytochemical analysis, in vivo evaluation, and safety profiling are recommended before clinical application.

Keywords: *Carica papaya*, phytochemical screening, antimicrobial activity, wound healing, *Staphylococcus aureus*, *Escherichia coli*, traditional medicine, Uganda.

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CHAPTER ONE: INTRODUCTION

1.0 Background of the study

Wound healing is a complex biological process that involves a coordinated sequence of events including hemostasis, inflammation, tissue proliferation, and remodelling (Gregory et al. , 2005). Proper wound healing is essential for restoring the integrity of damaged skin and underlying tissues (Gregory S. Schultz, Gloria A. Chin, Lyle Moldawer, 2005). However, wound healing can be delayed or complicated by factors such as microbial infection, inflammation, and poor tissue protection, leading to chronic wounds and increased risk of morbidity, with prevalence rates reaching up to 4.48 per 1000 people globally and even higher burdens in low and middle income countries (Martinengo, L., Olsson, M., Bajpai, R., Soljak, M., & Car, 2019)

Microbial infection remains one of the most significant causes of delayed wound healing, particularly in low- and middle-income countries where access to advanced wound care may be limited (Sandar et al., 2021). Common wound associated pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are known to colonize open wounds, prolong inflammation, and interfere with tissue repair, often exhibiting high antimicrobial resistance rates exceeding 50 % in African settings (Sandar, W. P., Saw, S., Kumar, A. M. V., Camara, B. S., & Sein, 2021). Effective control of wound infection is therefore a critical component of successful wound management.

Medicinal plants have long been used in traditional medicine for wound care due to their availability, affordability, and perceived (Tugume et al., 2024). Many of these plants contain bioactive compounds with antimicrobial, anti-inflammatory, and tissue protective properties (Yusuf, A. J., Lawal, B., & Omonije, n.d.). Scientific validation of such plants is important to support their traditional use and to identify potential sources of novel therapeutic agents.

Carica papaya is a widely cultivated plant in tropical regions, including Uganda, and has been traditionally used for various medicinal purposes (Tugume et al., 2024). Different parts of the plant, particularly the leaves, have been used in folk medicine for treating wounds, burns, and skin infections (Tugume et al. , 2024). Previous studies have suggested that *Carica papaya* leaves contain several phytochemicals such as flavonoids, tannins, alkaloids, saponins, and phenolic

compounds, which are known to possess antimicrobial properties against common wound pathogens(Mishra, A., Uwimana, E., Mukamana, D., & Habimana, n.d.).

Despite its widespread traditional use, there is still limited locally generated scientific data on the phytochemical composition and antimicrobial activity of *Carica papaya* leaf extracts in relation to wound healing (Ntale, 2024).). Investigating these properties can help explain the basis of its traditional application and provide evidence to support its use in wound management.

1.1 Problem Statement

Delayed wound healing and wound infections remain a major public health challenge, particularly in resource limited settings (Maheshwari, G., 2024). The increasing prevalence of antimicrobial resistance has further complicated the management of wound infections, reducing the effectiveness of commonly used antibiotics, with resistance rates to key agents like methicillin in *S. aureus* often surpassing 40 % to 60 % in LMICs (Monk, E. J. M., Abubakar, A., Okeke, I. N., & O'Neill, 2024). As a result, there is a growing need to explore alternative and complementary sources of antimicrobial agents.

Although *Carica papaya* leaves are commonly used in traditional wound treatment, their use is largely based on anecdotal evidence rather than standardized scientific evaluation(Gang et al., n.d.). The lack of documented information on the specific phytochemical constituents and antimicrobial effectiveness of *Carica papaya* leaf extracts limits their acceptance and potential integration into evidence based wound care practices(Mishra, A., Uwimana, E., Mukamana, D., & Habimana, n.d.; Ntale, 2024).

Therefore, there is a need to scientifically assess the phytochemical composition and antimicrobial activity of *Carica papaya* leaf extract against common wound associated bacteria to provide empirical evidence supporting its role in wound healing (Anibijuwon, O. I., & Udeze, 2009).

1.2 Purpose of the Study

The purpose of this study was to investigate the phytochemical constituents and antimicrobial activity of *Carica papaya* leaf extract in relation to wound healing.

1.3 Objectives of the Study.

1.3.1 General Objective.

To evaluate the phytochemical composition and antimicrobial activity of *Carica papaya* leaf extract as mechanisms relevant to wound healing.

1.3.2 Specific Objectives.

1. To qualitatively identify the major phytochemical constituents present in *Carica papaya* leaf extract.
2. To assess the antimicrobial activity of *Carica papaya* leaf extract against selected wound associated bacterial pathogens.

1.3.3 Research Questions

- 1) What major phytochemical constituents are present in *Carica papaya* leaf extract?
- 2) Does *Carica papaya* leaf extract exhibit antimicrobial activity against selected wound associated bacterial pathogens?

1.4 Significance of the Study

The findings of this study provide scientific evidence supporting the traditional use of *Carica papaya* leaves in wound management. By identifying the phytochemical constituents and evaluating antimicrobial activity, the study contributes to understanding the possible mechanisms through which the plant aids wound healing.

The results also serve as baseline data for future research, including quantitative phytochemical analysis, formulation of herbal wound treatments, and further pharmacological studies. Additionally, the study promotes the use of locally available medicinal plants as complementary approaches to wound care, especially in resource limited settings.

1.5 Scope of the Study.

This study was limited to the qualitative phytochemical screening and in vitro antimicrobial assessment of *Carica papaya* leaf extract. The antimicrobial activity was evaluated against selected wound associated bacterial pathogens using standard laboratory methods. The study did not involve antioxidant assays, cell culture studies, animal models, or clinical trials.

1.6 Operational Definitions of Key Terms

- **Wound healing:** The biological process by which damaged skin and tissues are repaired and restored.
- **Phytochemicals:** Naturally occurring bioactive compounds found in plants that may have medicinal properties.
- **Antimicrobial activity:** The ability of a substance to inhibit the growth of or destroy microorganisms.
- ***Carica papaya* leaf extract:** A crude preparation obtained from papaya leaves using a suitable solvent.
- **Wound associated pathogens:** Microorganisms commonly found in infected wounds that delay healing.

CHAPTER TWO: LITERATURE REVIEW

This chapter provides a comprehensive literature review on wound healing, infections, *Carica papaya* properties, and relevant methods, integrating with the study's focus on phytochemicals and antimicrobial assays.

2.0 Taxonomy of *Carica papaya*

The papaya is classified as follows: kingdom Plantae; sub-kingdom Tracheobionta, super division Spermatophyta, division Magnoliophyta, class Magnoliopsida, subclass Dilleniidae, order Violales, family Caricaceae, genus *Carica* and species *Carica papaya*. The classification of papaya has undergone many changes over the years. According to the most recent taxonomic revision, the family Caricaceae comprises six genera: *Carica*, *Cyclicomorpha*, *Jarilla*, *Jacaratia*, *Horovitzia* and *Vasconcellea*, with *Carica papaya* the only species in the genus *Carica*. (Koul et al., 2022)

2.1 Botanical Description of *Carica papaya*

Carica papaya commonly known as papaya or pawpaw is a tropical plant belonging to the family Caricaceae. It is a fast-growing soft-wooded herbaceous plant that is widely cultivated in tropical and subtropical regions. The plant generally develops a single straight and succulent stem with prominent leaf scars. Unlike typical woody trees, the stem is relatively soft and may be hollow internally. Papaya is therefore botanically described as a large herbaceous plant rather than a true woody tree.

The plant has large long-stalked leaves that are concentrated towards the upper part of the stem. The leaves are palmately lobed with several deep divisions extending towards the centre of the leaf blade. The large leaf surface enables the plant to carry out considerable photosynthetic activity. Different botanical descriptions report that the leaves may have several prominent lobes and long petioles which are characteristic features useful in identifying *C. papaya* (Sharma et al., 2020).

Carica papaya produces flowers that may occur as male, female or bisexual flowers depending on the plant type. The flowers are generally small and pale yellow to yellowish-white. Female and bisexual flowers develop into fruits after pollination. The fruits are fleshy berries that vary in size and shape depending on the variety. Mature fruits generally contain orange, yellow or reddish flesh surrounding numerous black seeds.

The seeds are enclosed within the central cavity of the fruit and possess a characteristic pungent or pepper-like flavour. Besides the fruit and seeds, the leaves, stem, roots and latex are also important plant parts because they contain various bioactive substances. Studies have reported phytochemical groups such as alkaloids, flavonoids, phenolic compounds, terpenoids, saponins and tannins in different parts of *C. papaya* (Kaur et al., 2019; Sharma et al., 2020).

The botanical characteristics of *C. papaya* are important to this study because the leaves are the plant material being investigated. Their abundance and accessibility make them suitable for collection and preparation of extracts for laboratory investigation. Furthermore, the reported presence of diverse phytochemicals in papaya leaves provides a scientific basis for investigating their possible antimicrobial activity.

2.2 Traditional Uses of *Carica papaya*

Carica papaya has a long history of use in traditional medicine in tropical regions. Different parts of the plant including the leaves, fruits, seeds, roots, stem, bark, flowers and latex, have been used traditionally for different health conditions. A review by (Kaur et al., 2019) reports that papaya has been incorporated into traditional medicinal systems in different parts of the world and that several parts of the plant have been associated with antibacterial, anti-inflammatory, antioxidant and wound-healing applications.

The leaves are among the most commonly investigated medicinal parts of the plant. Traditional preparations of papaya leaves have been used for conditions such as fever, digestive problems and inflammatory conditions. The leaves have also been associated with traditional treatment of wounds and infections (Ugbogu et al., 2023) reports that different parts of *C. papaya* are traditionally used for conditions including bacterial infections, wounds, inflammation, diarrhea and other illnesses.

Papaya fruit is predominantly used as food but also has traditional medicinal importance. Ripe fruits are consumed because of their nutritional value while unripe fruits have traditionally been incorporated into various food and medicinal preparations. The fruit is an important source of nutrients and bioactive compounds including vitamins, carotenoids and other phytochemicals (Ugbogu et al., 2023).

The seeds also have traditional uses. They have been consumed as a spice because of their pungent flavour and have been investigated for possible medicinal properties. The seeds contain several bioactive constituents including glucosinolates, phenolic compounds and lipids. However, some traditional medicinal claims concerning papaya seeds require further toxicological and clinical investigation before they can be recommended as established treatments.

Papaya latex has also been traditionally used because of its proteolytic enzymes particularly papain. The latex obtained from immature fruits has historically been used in traditional preparations and has subsequently attracted scientific and industrial interest because of its ability to break down proteins (Choudhary et al., 2025).

The traditional uses of *C. papaya* provide an important basis for scientific investigation. However, traditional use alone does not establish clinical effectiveness. Laboratory studies are necessary to determine whether particular plant preparations demonstrate measurable biological activities. In the present research, the traditional medicinal importance of papaya provides part of the justification for investigating the phytochemical constituents and antimicrobial activity of its leaves against *S. aureus* and *E. coli*.

2.3 Industrial Uses of *Carica papaya*

Carica papaya has considerable industrial importance because different parts of the plant can be utilised in food processing, pharmaceutical production, nutraceutical development and other value-added applications. The fruit is commercially consumed fresh and is also processed into products such as juices, jams, jellies, dried products and other food preparations. This makes papaya an economically important tropical crop (Sharma et al., 2020).

One of the most important industrial products obtained from *C. papaya* is papain a proteolytic enzyme found mainly in the latex of immature fruits. Papain has the ability to hydrolyse proteins and is therefore valuable in several industries. In the food industry, it is used particularly for meat tenderisation and other protein-processing applications. Its properties have also encouraged its use in pharmaceutical, biotechnology and other industrial processes (Choudhary et al., 2025).

The pharmaceutical industry has shown interest in *C. papaya* because of the biological activities associated with its phytochemicals and enzymes. Extracts from different plant parts have been investigated for potential antimicrobial, anti-inflammatory, antioxidant and wound-healing

applications. However, much of the evidence remains laboratory-based meaning that further research is required before many of these applications can be considered established therapeutic uses (Ugbogu et al., 2023).

Papaya also has potential in the nutraceutical and functional-food industries. The fruit, seeds and leaves contain nutrients and bioactive compounds including flavonoids, alkaloids, carotenoids, phenolic compounds and phytosterols. These constituents have generated interest in developing value-added food and nutraceutical products from papaya and its different plant (Dotto & Abihudi, 2021).

Another important industrial area is the utilisation of papaya processing waste. Seeds and peels are frequently discarded during fruit processing yet they contain potentially valuable nutrients, phytochemicals, fibres, lipids and other compounds. Research has therefore explored the conversion of papaya waste into functional food ingredients, bioactive products, bioenergy and other value-added materials (“Carica Papaya Biowaste Valorization: Biorefinery Advances and Extraction Optimization.”)

The industrial importance of *C. papaya* demonstrates that the plant is not only a food crop but also a source of potentially valuable biological materials. For the present study this is relevant because establishing the phytochemical composition and antimicrobial activity of papaya leaves may provide preliminary information that could contribute to future development of plant-based antimicrobial or pharmaceutical products. Such development would however, require additional studies on compound isolation, standardization, toxicity, formulation and efficacy

2.4 Wound Healing Process.

Wound healing follows four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Gregory et al., 2005). Hemostasis halts bleeding via platelet aggregation and fibrin clot formation, providing a provisional matrix (Rodrigues, M., & Kosciuczyk, 2018). Inflammation clears debris through neutrophils and macrophages, but prolongation stalls repair (Wilkinson, H. N., & Hardman, 2020).

Haemostasis begins immediately after injury and limits blood loss through vasoconstriction, platelet activation and formation of a fibrin clot. The clot provides temporary mechanical protection and a provisional matrix through which cells involved in repair can migrate. This early

response is therefore important not only for stopping bleeding but also for initiating subsequent tissue-repair events (Rodrigues & Kosciuczyk, 2018).

The inflammatory phase follows homeostasis and involves recruitment of immune cells to the injured site. Neutrophils contribute to early microbial control and removal of damaged material, while macrophages participate in clearance and regulation of the transition from inflammation to repair. Although inflammation is necessary, excessive or prolonged inflammation can damage newly developing tissue and delay healing (Wilkinson & Hardman, 2020).

During proliferation, fibroblasts migrate into the wound and produce extracellular-matrix components, while angiogenesis supplies developing tissue with oxygen and nutrients. Granulation tissue forms within the wound bed and epithelial cells migrate over the wound surface to restore the protective barrier. These processes progressively reduce the size of the wound and establish a foundation for later tissue strengthening.

Remodelling is the later stage in which newly deposited collagen and extracellular matrix are reorganised for tensile strength over months (Gregory S. Schultz, Gloria A. Chin, Lyle Moldawer, 2005). The repaired tissue gradually gains tensile strength, although it does not normally regain all the properties of uninjured tissue. Disturbance at any stage can contribute to chronic wounds, which impose a substantial health burden and may be particularly difficult to manage in resource-limited settings affecting 12 % of Western populations and higher in LMICs due to poor care access (Martinengo et al., 2017).

2.5 Factors Delaying Wound Healing

Infections dominate delays, especially in LMICs where chronic wounds harbor polymicrobial biofilms with *S. aureus*, *P. aeruginosa*, and *E. coli* (Monk, E. J. M., Abubakar, A., Okeke, I. N., & O'Neill, 2024; Sandar, W. P., Saw, S., Kumar, A. M. V., Camara, B. S., & Sein, 2021). These pathogens evoke persistent inflammation via proteases and toxins, impeding re epithelialization (Rodrigues, M., & Kosciuczyk, 2018).

AMR exacerbates issues, with MRSA and multidrug resistant *P. aeruginosa* prevalent in 20 % - 50 % of wound isolates from Africa and Asia (Mardourian, M., Sakr, C., Harb, A., & El Hajj, 2023; Monk, E. J. M., Abubakar, A., Okeke, I. N., & O'Neill, 2024). In Uganda, limited surveillance hinders management (Tugume et al., 2024).

2.6 Role of Medicinal Plants in Wound Care

Plants offer affordable antimicrobials and healers in resource poor areas; Ugandan ethnobotany documents 35+ species for wounds, including *C. papaya* leaves (Tabuti, J. R. S., Kukunda, C. B., & Waako, 2012; Tugume, P., Kakudidi, E. K., Buyinza, M., & Kamatenesi Mugisha, 2024). Bioactives like flavonoids reduce inflammation, while tannins promote hemostasis (Yahaya, T. A., Alabi, O. A., & Bello, 2017).

2.7 Phytochemicals in *Carica papaya* Leaves

C. papaya leaves yield alkaloids, flavonoids, saponins, tannins, phenols, and terpenoids via ethanolic maceration (Ogunmola, O. A., Adeola, S. A., & Akinwale, 2022; Yahaya, T. A., Alabi, O. A., & Bello, 2017). Alkaloids disrupt bacterial membranes; flavonoids scavenge radicals for anti-inflammation (Mishra, A., Uwimana, E., Mukamana, D., & Habimana, n.d.). Qualitative tests (e.g., Mayer's for alkaloids, Shinoda for flavonoids) confirm presence (+) or absence (-) (Harborne, 1998).

2.8 Pathogenic Bacteria Associated with Wound Infections

Pathogenic bacteria are microorganisms capable of causing disease when they enter susceptible tissues or overcome the body's normal defence mechanisms. In wounds, disruption of the skin barrier provides an opportunity for bacteria to colonize damaged tissue and when conditions permit establish infection. Wound infections can interfere with normal healing by sustaining inflammation, damaging tissue and delaying progression towards tissue repair. Common bacterial pathogens associated with wound infections include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* (Monk et al., 2024).

Staphylococcus aureus is particularly important in wound infections. It is a Gram-positive bacterium capable of colonizing the skin and causing infections when the protective skin barrier is damaged. A systematic review of wound, skin, soft-tissue and surgical-site infections in Central, Eastern, Southern and Western Africa found *S. aureus* to be the most frequently isolated organism accounting for approximately 29 % of pooled samples. Among infected wounds specifically, it was isolated in approximately 32 % of samples (Monk et al., 2024)

The importance of *S. aureus* is further increased by antimicrobial resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) can resist several commonly used β -lactam antibiotics making infections more difficult to treat. (Monk et al., 2024) reported methicillin resistance in more than 40 % of *S. aureus* isolates included in their African wound-infection review. This demonstrated the importance of investigating additional antimicrobial sources including compounds obtained from medicinal plants.

Escherichia coli is a Gram-negative bacterium that normally occurs as part of the intestinal microbiota but can cause opportunistic infections when introduced into susceptible sites. In wound infections, *E. coli* may be associated with contamination of damaged tissue and can contribute to persistent infection. In the African systematic review by (Monk et al., 2024) *E. coli* accounted for approximately 14 % of pooled isolates from wound, skin, soft-tissue and surgical site infections.

Antimicrobial resistance among *E. coli* is also an important concern. (Haindongo, 2023) in a systematic review of antimicrobial resistance among *E. coli* and *S. aureus* in Africa, found substantial resistance to several commonly used antibiotics. The authors reported pooled resistance of *E. coli* to cefotaxime at 42 % while resistance among *S. aureus* to cloxacillin was approximately 34 %.

The presence of pathogenic bacteria in wounds can create a cycle in which microbial growth promotes inflammation and tissue damage, while damaged tissue provides an environment that can support further microbial growth. Some wound pathogens also form biofilms which can help microorganisms persist and reduce susceptibility to antimicrobial treatment.

The problem is particularly relevant in Africa where antimicrobial resistance among wound-associated bacteria is substantial. (Monk et al., 2024) found high resistance across several major wound pathogens and highlighted gaps in microbiological diagnostic capacity. Their findings demonstrate the importance of identifying and investigating additional antimicrobial agents while strengthening appropriate antimicrobial use and surveillance.

In relation to this study *S. aureus* and *E. coli* are appropriate test organisms because they represent Gram-positive and Gram-negative bacteria, respectively. Testing *Carica papaya* leaf extract against both organisms provides an opportunity to determine whether the extract demonstrates antibacterial activity against bacteria with different structural characteristics. This supports the study's objective of evaluating the antimicrobial activity of *C. papaya* leaf extract.

2.9 Antimicrobial Activity of *C. papaya* Extracts

Ethanollic leaf extracts inhibit *S. aureus* (15-25 mm zones), *E. coli* (12-20 mm), and *P. aeruginosa* (10-18 mm) via agar well diffusion, rivaling ciprofloxacin at 100-200 mg/mL (Ogunmola et al., 2022; Yahaya et al., 2017) Mechanisms include membrane permeabilization and enzyme inhibition (Mishra et al., n.d.). In burn models, extracts accelerate closure by 30-50 % through debridement and angiogenesis (Anibijuwon, O. I., & Udeze, 2009).

2.10 In Vitro Antimicrobial Methods

Agar well diffusion standardizes screening: 6-8 mm wells receive 50-100 µL extract on Mueller Hinton agar seeded at 0.5 McFarland; zones measured post 37°C/24 hour incubation (Balouiri, M., Sadiki, M., & Ibensouda, 2016).). Controls validate: antibiotics positive, solvents negative. This suits plant extracts, correlating with MIC for wound pathogens (Asfa, B., Ahmed, R., Hassan, M., & Ali, 2025).

2.11 Gaps and Study Relevance

Despite promise, local Ugandan data on *C. papaya* leaf phytochemistry and activity against wound bacteria are sparse, relying on anecdotal use without quantification or in vivo validation (Tugume, P., Kakudidi, E. K., Buyinza, M., & Kamatenesi Mugisha, 2024). This study addresses this via qualitative screening and diffusion assays, informing chronic wound management amid AMR.

CHAPTER THREE: METHODOLOGY

3.0 Study Design

This study employed an experimental laboratory-based design to investigate the phytochemical constituents and antimicrobial activity of *Carica papaya* leaf extract in relation to wound healing. The study focused on qualitative phytochemical screening and in vitro antimicrobial assays against selected wound associated bacterial pathogens.

3.1 Plant Material Collection and Identification

Fresh leaves of *Carica papaya* were collected from Tororo Nagongera Town Council. The plant material was authenticated by the biology lab technician Mr. Moses Olowo from Busitema University, and a voucher specimen was retained for reference.

3.2 Preparation of Plant Extract

3.2.1 Drying and Pulverization

The collected leaves were washed thoroughly with clean distilled water to remove dirt and debris, then air dried at room temperature under shade for 14 days until completely dry. The dried leaves were ground into a fine powder using a clean grinder and stored in airtight containers until extraction.

3.2.2 Extraction Procedure (Ethanolic extraction):

A measured quantity (30 g) of the powdered leaf material was soaked in 70 % ethanol in a clean container and left to macerate for 72 hours with occasional stirring. The mixture was filtered using Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator at 70°C and the crude extract as obtained.

3.3 Phytochemical Screening

Qualitative phytochemical screening was carried out using standard methods to detect the presence of major bioactive compounds.

The phytochemicals tested included:

□ Alkaloids

1 mL of Wagner's reagent (iodine in potassium iodide) was added to the 1 mL of an acidic solution of crude extract and a reddish-brown precipitate was formed indicating the presence of alkaloids.

□ Flavonoids

A few drops of neutral ferric chloride solution was added to 1 mL of an alcoholic solution of a crude extract using a test tube and blackish red colour indicated the presence of flavonoids.

□ Saponins

A little part of the crude extract was mixed with 20 mL of distilled water and then agitate in a graduated cylinder for 10 minutes and Foam formation indicated the presence of saponins.

□ **Steroids**

1 mL of concentrated sulphuric acid was added to 5 mL of a chloroform solution of crude extract using a test tube. After shaking, the test tube was allowed to stand for 5 minutes. Lower layer turned into red in colour which indicated the presence of steroids

□ **Tannins**

Few drops of ferric chloride solution was added to 1 mL of aqueous solution of crude extract using a test tube and a blackish precipitate formed indicated the presence of tannins.

The presence of phytochemicals was indicated by characteristic color changes or precipitate formation and were recorded as present (+) or absent (–).

3.4 Test Microorganisms

The bacterial strains used in this study were common wound associated pathogens:

- Staphylococcus aureus
- Escherichia coli

3.5 Antimicrobial Activity Assay

3.5.1 Preparation of Inoculum

Bacterial cultures were grown overnight and standardized to match the 0.5 McFarland turbidity standard to ensure uniform inoculum density.

3.5.2 Agar Well Diffusion Method

Sterile Mueller Hinton agar plates were prepared and inoculated evenly with the standardized bacterial suspensions using sterile swabs. Wells were bored into the agar using a sterile cork borer.

Measured volumes of *Carica papaya* leaf extract at different concentrations were introduced into the wells. A standard antibiotic (e.g., ciprofloxacin) was used as a positive control, while the extraction solvent served as a negative control.

The plates were incubated at 40°C for 72 hours.

3.6 Measurement of Antimicrobial Activity

After incubation, zones of inhibition around the wells were measured in millimeters using a ruler. The antimicrobial activity of the extract was evaluated based on the diameter of the inhibition zones.

3.7 Data Analysis

Data obtained from phytochemical screening were presented in tables as presence or absence of compounds. Antimicrobial activity results were summarized using mean zone of inhibition values and presented in tables.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.0 Introduction

This chapter presents the findings of the study on the phytochemical composition and antimicrobial activity of *Carica papaya* leaf extract. The results are organized according to the specific objectives of the study: (1) qualitative identification of major phytochemical constituents, and (2) assessment of antimicrobial activity against selected wound-associated bacterial pathogens (*Staphylococcus aureus* and *Escherichia coli*).

4.1 Phytochemical Composition of *Carica papaya* Leaf Extract

Qualitative phytochemical screening was conducted using standard test methods as described in Chapter Three. The results revealed the presence of five major bioactive compounds in the ethanolic leaf extract of *Carica papaya*. **Table 4.1** summarizes the findings.

Table 4.1: Phytochemical Constituents of *Carica papaya* Leaf Extract

Phytochemical Constituent	Test Performed	Observation	Deduction
Flavonoids	Shinoda's test	Red to pink coloration	Present (+)
Steroids	Salkowski's test	Lower layer turned red	Present (+)
Saponins	Frothing test	Persistent foam formation	Present (+)
Alkaloids	Wagner's test	Red/brown precipitate	Present (+)
Tannins	Ferric chloride test	Black precipitate	Present (+)

Interpretation: The ethanolic extract of *Carica papaya* leaves tested positive for all five phytochemical groups: flavonoids, steroids, saponins, alkaloids, and tannins.

No phytochemical tested was absent. This indicates that the leaf extract is rich in diverse bioactive compounds potentially relevant to wound healing.

4.2 Antimicrobial Activity of *Carica papaya* Leaf Extract

The antimicrobial activity of the ethanolic leaf extract was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the agar well diffusion method. Eight different concentrations of the extract (30 mg/ml to 100 mg/ml) were tested in triplicate. Ciprofloxacin (1 mg/ml) served as the positive control, while 70% ethanol served as the negative control. Zones of inhibition were measured in millimeters (mm). **Table 4.2** presents the complete results.

Table 4.2: Zones of Inhibition (mm) of *Carica papaya* Leaf Extract Against *S. aureus* (mm)

	Staphylococcus aureus (mm)			
Concentration (mg/ml)	Trial 1	Trial	Trial 3	Average (mm)
100	6.0	5.5	6.0	5.83
90	5.0	4.0	5.0	4.67
80	3.5	4.0	3.5	3.67
70	3.0	3.5	2.0	2.83
60	3.0	1.5	3.5	2.67
50	2.5	2.0	1.0	1.83
40	2.0	3.0	1.0	2.00
30	1.5	2.0	1.0	1.50
Ciprofloxacin (1 mg/ml)	10.0	12.0	9.5	10.50
Solvent (70% Ethanol)	0.0	0.0	0.0	0.00

Table4.3:Inhibition (mm) of *Carica papaya* Leaf Extract Against *E. coli* (mm)

	Escherichia coli (mm)			
Concentration (mg/ml)	Trial	Trial	Trial 3	Average (mm)
100	6.5	6.0	7.0	6.50
90	5.0	6.0	5.5	5.50

80	5.0	4.0	5.0	4.67
70	5.0	4.5	5.0	4.83
60	4.0	6.0	4.5	4.83
50	4.0	3.0	3.0	3.33
40	3.0	1.0	1.5	1.83
30	2.0	1.0	1.5	1.50
Ciprofloxacin (1 mg/ml)	12.0	13.0	9.0	11.33
Solvent (70% Ethanol)	0.0	0.0	0.0	0.00

Summary of Antimicrobial Activity

4.3.1 Activity Against *Staphylococcus aureus*

The extract exhibited concentration-dependent antibacterial activity against *S. aureus*.

The highest inhibition zone (5.83 mm) was recorded at 100 mg/ml as shown in **Table 4.2**.

Activity progressively decreased with lower concentrations, with the lowest inhibition (1.50 mm) at 30 mg/ml.

The positive control (ciprofloxacin) produced an average zone of 10.50 mm, while the negative control showed no activity (0 mm).

4.3.2 Activity Against *Escherichia coli*

The extract also demonstrated concentration-dependent activity against *E. coli*, though with slightly larger inhibition zones compared to *S. aureus* at most concentrations as observed in **Table 4.3**.

The highest inhibition (6.50 mm) was recorded at 100 mg/ml.

Activity remained moderate at concentrations between 60-80 mg/ml (4.67-4.83 mm) before declining at lower concentrations.

Ciprofloxacin produced an average zone of 11.33 mm, while the solvent control showed no activity.

4.3.3 Comparative Observations

E. coli appeared slightly more susceptible to the extract at concentrations of 70-100 mg/ml compared to *S. aureus*.

Both pathogens showed negligible inhibition (≤ 2 mm) at concentrations below 40 mg/ml as it can be seen in both tables.

The extract was less potent than ciprofloxacin at all tested concentrations, which is expected for a crude plant extract compared to a purified antibiotic.

4.4 Discussion of Phytochemical Findings

The qualitative phytochemical screening revealed that *Carica papaya* leaf extract contains flavonoids, steroids, saponins, alkaloids, and tannins. These findings are consistent with previous studies conducted in other geographical contexts i.e. (Ogunmola et al. ,2022) and (Yahaya et al. , 2017) similarly reported the presence of these phytochemicals in ethanolic extracts of *C. papaya* leaves.

The presence of these bioactive compounds is significant for wound healing for several reasons:

Flavonoids are well documented for their antioxidant and anti-inflammatory properties. In the context of wound healing, flavonoids can reduce oxidative stress, modulate inflammatory responses, and promote tissue repair (Yusuf, Lawal, & Omonije, n.d.). By reducing prolonged inflammation, flavonoids may help prevent the transition from acute to chronic wounds.

Tannins possess caustic properties that can promote hemostasis by precipitating proteins and contracting tissue. This action may help control bleeding in fresh wounds and create a protective barrier against microbial colonization (Yahaya et al., 2017).

Alkaloids have demonstrated antimicrobial activity through mechanisms such as bacterial membrane disruption and interference with microbial DNA replication (Mishra, Uwimana, Mukamana, & Habimana, n.d.). This supports their potential role in controlling wound infections.

Saponins exhibit surface-active properties that may enhance membrane permeability, potentially contributing to antimicrobial effects. Additionally, some saponins have been associated with immune-modulatory activities that could support wound healing (Tabuti et al. , 2012).

Steroids possess anti-inflammatory properties that may help modulate the inflammatory phase of wound healing, potentially reducing excessive inflammation that can delay tissue repair.

The presence of all five phytochemical groups in the extract suggests that *Carica papaya* leaves may exert multi-faceted mechanisms on wound healing, including antimicrobial, antiinflammatory, antioxidant, and hemostatic actions.

This polypharmacological profile is typical of many medicinal plants used in traditional wound care (Tugume et al., 2024).

4.5 Discussion of Antimicrobial Activity

4.5.1 Evidence of Antibacterial Effect

The results demonstrate that *Carica papaya* leaf ethanolic extract possesses measurable antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) wound-associated pathogens. The concentration-dependent nature of this activity (higher inhibition zones at 100 mg/ml compared to lower concentrations) indicates a genuine dose-response relationship, strengthening the validity of the findings.

The absence of activity in the solvent control (70% ethanol) confirms that the observed inhibition was due to the phytochemical constituents of the extract rather than the solvent itself. The positive control (ciprofloxacin) produced substantially larger zones (10.5-11.33 mm), which is expected given that it is a purified pharmaceutical antibiotic.

4.5.2 Comparison with Previous Studies

The inhibition zones observed in this study (5.83 mm for *S. aureus* and 6.50 mm for *E. coli* at 100 mg/ml) are somewhat lower than those reported by some previous researchers. It can be noted that (Ogunmola et al., 2022) reported zones of 15-25 mm for *S. aureus* and 12-20 mm for *E. coli* using similar extract concentrations. Also (Yahaya et al., 2017) reported zones of 10-18 mm for *P. aeruginosa*.

Despite these quantitative differences, the qualitative finding of antibacterial activity is consistent with the broader literature. The presence of active compounds such as alkaloids and tannins, both known for antimicrobial properties, supports the biological plausibility of the observed effects.

4.5.3 Differential Susceptibility: *S. aureus* against *E. coli*

At concentrations of 70-100 mg/ml, *E. coli* appeared slightly more susceptible than *S. aureus* (average zones: 4.83-6.50 mm for *E. coli* vs. 2.83-5.83 mm for *S. aureus*).

This finding is notable because Gram-negative bacteria like *E. coli* are generally considered more resistant to antimicrobial agents due to their outer membrane, which acts as an additional permeability barrier.

Several explanations are possible:

The specific phytochemical profile of this extract may contain compounds (such as certain alkaloids or saponins) that are particularly effective at disrupting the Gram-negative outer membrane.

The moderate activity against *S. aureus* suggests that while the extract has some effect, it may not be the optimal plant source for targeting this specific pathogen.

Alternatively, the *S. aureus* isolate used in this study may have been relatively less susceptible due to local resistance patterns.

From a wound healing perspective, activity against both Gram-positive and Gram-negative pathogens is advantageous, as chronic wounds often harbor polymicrobial biofilms containing both types of organisms (Sandar et al., 2021).

4.5.4 Clinical Relevance of Inhibition Zone Sizes

Interpretation of inhibition zones from plant extracts requires caution. Unlike standardized antibiotic disc diffusion tests (e.g., CLSI guidelines), there are no established breakpoints for crude plant extracts. Zones of 6-7 mm, while modest compared to ciprofloxacin, may still be biologically meaningful. In the wound environment, even partial inhibition of bacterial growth can reduce biofilm formation, lower bacterial load, and create conditions favorable for the host immune system and tissue repair processes (Asfa et al., 2025).

Furthermore, plant extracts may exert effects beyond direct bactericidal activity, including antiinflammatory, antioxidant, and vascular- growth promoting effects that are not captured by antimicrobial assays alone. The presence of flavonoids and steroids (both with anti-inflammatory properties) suggests that *C. papaya* leaf extract may support wound healing through multiple concurrent mechanisms.

4.6 Relationship to Wound Healing

The findings of this study support the traditional use of *Carica papaya* leaves in wound management through at least two plausible mechanisms:

Mechanism 1: Infection Control. The demonstrated antibacterial activity against *S. aureus* and *E. coli* suggests that the extract may help reduce microbial load in colonized or infected wounds.

Since bacterial infection is a major cause of delayed wound healing (Monk et al., 2024) any antibacterial effect could be clinically beneficial.

Mechanism 2: Anti-inflammatory and Tissue Protective Effects. The presence of flavonoids, steroids, and tannins all of which possess anti-inflammatory, antioxidant, or hemostatic properties suggests that the extract may also modulate the wound environment in ways that support healing beyond simple infection control.

These findings align with the ethnobotanical documentation by as observed by (Tugume et al. ,2024 and Tabuti et al. ,2012), who reported widespread use of *C. papaya* leaves for wound healing in Ugandan traditional medicine.

4.7 Limitations of the Study

The following limitations should be considered when interpreting the results:

- 1. Qualitative phytochemical analysis only:** The study did not quantify the concentrations of individual phytochemicals. Quantitative analysis (e.g., using HPLC or spectrophotometry) would provide more precise information.
- 2. Limited bacterial species tested:** Only two bacterial species (*S. aureus* and *E. coli*) were tested. Other wound-associated pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus mirabilis* were not included. *P. aeruginosa* is particularly important in chronic wounds and burn injuries.
- 3. In vitro design only:** The study did not include in vivo (animal) models or clinical trials. In vitro antimicrobial activity does not always translate to clinical effectiveness due to factors such as protein binding, tissue penetration, and host immune interactions.

4. **Crude extract used:** The study used a crude extract rather than isolated bioactive compounds. While this reflects traditional use, it does not identify which specific compound(s) are responsible for the observed activity.
5. **No toxicity assessment:** The study did not evaluate the safety or cytotoxicity of the extract. Medicinal plants, while traditionally considered safe, can have adverse effects such as contact dermatitis or systemic toxicity if absorbed through wounds.
6. **Incubation conditions:** The protocol specified incubation at 40°C for 72 hours, which differs from standard bacteriological practice (37°C for 24 hours). This may have affected bacterial growth and inhibition zone sizes, potentially limiting comparability with other studies.
7. **Modest inhibition zones:** The observed zones of inhibition were relatively small (≤ 6.5 mm at the highest concentration). While biologically meaningful, clinical relevance requires further investigation.

4.8 Conclusion of Results

The ethanolic leaf extract of *Carica papaya* contains at least five major phytochemical groups (flavonoids, steroids, saponins, alkaloids, and tannins) and demonstrates measurable antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* in a concentration-dependent manner. These findings provide preliminary scientific evidence supporting the traditional use of *Carica papaya* leaves in wound management.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.0 Introduction:

This chapter interprets the findings presented in Chapter Four in relation to the study objectives, existing literature, and the broader context of wound healing. The chapter also presents the conclusions drawn from the study, outlines limitations, and offers recommendations for practice and future research.

5.1 Conclusions:

Based on the findings of this study, the following conclusions are drawn:

- 1. Phytochemical composition:** *Carica papaya* leaf ethanolic extract contains at least five major phytochemical groups: flavonoids, steroids, saponins, alkaloids, and tannins. This rich phytochemical profile provides a plausible biochemical basis for the traditional use of the plant in wound healing.
- 2. Antimicrobial activity:** The extract exhibits measurable, concentration-dependent antibacterial activity against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), two common wound-associated pathogens.
- 3. Mechanistic relevance to wound healing:** The combination of antimicrobial, antiinflammatory (flavonoids, steroids), and hemostatic (tannins) properties suggests that *Carica papaya* leaf extract may support wound healing through multiple mechanisms, not solely through infection control.
- 4. Support for traditional use:** The findings provide preliminary scientific evidence consistent with the ethnobotanical documentation of *Carica papaya* leaf use for wound management in Uganda and other tropical regions.
- 5. Need for further research:** While promising, these findings are preliminary. Translation to clinical practice requires additional studies, including quantitative phytochemical analysis, in vivo efficacy testing, safety assessments, and eventually, controlled clinical trials.

5.2 Recommendations

Based on the findings and limitations of this study, the following recommendations are offered:

5.2.1 Recommendations for Practice

1. **Continued traditional use with caution:** The findings support, but do not definitively prove, the effectiveness of *Carica papaya* leaf preparations for wound care. Traditional users should continue to apply these remedies with awareness that they are complementary rather than replacements for standard wound care (cleaning, debridement, and appropriate medical evaluation).
2. **Hygienic preparation:** If used, leaf preparations should be made under clean conditions to avoid introducing additional pathogens into wounds.
3. **Not a substitute for antibiotics:** For clinically infected wounds (signs of purulence, cellulitis, systemic symptoms), formal medical evaluation and antibiotic therapy should not be delayed.

5.2.2 Recommendations for Further Research

Immediate follow-up studies:

1. **Quantitative phytochemical analysis:** Conduct HPLC or spectrophotometric quantification of key compounds (e.g., total phenolic, flavonoid, and tannin content) to enable standardization.
2. **Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination:** Broth microdilution methods should be used to determine MIC and MBC values for comparison with other studies.
3. **Test additional pathogens:** Include *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus mirabilis* in future antimicrobial assays.
4. **Cytotoxicity and safety testing:** Evaluate the extract's safety using in vitro cytotoxicity assays (e.g., on fibroblast or keratinocyte cell lines) to assess potential harm to healing tissues.
5. **Antioxidant assays:** Conduct DPPH or FRAP assays to quantify free radical scavenging activity, which is relevant to wound healing.

Intermediate studies:

6. **In vivo wound healing model:** Evaluate the extract in an animal model (e.g., excision or burn wound model in rats or mice) to assess wound closure rate, histology, and microbial load.
7. **Formulation development:** Prepare and test simple topical formulations (e.g., ointments, gels, or creams) containing standardized *C. papaya* leaf extract.
8. **Comparative study:** Compare the antimicrobial activity of *C. papaya* leaf extract with other medicinal plants used for wound healing in Uganda (e.g., *Aloe vera*, *Vernonia amygdalina*).

Long-term studies:

9. **Clinical trial:** If preclinical studies demonstrate safety and efficacy, conduct a randomized controlled trial comparing *C. papaya* leaf formulation to standard care (e.g., silver sulfadiazine or iodine) for superficial wounds.
10. **Bioactivity-guided fractionation:** Isolate and characterize the specific compounds responsible for antimicrobial and anti-inflammatory activities to identify potential lead compounds for drug development.

5.3 Contribution to Knowledge

This study makes the following contributions to the existing body of knowledge:

1. Provides locally generated scientific data on the phytochemical composition of *Carica papaya* leaves from Eastern Uganda, addressing the gap noted by (Ntale, 2024) and (Tugume et al., 2024).
2. Confirms that *C. papaya* leaf extract from this region demonstrates antibacterial activity against wound-associated pathogens, consistent with studies from other geographical contexts.
3. Contributes baseline data that can inform future research on medicinal plants for wound care in resource-limited settings, where antimicrobial resistance is increasingly problematic (Monk et al., 2024).
4. Supports the growing body of evidence that traditional medicinal plants warrant scientific investigation as potential complementary approaches to wound management.

5.4 Final Remarks

The search for effective, affordable, and accessible wound care interventions remains urgent, particularly in low- and middle-income countries where the burden of chronic wounds is high and antimicrobial resistance is rising. *Carica papaya* leaf extract, as investigated in this study, demonstrates phytochemical richness and antibacterial activity that aligns with its traditional use in wound healing.

However, scientific validation is an iterative process. The findings presented here represent an early but important step moving from unreliable use to preliminary laboratory evidence. The modest inhibition zones, the absence of safety data, and the in vitro-only design mean that these results should be interpreted as promising rather than conclusive.

Future research, as outlined above, will determine whether *Carica papaya* leaf extract can be developed into a standardized, safe, and effective wound care product suitable for integration into evidence-based practice. For now, this study adds to the justification for continued research into locally available medicinal plants as part of a comprehensive approach to wound management in Uganda and beyond.

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6.0 APPENDICES



Figure 1; Washing and preparation of Carica papaya Leaves before drying.



Figure 2; Air-dried Carica papaya leaves prepared for pulverization.



Figure 3; Rotary evaporator concentrating the plant extract by removing the solvent under reduced pressure.



Figure 4; Qualitative Phytochemical screening of Carica papaya leaf extract.



Figure 5; Preparation of the different concentrations used in the antibacterial activity.



Figure 6; Antibacterial activity