ANTIMICROBIAL ACTIVITY OF THE METHANOLIC EXTRACT OF *CASSIA ALATA* LINN (FABACEAE) FORMULATED AS SHAMPOO

BY

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OCTOBER, 2025

CERTIFICATION

This is to certify that this work was carried out by Orizu Sandra CHUKWUPUTAOBIE in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, in partial fulfillment of the requirements for the award of the Pharm. D degree from the university.

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DEDICATION

This project is dedicated to the Almighty God who has kept me through pharmacy school and provided the resources to make this project work a success**.**

ACKNOWLEDGEMENTS

My sincere gratitude to God Almighty, who has been a source of strength, provision, and inspiration to complete this project.

My deep appreciation goes to my amiable Dr Abere Tavs for his support and guidance during the course of this project. Thank you for being a phenomenal supervisor.

My utmost appreciation goes to my mum, Mrs Orizu Chinenye, for her constant support, for always inspiring me to give my best at all times, and for her encouragement throughout pharmacy school and even in the course of this project. To my siblings, Obianuju, Jennifer, Juliet, Chinenye, and Chukwuemeka, I love you all. Thank you for standing by me.

I also appreciate my friends Olulade Victoria, Pharm Ali Uche Ohanado Ikechukwu, Oronsaye Esosa, Osakue Eloghosa, Ejimofor Chiamaka, and Igwebuike Oluchi for their contributions to the success of this project and all my colleagues who have made pharmacy school a worthwhile journey.

My project colleagues are not left out, Omotshola Jackson. It was really nice working with you.

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ABSTRACT

Background: The rise in resistance to synthetic antimicrobial agents has encouraged interest in herbal formulations. *Cassia alata* Linn. (Caesalpiniaceae), commonly known as the candle bush, is traditionally used for treating skin infections due to its rich phytochemical constituents. This study focuses on formulating an herbal shampoo using Cassia alata leaf extract and evaluating its antimicrobial activities against selected bacterial and fungal pathogens.

Objectives: To formulate the leaf extract of *Cassia alata* into a shampoo and investigate its antimicrobial profiles.

Methods: Standard procedures were followed to evaluate the presence of phytochemicals. The methanol extract was incorporated into a shampoo base prepared using stearic acid, sodium hydroxide, lanolin, sodium lauryl sulfate and additives. Antimicrobial activity was evaluated by the agar well diffusion method against the following test organisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella aerogenes, Aspergillus niger,* and *Candida albicans*) at extract concentrations of 100-500 mg/ml. Zones of inhibition were compared with ciprofloxacin (bacterial control) and Ketoconazole (fungal control).

Results: The phytochemical screening revealed the presence of Anthraquinones, Saponins, Tannins and Alkaloids. The shampoo formulation was clear, exhibiting good foam formation, with no signs of phase separation, indicative of its stability. There was no skin irritation. The formulated herbal shampoo, containing the leaf extract of *C. alata* showed marked reduced antimicrobial activity at 500 mg/ml when compared to the standard antimicrobial agents.

Conclusion: *Cassia alata* herbal shampoo formulation has shown promising prospect as a potent source of phytonutrients and can be used in managing microbial activities of the skin.

Keywords: *Cassia alata,* Caesalpiniaceae, Herbal, Shampoo, Antimicrobial

CHAPTER ONE

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Background of the Study

Over 13 million deaths worldwide are attributed to the appearance of new infectious diseases or the resurgence of once-controlled pathogens annually (Abreu *et al*., 2017). Bacterial infections remain a major public health concern, as many harmful bacteria rapidly acquire resistance to multiple antibiotics (Xu *et al*., 2018; Pacheco *et al*., 2022; Tiseo *et al*., 2022). Several factors contribute to this increasing antibiotic resistance, including excessive prescribing by healthcare professionals and the practice of self-medication. The widespread use of antibiotics fosters both acquired and natural resistance, leading to numerous treatment failures across the globe (Adodo *et al*., 2024). In response, researchers are prioritizing the development of safe and effective natural antimicrobial agents (Nourbakhsh *et al*., 2022), particularly as the ecological and health risks linked to synthetic antimicrobials become more concerning (Liang *et al*., 2020). To address this growing issue, significant efforts are being directed toward the large-scale screening of medicinal plants traditionally used in healing practices (Pathak and Gadela, 2024; Ibrahim and Kebede, 2020).

Medicinal plants have been used for thousands of years across cultures as one of the earliest forms of treatment. Knowledge of their healing properties has been preserved and passed down through generations. Natural compounds play a crucial role in pharmaceutical development, with many modern medications derived from traditional herbal remedies. Extensive research has explored the biological properties of various plant species and their active components in medicine (Marrelli, 2021). For centuries, medicinal plants have also been widely used to treat microbial infections (Safarzadeh *et al*., 2022; Alfuraydi *et al*., 2024). In 2022, the global herbal medicine market was valued at $170 billion, with projections suggesting it could reach $600 billion by 2033, reflecting a compound annual growth rate of 15% from 2023 to 2033 (Sarkar *et al*., 2024). Recent research has increasingly focused on plant extracts' ability to inhibit pathogenic bacterial growth, particularly for their potential in combating bacterial drug resistance (Zouine *et al*., 2024).

Cassia alata, a plant native to Argentina, is commonly known by various names, including Candle Brush, Candlestick, and *Senna alata* (Lim, 2012). In Indonesia, it is referred to as “ketepeng china.” Across South Asia, *C. alata* is widely used as a herbal remedy for various ailments, such as rheumatism, as well as a laxative. Its seeds and leaves possess strong

antifungal properties and are used in India to manage eczema. Additionally, *C. alata* is known for alleviating stomach pain during pregnancy, headaches, and paralysis. Extracts from the plant are commonly utilized in traditional medicine to treat skin diseases in several countries (Fatmawati *et al*., 2020).

Various types of formulations exist for managing skin diseases, including shampoos, soaps, ointments, and creams, which can also be formulated from herbal medicinal plants. A shampoo refers to any formulation containing a surfactant, or surface-active agent, available in various forms such as liquid, solid, or powder. However, the prolonged use of these surface-active agents poses potential risks to both human health and the environment. Many synthetic compounds, chemicals, dyes, and their derivatives have been linked to skin diseases and various adverse effects. In contrast, herbal products are widely regarded as a safer alternative, making herbal cosmetics increasingly popular. As a result, there is growing consumer interest in herbal-based personal care products, with a wide range now readily available (Namita, 2012).

Therefore, this study explores the formulation and evaluation of *C. alata*-based shampoo, focusing on its antimicrobial efficacy against common pathogens. By investigating its effectiveness in inhibiting bacterial and fungal growth, this study aims to validate the use of *C. alata* as a key ingredient in herbal shampoos.

1.2. *Cassia alata* Linn

C. alata Linn., commonly known as the ringworm bush, belongs to the family Fabaceae and the subfamily Caesalpinioideae. It is a tropical, upright shrub that typically grows between 2 and 3 meters but can reach up to 6 and 8 feet. Characterized by its compound leathery leaves and soft wooden structure, it thrives in open wastelands near water sources. This plant is widely distributed across tropical regions, including America, India, Fiji, Indonesia, Malaysia, Brazil, and Africa (Meenupriya *et al*., 2014). The plant is recognized by various names worldwide, including King of the Forest, Emperor's Candlesticks, Candle Bush, Candelabra Bush, Christmas Candle, Ringworm Tree, and Impetigo Bush (Oladeji *et al*., 2020). It holds historical significance as one of the oldest medicinal plants in Central America and is well-known in the Asia-Pacific region for its therapeutic properties. Traditionally, it has been used to treat a range of ailments, particularly skin-related conditions such as ringworm, scabies, blemishes, and fungal infections (Turista *et al*., 2019). Furthermore, it has been utilized for

managing fevers, insect bites, hookworm infestations, sexually transmitted diseases, and constipation (Kavipriya *et al*., 2018; Dewia *et al.*, 2019).

1.2.1. Scientific Classification

- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Fabales
- **Family:** Fabaceae
- **Subfamily:** Caesalpinioideae
- **Tribe:** Cassieae
- **Subtribe:** Cassiinae
- **Genus:** *Cassia*
- **Species:** *C. alata* (Keng *et al*., 2024)

The Fabaceae, or legume family, is one of the world's largest and most diverse families of flowering plants, comprising nearly 20,000 species across ~800 genera, and is of major ecological and economic importance owing to its roles in food production, nitrogen-fixation, and habitat formation (Fang, 2024). The genus *Cassia* falls under the tribe Cassieae within the subfamily Caesalpinioideae; recent molecular and seed-morphological studies (Cai *et al*., 2025) have helped clarify relationships within Cassieae, distinguishing *Cassia* and its close relatives and aiding taxonomic revisions. Species of *Cassia* are well-known in traditional medicine and have been shown in recent analyses to contain multiple bioactive compounds, including anthraquinones, flavonoids, terpenes, and essential oils, which contribute to antimicrobial, antioxidant, and wound-healing activities (El-Hawary *et al*., 2022).

1.2.2. Botanical Description of *Cassia alata*

C. alata, commonly found in tropical and subtropical regions, thrives in diverse environments such as roadsides, riverbanks, rainforest edges, lakeshores, and open forests. It is widespread

in countries including India, Pakistan, Burma, Sri Lanka, the Philippines, and various African nations (Oluwole *et al*., 2020). The plant grows at elevations up to 1,400 meters and is known for its rapid growth, although it has a relatively short lifespan. It flourishes in moist tropical climates with annual rainfall ranging from 600 to 4,300 mm and temperatures between 15°C and 30°C. Adaptable to various soil types, *C. alata* can grow in soils with acidity levels ranging from acidic to slightly alkaline, including heavy and sandy soils. It is also tolerant of both drought and waterlogged conditions, making it resilient in wastelands and floodplains (Kavipriya *et al*., 2018).

This upright shrub typically grows to a height of 3 to 4 meters. It has pinnately compound leaves, each measuring 30 to 60 cm in length, with 6 to 12 pairs of oblong, thinly leathery leaflets ranging from 6 to 15 cm in length and 3.5 to 7.5 cm in width. The flowers are arranged in vertical clusters resembling candles, with bright yellow blossoms emerging from the base while unopened buds at the top remain covered by orange bracts. The plant produces smooth, four-sided, dark purple to black winged pods, each containing 50 to 60 flattened, triangular to squarish seeds (Kavipriya *et al*., 2018).

Figure 1a. *C. alata* plant (Yon *et al*., 2022).

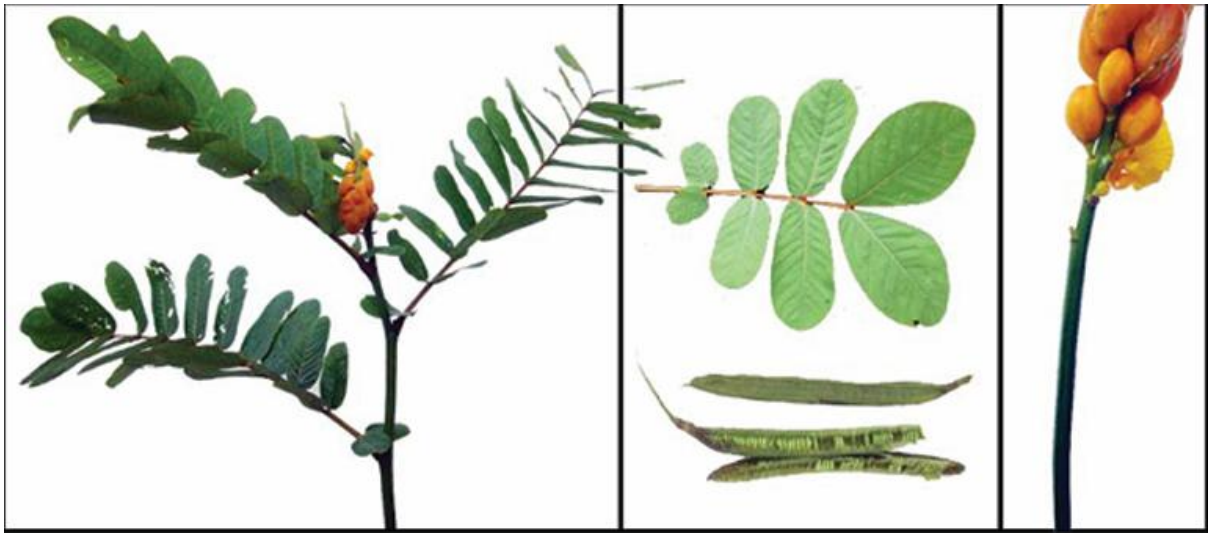

Figure 1b. *C. alata* whole plant, flowers, and seeds (Ali and Shah, 2019).

1.2.3. Ethnomedicinal Uses

C. alata has been widely utilized in traditional medicine across various cultures for treating numerous ailments. Different parts of the plant, including the leaves, flowers, seeds, and wood, possess therapeutic properties that have been applied in tropical and subtropical regions (Chatterjee *et al*., 2012; Oladeji *et al*., 2020). In Cuba, the whole plant is commonly used for its diuretic properties and as a natural laxative for constipation. It is also applied in the treatment of skin infections, particularly herpes ulcers. Similarly, in Guatemala, Brazil, and Guinea, *C. alata* is used to manage flu symptoms and malaria (Schmelzer and Gurib-Fakim, 2008).

The leaves of *C. alata* are widely used in traditional medicine due to their antifungal, antiviral, anti-inflammatory, and immunomodulatory effects (Abile, 2022). In Tanzania, Ghana, India, Indonesia, and several African countries, leaf infusions and decoctions are prepared to relieve constipation (Fadholly *et al*., 2025). The topical application of the leaves is a common remedy for skin conditions such as ringworm and white-spot fungal infections (Yon *et al*., 2022). *C. alata* leaves also exhibit antiviral properties, making them effective against the herpes simplex virus. Their immunomodulatory function helps regulate white blood cell production, which may be beneficial in managing conditions like psoriasis (Gritsanapan and Mangmeesri, 2009). In Nigeria, fresh leaf sap is directly applied to the skin to treat fungal infections such as ringworm, while a decoction of the leaves is used to manage chronic lichen dermatosis (Oladeji *et al*., 2020).

In the Philippines, *C. alata* leaves are utilized for their antibacterial, anti-inflammatory, analgesic, and antifungal properties, as well as for their potential to lower blood glucose levels (Eusebio-Alpapara *et al*., 2022). In Togo and Gabon, crushed leaves mixed with palm oil are applied to the skin to treat general skin disorders (Chew *et al*., 2023). Indian traditional medicine utilizes leaf decoctions as expectorants for respiratory conditions such as bronchitis and shortness of breath. They also serve as astringents, skin washes for eczema, and remedies for haemorrhoids. *C. alata* leaves are also employed in India for gastrointestinal parasite control and blood glucose regulation (Chatterjee *et al*., 2012). In Brazil, the plant is believed to improve blood circulation in female reproductive organs and stimulate menstruation, while in Egypt, leaf decoctions are used as a laxative to relieve constipation (Elshershaby *et al*., 2025; Gritsanapan and Mangmeesri, 2009). Similarly, in Sierra Leone, the leaves are traditionally prepared to alleviate pain associated with childbirth and abortion (Oladeji *et al*., 2016). In Thailand, a leaf decoction containing at least 0.5%

hydroxyanthracene derivatives is used to relieve constipation (Gritsanapan and Mangmeesri, 2009).

The flowers of *C. alata* also hold medicinal significance. In Peru, a flower infusion is traditionally prepared as a diuretic and for treating urinary tract infections. Among the Tikuna Indians of the Amazon, a decoction of the flowers is consumed daily to relieve constipation (Abile, 2012). The seeds of *C. alata* are utilized in Chinese medicine, where they are brewed into tea to enhance eyesight and provide relief for asthma (Oladeji *et al*., 2020). Additionally, the wood of the plant has been used in herbal medicine to counteract liver damage caused by hepatotoxins and to treat gastrointestinal conditions such as loss of appetite (Adedoyin *et al*., 2015; Al-Malki *et al*., 2025). In Africa, powdered leaves are applied to the skin to combat fungal infections (Yon *et al*., 2022).

C. alata plays a significant role in African traditional medicine, where various plant parts are used to relieve stomach aches during pregnancy, treat dysentery, and manage haemorrhoids (Chatterjee *et al*., 2012). Its widespread use in traditional healing practices highlights its importance as a medicinal plant with diverse therapeutic applications.

1.2.4. Phytochemical Constituents

The phytochemical composition of *C. alata* includes a diverse range of bioactive compounds that contribute to its medicinal properties. Various parts of the plant, including the leaves, seeds, stems, twigs, and flowers, contain essential phytochemicals such as carotenoids, polyphenols, flavonoids, alkaloids, terpenoids, anthraquinones, fatty acids, and phytosterols (Abile, 2012; Chatterjee *et al*., 2012; Oladeji *et al*., 2020; Yon *et al*., 2022).

Carotenoids, particularly beta-carotene, are present in *C. alata* leaves and contribute to its antioxidant properties. These compounds play a significant role in neutralizing free radicals and preventing oxidative stress (Oladeji *et al*., 2020). Polyphenols such as phenolic acids, including gallic acid, caffeic acid, and chlorogenic acid, are abundant in the leaves. These compounds exhibit strong antioxidant, anti-inflammatory, and antimicrobial properties, making them beneficial for treating various infections and inflammatory disorders (Chatterjee *et al*., 2012).

Flavonoids are among the most significant bioactive constituents found in multiple parts of *C. alata*, including the leaves, seeds, twigs, and roots (Fatmawati *et al*., 2020). Key flavonoids

identified include kaempferol, quercetin, luteolin, apigenin, and naringenin. Kaempferol and its derivatives, such as kaempferol-3-O-glucoside and kaempferol-3-O-gentiobioside, exhibit potent antioxidant and anti-inflammatory properties. Similarly, quercetin and luteolin contribute to the plant's antiviral and antifungal effects. Other flavonoid derivatives, including chrysoeriol-7-O-(2”-O-β-D-mannopyranosyl)-β-D-allopyranoside and rhamnetin-3-O-(2”-O-β-D-mannopyranosyl)-β-D-allopyranoside, further enhance the medicinal value of *C. alata* (Abile, 2012; Oladeji *et al*., 2020).

The alkaloid content of *C. alata* includes adenine and cannabinoid alkaloids, such as 4-butylamine-10-methyl-6-hydroxy-cannabinoid dronabinol, primarily found in the leaves. These alkaloids are known for their analgesic, anti-inflammatory, and neuroprotective effects (Yon *et al*., 2022). The plant contains various terpenoids, including caryophyllene, germacrene, selinene, bicyclogermacrene, limonene, phellandrene, and bulnesene, which contribute to its antifungal and antibacterial activities (Chatterjee *et al*., 2012). Anthraquinone derivatives, including aloe-emodin, rhein, emodin, chrysophanol, and physcion, are highly concentrated in the leaves and stems of *C. alata*. These compounds possess laxative, antimicrobial, and anticancer properties. Sennosides A, B, C, and D, which are responsible for the plant’s purgative effects, are also present in the leaves and stem (Oladeji *et al*., 2020). Additionally, glycosides such as chrysoeriol-7-O-(2”-O-β-D-mannopyranosyl)-β-D-allopyranoside and rhamnetin-3-O-(2”-O-β-D-mannopyranosyl)-β-D-allopyranoside have been isolated from the seeds, stem, and leaves, demonstrating hepatoprotective and cardiovascular benefits (Abile, 2012; Yon *et al*., 2022).

The plant is also a rich source of essential fatty acids, which are predominantly found in its seeds, leaves, and flowers. Key fatty acids include palmitic acid, linoleic acid, oleic acid, stearic acid, and arachidic acid. These compounds contribute to the plant’s anti-inflammatory and skin-protective properties. Other fatty acids such as heptadecanoic acid, tricosanoic acid, tetracosanoic acid, and behenic acid have been reported to support metabolic health and lipid regulation (Chatterjee *et al*., 2012).

Phytosterols, including β-sitosterol and stigmasterol, are primarily found in the leaves and contribute to cholesterol-lowering and anti-inflammatory effects. These sterols are known to aid in cardiovascular health and immune modulation (Oladeji *et al*., 2020). Gas Chromatography-Mass Spectrometry (GC-MS) analysis study of *C. alata* by Kavipriya *et al*. (2018) identified thirteen bioactive compounds, each with unique pharmacological properties.

- **Oleic Acid**: Known for its anti-cancer, anti-inflammatory, and immune-boosting properties, it also aids in wound healing and helps combat bacterial and fungal infections.
- **Vitamin E Acetate** (Retention Time: 14.709 RT): A potent antioxidant that helps neutralize free radicals, attributed to its phenolic hydrogen structure.
- **10-Methyl-E-11-tridecen-1-ol propionate** (Retention Time: 10.607 RT): Lacks significant bioactivity.
- **l-(+)-Ascorbic Acid 2,6-Dihexadecanoate** (Retention Time: 10.176 RT): Exhibits antioxidant, anti-inflammatory, antiscorbutic, anti-mutagenic, antinociceptive, and wound-healing properties (Kavipriya *et al*., 2018).

Other notable compounds include:

- **Oxirane** (Retention Times: 9.669, 9.783, 9.841 RT): Demonstrates bactericidal, fungicidal, and sporicidal activities. It is widely used as a sterilizing and antimicrobial agent effective against bacteria, fungi, and viruses.
- **6-Anhydrohexopyranoses** (Retention Time: 7.672 RT): Used in the synthesis of biologically significant compounds such as rifamycin S, indanomycin, thromboxane B2, (+)-biotin, tetrodotoxin, quinones, macrolide antibiotics, and modified sugars.
- **1,2-Bis(trimethylsilyl)benzene** (Retention Times: 15.651, 16.055 RT): Possesses antioxidant, antimicrobial, anticancer, and antitumor properties (Kavipriya *et al*., 2018).

Overall, the phytochemical diversity of *C. alata* highlights its significant pharmacological potential. The presence of flavonoids, alkaloids, polyphenols, and anthraquinones supports its use in traditional medicine for treating infections, inflammation, and gastrointestinal disorders. The bioactive compounds found in different plant parts contribute to their wide range of therapeutic applications (Abile, 2012; Chatterjee *et al*., 2012; Oladeji *et al*., 2020; Yon *et al*., 2022).

1.2.5. Pharmacological Properties

C. alata exhibits a range of pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and wound-healing effects, which are beneficial in managing atopic

dermatitis (AD). Its antimicrobial properties help prevent secondary infections, particularly those caused by *Staphylococcus aureus*, aiding in wound healing and recovery of AD-related skin lesions. Additionally, the plant has anti-inflammatory effects by suppressing pro-inflammatory cytokines and enhancing anti-inflammatory responses. Antioxidants such as phenolics, flavonoids, and anthraquinones protect the skin, neutralize free radicals, mitigate oxidative stress, and reduce inflammation (Yon *et al*., 2022).

1.2.5.1. Antimicrobial Properties

Individuals with AD often have compromised skin barriers, allowing microorganisms and allergens to penetrate and worsen the condition through secondary infections. *S. aureus* is the most prevalent microorganism associated with AD, exacerbating inflammation by releasing allergens (Ogonowska *et al*., 2020). While antibiotic therapy is essential for managing AD, excessive antibiotic use can lead to resistance. As a result, interest in plant-based antimicrobial agents has increased due to their rich composition of bioactive compounds such as tannins, terpenoids, alkaloids, and flavonoids.

C. alata has been used to treat microbial infections, with research confirming its antimicrobial efficacy against *S. aureus*. Extracts from various parts of the plant, including roots, bark, stems, and leaves, have demonstrated moderate antimicrobial activity (Alam *et al*., 2009; Somchit *et al*., 2003; El-Mahmood and Doughari, 2008). A study by Iraqui *et al*. formulated a hydrogel containing *C. alata* leaf extracts, which showed superior antimicrobial activity compared to commercial formulations such as Renicol and Daktarin. The hydrogel significantly reduced microbial populations in treated wounds, accelerating healing compared to standard treatments (Iraqui *et al*., 2019).

Several phytochemicals in *C. alata*, including kaempferol and aloe emodin, exhibit strong antimicrobial effects against multidrug-resistant *S. aureus* (Hazni *et al*., 2008). Other compounds such as gallic acid, caffeic acid, and rhein also contribute to its antibacterial activity (Yon *et al*., 2022). Studies indicate that phenolic acids in *C. alata* enhance bacterial susceptibility by altering membrane integrity, leading to cell death (Andrade *et al*., 2020; Chew *et al*., 2020). The antimicrobial potency of these compounds is influenced by structural factors, such as alkyl chain length and hydroxyl group positioning (Kępa *et al*., 2018; Chew *et al*., 2018; Shi *et al*., 2021). Quercetin, another compound in *C. alata*, disrupts bacterial cell walls and membranes, further contributing to its antimicrobial effects (Yang *et al*., 2020).

Rhein, found in *C. alata*, interferes with bacterial metabolic pathways, inhibits respiration, and regulates gene expression involved in bacterial survival, ultimately suppressing *S. aureus* growth (Yu *et al*., 2008).

1.2.5.2. Wound Healing

Wound healing is a natural process that involves the regeneration of dermal and epidermal tissues. It occurs in three phases: inflammatory, proliferative, and remodeling (Mathew-Steiner *et al*., 2021). The process begins when platelets come into contact with exposed collagen at the injury site, initiating the body's repair mechanisms. However, individuals with atopic dermatitis (AD) often experience an itch-scratch cycle that leads to skin damage and delayed wound healing (Tokura, 2010). Open wounds increase susceptibility to bacterial, viral, and fungal infections, making recovery more challenging for AD patients.

Wound healing is closely linked to antimicrobial activity. Caffeic acid, present in *C. alata*, exhibits antibacterial properties effective against both Gram-positive and Gram-negative bacteria, which supports the healing process (Kanedi *et al*., 2016). A study by Palanichamy *et al*. (1991) evaluated the antimicrobial effects of *C. alata* ethanolic leaf extracts using a rabbit wound model. The wounds were inoculated with *Staphylococcus aureus*, and an ointment containing the extract was applied for 21 days. The results showed a significant reduction in wound surface area, with healing rates of 58.8%, 79.0%, and 88.9% after 7, 14, and 21 days, respectively. In contrast, the negative control group, which received an ointment without antimicrobial agents, showed only a 57.1% reduction in wound size over the same period. Meanwhile, the positive control group treated with 0.2% nitrofurazone ointment achieved a 98.8% wound reduction within 14 days. Though *C. alata* was not as effective as nitrofurazone, it demonstrated promising natural antimicrobial activity for AD wound management.

Another study by Midawa *et al*. (2010) further supported the wound-healing potential of *C. alata* in animal models. Rats with excision wounds were treated with varying doses (125, 250, and 500 mg) of the leaf extract twice daily until complete wound closure. The healing process was significantly improved, with a shorter epithelialization period (16 days) compared to the negative control group (23 days). This study, along with findings by Iraqui *et al*. (2019), suggested that *C. alata* promotes wound healing through its antimicrobial effects.

The wound-healing properties of *C. alata* are attributed to bioactive compounds such as alkaloids, terpenoids, flavonoids, anthraquinones, and tannins. These compounds enhance the activity of fibroblasts and keratinocytes, stimulate collagen synthesis, and regulate the expression of cytokines and growth factors (Chew *et al*., 2022). Oleic and linoleic acids in *C. alata* leaves modulate inflammatory responses, inhibit leukocyte accumulation, regulate metalloproteinase (MMP) and tissue inhibitor of MMP (TIMP) expression, and promote wound closure (Cardoso *et al*., 2011; Mena *et al*., 2016). Oleic acid also supports angiogenesis, increases collagen III production during the final inflammatory phase, and accelerates wound healing (Cardoso *et al*., 2011). These properties make wound healing a crucial aspect of AD management.

1.2.5.3. Anti-Inflammatory Properties

C. alata exhibits significant anti-inflammatory activity in both *in vitro* and *in vivo* models. In a carrageenan-induced mouse paw edema study, oral administration of *C. alata* extract (5 mg/20g body weight) led to a notable reduction in inflammation (Villaseñor *et al*., 2002). Similarly, Lewis *et al*. (2011) investigated the anti-inflammatory effects of *C. alata* in a rheumatoid arthritis model and found that the leaves inhibited tumor necrosis factor-alpha (TNF-α) production by immature dendritic cells in a dose-dependent manner. TNF-α is a key regulator of pro-inflammatory cytokines and lipid mediators, playing a significant role in chronic inflammatory diseases.

Astragalin, a flavonoid found in *C. alata* leaves, regulates anti-inflammatory pathways by modulating transcription factors, enzymes, and cytokines such as TNF-α, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), and matrix metalloproteinases (MMP-1, MMP-3) (Riaz *et al*., 2018). Additionally, it influences interleukin levels (IL-1β, IL-4, IL-6, IL-8, IL-13) and interferon-gamma (IFN-γ), reducing inflammatory responses. Studies using *in vitro* models such as RAW 264.7 macrophages, human gingival epithelial cells, and mouse uterine endometrial epithelial cells further support its anti-inflammatory activity (Yon *et al*., 2022).

1.2.5.4. Antioxidant Activities

Oxidative stress and reduced antioxidant levels contribute significantly to AD pathogenesis (Sivaranjani *et al*., 2018). Free radicals disrupt the skin's defense mechanisms, increasing

lipid peroxidation and depleting antioxidant reserves, which in turn exacerbate skin damage and inflammation. The inclusion of natural antioxidants, such as polyphenols, in AD treatment may help protect the skin, mitigate oxidative stress, and suppress inflammatory responses. *C. alata* is rich in polyphenols, including phenolic acids, flavonoids, anthocyanins, and anthraquinones, which exhibit potent free radical scavenging activity. These compounds effectively neutralize reactive oxygen species (ROS) such as nitric oxide, hydrogen peroxide, superoxide anions, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Fatmawati *et al*., 2020; Chatterjee *et al*., 2013; Sagnia *et al*., 2014; Casetti *et al*., 2012; Chua *et al*., 2019).

An aqueous extract of *C. alata* demonstrated remarkable antioxidant activity, inhibiting 50% of DPPH radicals at an IC50 value of 2.25 ± 0.28 µg/mL. This activity surpassed that of antioxidant standards such as ascorbic acid (IC50 = 3.99 ± 0.09 µg/mL) and Trolox (IC50 = 4.50 ± 0.08 µg/mL) (Yon *et al*., 2022). In addition to free radical scavenging, studies indicate that *C. alata* possesses strong reducing power and lipid peroxidation inhibition capabilities (Chua *et al*., 2022; Fatmawati *et al*., 2020). Since oxidative stress can overwhelm the skin's defenses and contribute to dermatological conditions, the antioxidant properties of *C. alata* may provide therapeutic benefits for skin disorders (Yon *et al*., 2022).

1.2.5.5. Anti-Obesity Effects

C. alata has been shown to possess anti-obesity properties. In a study by Chichioco-Hern and Leonido (2011), extracts from *C. alata* significantly reduced the body weight of mice. The selection of these plants was based on their phytochemical composition. Specifically, the methanol extract of *Senna alata* was assessed for its lipid-lowering (hypolipidemic) effects in mice with diet-induced lipedema. The study further reported a dose-dependent reduction in the parametrial fat weight of the mice. In another study, Naowaboot and Piyabhan (2017) demonstrated that *C. alata* leaf extract improved insulin sensitivity in obese mice fed a high-fat diet, and also reduced both epididymal fat weight and adipocyte (fat cell) size.

1.2.5.6. Hepatoprotective Effects

The hepatoprotective potential of an alcoholic extract (95%) derived from dried leaves of *C. alata* (ECA) was evaluated against paracetamol-induced liver damage in albino rats. Pre-treatment with ECA significantly lowered biochemical markers of liver injury, such as serum

glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, and gamma glutamate transpeptidase (GGTP). Histological analysis also showed that ECA provided notable protection against liver damage (Ali and Shah, 2019). The hepatoprotective effect is believed to be due to the presence of flavonoids in the leaves. Ramasamy *et al*. (2009) confirmed that the hepatoprotective activity of ECA is linked to bioactive compounds, especially flavonoids, tannins, and other polyphenolic constituents.

1.2.5.7. Anticancer and Cytotoxic Effects

The cytotoxic effects of the chloroform fraction of *C. alata* leaves were tested using the MTT assay on three human cancer cell lines: MDA-MB-231 (breast cancer), HepG2 (liver cancer), and Caco-2 (colon cancer) (Mohammed *et al*., 2017). The extract exhibited significant cytotoxicity against HepG2 cells with an IC50 of 37.4 µg/ml after 48 hours, but showed weaker activity against MDA-MB-231 and Caco-2 cells (IC50 >100 µg/ml). The anticancer effect against HepG2 cells was likely due to the presence of anthraquinones (Ali and Shah, 2019).

Levy and Lewis (2011) also studied the cytotoxic potential of the hexane extract from *C. alata* leaves against A549 lung cancer cells using the MTT assay, reporting an IC50 of 143 µg/ml. The cytotoxicity was mediated by the activation of caspase-8, potentially due to the presence of kaempferol. Similarly, Adebesin *et al*. (2013) evaluated the cytotoxicity of hydromethanolic leaf extract (HMLE) of *C. alata* using the WST-1 assay on K562 leukemia cell lines, confirming its anticancer potential.

1.3. Shampoos and Herbal Shampoos

Hair often serves as an external indicator of the body's internal health. Among the various hair care practices, shampooing is the most widely used method. Its primary purpose is to cleanse the hair by removing accumulated sebum, dust, and debris from the scalp. Shampoo formulations vary depending on hair type, hair care routines, and specific hair concerns such as oily scalp, dandruff, or androgenic alopecia. Typically, shampoos are prepared in liquid, creamy, or gel forms. Their consistency is influenced by the presence of traditional soaps enriched with glycerides, natural or synthetic fatty alcohols, or thickening agents like gums, resins, and polyethylene glycol (PEG). In India, herbal cleansers such as shikkakai and reetha

are commonly used by women due to their natural cleansing properties and minimal side effects (Preethi *et al*., 2013).

Primarily regarded as cosmetic products, shampoos play an essential role in daily hair care, functioning as cleaning agents for the scalp and hair. They are generally viscous solutions composed of detergents along with additives, preservatives, and active ingredients. The application involves massaging the shampoo into wet hair and then rinsing it off with water. The main goal is to eliminate dirt and impurities without excessively removing the natural oils (sebum) from the hair. Presently, both medicated and non-medicated synthetic shampoos are available in the market. However, herbal shampoos have gained significant popularity due to their natural composition, safety profile, absence of adverse effects, and growing consumer preference (Vijayalakshmi *et al*., 2018; Potluri *et al*., 2013).

A shampoo is a formulation containing surfactants in various forms, such as liquid, solid, or powder. When applied under recommended conditions, it effectively removes surface grease, dirt, and dead skin cells from the hair shaft without causing harm or discomfort to the user (Preethi *et al*., 2013).

1.3.1. Ideal Characteristics of a Shampoo

An ideal shampoo should meet the following requirements (Preethi *et al*., 2013; Barve *et al*., 2016):

- Efficiently and completely eliminate dust, excess sebum, and other impurities.
- Thoroughly cleanse the hair.
- Produce sufficient and stable foam.
- Be easily rinsed off with water without leaving residues.
- Leave the hair soft, non-dry, shiny, and manageable.
- Impart a pleasant and lasting fragrance to the hair.
- Be gentle on the skin and hands, avoiding roughness or chapping.
- Be non-irritating to the skin and eyes and free from adverse effects.

1.3.2. Basic Composition of Shampoo

A typical shampoo formulation comprises the following components (Preethi *et al*., 2013; Barve *et al*., 2016):

- **Principal Surfactants**: Serve as the main cleansing agents responsible for detergency and foam production (mainly anionic surfactants).
- **Secondary Surfactants**: Enhance foam quality, detergency, and contribute to mild conditioning.
- **Conditioning Agents**: Include lanolin, mineral oils, fenugreek, herbal extracts, henna, and egg derivatives to improve hair texture.
- **Foam Builders**: Example - Shikakai, to enhance lathering.
- **Viscosity Modifiers**: Electrolytes such as ammonium chloride (NH_4Cl) and sodium chloride (NaCl).
- **Natural Gums**: Gum karaya, tragacanth, and alginates to stabilize viscosity.
- **Cellulose Derivatives**: Such as hydroxyethyl cellulose and methylcellulose to modify texture.
- **Carboxy Vinyl Polymers**: Example - Carbopol 934 for thickening.
- **Other Additives**: Polyvinylpyrrolidone (PVP), phosphate esters.
- **Sequestering Agents**: Ethylenediaminetetraacetic acid (EDTA) to prevent mineral deposits.
- **Opacifying Agents**: Alkanolamides of higher fatty acids, propylene glycol, and metal salts like magnesium, calcium, and zinc stearates.
- **Clarifying Agents**: Include solubilizing alcohols (ethanol, isopropanol), phosphates, and non-ionic solubilizers like polyethoxylated alcohols and esters.
- **Fragrances**: Herbal, fruity, or floral scents.
- **Preservatives**: Such as methyl paraben, propyl paraben, and formaldehyde.
- **Antidandruff Agents**: Natural products like shikakai, neem, and thulasi (Preethi *et al*., 2013; Barve *et al*., 2016).

1.3.3. Types of Shampoo

Shampoos are available in different forms depending on the formulation and purpose (Preethi *et al*., 2013; Barve *et al*., 2016):

1. Liquid Shampoo
2. Solid Cream Shampoo
3. Jelly Shampoo
4. Powder Shampoo
5. Lotion Shampoo
6. Aerosol Foam Shampoo
7. Specialized Shampoos: Including conditioning, anti-dandruff, baby, and two-layer shampoos.

1.3.4. Herbal-Based Shampoos

Many plant-derived active compounds, such as phytohormones, flavonoids, hydroxyl acids (commonly known as fruit acids), glycosides like saponins, enzymes, and essential oils, are valuable ingredients in cosmetic formulations. Among these, saponins play a key role due to their ability to produce foam and cleanse the scalp effectively (Barve *et al*., 2016).

1.3.4.1. Saponins

The word "saponin" originates from the Latin word *sapo*, meaning soap. Saponins are known for their frothing ability when mixed with water, earning them the nickname "natural detergents." Chemically, saponins are a diverse group of molecules, primarily consisting of glycosylated steroids, steroidal alkaloids, and triterpenoids. These compounds share several common properties (Barve *et al*., 2016):

1. **Surfactant Property**: The combination of a hydrophobic aglycone and hydrophilic sugar components makes saponins amphiphilic, enabling them to lower surface tension in water and create stable foams.

2. **Hemolytic Activity**: The extent of red blood cell lysis caused by saponins varies based on their structure. Bisdesmosides generally exhibit lower hemolytic activity compared to monodesmosides.
3. **Steroid-Binding Ability**: This property is more pronounced in steroidal saponins and glycoalkaloids than in triterpenoid saponins.
4. **Biocidal Action**: Their ability to disrupt cell membranes contributes to their toxic effect on certain cells, giving them antimicrobial and pesticidal properties.

Saponin-rich plants have been traditionally used in household cleaning products (Sparg *et al*., 2004). A notable example is *Saponaria officinalis* (soapwort), which has been used for centuries as a natural detergent. Triterpenoid saponins also find applications in the production of foam-based fire extinguishers, toothpaste, foamed beverages (such as beer and soft drinks), shampoos, liquid soaps, and other cosmetic products. Several plants known for their cleansing properties can be incorporated into shampoo formulations due to their natural detergent action (Barve *et al*., 2016).

1.4. Statement of the Problem

Conventional shampoos often contain synthetic chemicals, surfactants, and preservatives that may cause adverse effects on human health, including scalp irritation, allergic reactions, and long-term dermatological issues. Furthermore, their frequent usage contributes to environmental pollution due to non-biodegradable compounds. The growing prevalence of microbial infections affecting the scalp, such as dandruff, seborrheic dermatitis, and folliculitis, further highlights the need for safe and effective alternatives. Despite the well-documented ethnomedicinal uses of *C. alata* for treating skin infections, its potential as an active antimicrobial agent in cosmetic formulations like shampoos remains underexplored. There is a gap in research concerning the development and evaluation of *C. alata*-based shampoos for antimicrobial efficacy, safety, and consumer suitability.

1.5. Justification of the Study

Given the increasing interest in natural and herbal products for personal care, this study is timely and relevant. *C. alata*, a tropical plant widely known for its antimicrobial, antifungal, and anti-inflammatory properties, presents a promising alternative to synthetic ingredients in

shampoo formulations. Utilizing its bioactive constituents in a shampoo could offer a dual benefit of cleansing and therapeutic action, especially against microbial scalp infections. Moreover, the development of a plant-based, eco-friendly shampoo aligns with global efforts toward sustainable and safe cosmetic practices. This study not only seeks to validate the traditional use of *C. alata* but also contributes to the advancement of herbal cosmetic science by formulating and evaluating a functional product with antimicrobial properties.

1.6. Objectives of the Study

The primary aim of this study is to formulate and evaluate an herbal shampoo containing *C. alata* extract and assess its antimicrobial activity.

Specific Objectives:

1. To extract and characterize the phytochemical constituents of *C. alata* leaves.
2. To develop a shampoo formulation incorporating *C. alata* extract.
3. To evaluate the physicochemical properties of the formulated shampoo (e.g., pH, foam stability, viscosity).
4. To assess the antimicrobial activity of the *C. alata*-based shampoo against selected microbial strains.
5. To compare the antimicrobial efficacy of the herbal shampoo with commercial shampoo products.

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Consumables and Reagents

Nutrient agar, nutrient broth, Sabouraud's agar, dimethyl sulphoxide (DMSO), absolute ethanol, methanol, chloroform, calcium carbonate (SRL Pharma GmbH, India), glycerin (Anhui Elite Industrial Co. Ltd., China), distilled water, saccharin, sodium lauryl sulphate (SLS), peppermint, FDC green dye, ciprofloxacin, ketoconazole, bacterial incubator, Pasteur pipettes, Fehling's solution, Dragendorff's reagent, Meyer's reagent, Hager's reagent, Wagner's reagent, swab sticks, paper masking tapes, syringes, cotton wool, nose masks, latex gloves, and aluminium foil were used for the study.

The microbial strains employed included *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Candida albicans*, and *Aspergillus niger*.

2.1.2. Glassware and Equipment

Mortar and pestle, conical flasks, measuring cylinders, micropipettes, mechanical and electric blenders, viscometer (NCJ-55 Digital Viscometer, Shang), autoclave (Health Team Instruments, England), pH meter (Oaklon pH Meter Model 1100), water bath (Electronic Thermostat Water Tank, Model No. HH-W21-CR4211), analytical weighing balance (Ohaus Corp., Pine Brook, NJ, USA), Petri dishes, cotton wool, masking tape, aluminium foil, sterile wire loop, refrigerator, incubator (ESCO-Isotherm, Singapore), hot air oven (Spring Efeilf Instrument, England), stirring rod, and dispensing bottles were utilized.

2.2. Methods

2.2.1. Plant Collection and Identification

Fresh leaves of *C. alata* were collected from the environs of the Faculty of Pharmacy, University of Benin, Edo State, Nigeria. The plant was authenticated by the Curator of the Herbarium, Department of Pharmacognosy, University of Benin, and a voucher specimen was deposited for reference.

A 500g quantity of the powdered plant material was macerated with agitation for 72 h. The mouth of the conical flask was then covered with aluminum foil to prevent solvent evaporation. After 6 days, the mixture was filtered properly to separate the marc from the extract. Subsequently, the filtrate was further filtered using a filter paper. Another 1500ml volume of ethanol was measured and then added to the plant marc and swirled several times. The final filtrate was transferred to a beaker and concentrated in a water bath at 450°C until all the solvents had evaporated, leaving the extract. The percentage yield was calculated using the following equation:

Percentage yield = (Weight of extract in grams/initial weight of plant material) × 100

2.2.2. Phytochemical Tests

The following phytochemical tests were carried out based on procedures outlined by Harbourne (1973) and Evans (2009). This was carried out to identify the presence of active phytochemical constituents.

2.2.2.1. General Test for Glycosides

Molisch Test

The methanol extract of *C. alata* (2ml) was mixed with 2ml of a 10% alcoholic solution of α-naphthol in a test tube. The test tube was inclined at 45 degrees, and 2 mL of concentrated sulphuric acid was cautiously poured down the sides of the tube to form a layer below the interface of the two liquids, indicating the presence of sugars.

Fehling's Test

Reducing sugars such as glucose, fructose, and lactose will reduce Fehling's solution from deep blue to green, yellow, or red coloration owing to the formation of red precipitate of cuprous oxide. Non-reducing sugars such as sucrose and polysaccharides such as starch will reduce Fehling's solution only when they are hydrolyzed with acids on boiling. But the acid must be neutralized before testing with Fehling's solution.

The methanol extract of *C. alata* (4 mL) and a mixture of Fehling's A and B were boiled in separate test tubes. 5 mL of the extract was added to Fehling's solution (A and B), and the result was noted.

To the remaining extract, 5 mL of dilute H_2SO_4 was added, the mixture was gently boiled for 5 min, and then filtered. The filtrate was made slightly alkaline with $NaHCO_3$, and Fehling's solution, that has already been boiled, was added. Any change in color from blue to green, yellow, or red indicated a positive reduction test.

2.2.2.2. Test for Saponins

Frothing test

The methanol extract of *C. alata* (0.5g) was placed in a test tube, and 4 mL of distilled water was added. The extract was diluted with water and shaken vigorously. Thick, persistent frothing indicated the presence of saponins.

2.2.2.3. Test for Anthraquinone Derivatives

The Methanol extract *of C. alata (0.5g) was shaken with 15 mL* of chloroform to extract. It was filtered, and 5 mL of extract was shaken with 5 mL of dilute ammonia. A pink color indicated the presence of anthraquinone glycosides in the extract.

2.2.2.4. Test for Cyanogenetic Glycosides

The methanol extract of *C. alata* (0.1g) was placed in three test tubes labeled A, B, and C. The Extract in test tubes A and B was mixed with a little water, and sodium picrate test paper was placed in each test tube. The test tubes were stoppered immediately, and tube B was placed in boiling water for 5 min while tubes A and C were kept at room temperature. After 30 min, changes in colour of the sodium picrate test papers were noted.

2.2.2.5. Test for Cardiac Glycosides

Salkowski's Test

The methanol extract of *C. alata* (0.5g) is dissolved in 2 mL of chloroform, and concentrated H2SO4 is added carefully to form a layer. A reddish brown colouration at the interface indicates the presence of a steroidal nucleus (aglycone of steroidal glycoside)

2.2.2.6. Test for Tannins

General Test

The methanol extract of *C. alata* was put in a test tube, and 5 mL of distilled water was added to it. A few drops of aqueous ferric chloride were added. A blue-black precipitate indicated the presence of gallitannins and ellagitannins, while a yellow precipitate indicated the presence of condensed tannins.

2.2.2.7. Test for Alkaloids

Alkaloids are naturally occurring organic compounds that have a cyclic nitrogenous nucleus exhibiting basic properties. They react with a number of substances to give characteristic precipitates. It is important to note that only aqueous solutions or extracts can be tested directly with alkaloidal reagents, which include Mayer's, Wagner's, Hager's, and Dragendorff's reagents. When non-polar or semi-polar solvents are used for extraction, it is removed by evaporation, and the dried extract is re-dissolved in 1% H_2SO_4 to extract the alkaloidal salts present in the sample.

Extraction and Detection of Alkaloids

For alkaloid detection, 5 mL of the extract was mixed separately with 5 mL of each of the following reagents: Dragendorff's, Wagner's, Hager's, and Mayer's reagents. The formation of characteristic precipitates indicated the presence of alkaloids. A reference test using quinine salt in place of the extract was performed for each reagent as a control. A reference test using Quinine salt in place of the extract for each of the four alkaloidal reagents was carried out to serve as a control.

Extraction Using Polar Solvent (Methanol)

A powdered sample of leaves of *C. alata* (5 g) was boiled with 50ml of methanol in a beaker over a water bath for 15 min. It was filtered, and the filtrate evaporated to dryness in an evaporating dish over a water bath. The residue was dissolved in 5 mL of 1% H_2SO_4 and filtered. The filtrate was then used to test for the presence of alkaloid using alkaloid reagents, and reference was made to the blank test (control).

2.2.3. Formulation of Herbal Shampoo

The British Pharmacopeia method (BP,1993) was adopted. A 2.0 g quantity of stearic acid was melted on a water bath, and 0.01 g of sodium hydroxide, dissolved in a small volume of water, was heated and added to the molten stearic acid. On complete saponification, 0.25 g of Lanolin was added. A paste of sodium lauryl sulfate and 5 g of the aqueous methanol extract was made in water and mixed with the soap formed.

Table 1. Materials and the quantities used for shampoo formulation.

Materials used	**Quantity (g)**
Stearic acid	2.0
Sodium hydroxide	0.01
Lanolin	0.25
Sodium lauryl sulfate	1
Aqueous methanol extract	5

2.2.4. Antimicrobial Assay

2.2.4.1. Microbial Isolation

Clinical isolates of five bacteria comprising three Gram-negative bacteria (E. coli, P. aeruginosa, and K. aerogenes) and two Gram-positive bacteria (*B. subtillis* and *S. aureus)* were used for the antibacterial assay. Two fungi (*C. albicans and A. niger)* were used for the antifungal assay. The organisms were obtained from the Department of Pharmaceutical

Microbiology and Biotechnology Laboratory at the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The purity of the culture was confirmed by the conventional cultural, morphological, and biochemical methods prior to use. The microbial culture was maintained in nutrient agar and Sabouraud dextrose agar for bacteria and fungi, respectively, at 4°C.

2.2.4.2. Preparation of Inoculum

An overnight culture was used for the preparation of microbial suspension with a turbidity equivalent to that of 0.5 Meforlard's standard.

2.2.4.3. Agar Well Diffusion Method

The media was prepared and sterilized at 121°C for 15 min. A total of 30 mL of nutrient agar was seeded with the bacterial culture and allowed to solidify, and on each plate, wells of 10 mm in diameter were made. The open wells were filled with different concentrations of the extract and herbal formulation, ranging from 100mg/mL to 500mg/mL, and incubated at 37°C for 24 h. For the antifungal assay, Sabouraud dextrose agar was used in place of Nutrient agar, and the medium was incubated at 280°C for 2 days. All tests were carried out in triplicate. The inhibition zone diameter was measured and compared with those obtained using Ciprofloxacin and Ketoconazole for antibacterial and antifungal assays, respectively (Collins *et al*., 1995).

2.2.4.4. Statistical Analysis

All data are expressed as mean ± standard error of mean (SEM), and statistical significance was evaluated using the Student's t-test. P- value of <0.05 was considered statistically significant.

CHAPTER THREE

3. RESULTS

3.1. Extraction Yield

The extraction of *C. alata* leaves with ethanol yielded a concentrated, greenish-brown residue after solvent evaporation. The weight of the extract obtained was recorded, and the percentage yield was calculated using:

Percentage yield = (Weight of extract (g) / Initial weight of plant material (g)) × 100

Example:

- Weight of powdered leaves = 500 g
- Weight of extract = 77.3 g
- Percentage yield = 15.46 %

3.2. Phytochemical Screening

The qualitative phytochemical analysis of the methanolic extract of *C. alata* revealed the presence of several bioactive compounds, as summarized below.

Test for Carbohydrates

Table 2. Test for glycosides.

Test	Observation	Inferences
Molisch's Test Methanol extract + 2ml 10% of alpha naphthol + 3ml of concentrated H_2SO_4	Violet ring formed at interface	Glycoside present
Fehling's Test Methanol extract + 1ml of Fehling's solution A +1ml of Fehling's solution B + heat	Reddish brown colouration formed	Reducing sugar present

Table 3. Test for saponins.

Test	Observation	Inference
Methanol extract + 5ml of water and shaken vigorously	Persistent frothing	Saponin present

Table 4. Test for cardiac glycosides.

Test	Observation	Inference
Methanol extract + 2ml of glacial acetic acid + Few drops of ferric chloride solution	A reddish- brown ring forms at the junction of the two liquids A bluish green colour appears in acetic acid layer	Deoxy-sugar present Cardiac glycosides confirmed

Table 5. Test for anthraquinones.

Test	Observation	Inference
Borntrager's test for free anthraquinone glycosides	No pink colouration	Free anthraquinone glycosides absent
Borntrager's test for combined anthraquinone glycosides. (i) Hydrolysis with water (ii) Hydrolysis with dilute acid (iii) Hydrolysis with dilute acid and oxidation with H_2SO_4	Pink colouration observed Pink colouration observed Pink colouration observed	Anthraquinone glycosides present Anthraquinone glycosides present Anthraquinone glycosides present Anthraquinone glycosides

(iv) Oxidative hydrolysis with Ferric chloride as catalysts	Pink colouration observed	present

Table 6. Test for alkaloids.

Test	Observation	Inference
<u>Polar solvent (using water to extract)</u> *Cassia alata* + H_2O + Dragendoff's solution	Brown precipitate	Alkaloids salt present
Cassia alata + H_2O + Wagner's reagent	Reddish precipitate	Alkaloids salt present
Cassia alata + H_2O + Hager's solution	Yellow precipitate	Alkaloids salt present
Cassia alata + H_2O + Mayer's solution	Brick - red precipitate	Alkaloids salt present
<u>Polar solvent (using H_2SO_4)</u> *Cassia alata* + dilute H_2SO_4 + Dragendoff's solution	Brown precipitate	Alkaloids salt present
Cassia alata + dilute H_2SO_4 + Wagner's solution	Reddish-brown precipitate	Alkaloids salt present
Cassia alata + dilute H_2SO_4 + Hager's	Yellow precipitate	Alkaloids salt present
Cassia alata + dilute H_2SO_4 + Mayer's solution	Brick - red precipitate	Alkaloids salt present

Semi- polar solvent (using Methanol to extract) *Cassia alata* + Methanol +Dragendoff's solution	Brown precipitate	Alkaloids present
Cassia alata + Methanol + Wagner's solution	Reddish brown precipitate	Alkaloids present
Cassia alata + Methanol + Hager's solution	Yellow precipitate	Alkaloids present
Cassia alata + Methanol +Mayer's solution	Brick - red precipitate	Alkaloids present
Non- Polar solvent (using chloroform to extract) *Cassia alata* + Chloroform +Dragendoff's solution	Light brown precipitate	Alkaloidal base present
Cassia alata + Chloroform +Wagner's solution	Brown precipitate	Alkaloidal base present
Cassia alata + Chloroform +Hagar's solution	Yellow precipitate	Alkaloidal base present
Cassia alata + Chloroform +Mayer's solution	Brick- red precipitate	Alkaloidal base present

Table 7. Test for tannins.

Test	Observation	Inference
General test for Phenolic compounds with aqueous ferric chloride	Blue- black precipitate	Phenolic compound (s) present

Phenazone test	A reddish-brown precipitate	Tannins present
Iron complex test	A blackish bulky precipitate which is insoluble in hot water	Pseudo tannins present
Formaldehyde test	A bulky precipitate present	Phlobatannins present
Modified iron complex test	A blackish precipitate which is insoluble in hot water, alcohol and dilute ammonia.	Hydrolysable Tannins present

Table 8: Test for Cyanogenetic glycosides

Test	Observation	Inference
Test for cyanogenetic glycosides (for test tube A, B and C)	Yellow colour of test paper retained	Cyanogenetic glycosides absent

3.3. Formulation of Herbal Shampoo

A herbal shampoo was successfully formulated using the ethanol extract of *C. alata*, sodium lauryl sulfate, lanolin and stearic acid. The product obtained was a green, homogenous, foaming shampoo with a characteristic herbal scent.

Table 9: Physical evaluation of the herbal shampoo.

Parameter	Observation
Appearance	Smooth, greenish, viscous liquid
Odor	Pleasant herbal smell
pH	6.8 ± 0.2
Foam stability	Stable for 5 min

Dirt dispersion	Uniform

3.4. Antimicrobial Activity

The antimicrobial efficacy of the Cassia alata extract and the formulated herbal shampoo was evaluated against selected bacterial and fungal isolates.

3.4.1. Zone of Inhibition (mm) (Methanol Extract of *Cassia alata*)

Table 10. Antimicrobial activity of the extract.

Microorganism	Extract (100 mg/ml)	Extract (200 mg/ml)	Extract (300 mg/ml)	Extract (400 mg/ml)	Extract (500 mg/ml)	Standard (Ciprofloxacin)	Standard (Ketoconazole)
Staphylococcus aureus	G	G	G	15 ± 0.38	17 ± 0.27	31 ± 0.33	ND
Bacillus subtilis	G	G	G	16 ± 0.62	18 ± 0.17	35 ± 0.26	ND
Escherichia coli	G	G	G	15± 0.38	17± 0.27	31 ± 0.33	ND
Pseudomonas aeruginosa	G	G	G	13. ± 0.45	15. ± 0.51	30 ± 0.38	ND
Klebsiella aerogenes	G	G	G	12 ± 0.21	15.0 ± 0.6	32 ± 0.38	ND
Candida albicans	G	13 ± 0.12	14 ± 0.35	16 ± 0.46	18 ± 0.44	26.0 ± 0.3	25 ± 0.22
Aspergillus niger	G	12 ± 0.3	13 ± 0.31	15 ± 0.22	19 ± 0.32	ND	24 ± 0.41

3.4.2. Zone of Inhibition (mm) (Herbal shampoo formulation of *Cassia Alata*)

Table 11. Antimicrobial activity of the formulation.

Microorganism	formulation (100 mg/ml)	formulation (200 mg/ml)	formulation (300 mg/ml)	formulation (400 mg/ml)	formulation (500 mg/ml)	Standard (Ciprofloxacin)	Standard (Ketoconazole)
Staphylococcus aureus	G	G	G	G	12 ± 0.23	32 ± 0.17	ND
Bacillus subtilis	G	G	G	G	15 ± 0.32	35 ± 0.22	ND
Escherichia coli	G	G	G	G	15± 0.28	30 ± 0.30	ND
Pseudomonas aeruginosa	G	G	G	G	14 ± 0.15	31 ± 14	ND
Klebsiella aerogenes	G	G	G	G	12 ± 0.13	33 ± 0.18	ND
Candida albicans	G	G	12 ± 0.22	14 ± 0.14	16 ± 0.26	ND	26 ± 0.22
Aspergillus	G	G	11 ±	13 ± 0.4	15 ±	ND	24 ±

niger			0.43		0.32		0.41

All data expressed as mean ± SEM (n = 3). Statistical significance was determined using Student's t-test, and $p < 0.05$ was considered significant. ND indicates not determined, G indicates no inhibition zone.

CHAPTER FOUR

4. DISCUSSION AND LIMITATIONS

C. alata, also known as Candle Brush or *Senna alata*, is a medicinal plant traditionally used across tropical regions for treating various ailments, particularly skin diseases, due to its potent antifungal and healing properties (Lim, 2012; Fatmawati *et al*., 2020). With growing concerns about the adverse effects of synthetic surfactants and chemicals in cosmetic formulations, there is increasing interest in developing safer, plant-based alternatives such as herbal shampoos and other personal care products (Namita, 2012). This study investigated the antimicrobial activity of *C. alata* leaf extract and its incorporation into a herbal shampoo formulation, with the aim of evaluating the extract's phytochemical constituents, physicochemical properties, and efficacy against selected bacterial and fungal pathogens to explore its potential as a natural antimicrobial ingredient. The study was driven by the growing demand for plant-based alternatives to synthetic agents in personal care products, particularly for managing scalp infections and maintaining hygiene.

In the present study, a 15.46% yield of *C. alata* extract was obtained from methanol, indicating a high recovery of soluble constituents. This is substantially greater than yields reported in the literature; for example, Edegb*o et al*. (2023) reported only approximately 4.8% yield with ethanol (compared with the approximately 15% obtained in the present study). Differences in extraction yield can arise from solvent strength, plant preparation (e.g., particle size and drying), extraction time, and plant chemotype (Fatmawati *et al*., 2020). A high yield suggests that the *C. alata* leaves contained abundant extractable compounds under our conditions, which is promising for obtaining sufficient bioactive material.

4.1. Phytochemical Evaluation of *Cassia alata* Extract

Qualitative screening confirmed that the methanol extract was rich in diverse bioactive classes. Phytoconstituents, including glycosides (anthraquinones, saponins, cardiac glycoside), tannins, alkaloids were detected. This aligns with prior analyses of *C. alata*: for instance, Edegbo *et al*. (2023) reported that *C. alata* leaf extracts contained "*anthraquinone, cardiac glycosides, steroids, alkaloids, phlobatannin, phenols, saponins, tannins, flavonoids, and carbohydrates."* Likewise, Shohidul Islam (2023) identified specific anthraquinones (aloe-emodin, rhein, emodin, chrysophanol) and their glycosides (sennosides) in *C. alata*.

The presence of these classes likely underpins the observed bioactivities. For example, anthraquinones (a major *C. alata* component) are known to exert antimicrobial effects by intercalating microbial DNA and generating reactive oxygen species (Panichayupakaranant *et al*., 2008), while saponins can solubilize and disrupt microbial cell membranes (Bodet *et al*., 2000). Tannins can precipitate proteins and inhibit enzymes, and alkaloids often target nucleic acids or membrane integrity (Kurtzman, 2010). Therefore, the rich phytochemical profile provides a plausible chemical basis for antimicrobial action. In summary, our extract's composition is consistent with literature: an array of phenolic and terpenoid glycosides, alkaloids, and anthraquinones (Edegbo *et al*., 2023; Fatmawati *et al*., 2020), which have been repeatedly linked to the plant's antibacterial and antifungal properties.

4.2. Formulated Shampoo Properties

The *C. alata* extract was successfully incorporated into a herbal shampoo that was smooth, viscous, greenish in color (owing to chlorophyll and phenolics in the extract), with a pleasing odor. It produced a stable foam for at least 5 min and dispersed a sample of residue uniformly, indicating good cleansing performance. The measured pH was 6.8 ± 0.2, indicating a mildly acidic to near-neutral pH. While many scalp-care recommendations prefer a slightly acidic shampoo (pH: approximately 5–5.5) to match the skin's natural pH and minimize irritation (D'Souza and Rathi, 2015; Dias *et al*., 2014), the pH of the formulation in this study is somewhat higher. In practice, shampoos are often adjusted (e.g., with citric acid) to a pH of about 5.5 to keep hair cuticles flat and prevent damage (Draelos, 2013). Thus, for optimal scalp compatibility, the pH of the herbal shampoo could be fine-tuned downward in future work. Overall, the shampoo's physical characteristics (foamability, scent) are consistent with consumer expectations for herbal shampoos: Kongtunjanphuk (2025) notes that successful plant-based shampoos should have a pleasant color and foam and be microbiologically active. In that context, our product's creaminess and froth suggest it could perform well in use, while providing the added benefit of the *C. alata* extract.

4.3. Antimicrobial Activity of *Cassia alata* Extract

4.3.1. Bacterial Activity

The crude methanol extract showed moderate antibacterial activity at high concentrations. No inhibition zones were seen below 400 mg/mL, but at 500 mg/mL, zones approximately 15–18

mm were observed for *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. *S. aureus* and *B. subtilis* (Gram-positive) were slightly more sensitive (17–18 mm) than were *Klebsiella* or *Pseudomonas* (Gram-negative, 15 mm). This pattern, Gram-positives being somewhat more inhibited than Gram-negatives, is commonly reported for plant extracts and likely reflects the protective outer membrane of Gram-negative bacteria (Naeem *et al*., 2010). Comparisons with other studies indicate that *C. alata* can be quite active: for example, Islam (2023) found zones up to 28 mm for *E. coli* and 19–21 mm for *S. aureus* and *Klebsiella* at only 100 µg per disk. Absolute values are not directly comparable (µg/disc vs. mg/mL extracts); however, the trend is similar. Indeed, Islam reported a clear dose–response, reaching 28 mm on *E. coli* at 100 µg/disc. Our extract yielded smaller zones, reflecting that a much larger dose (500 mg/mL) was needed to achieve inhibition zones of approximately 15–18 mm. One reason may be that aqueous or less-purified extracts often show weaker diffusion. Nevertheless, the fact that *C. alata* extract inhibited both Gram-positive and Gram-negative pathogens (albeit at high doses) corroborates its broad-spectrum potential. Similar to our findings, Aminuddin *et al*. (2016) reported that an C. alata soap showed no significant antibacterial activity against *E. coli, P. aeruginosa, B. subtilis, or S. aureus* under their test conditions, suggesting that only highly concentrated or specific extracts exhibit antibacterial activity. In our case, only the highest extract concentration was antimicrobial, which is consistent with the need for high doses of crude extracts to approach antibiotic potency.

It is likely that no single compound is responsible; rather, a synergistic cocktail causes membrane damage and metabolic blockade. The moderate zones observed in this study (12–18 mm) suggest a bacteriostatic effect at 500 mg/mL. In practice, these values are lower than the >30 mm zones produced by potent antibiotics (the ciprofloxacin control used in this study was 30–35 mm). Thus, while the *C. alata* extract has genuine antibacterial constituents, its potency is lower than that of pure drugs. This matches the ethnomedicinal use: *C. alata* is traditionally applied to skin infections (often fungal) (Aminuddin *et al*., 2016) rather than as a systemic antibacterial (Trüeb, 2014).

4.3.2. Antifungal Activity

In the present study, the *C. alata* extract showed notable antifungal activity. *C. albicans* had an 18 mm inhibition zone at 500 mg/mL and *A. niger* had a 19 mm zone at 500 mg/mL. By comparison, the antifungal drug ketoconazole produced larger zones (25–26 mm) at standard

disc doses. Nevertheless, an 18–19 mm zone with the 500 mg/mL extract is meaningful, indicating that the extract contains fungistatic agents. In fact, the literature emphasizes *C. alata*'s potency against fungi: Edegbo *et al*. (2023) reported that ethanolic and methanolic leaf extracts had minimum inhibitory concentrations as low as 0.78–1.56 mg/mL against dermatophytes, which implies strong activity. Likewise, Prabhu *et al*. (2020) demonstrated that *C. alata* flower extract (with chrysine and anthracenedione) inhibited the dandruff yeast *Malassezia furfur* at low micromolar concentrations. These anthracenedione compounds (structurally related to emodin) are known antifungal agents, likely damaging fungal cell membranes or inhibiting sterol synthesis. In the tests conducted in the present study, the trend that fungi were inhibited at lower relative concentrations than those with bacteria echoes other findings: for instance, Aminuddin *et al*. found that *C. alata* aqueous extract had clear antifungal activity (against *Saccharomyces cerevisiae*) but no antibacterial effect at comparable conditions. The findings of this study align with this; the extract required much lower *relative* active quantity to affect the yeast and mold than to inhibit bacteria.

The raw extract's antifungal efficacy was considerably stronger (on a per-mass basis) than its antibacterial effect, consistent with *C. alata*'s folk use as an antifungal ("Ringworm plant" for dermatophytes (Edegbo *et al*., 2023). Even though the *Candida* and *Aspergillus* zones observed in this study did not match those with ketoconazole, they were substantial. For example, an 18 mm zone against *C. albicans* at 500 mg/mL was observed; by comparison, Islam (2023) reported a similarly sized zone (approximately 19 mm) against *E. coli* at just 100 µg. This highlights that Cassia's fungitoxic compounds (likely anthraquinones, phenolics, among others) are effective but require higher doses when crude. Importantly, the absence of any zone ("G" or "no inhibition") at concentrations ≤300 mg/mL indicates that the active threshold is relatively high, suggesting the need for concentration or purification for practical use.

4.4. Antimicrobial Activity of *Cassia alata* Shampoo Formulation

Incorporation into a shampoo base attenuated the antimicrobial effects of the extract. In the agar diffusion tests, the *Cassia alata* herbal shampoo showed no inhibition of any organism up to 400 mg/mL. Only at 500 mg/mL did small zones appear (12–16 mm) against each microbe, and these were smaller than the equivalent zones produced by the raw extract. For example, *S. aureus* was inhibited by 17 mm when treated with the pure extract at 500 mg/mL;

however, only 12 mm by the shampoo at the same concentration. This reduction likely reflects the dilution of active compounds by the surfactant matrix—comprising sodium lauryl sulfate, stearic acid, and lanolin—and the possible binding or encapsulation of phytochemicals within micelles or emulsified phases, which can reduce their availability for antimicrobial action (Nielsen, Kjrulff, and Madsen, 2016; Bahr *et al*., 2019; Kiokias, Proestos, and Oreopoulou, 2022). The presence of surfactants, such as sodium lauryl sulfate, is known to form micellar structures that trap hydrophobic phytochemicals, thereby decreasing their diffusion and biological effectiveness (Oh, Sung, and Kim, 2011).

In practical terms, the formulated shampoo demonstrated weaker antimicrobial potency than the pure extract, which is expected since the extract becomes less concentrated per unit volume upon incorporation into the shampoo base (Godeto *et al*., 2023; Arhin *et al.*, 2024). Similar reductions in antimicrobial activity have been observed following formulation in other herbal preparations, such as soaps and shampoos, where the inclusion of surfactants and stabilizers diluted or impeded the diffusion of bioactive molecules (Kong *et al*., 2025). Additionally, viscosity and the physicochemical nature of the formulation can significantly restrict the radial movement of active molecules in agar diffusion assays, leading to smaller inhibition zones despite inherent antimicrobial potency (Hossain, Bhadra, and Hossain, 2022; Hernández-Lepe, Varela-Camarena, and González-Huerta, 2024; Yang, Low, and Yap, 2017). This observation aligns with the established understanding that agar-based diffusion assays depend not only on antimicrobial strength but also on the compound's solubility and diffusion rate within the agar medium (American Society for Microbiology, 2024; Gattinger *et al*., 2023).

Interestingly, even the shampoo's modest zones were primarily antifungal. At 500 mg/mL, it inhibited *C. albicans* (16 mm) and *A. niger* (15 mm) similarly to *Streptococcus* (14–15 mm). In contrast, none of the bacteria were inhibited at lower doses. This pattern matches the soap formulation results of Aminuddin *et al*. (2016): they observed their C. *alata* soap had *antifungal* activity (against a test yeast) but *no antibacterial* effect. Thus, the shampoo seems to retain the extract's antifungal bias. The antifungal zones (15–16 mm) at 500 mg/mL are still appreciable; they suggest the shampoo might help control yeast- or fungal-related scalp infections (like dandruff or ringworm), especially if used undiluted. However, the relatively small zones also signal that, as a finished product, the shampoo alone is not as potent as conventional antifungal shampoos (including ketoconazole-based shampoos). Enhancing the release of actives (via emulsifiers or by adjusting pH) might improve efficacy.

Any antimicrobial action of the shampoo reflects a combination of factors. The surfactant sodium lauryl sulfate has some antiseptic properties of its own, but it is not highly selective. More importantly, Cassia compounds (such as anthraquinones and saponins) remained present and could disrupt fungal membranes. It is possible that the slight acidity of the shampoo (pH: approximately 6.8) aided the release of phenolics. Nonetheless, the data imply that *C. alata*'s actives are partially sequestered in the formulation, reducing their effective concentration. The fact that any zone was still observed indicates they do diffuse out, and the zones against Candida and Aspergillus at 500 mg/mL show that the shampoo is not devoid of antifungal activity. In practical terms, this suggests that a *C. alata* herbal shampoo could provide prophylactic or mild therapeutic benefit against scalp fungi, but may need higher strength or complementary actives (such as other plant extracts or conventional antifungals) for robust action.

4.5. Implications and Applications

The findings of this study reinforce that *C. alata* is a promising herbal antimicrobial agent, especially against fungi. The rich phytochemical profile identified in the extract likely acts synergistically to inhibit pathogens. In herbal shampoo form, *C. alata* contributes to broad cleansing and antifungal properties: it may help manage dandruff and seborrheic scalp conditions, which are often fungal in origin. Indeed, herbal shampoo formulations with bioactive plants are sought for their multifunctional benefits (Kongtunjanphuk, 2025). Besides antimicrobial effects, *C. alata* contains antioxidants and flavonoids that could soothe scalp inflammation and strengthen hair, similar to other botanical ingredients used in hair care (Kongtunjanphuk, 2025).

However, practical use requires consideration of potency. The results of this study suggest that while *C. alata* extract has activity, it is less potent than standard drugs and works at high concentrations. To translate this into a consumer product, the formulation could be optimized. For instance, one might increase the extract dose in the shampoo, or use extracts concentrated on the key antifungal molecules. Alternatively, combining *C. alata* with other synergistic herbs (such as *Lawsonia inermis* or *Curcuma longa*, as in Kongtunjanphuk's study) could amplify the effect. Additionally, ensuring the shampoo pH is adjusted to the slightly acidic range (approximately 5.0–6.0) would improve scalp compatibility and might enhance microbial inhibition (Trüeb, 2014).

Collectively, this study's results are broadly consistent with existing literature: *C. alata* contains diverse antimicrobials and can inhibit both bacteria and fungi, with a stronger effect on fungi. The data explain why it has been used traditionally for fungal skin diseases (Edegbo *et al*., 2023) and why modern work finds anti-dermatophyte activity (Edegbo *et al*., 2023). The formulated shampoo retained some of these benefits, validating the concept of a *C. alata* herbal shampoo. For future research, it would be valuable to test the shampoo against dandruff-causing *Malassezia* or dermatophytes, and to compare it with commercial anti-dandruff shampoos. Optimization of the extract preparation (such as fractionation of actives) and shampoo formulation (including emulsifiers, pH) could further improve efficacy.

In summary, the high extraction yield and rich phytochemistry of *C. alata* underpin its antimicrobial activity. Our study showed the raw extract inhibits various pathogens (most strongly fungi) at high concentrations, and the formulated shampoo retains a modest antifungal effect. These findings support the potential of *C. alata* as a herbal shampoo ingredient for scalp health, while also highlighting the need to refine formulations to match conventional antimicrobial strength.

4.6. Limitations

The findings of this study highlight the antimicrobial potential of *C. alata* and show its successful incorporation into a herbal shampoo formulation; however, several important limitations should be acknowledged. First, the antimicrobial evaluation was limited to a few bacterial and fungal strains, including *S. aureus*, *B. subtilis*, *E coli*, *P. aeruginosa*, *K. aerogenes*, *C. albicans*, and *A. niger*. While these organisms represent common skin and scalp pathogens, they do not encompass the full range of microorganisms responsible for dermatological infections, particularly *Malassezia* species, which are key causative agents of dandruff and seborrheic dermatitis. A broader panel of microbial isolates, including resistant strains, would have provided a more comprehensive evaluation of antimicrobial efficacy.

Second, the study employed a crude methanolic extract of *C. alata* without further fractionation or purification. This approach mirrors traditional use; nonetheless, it limits understanding of which specific phytochemicals contribute most significantly to the observed bioactivity. The extract's complex mixture of secondary metabolites, such as anthraquinones, flavonoids, alkaloids, tannins, and saponins, likely acts synergistically, but isolating and quantifying individual components could help optimize formulations and enhance

consistency. Furthermore, variability in extraction yield and composition due to plant age, environmental factors, and solvent polarity could affect reproducibility and scalability.

A further limitation concerns the *in vitro* nature of the antimicrobial tests. Agar diffusion methods provide useful preliminary data, but they do not fully simulate the dynamic conditions of scalp application, where factors such as sebum, pH, and contact time influence activity. The inhibitory concentrations observed in the laboratory may not directly translate to clinical or consumer settings. Additionally, the formulation's physicochemical characteristics, such as long-term stability, viscosity over time, color retention, and pH stability, were not extensively studied. Similarly, dermatological safety, irritation potential, and allergenicity were not evaluated, all of which are essential for product development.

Moreover, the shampoo's performance was not compared with that of commercially available synthetic or herbal shampoos. Such a comparison could have contextualized the antimicrobial potency and provided a benchmark for improvement. Lastly, while the product demonstrated acceptable foaming and appearance, consumer acceptability studies were not performed to assess user satisfaction, fragrance preference, or hair conditioning effects.

In summary, while this study establishes foundational evidence for the antimicrobial utility of *C. alata* in a shampoo formulation, its limitations underscore the need for future research. Comprehensive chemical characterization, *in vivo* efficacy and safety testing, stability assessments, and consumer trials are necessary to fully validate and optimize the formulation for practical and commercial application.

CONCLUSION

This study investigated the antimicrobial activity of *C. alata* leaf extract and its formulation into a herbal shampoo, aiming to develop a natural and effective alternative to synthetic antimicrobial agents commonly used in personal care products. The methanolic extract of *C. alata* demonstrated broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as selected fungal species, confirming the plant's traditional use in the treatment of skin and scalp infections. The formulated shampoo also retained significant antimicrobial efficacy, suggesting that the bioactive compounds within the extract remained stable and functional after formulation.

The phytochemical analysis revealed the presence of secondary metabolites, including alkaloids, tannins, saponins, and anthraquinones, which are known to possess antimicrobial, antioxidant, and anti-inflammatory properties. These constituents likely synergistically contributed to the observed inhibitory effects on the test organisms. Furthermore, the formulated shampoo displayed acceptable physicochemical properties, including appropriate pH, foam stability, and appearance, indicating its suitability for topical application.

The findings underscore the potential of *C. alata* as a valuable natural source of antimicrobial agents for use in herbal shampoo formulations. Such plant-based alternatives are particularly relevant given the increasing consumer demand for safer, environmentally friendly, and sustainable cosmetic products. However, to move from experimental validation to practical application, further work is required. Future studies should include detailed chemical profiling of active constituents, *in vivo* assessments of antimicrobial effectiveness, toxicity, and irritancy testing, and stability evaluations over prolonged storage periods.

In conclusion, this study provides scientific support for the traditional medicinal use of *C. alata* and shows its promise as a functional ingredient in herbal shampoo formulations. The development of such natural antimicrobial products may significantly improve scalp hygiene and reduce reliance on synthetic chemicals, thereby promoting public health and environmental sustainability.

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