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Investigation into the Antimicrobial Potentials and Phytochemical Composition of Cassia Siamea

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DECLARATION

I declare that this research project is the result of my independent investigation and has not been submitted for any other academic qualification.

Signature:

Name: Abdulai Turay Date: October 2020

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CERTIFICATION

This is to certify that the research project titled "Phytochemical Screening and Antimicrobial Activity of Cassia siamea Leaves and Stem Bark Extracts Against Selected Pathogens" was carried out by under our supervision and is approved for submission.

Signature:

Supervisor: Dr. Eugene B. S Conteh

Head of Department: Dr. Eugene B. S Conteh, Pharmaceutical Chemistry.

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DEDICATION

This work is dedicated to my beloved family and all who supported me during this research.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
C. siamea	*Cassia siamea*
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NaCl	Sodium Chloride
CFU	Colony Forming Unit
°C	Degrees Celsius
mg/ml	Milligrams per milliliter
mL	Milliliter
mm	Millimeter
g	Gram
h	Hour(s)
WHO	World Health Organization

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ABSTRACT

The rise in antibiotic-resistant pathogens poses a serious global health threat, necessitating the search for alternative therapies. This study investigates the antimicrobial potential and phytochemical constituents of *Cassia siamea*, a medicinal plant traditionally used across various cultures for its healing properties. Crude extracts from the leaves and stem bark were prepared using methanol and aqueous solvents, then tested for antimicrobial activity against three significant pathogens: *Streptococcus pyogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus*.

Phytochemical screening revealed the presence of flavonoids, tannins, glycosides, saponins, steroids, and phenols, with flavonoids and glycosides strongly present in both plant parts. Alkaloids, anthraquinones, and terpenoids were absent. Antimicrobial testing using the well diffusion method showed measurable zones of inhibition in all extracts, with aqueous leaf extract displaying the highest activity against *Staphylococcus aureus* (15.00 mm). Conversely, methanolic stem bark extract demonstrated significant activity against *Streptococcus pyogenes* (14.00 mm) and *Staphylococcus aureus* (16.00 mm), while aqueous stem bark extract showed minimal effect. Minimum inhibitory concentration (MIC) results corroborated these findings, with the lowest MIC values observed for aqueous leaf extract against *S. aureus* and *S. typhi* (2.5 mg/ml). The observed antimicrobial activity is likely due to the synergistic effects of bioactive compounds, which may disrupt microbial cell membranes or inhibit critical metabolic functions.

This research affirms the therapeutic potential of *Cassia siamea* and highlights its promise as a source of novel antimicrobial agents, particularly in regions with limited access to conventional pharmaceuticals. The findings support further investigation and possible development of phytomedicine based on this species.

***Keywords**: Cassia Siamea, Phytochemicals, Antimicrobial Activity, Medicinal Plants, Antibiotic Resistance, MIC.*

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CHAPTER ONE
INTRODUCTION

➢ *Background of Study*

Herbalism is a time-honored practice that emphasizes the use of plants and their extracts for therapeutic purposes. This holistic approach has persisted in millennia, rooted in ancient traditions of healing that recognize the profound connection between nature and human health. In addition to plant-based remedies, herbalism often encompasses the use of fungi, bee products, minerals, shells, and certain animal parts, reflecting the diverse resources that nature offers (Doughari et al., 2009). The foundation of herbalism lies in the use of natural substances, which have been integral to human health for millennia. Historically, cultures around the world have relied on herbal remedies to treat ailments, enhance well-being, and maintain health. Knowledge of these remedies has been passed down through generations, and modern scientific research increasingly validates many of these traditional uses. Indeed, an impressive number of contemporary pharmaceuticals have their origins in natural sources, illustrating the enduring relevance of herbalism in the field of medicine(Doughari et al., 2009). For instance, the use of willow bark as a pain reliever led to the synthesis of acetylsalicylic acid, better known as aspirin. Similarly, the discovery of taxol, derived from the Pacific yew tree, has revolutionized cancer treatment. These examples underscore the potential of botanical compounds in drug development and highlight the significant role that herbalism plays within the larger framework of healthcare. (Doughari et al., 2009). Moreover, the growing interest in herbalism can be seen as a reflection of a collective shift towards integrative approaches to health. Many individuals seek out herbal remedies as complementary treatments alongside conventional medicine, drawn by the allure of natural solutions and a desire for holistic wellness. As awareness and education around herbalism increase, practitioners are better equipped to utilize these resources safely and effectively, balancing traditional wisdom with modern scientific inquiry(Doughari et al., 2009)

Plant-based traditional medicine has remained a cornerstone of health care for both humans and animals throughout history. As societies evolve, the resurgence of interest in these natural remedies underscores their critical role in contemporary health practices. The compounds derived from various plants exhibit potent therapeutic applications against a range of pathogens, including bacteria, fungi, and viruses. This versatility not only highlights the intrinsic value of plant compounds but also illustrates their potential to complement modern medical treatments. Over the past decade, there has been a marked increase in the consumption of medicinal herbs, driven by a growing desire for alternative approaches to health and wellness. This shift reflects a broader trend towards holistic and preventive care, wherein individuals seek to enhance their quality of life and maintain optimal health through natural means. The popularity of herbal supplements and therapies emphasizes the need for rigorous scientific inquiry into their applications, effectiveness, and safety. Despite the promising benefits of herbal medicines, extensive research into their adverse effects is imperative. The potential for interactions with conventional medications, as well as varying individual responses, necessitates thorough investigation. By ensuring the efficiency and quality of these herbal products, practitioners can promote their safe use, ultimately enhancing public trust in plant-based therapies (Umer et al., 2013).

Cassia siamea, belonging to the Leguminosae family and the Caesalpinoideae subfamily, has garnered attention in the realm of medicinal botany due to its diverse therapeutic applications. The plant’s leaf, stem bark, and root are integral to various traditional medicinal practices, particularly in treating ailments such as indigestion, conjunctivitis, heartburn, and skin disorders arising from menstrual issues (Vijayaram et al., 2016). The combination of extracts from the root, leaf, and flowers serves not only as a remedy for these conditions but also highlights the importance of *Cassia siamea* in pharmacological research. The significance of *Cassia siamea* extends beyond its traditional uses; it is particularly notable for its array of phytochemicals. These bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and phenols, are produced by plants as natural protectants (Kulkarni & Koka, n.d.). Recent findings underscore their potential in managing human diseases, particularly infectious ailments caused by pathogens such as *Streptococcus pyogenes*, *Salmonella typhi*, and *Staphylococcus aureus*. The compelling evidence of phytochemicals exhibiting antioxidant, antimicrobial, and anti-inflammatory properties has escalated interest in their therapeutic applications, especially in an era where antibiotic resistance is a burgeoning concern. Research has demonstrated that the medicinal efficacy of *Cassia siamea* may vary significantly based on factors such as species, part of the plant used, environmental conditions, and the plant's age. These variabilities present both challenges and opportunities in the pursuit of synthesizing complex medicinal molecules (Oo, n.d.).

This comprehensive study was focused on the extraction methods, such as using methanol and water, which are essential for elucidating the phytoconstituent profiles of *Cassia siamea*, thereby advancing our understanding of its therapeutic modalities.

➢ *Statement of the Problem*

Antibiotic resistance has emerged as a critical global health crisis, significantly reducing the efficacy of commonly used antimicrobial medications. Overuse and misuse of antibiotics have accelerated the evolution of resistant pathogens, leaving medical professionals struggling to treat drug-resistant infections. This predicament has prompted an urgent search for alternative therapeutic approaches, especially from natural sources. Plants, as bio-diverse repositories of secondary metabolites, have shown promising antimicrobial properties, offering a potential solution to this pressing problem (Sibanda & ., 2007).

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Cassia siamea, a tropical plant native to Southeast Asia and widely distributed across various regions, is a noteworthy example of flora with significant antimicrobial activity. Research into the plant's phytochemical composition has revealed the presence of bioactive compounds such as phenolics, flavonoids, and alkaloids, which exhibit potent antibacterial effects. These compounds have demonstrated efficacy against a spectrum of pathogenic microorganisms, including drug- resistant strains (Sibanda & ., 2007). Additionally, *Cassia siamea* has been recognized for its medicinal properties, including its use as an antimalarial and anti-inflammatory agent, further reinforcing its therapeutic potential.

Antibiotic resistance is one of the most pressing global health challenges of our time. The increasing ineffectiveness of conventional antibiotics against a backdrop of emerging and re-emerging diseases necessitates a paradigm shift in our approach to infection control and treatment. As pathogenic microorganisms evolve and develop resistance, the search for alternative therapeutic agents becomes imperative. In this context, the potential of medicinal plants warrants critical examination, particularly given their historical use in traditional medicine and the perception that they may offer fewer side effects compared to synthetic drugs. The phenomenon of antibiotic resistance arises from various factors, including over-prescription of antibiotics, misuse in agriculture, and poor infection control practices. As a result, common infections are becoming harder to treat, leading to prolonged illness, increased medical costs, and heightened mortality rates. Concurrently, the emergence of new diseases, often linked to environmental changes and globalization, further complicates the medical landscape. In response to these challenges, there is a growing body of evidence suggesting that phytochemicals derived from medicinal plants possess antimicrobial properties that can complement or even replace conventional antibiotics. Numerous studies have identified compounds such as alkaloids, flavonoids, and essential oils with demonstrated efficacy against a wide range of pathogens, including bacteria, fungi, and viruses. Notably, these natural substances often exhibit a unique mechanism of action that could circumvent established resistance pathways. Furthermore, the integration of plant-based antimicrobial agents into existing therapeutic regimens can enhance efficacy while potentially mitigating side effects associated with synthetic drugs. The holistic approach offered by traditional medicine, which often combines various plant extracts, could lead to synergistic effects that improve treatment outcomes. Moreover, the economic advantage of utilizing locally available medicinal plants could make healthcare more accessible, especially in low-resource settings where traditional antibiotics may be less available. In conclusion, the global crisis of antibiotic resistance, coupled with the rise of new and re-emerging diseases, underscores the need for innovative solutions in infectious disease management. The exploration of medicinal plants as alternative antimicrobial agents presents a promising avenue for research and application. By investing in the study and standardization of these natural remedies, we may discover effective, safe, and sustainable options to complement the aisle of synthetic antibiotics, ultimately improving global health outcomes. (*In Vitro Anti-Listerial Activities of Crude n-Hexane and Aqueous Extracts of Garcinia Kola (Heckel) Seeds - PMC*, n.d.)

➢ *Aim of the Problem.*

The goal of this study is to assess how crude extracts from the leaves and stem bark of *Cassia siamea* impact *Streptococcus pyogenes, Salmonella typhimurium,* and *Staphylococcus aureus*. Additionally, it aims to perform a phytochemical analysis of these crude extracts.

➢ *Hypothesis*

Cassia siamea demonstrates significant antimicrobial efficacy against *Streptococcus pyogenes*, *Salmonella typhi*, and *Staphylococcus aureus.*

➢ *Objectives of the Study*

- *Main*

The primary objective of this study is to investigate the bioactivity of the leaves and stem bark of Cassia siamea against selected bacterial pathogens.

- *Specific*

✓ To collect and authenticate the leaves and stem bark of *Cassia siamea*.
✓ To extract the compounds from the leaves and stem bark of Cassia siamea utilizing methanol and water.
✓ To conduct sterility testing on the plant extracts.
✓ To identify the phytochemicals, present in the crude extracts obtained from the leaves and stem bark of Cassia siamea.
✓ To evaluate the antimicrobial activity of the crude extracts derived from both the leaves and stem bark of *Cassia siamea*.
✓ To ascertain the minimum inhibitory concentration (MIC) of the plant extracts.

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➢ *Justification of the Study*

The exploration of phytochemicals in medicinal plants has gained momentum due to the increasing global concern regarding antibiotic resistance and the urgent need for alternative therapeutic agents. *Cassia siamea*, commonly known as yellow *cassia*, is a leguminous tree native to tropical regions and well-documented for its medicinal properties. However, despite its prevalence, a comprehensive phytochemical screening of the leaves and stem bark of the Sierra Leonean variety, as well as an assessment of its bioactivity against selected bacterial pathogens, remains an uncharted territory.

Phytochemical screening is pivotal in identifying the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which are known for their antimicrobial properties. The Sierra Leonean variety of *Cassia siamea* warrants investigation, as regional variations in plant constituents may lead to differing therapeutic potentials. Initial studies suggest that this species may harbor a rich array of these phytochemicals, which could play a significant role in combating pathogenic bacteria that threatens public health.

The bioactivity of plant extracts against bacterial pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, is of paramount importance in pharmacological research. Traditional practices in many Sierra Leoneans communities utilize *Cassia siamea* for its perceived healing properties, yet empirical data substantiating these claims have been sparse. The lack of comprehensive studies hinders the potential development of sustainable, plant-based antibacterial agents derived from this species.

In conclusion, the phytochemical screening of the leaves and stem bark of *Cassia siamea* from Sierra Leone represents a significant gap in current research that warrants immediate attention. Understanding the phytochemical profile and bioactivity against specific bacterial pathogens could unveil novel therapeutic agents, fostering an integrative approach to healthcare. The implications of such research extend beyond local medicinal practices, potentially enriching the global pharmacological landscape in the fight against multidrug-resistant bacteria. Thus, the pursuit of this inquiry is not only scientifically relevant but also imperative for harnessing the true potential of native flora in health sciences.

➢ *Limitation of Study*

- The COVID-19 pandemic has significantly impacted the timeline for conducting this study.
- There were delays in acquiring certain reagents and equipment essential for the research.
- A confirmed COVID-19 case involving one of the personnel at the Pharmacy Board microbiology laboratory has also affected the progress of the study.

➢ *Study Location*

The research was conducted in the Department of Pharmaceutical Sciences Laboratory at College of Medicine and Allied Health Sciences, University of Sierra Leone and the Microbiology Laboratory at the Pharmacy Board of Sierra Leone.

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CHAPTER TWO
LITERATURE REVIEW

➢ *The Importance of Medicinal Plants in the Search for New Antimicrobial Agents.*

The emergence of drug-resistant microorganisms poses a significant threat to global health, necessitating an urgent search for alternative treatment options. Phytochemicals derived from medicinal plants represent a promising direction for this endeavor. Historically, these plants have served as vital sources of healing compounds, particularly in regions where access to modern medicine is limited. As research progresses, a renewed focus on these natural products is essential for developing effective therapies against infectious diseases. (Ncube et al., 2008)

Medicinal plants harbor a diverse array of phytochemicals, which have been utilized in traditional medicine for centuries. These compounds often exhibit therapeutic properties that can be harnessed to combat various ailments. For instance, recent studies have indicated that natural extracts can be more effective than conventional pharmaceuticals, particularly against drug-resistant strains of pathogens. The practical advantages of using phytochemicals including fewer side effects and cost- effectiveness reinforce the case for their integration into modern medicinal practices.

As noted by the World Health Organization, approximately 80% of the global population relies on traditional medicine for their healthcare needs. This statistic underscores the relevance of herbal remedies in contemporary health systems, especially in developing countries. Scientific inquiry into these natural substances not only provides insight into their potential therapeutic applications but also validates the knowledge of traditional healers who have relied on these plants for generations (Ncube et al., 2008)

Recent investigations into African medicinal plants, such as *Cassia siamea*, illustrate the significant biological activities that various isolated compounds can offer. Through advanced techniques in biochemistry and pharmacology, researchers can concentrate and elucidate the active components within these plants. This scientific approach facilitates the development of new pharmaceuticals that are grounded in the rich heritage of traditional medicine (Ncube et al., 2008). In conclusion, the search for phytochemicals from medicinal plants is more than an academic pursuit; it is a vital strategy for addressing the challenges posed by antimicrobial resistance. By leveraging the historical use of these plants and their biochemical potential, scientists can pave the way for innovative treatments that are not only effective but also accessible, thereby enhancing public health worldwide. As the quest for new antimicrobial agents continues, it is imperative that the contributions of traditional medicine are both recognized and integrated into modern healthcare solutions (Ncube et al., 2008).

➢ *The Multifaceted Value of Cassia Siamea*

Cassia siamea, synonymously known as Senna siamea, is a remarkable angiosperm native to Southeast Asia, including countries such as Burma, Sri Lanka, and Malaysia. Over the years, its distribution has expanded significantly, finding a place in various ecosystems across Africa, Latin America, and Oceania. With its scientifically recognized family lineage, having transitioned from the Caesalpinoideae to the Leguminosae, *Cassia siamea* stands as a testament to the rich biodiversity of tropical regions (*Determination of Barakol Extracted from Cassia Siamea by HPLC with Electrochemical Detection - PubMed*, n.d.). Characterized by its medium height, typically between 10 to 12 meters, and occasionally reaching 20 meters, *Cassia siamea* exhibits a dense and rounded crown in its youth, which gradually matures to a more irregular, spreading shape. The plant's aesthetics are complemented by its young grey, smooth bark that develops longitudinal fissures with age. The leaves, which can measure between 15 to 30 cm in length, are alternate and compound, featuring 6 to 14 leaflets, each terminating in a slight bristle. Notably, the plant produces striking bright yellow flowers arranged in large, upright, pyramid-shaped panicles that can extend up to 60 cm, creating a vibrant spectacle in its natural habitat (*TREES COMMONLY CULTIVATED IN SOUTHEAST ASIA*, n.d.).

The fruit of Cassia siamea takes the form of flat, indehiscent pods that range from 5 to 30 cm in length and are constricted between seeds, which are typically bean-shaped, greenish-brown, and measure 8 to 15 mm long. Each pod contains approximately 20 seeds, showcasing the plant's reproductive efficiency and potential for propagation (*Plant Resources of South-East Asia*, 1990) Historically, Cassia siamea has garnered attention for its various medicinal properties, making it a valuable resource in traditional medicine among tropical populations. Its utility extends beyond health applications; the plant finds significant roles in cattle rearing, agricultural practices, and environmental restoration. Additionally, its wood is prized for furniture-making, tapping into a market that values sustainability and ecological balance. (*Plant Resources of South-East Asia*, 1990). In conclusion, the significance of *Cassia siamea* transcends its biological characteristics. Its expansive range, aesthetic appeal, medicinal virtues, and practical uses in agriculture and industry underscore its importance as a multifaceted asset in both local and global contexts.

Recognizing and harnessing the potential of this versatile species is essential for biodiversity conservation and sustainable development in the regions it inhabits (Sahni, 1998)

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1- Stem, 2- Leaves, 3- Flowering Branch and Flowers, 4- Pods, 5- Stem Bark.
Fig 1 Different Parts of *Cassia Siamea*

➢ *Taxonomic Classification of Cassia Siamea*

The taxonomic classification of *Cassia siamea*, commonly known as the Siamese senna, illustrates the systematic organization of living organisms within the plant kingdom. This classification is hierarchical, reflecting the evolutionary relationships among species and providing insight into their characteristics and ecological significance.

At the highest level, *Cassia siamea* resides within the Kingdom Plantae, which encompasses all plants. Within this kingdom, it further belongs to the Subkingdom Tracheobionta, identifying it as a vascular plant characterized by specialized tissues for the conduction of water and nutrients. This classification leads us to the Super division Spermatophyta, indicating that *Cassia siamea* is a seed plant, which reproduces through seeds as opposed to spores.

Diving deeper, *Cassia siamea* is classified under the Division Magnoliophyta, denoting its status as a flowering plant. This division comprises an array of species that exhibit flowering characteristics for sexual reproduction. Within Magnoliophyta, it is categorized under the Class Magnoliopsida, which represents the dicotyledons, a group of flowering plants that typically feature two embryonic leaves or cotyledons.

Further refinement of its classification reveals that *Cassia siamea* belongs to the Subclass Rosidae, positioning it alongside a diverse collection of flowering plants characterized by certain morphological traits. It is then classified under the Order Fabales, encompassing legumes and their relatives, known for their unique seed structures and symbiotic relationships with nitrogen-fixing bacteria.

In the family hierarchy, *Cassia siamea* is placed within the Family Leguminosae (also known as Fabaceae), which includes a multitude of economically and ecologically important plants. This family is recognized for its significant contribution to agriculture and the environment. Within this family, *Cassia siamea* is grouped under the Subfamily Caesalpinoideae, which features many tropical and

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subtropical species.

Finally, the classification reaches the Genus level, with *Cassia* as the genus that unites several species of plants with similar characteristics. The species designation, *siamea*, highlights its specific identity within this genius, distinguishing it from other related plants.

In conclusion, the taxonomic classification of *Cassia siamea* provides a comprehensive framework for understanding its place in the plant kingdom. This classification not only aids in plant identification but also emphasizes the ecological roles and evolutionary lineage of this species, contributing to our broader understanding of biodiversity.

Table 1 Taxonomic Classification of *Cassia siamea*"

Rank	**Classification**
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Super Division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Leguminosae
Subfamily	Caesalpinoideae
Genus	Cassia
Species	Cassia siamea

➤ *Vernacular Names of C. Siamea: A Reflection of Cultural Diversity*

The plant known scientifically as C. siamea possesses a rich tapestry of vernacular names that reflect the cultural diversity across various localities. Table 1 presents a compilation of these names sourced from different regions, demonstrating the unique linguistic and cultural identities associated with this species.

In Sierra Leone, *C. siamea* is referred to as "Sheku Toure," a name that likely echoes the local heritage and perhaps pays homage to a notable figure in the region. Moving westward to Benin, it assumes the names "Kassia" and "cassiain," illustrating the integration of the plant into traditional practices and local nomenclature. Similarly, in Burkina Faso, it is known as "Kasse tiiga," further underscoring the variations in dialect and language tied to the plant.

Continuing through the African continent, Ghana identifies *C. siamea* as "Zangara tsi," while in Kenya, it bears the names "Ndek obino," "Oyieko," and "Ndege owinu." Each of these names encapsulates the local usage and significance of the plant, showcasing its importance not only in daily life but also in cultural narratives and folklore.

Beyond Africa, the name variations expand to Southeast Asia. In Malaysia, it is called "Sebusok," "guah Hitam," and "juah," among others, indicating a complex interplay of languages and cultures. The Indonesian names "Bujuk," "dulang," and "johar" further exemplify this diversity, alluding to regional characteristics and uses.

In the Indian subcontinent, C. siamea is known by several names, including "Minjri," "kassod," and "ponavari," each reflecting the local dialects and traditional knowledge systems. The proliferation of names in India signals the plant's versatility and significance in various contexts, from ecological to cultural.

Lastly, names in Nepal and Thailand, such as "Criminal" and "Kassod tree," respectively, highlight the continued relevance of the plant across different landscapes and cultures. The use of terms like "yellow cassia" and "thai pod copper" in Thailand indicates specific attributes of the plant that are recognizable to local communities.

In summary, the diverse vernacular names of C. siamea encapsulate a wealth of cultural heritage and ecological understanding unique to each locality. This nomenclatural variety not only fosters a deeper appreciation for the plant itself but also serves as a testament to the intricate relationship between people, language, and nature. The study of these names provides valuable insights into local traditions and the significance of biodiversity, reinforcing the importance of preserving both linguistic and botanical diversity globally.

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Table 2 Vernacular Names of the Plant Species across Different Regions"

Locality	Vernacular Names	Reference
Sierra Leone	Sheku Toure	-
Benin	Kassia, Cassiatin	(Allabi et al., 2011)
Burkina Faso	Kasse tiiga	(Nadembega et al., 2011)
Ghana	Zangara tsi	(Asase et al., 2010)
Kenya	Ndek obino, Oyieko, Ndege owinu	(Asase et al., 2010)
Malaysia	Sebusok, Guah Hitam, Juah, Petai Belalang, Johor	(Al-Adhroey et al., 2010)
Togo	Zangalati	(Koudouvo et al., 2011)
Spain	Flamboyan Amarillo	(Kolawole et al., 2010)
India	Minjri, Manjekonna, Kassod, Ponavari, Vakai, Simaiavari, Kilek, Nela T	(Kolawole et al., 2010)
Indonesia	Bujuk, Dulang, Johar	-
Nepal	Criminal	-
Thailand	Kassod tree, yellow cassia, Showers Thailand, Thai pod copper,	-

➢ *Uses of Cassia Siamea in Ethnomedicine*

Cassia siamea, commonly known as the Siamese senna, is a plant esteemed in various traditional medicinal practices, particularly across tropical regions where it plays a crucial role in ethnomedicine. The versatility of this plant is reflected in its utilization of multiple parts, including leaves, stems, roots, flowers, and seeds *("(PDF) Ethnoveterinary Practices in the Control of Helminthosis and Ticks of Livestock amongst Pastoralists in Karamoja Region, Uganda," n.d.).* Each component is believed to carry distinct therapeutic properties, making a valuable resource in the treatment of numerous health conditions.

One of the most significant applications of *Cassia siamea* is in the management of malaria, a pervasive tropical disease responsible for considerable morbidity and mortality. The plant's efficacy in combating malaria has been recognized in various ethnic medicine traditions, where it is often used either in isolation or in conjunction with other herbal remedies. Such combinations may enhance therapeutic outcomes, addressing the multifactorial nature of the disease, which involves a complex interaction between the pathogen and host immunity (*International Journal of Research in Ayurveda and Pharmacy*, n.d.).

The preparation of *C. siamea* for medicinal use typically involves decoction, a method that extracts the active compounds through boiling, which is believed to maximize the bioavailability of these therapeutic agents. Traditional knowledge guides the choice of plant parts and accompanying substances, tailored to the specific cultural practices and health needs of different populations. Most commonly, these preparations are administered orally, allowing for systemic absorption of the bioactive constituents (*Research in Ayurveda and Pharmacy*, n.d.).

In conclusion, *Cassia siamea* embodies the rich tapestry of ethnomedicine, with its multifaceted uses rooted in local knowledge systems. Its contributions extend beyond the treatment of malaria, as ongoing research continues to explore its broader pharmacological potential, ensuring that this traditional medicinal resource remains relevant in contemporary health discourse.

➢ *The Multifaceted uses of Leaves in Herbal Remedies across Cultures*

Leaves have long been integral to traditional medicine systems, particularly among African and Asian populations. Their versatility and efficacy render them the most utilized part of various plants, often exploited for their therapeutic properties in herbal remedies. A closer examination of the various applications reveals the culturally significant practices that underscore the importance of leaves in treating various ailments.

In Burkina Faso, the decoction of fresh and dried leaves is prepared by boiling for 20 minutes in water and is consumed either with lemon juice or used for body baths as a remedy for malaria and liver disorders. This highlights the innovative use of local flora in combating serious health challenges. Similarly, Côte d'Ivoire employs a different approach, where a daily oral dosage of leaf decoction is administered to alleviate cough, stomach pains, and malaria (*Zambian Plants Used as Traditional Fever Cures | The Kew Shop*, n.d.).

The use of leaves as a herbal remedy is not confined to specific regions; it extends to countries like Sierra Leone and Togo, where leaf decoction serves as a preventive measure against malaria and boasts antimicrobial properties. In Nigeria, traditional practices involve mixing dried leaves with those of lemon, pawpaw, and lime, boiling them into a "tea" that is consumed as a treatment for malaria. This communal knowledge underscores the significance of plant-based remedies in the local healthcare systems (Lemaire & Adosraku, 2002). Furthermore, in Uganda, the therapeutic potential of leaves is realized through a more tactile approach where cleaned leaves are chewed, with the liquid swallowed to alleviate abdominal pain (Mbatchi et al., 2006a). Such practices exemplify the close relationship that local populations maintain with their herbal traditions. In India, the process of creating a herbal decoction involves thorough cleaning and

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boiling of leaves, with honey added to enhance flavor before consumption, demonstrating the dual role of taste and health in traditional medicine. Meanwhile, in Laos, leaves are ingeniously prepared to reduce bitterness before being crushed and incorporated into a dish, "chi om leck," which serves not only as a meal but also as a source of sedative and euphoric effects. This culinary application underscores the blending of nutrition and medicine in traditional practices. In summary, the diverse applications of leaves in herbal remedies across different cultures emphasize their importance in local healthcare paradigms. The unique methods of preparation and consumption reflect a rich tapestry of traditions and knowledge, highlighting the vital role that leaves play in addressing health issues in Africa and Asia. As global health practitioners seek to integrate traditional knowledge into modern healthcare, the study of these practices may contribute to a holistic understanding of medicinal plants and their potential benefits(Kamatenesi et al., 2011a).

- *The Therapeutic Applications of Roots in Traditional Medicine across Africa*

Roots have served as a cornerstone of traditional medicine practices across various cultures in Africa, offering a wealth of healing properties that address numerous health concerns. In Benin, for instance, root decoction has emerged as a common remedy for ailments such as fever, constipation, hypertension, and insomnia. The simplicity of brewing roots into a decoction demonstrates a profound understanding of the therapeutic benefits these natural substances can provide (Sati et al., 2010).

In Kenya, the synergistic use of roots from *C. siamea* and *Zanthoxylum chalybeum* underscores the rich knowledge of local herbalists. A mixture prepared through infusion, decoction, or maceration acts as an antidote for snake bites, highlighting the critical role traditional remedies play in emergency health interventions. This traditional knowledge is invaluable, especially in areas where access to conventional medicine may be limited (*Dokumen.Tips | 522: Connection Timed Out*, n.d.).

Further south, in Southeast and Sub-Saharan Africa, the use of root decoctions extends to managing diabetes mellitus, indicating a recognition of the medicinal potential of these natural resources. The practice of crushing roots to create an aqueous extract for easing sore throat symptoms also reflects a community-based understanding of health and wellness, utilizing locally available materials to tackle common ailments (*The Use of Medicinal Plants in Self-Care in the Agonlin Region of Benin - PubMed*, n.d.).

In Côte d'Ivoire, the repeated administration of small doses of root maceration or decoction demonstrates a refined application of traditional practices in treating conditions such as angina and malaria. This method speaks to the strategic consideration of dosage and the cumulative effects of herbal treatments, principles that are often overlooked in modern therapeutic regimens.

Overall, the utilization of roots in traditional African medicine reveals a testament to the ingenuity and resourcefulness of local populations. By harnessing the healing properties of native plants, these communities continue to preserve and enhance their health practices, laying the groundwork for potential integration with modern medicine for improved healthcare outcomes (*Kenyan Medicinal Plants Used as Antivenin: A Comparison of Plant Usage | Journal of Ethnobiology and Ethnomedicine | Full Text*, n.d.).

- *The Medicinal uses of Stems in Traditional Practices*

The medicinal applications of plant stems, particularly in regions such as Burkina Faso, Ghana, Nigeria, and Malaysia, highlight the rich traditional knowledge surrounding herbal remedies. In these areas, the decoction of whole stems or stem bark is commonly utilized as a treatment for malaria and liver disorders, illustrating the significant role that local flora plays in addressing prevalent health concerns.

In particular, the stem of *Cassia siamea*, when combined with the fruit of Xylopia aethiopica, serves as an effective laxative. This demonstrates the diverse pharmacological properties attributed to different parts of a plant, underscoring the importance of phytochemistry in herbal medicine. Moreover, the decoction of *C. siamea's* stem bark is also employed in the management of diabetes, providing a natural alternative for those seeking holistic treatment options (Odugbemi et al., 2006). In Japan, the same decoction is noted for its mild, pleasant flavor and safety profile, making it an accessible purgative for various ailments. The versatility of stem preparations is evident, as they are not only utilized for gastrointestinal issues but also for dermatological and urogenital disorders in Cambodia, where traditional remedies are employed to treat conditions such as scabies, herpes, and rhinitis.

Collectively, these practices emphasize the need for further scientific inquiry into the pharmacological potential contained within plant stems. Research may validate and expand upon traditional uses, ensuring that these valuable natural resources are preserved and integrated into modern medicine. This cross-cultural utilization of stem-derived remedies illustrates the profound connection between local practices and the broader field of ethnobotany, warranting continued exploration and appreciation of traditional healing methodologies (*Ethnobotanical Study on Some Malaysian Anti-Malarial Plants: A Community Based Survey - PubMed*, n.d.).

- *The Diverse uses of Flowers and Seeds in Traditional Medicine*

In various cultures, particularly in regions such as Burkina Faso and Southeast Asia, the use of flowers and seeds extends beyond mere decoration, serving as integral components of traditional medicine. In Burkina Faso, for example, decoctions made from local

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flowers are consumed or applied in body baths to combat ailments such as malaria and liver disorders. These preparations are not solely limited to physical health; they are also noted for their efficacy against insomnia and asthma, demonstrating the holistic approach of traditional remedies to both mental and physical well-being (Kamatenesi et al., 2011b).

The seeds of certain plants in this region also play a crucial role in health. They are employed to expel intestinal worms and function as antidotes for snake and scorpion bites, showcasing the diverse therapeutic properties that lie within these natural resources. Additionally, the combination of C. siamea and Ficus thonnigii fruits is used in decoctions aimed at preventing convulsions in children and treating typhoid fever, indicating a deeper understanding of plant synergies in traditional medicine(Mbatchi et al., 2006b).

In contrast, the culinary uses of flowers and young fruits are prominent in countries like Sri Lanka and Thailand. Here, these plant parts are regularly incorporated into dishes and curries, not only providing flavor but also endowing laxative and sedative properties. The consumption of these ingredients has been linked to anxiolytic effects and relief from dysuria, further illustrating the intersection of food and health in traditional practices.

In summary, the versatile applications of flowers and seeds highlight the profound relationship between nature and health within various cultures. Whether used in decoctions for serious medical conditions or as food that promotes wellness, these botanical elements reflect an enduring reliance on nature's remedies in traditional medicine practices across the globe.

- *Antibacterial Effects of Cassia Siamea Leaf Extracts*

Cassia siamea, commonly known for its traditional use in treating infectious diseases, has gained significant scientific interest due to its antibacterial properties. Recent studies utilizing cylinder plate assays have thoroughly investigated the antibacterial efficacy of methanol and other solvent extracts of C. siamea leaves against a range of Gram-positive and Gram-negative bacterial species, offering valuable insights into its potential as a natural antimicrobial agent (Fonmboh et al., 2020). The methanol leaf extract of *Cassia siamea* demonstrated strong antibacterial activity against *Bacillus cereus* and *Listeria monocytogenes*, with IC50 values of 5.2 mg/mL and 20.8 mg/mL, respectively, after 24 hours of exposure at 37°C. However, the extract exhibited lower efficacy against other tested pathogens, including Escherichia coli and Klebsiella pneumoniae, with IC50 values reaching as high as 166.7 mg/mL. This marked difference in antibacterial activity against specific bacteria highlights the necessity for further exploration of the extract's mechanisms and its active components(Bukar et al., 2009).

In terms of efficacy, the hexane extract of *C. siamea* revealed strong activity against Corynebacterium diphtheria, Salmonella typhi, and Shigella species, exhibiting promising results within the same exposure conditions. Interestingly, certain bacterial strains, such as *Staphylococcus aureus* and *Staphylococcus pyogenes*, showed resistance, suggesting a selective antibacterial profile that could be harnessed in targeted therapeutic applications.

Furthermore, the ethanol leaf extracts outperformed ciprofloxacin (30 µg/disc) against Staphylococcus aureus, revealing promising antibacterial potential. Notably, at a concentration of 40 mg/mL, the ethanol and acetone extracts displayed significant inhibitory action against Salmonella typhi, with inhibition zone values indicating substantial activity compared to standard antibiotics such as ampicillin and chloramphenicol(Abdisa & Kenea, 2020).

The observed antibacterial properties of *C. siamea* leaves can be attributed to various phytochemicals present in the extracts, including alkaloids, phenolics, and sterols. Specific compounds like barakol have been identified as influential in eliciting antibacterial responses against both Gram-positive and Gram-negative bacteria. Moreover, the combination of C. siamea extracts with other botanicals, such as Momordica charantia Linn, has demonstrated synergistic effects, amplifying its antibacterial activity against several pathogens.

In conclusion, the antibacterial effects of *Cassia siamea* leaf extracts present a compelling case for its use in developing alternative therapeutic strategies against infective agents. Continued investigation into its bioactive compounds and mechanisms of action will be crucial in determining its full potential within the realm of herbal medicine and antibiotic alternatives(Lemaire & Adosraku, 2002).

- *The Chemistry of Cassia Siamea*

Cassia siamea, commonly known as *Siamese cassia*, has become a focal point in phytochemical research due to its extensive range of bioactive compounds and their therapeutic potential, particularly in the domain of antimalarial activity. Preliminary phytochemical screenings have revealed a rich composition of secondary metabolites, including chromones, polyphenols, alkaloids, saponins, and various vitamins and minerals. Among these, the presence of chromones and their derivatives, such as chromone alkaloids and bischromones, has significant implications for the plant's medicinal properties (Lemaire & Adosraku, 2002).

The alkaloid fraction obtained from the leaves of *Cassia siamea* has demonstrated superior antiplasmodial activity compared to

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other extracts. Notably, cassiarin A, an isolated compound with potent biological effects, exhibits an IC50 value of 0.005 µg/mL against the 3D7 strain of Plasmodium falciparum, indicating its efficacy is comparable to that of the conventional antimalarial drug chloroquine. This remarkable activity highlights the potential of cassiarin A as a promising candidate for developing new malaria treatments.

Furthermore, other compounds derived from *C. siamea*, such as cassiarin J and K, also show noteworthy antiplasmodial activities, albeit at higher IC50 values. The variability in the strength of these compounds underscores the importance of further research into their pharmacological properties. Studies have also indicated that the efficacy of these compounds extends beyond antimalarial applications, with activities noted against bacterial strains such as Escherichia coli.

In addition to its bioactive alkaloids, the phytochemical profile of *Cassia siamea* encompasses a variety of polyphenolic compounds. These include flavonoids and tannins, which possess well- documented antioxidant properties, further contributing to the plant's therapeutic potential. Emodin and lupeol, derived from the ethyl acetate fraction of *Cassia siamea*, have also demonstrated significant antibacterial activity (Su et al., 2021)

In conclusion, the chemistry of *Cassia siamea* is marked by its diverse array of phytochemicals, with alkaloids playing a critical role in its antiplasmodial and antibacterial properties. The promising results from studies on compounds like cassiarin A pave the way for future pharmaceutical applications. Continued exploration of the plant's phytochemistry is essential for unlocking its full potential as a source of novel therapeutics, particularly in the fight against malaria and bacterial infections.

➢ *Chemical Composition of Cassia Siamea*

Cassia siamea, commonly known as the *Siamese cassia*, is a plant of considerable interest in phytochemical research due to its diverse bioactive compounds. Various parts of the plant, including leaves, stem bark, root bark, flowers, and seeds, yield a complex array of molecular groups that contribute to its pharmacological profile. This essay summarizes the chemical constituents found in the leaves, stem bark, root bark, flowers, and seeds, as highlighted in Table The leaves of *Cassia siamea* are rich in chromone alkaloids such as Barakol and cassiarins, alongside a plethora of other molecular groups including anthraquinones, bianthraquinones, and flavonoids. Noteworthy compounds identified in methanolic extracts include Anhydrobarakol, multiple hydroxychromones, and various anthraquinones like chrysophanol and emodin. The leaves also contain essential vitamins (A, C, and E) and carotenoids, indicative of their potential antioxidant properties (Su et al., 2021).

In contrast, the stem bark demonstrates a robust mineral profile, featuring essential elements such as iron, magnesium, and potassium. The stem bark's methanolic extracts reveal multiple bianthraquinones and flavonoids, which may contribute to the therapeutic properties of the plant. Significant compounds include lupeol and friedelin, both known for their biological activities.

Root bark extracts are highlighted for their content of anthraquinones and bianthraquinones, showcasing compounds like chrysophanol and various forms of cassiamin. This composition suggests potential for medicinal applications, particularly in traditional remedies. (Lemaire & Adosraku, 2002).

Flowers of *Cassia siamea* present diverse phytochemicals primarily in chloroform and methanolic extracts, featuring alkaloids such as Barakol and various phenolic acids. Flavonoids such as rutin and myricetin are reported, adding to the flower's nutritional and medicinal value.

Lastly, the seeds are notably rich in fatty acids and sterols. Compounds such as cholesterol and β- sitosterol, along with significant fatty acids like oleic and linoleic acids, suggest potential health benefits, including anti-cholesterol properties.

In summary, *Cassia siamea* is a repository of various bioactive compounds across its different plant parts, with implications in both traditional medicine and modern therapeutics. The breadth of its chemical composition underscores the necessity for further research to fully elucidate its pharmacological potential. Such investigations can pave the way for innovative applications in health and wellness sectors.

Table 3 Chemical Composition of *Cassia siamea* by Plant Part and Extract Type

Plant Part	Extract Type	Compound Class	Specific Molecules
Leaves	Chloroform	Chromone alkaloids	Barakol
	Methanol	Chromones	Anhydrobarakol; 5-acetonyl-7-hydroxy-2-methylchromone; 5-acetonyl-7-hydroxy-2- hydroxymethylchromone
		Chromone alkaloids	Cassiarin A, Cassiarin B

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		Anthraquinones	Chrysophanol; Emodin; Physcion; Rhein; Sennosides
		Bianthraquinones	Cassiamin A, Cassiamin B
		Bischromones	Chrobisiamone A; Resins
	Ethanol	Triterpenoid	Lupeol
		Flavonoids	D-pinitol; Luteolin
		Dihydronaphthalenone	4-(trans)-acetyl-3,6,8-trihydroxy-3-methyl-DHN; 4-(cis)-acetyl-3,6,8-trihydroxy-3-methyl-DHN
	Hydroalcoholic	Steroids	β- and γ-sitosterol
		Carotenoids	Carotenes; Xanthophylls
		Vitamins	Vitamin A, C, E
	Aqueous	Isoflavone glycoside	2',4',5,7-tetrahydroxy-8-C-glucosylisoflavone
Stem Bark	Hexane	Minerals	Fe, Mg, Mn, K, Ca, Na, Cu, Cd, Pb, P
	Methanol	Bianthraquinones	Various cassiamins; Madagascarin
		Anthraquinones	Chrysophanol; Emodin; Physcion; Glycosylated anthraquinones
		Flavonoids	Piceatannol
		Triterpenoid glycoside	19,24-dihydroxy-12-ene-28-oic acid-3-O-D-xylopyranoside
		Triterpenoids	Lupeol; Friedelin
		Minerals	Fe, Mg, Mn, K, Ca, Na, Cu, Pb, Cr, Ni, Zn
	Chloroform	Triterpenoid	Betulinic acid
		Phenolic	Coumarin
		Chromones	Siamchromones A–G
	n-Butanol	Chromone glycosides	Two chromone-glucosides with hydroxypropyl and propyl substituents
		Flavonoid	Kaempferol
Root Bark	Methanol	Anthraquinones	Chrysophanol; Emodin
		Bianthraquinones	Cassiamin A, B; other complex bianthracenes
Flowers	Chloroform	Chromone alkaloids	Barakol; 10,11-dihydroanhydrobarakol; Cassiarin C–F
	Methanol	Chromone alkaloids	Cassiadinine
		Phenolic acids	Gallic, Protocatechuic, p-Hydroxybenzoic, Chlorogenic, Vanillic, Caffeic, Syringic, p-Coumaric, Ferulic, Sinapic acids
		Flavonoids	Rutin; Myricetin; Quercetin; Kaempferol
Seeds	Hexane	Steroids	Cholesterol; Stigmasterol; β-sitosterol
		Fatty acids	Palmitic; Stearic; Oleic; Linoleic acids
	Aqueous	Anthraquinones	Aloe-emodin; Sennosides A1

➢ *Structural Description of the Phytoconstituents of Cassia siamea*

Cassia siamea, commonly known as the yellow *cassia or Siamese cassia*, is a plant renowned for its rich phytochemical profile. Among its assorted constituents, chromones and their derivatives stand out for their diverse biological activities and potential therapeutic applications. This essay will explore the structural characteristics of notable chromones, and derivatives isolated from *Cassia siamea*, emphasizing their unique molecular frameworks.

- *Chromones and Their Derivatives*

Chromones are a class of compounds characterized by a chromone nucleus, which consists of a benzopyran structure. The derivatives of chromones found in *Cassia siamea* exhibit various substitutions that influence their biological activity. Key examples include Barakol and the cassiarin series (A, B, J, G, and K). For instance, Barakol is recognized for its anti-inflammatory properties and showcases a hydroxy and acyl substituent arrangement that is vital for its activity. The cassiarin derivatives, such as cassiarin A through K, demonstrate diverse substitution patterns on the chromone nucleus. These variations in R groups, particularly at positions 5, 6, 7, and 12, result in altered biochemical interactions and activities. For instance, the presence of hydroxyl groups tends to enhance antioxidant activity, while methyl and acyl groups may contribute to anti-microbial properties.

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Among the derivatives, compounds such as Cassiadinine and chloro-substituted cassiarins emphasize the structural diversity that contributes to the pharmacological potential of these phytochemicals. The inclusion of acetate and hydroxymethyl groups in some derivatives further highlights their complex structures, facilitating different pharmacodynamic profiles.

- *Anthraquinones*

In addition to chromones, Cassia siamea is also rich in anthraquinones. The structural features of anthraquinones, which consist of a three-ringed system linked to a central quinone moiety, enhance their therapeutic potential. Notable anthraquinones isolated from Cassia siamea include cassiamins A, B, and C, each bearing multiple hydroxyl groups which are crucial for their biochemical activity. Madagascarin, another significant anthraquinone, exhibits structural similarities yet distinct hydroxyl substitutions that can influence its solubility and efficacy.

- *Flavonoids and Triterpenoids*

The flavonoids luteolin and kaempferol, along with the triterpenoid lupeol, further enrich the phytochemical profile of Cassia siamea. Luteolin and kaempferol, both possessing multiple hydroxyl groups, are well-known for their antioxidant and anti-inflammatory properties. Lupeol, on the other hand, is a pentacyclic triterpene with demonstrated anti-cancer attributes, showcasing the versatility of the phytoconstituents present in this plant.

The phytoconstituents of Cassia siamea illustrate a remarkable array of structural variations that contribute to their potential therapeutic benefits. Chromones, particularly in the cassiarin series, exemplify the complexity and versatility of this plant's bioactive molecules. Understanding the structural nuances of these compounds paves the way for further exploration into their applications in medicine and nutrition, reflecting the broader importance of plant-derived substances in contemporary science. The ongoing research into the phytochemical landscape of Cassia siamea holds promise for the development of novel therapeutic agents.

Table 4 Chromones and Derivatives

Compound Name	Notes
Barakol	Chromone alkaloid
Cassiarin A	Chromone alkaloid
Cassiarin B	Chromone alkaloid
Cassiarin J	Chromone derivative
Cassiarin K	Chromone derivative
Cassiarin H	Chromone derivative
Cassiarin G	Chromone derivative
Anhydrobarakol	Chromone derivative
Cassiadinine	Chromone alkaloid
Chrobisiamone A	Bischromone
5-acetonyl-7-hydroxy-2-methylchromone	Chromone
5-acetonyl-7-hydroxy-2-hydroxymethylchromone	Chromone
2-methyl-5-propyl-7,12-dihydroxy-chromone-12-O-β-D- glucopyranoside	Chromone glycoside

Table 5 Chromone Substitution

Compound No.	R1	R2	R3	R4	R5
5	H	OH	CH_3	CH_3	H
6	H	OH	CH_3	CH_3	OH
7	H	H	CH_3	CH_3	H
8	H	CH_3	OH	CH_3	OH
9	H	CH_3	OH	CH_3	H
10	Cl	OH	CH_3	CH_3	H
11	H	H	CH_2OH	CH_2OH	H
12	H	CH_3	OH	CH_2OH	H

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Table 6 Anthraquinones and Bianthraquinones

Compound Name	Structure Type
Cassiamin A (5)	Bianthraquinone
Cassiamin B (6)	Bianthraquinone
Cassiamin C (7)	Bianthraquinone
Madagascarin (8)	Bianthraquinone
1,1’,3,8,8’-pentahydroxy-3’,6-dimethyl-[2,2’-bianthracene]- 9,9’,10’-tetrone (9)	Bianthracene
7-chloro-1,1’,6,8,8’-pentahydroxy-3,3’-dimethyl-[2,2’- bianthracene]-9,9’,10,10’-tetrone (10)	Chlorinated bianthracene
1,1’,8,8’-tetrahydroxy-3,3’-dihydroxymethyl-[2,2’- bianthracene]-9,9’,10,10’-tetrone (11)	Hydroxymethyl bianthracene
1,1’,3,8,8’-pentahydroxy-3-hydroxymethyl-6-methyl-[2,2’- bianthracene]-9,9’,10,10’-tetrone (12)	Methyl-hydroxymethyl bianthracene
4,4-bis(1,3-dihydroxy-2-methyl-6,8-dimethoxyanthraquinone)	Bianthraquinone

Table 7 Flavonoids and Triterpenoids

Compound Name	Type
Luteolin	Flavonoid
Kaempferol	Flavonoid
Lupeol	Triterpenoid

➢ *Solvents for Plant Extraction: Implications for Bioactivity*

The extraction of bioactive compounds from plant sources is a crucial step in the development of natural products, particularly in the fields of pharmaceuticals and nutraceuticals. The type of solvent utilized in this process significantly influences both the nature of the compounds extracted and the resultant biological activity. The polarity of solvents non-polar, polar, and less polar plays a pivotal role in determining the efficiency of extraction and the types of bioactive compounds that can be isolated. Consequently, selecting an appropriate solvent is essential for optimizing extraction protocols to maximize the yield of potential active constituents.

Several factors contribute to the efficacy of solvent extraction, including the rate of extraction, the quantity of compounds retrieved, the handling of extracts, and the toxicity of the solvents themselves. These factors must be thoroughly evaluated to ascertain the value of a particular solvent. Research has shown that a variety of solvents yield different extraction outcomes. For instance, methanol has been reported to facilitate superior antimicrobial activity when extracting compounds from the rhizome and leaves of plants, while hexane demonstrated no such activity in certain studies. Specific examples include petroleum ether and methanolic extracts of *Cassia occidentalis* leaves, which exhibited effectiveness against *E. coli* at a concentration of 400 mg/mL, showcasing inhibition zones of 5 to 11 mm.

Water, often referred to as a universal solvent, is frequently employed in traditional medicine preparations due to its ability to dissolve numerous natural products, including pigments and bioactive components. Despite its high yield potential, water extracts have sometimes exhibited lower antimicrobial activities. Conversely, solvents such as acetone have proven effective for both polar and non-polar compounds, making them popular choices in extraction processes. For example, n-hexane extracts from *G. kola* seeds displayed remarkable activity against Vibrio species, while ethyl acetate extractions revealed a broad spectrum of effectiveness against both gram-positive and gram-negative bacteria *(“(PDF) Antimicrobial Activity of Leaf Extracts of Senna Obtusifolia (L),” n.d.).*

Recent investigations into the leaves and stem bark of *Cassia siamea* using methanol and distilled water indicate that organic solvent extracts generally show promising antibacterial activity. This underscores the critical role of solvent selection in the extraction process, as different solvents can significantly affect the bioactivity of the final extract. In conclusion, optimizing extraction efficiency through careful solvent choice is essential for harnessing the full potential of plant- derived bioactive compounds, ultimately aiding in the development of effective antimicrobial agents.(*Vol. 7 No. 20 (2008) | African Journal of Biotechnology*, n.d.)

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CHAPTER THREE
METHODS AND RESULTS

➢ *Collection of Plant Material: Cassia siamea*

The collection of plant material is a crucial step in botanical research, serving as the foundation for various scientific inquiries, including pharmacological studies, ecological assessments, and biodiversity conservation. In April 2020, leaves and stem bark of Cassia siamea were meticulously gathered during the morning hours in a forest located in the village of Makotha, Marampa Chiefdom, Port Loko District.

This collection process was conducted with due regard for ethical and sustainable harvesting practices, ensuring minimal disturbance to the surrounding ecosystem. Cassia siamea, a species known for its medicinal properties and ecological significance, necessitates careful handling and proper identification to maintain the integrity of the samples. Following collection, the plant material was transported to the Department of Botany at Fourah Bay College, University of Sierra Leone, where it underwent a thorough identification and authentication process. This step is vital in confirming the species' identity and ensuring that subsequent research is based on accurate and valid data.

The authentication, conducted by botanists with expertise in local flora, not only reinforces the scientific validity of the collected samples but also contributes to a broader understanding of Cassia siamea within its natural habitat. It emphasizes the importance of rigorous methodologies in botanical studies and sets a precedent for future research endeavors in the region. Through such meticulous processes, we can enhance our knowledge of plant species and their potential applications, thus fostering greater appreciation and conservation of biodiversity.

➢ *Sources of Microorganisms: A Focus on Pathogenic Strains*

Microorganisms play a crucial role in various biological processes, but certain strains pose significant threats to human health, particularly in healthcare settings. This essay examines three prominent pathogens: *Streptococcus pyogenes, Staphylococcus aureus*, and *Salmonella typhimurium*. These organisms were selected for study due to their prevalence and alarming trend of increasing antibiotic resistance.

Streptococcus pyogenes is an important human pathogen responsible for a range of illnesses, from mild infections such as pharyngitis to more severe conditions like necrotizing fasciitis. Its capacity to develop resistance to common antibiotics makes it a significant challenge for healthcare practitioners. The organism's frequency in clinical infections emphasizes the need for ongoing surveillance and research.

Staphylococcus aureus, particularly methicillin-resistant strains (MRSA), has become a leading cause of nosocomial infections. This bacterium's ability to survive in diverse environments and its pathogenic potential have made it a focus of concern in hospital settings. The increasing rate of antibiotic resistance in *Staphylococcus aureus* necessitates new therapeutic strategies and stringent infection control measures.

Salmonella typhimurium, known for causing gastroenteritis, is another major concern, especially in the context of foodborne illness. It has demonstrated increasing resistance to multiple antibiotics, complicating treatment options and prolonging illness duration. Studies on this organism are particularly relevant as they address not only individual health impacts but also public health implications stemming from food safety.

The strains selected for this study were sourced from the Microbiology Laboratory at Connaught Teaching Complex Hospital in Freetown. This setting provides a pertinent backdrop for isolating and analyzing these pathogens, given the hospital's role in treating numerous cases of infections caused by these microorganisms.

➢ *Extraction and Preparation of Plant Materials for Phytochemical Analysis*

The extraction of phytochemicals from plant materials is a vital process in botanical research, contributing significantly to the understanding of their biological activities and potential therapeutic applications. This essay discusses a systematic approach to preparing leaf and stem bark samples for such analyses.

Initially, the leaf and stem bark were harvested from the selected plant and subsequently subjected to a rigorous cleaning process. This involved washing the samples to remove any surface debris or contaminants, ensuring that the integrity of the plant material was maintained. Following the washing procedure, the samples were air-dried for a period of five days at room temperature. This drying phase is crucial, as it facilitates the removal of moisture, thus minimizing the risk of microbial growth and degradation of the phytochemicals of interest.

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Once thoroughly dried, the leaf and stem bark were pounded using a pestle and mortar, resulting in a powdered form that increases the surface area of the plant material. This step is instrumental in enhancing the efficiency of subsequent extraction processes, as it allows for better solvent penetration and improved release of the bioactive compounds from the plant matrix.

The powdered samples were then subjected to maceration in a solvent mixture of methanol and water. The choice of solvent is critical, as it influences the solubility of specific phytochemicals. Methanol is known for its ability to extract a wide range of polar and non-polar compounds, making it an ideal choice for this type of extraction. The maceration process allows the phytochemicals to dissolve in the solvent over an extended period, leading to optimal extraction yields.

Following maceration, the mixture was filtered to separate the liquid extract from the solid residues. This step is paramount in isolating the desired phytochemical components for further analysis. To concentrate the extract, a portion of the filtrate underwent evaporation using a rotary evaporator. This technique enables the removal of the solvent under reduced pressure, allowing the extracted phytochemicals to remain intact without degradation caused by excessive heat.

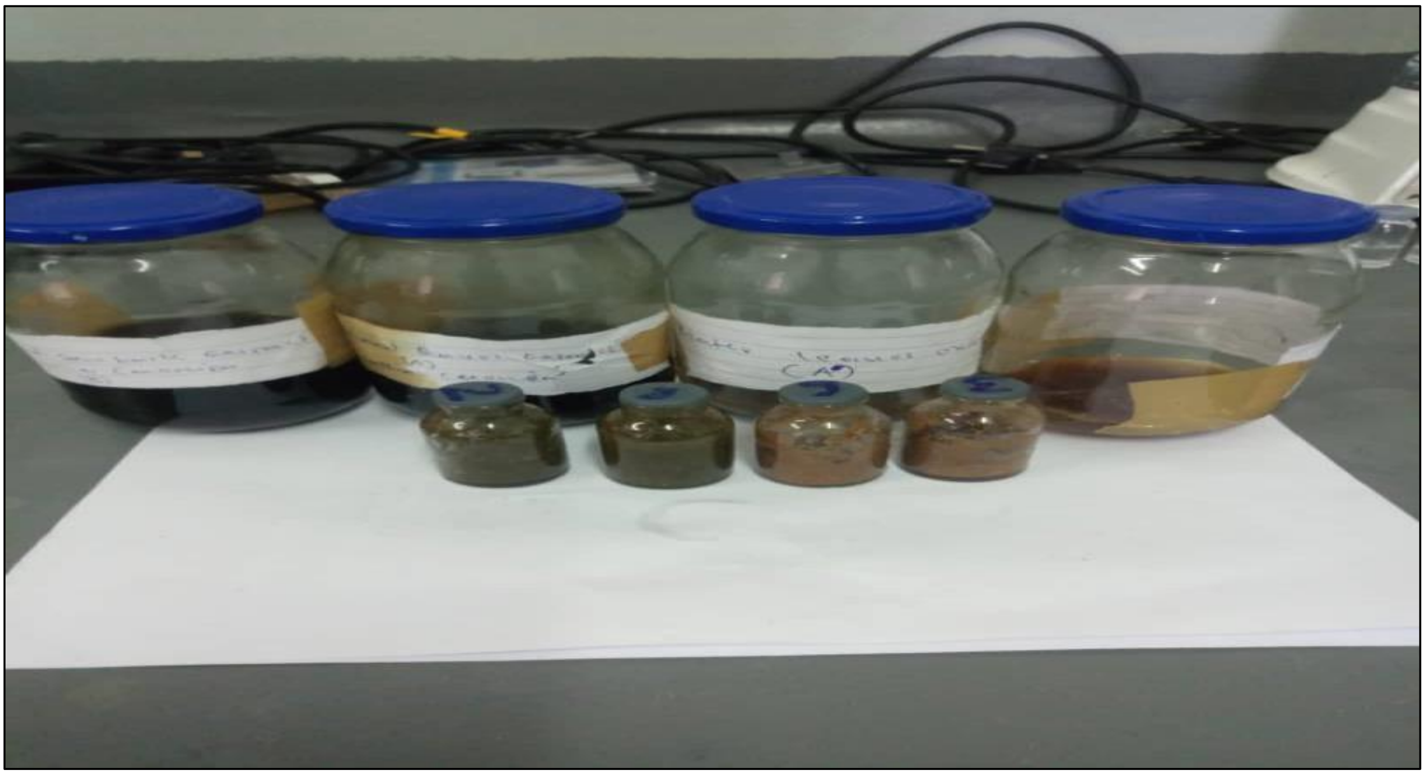

Fig 2 Filtrate and Crude Extracts Derived from the Leaf and Stem Bark of *Cassia siamea*.

➢ *Phytochemical Analysis: A Key to Understanding Plant Constituents*

Phytochemical analysis is a crucial step in the exploration and characterization of plant-derived compounds, offering insights into their potential therapeutic and nutritional benefits. This analytical approach involves the systematic examination of plant extracts to identify various bioactive constituents, including alkaloids, flavonoids, tannins, glycosides, and saponins, among others. Utilizing established methodologies, such as those outlined by Trease and Evans and Sofowora, researchers can uncover the rich chemical diversity present in different plant species(*Guidelines for the Use and Interpretation of Assays for Monitoring Autophagy (3rd Edition) - PubMed*, n.d.).

In conducting phytochemical analysis, crude extracts are prepared from selected plant materials, which are then subjected to specific chemical tests aimed at identifying the presence of phytochemicals. These tests often rely on color change reactions, precipitation, and other observable phenomena that indicate the presence of targeted compounds. For instance, the detection of flavonoids may involve the formation of a distinct color upon the addition of certain reagents, while alkaloids can be identified through the formation of complex salts.

The methodologies put forth by Trease and Evans, as well as Sofowora, serve as vital references in this domain, ensuring that the analysis adheres to recognized scientific standards. Their detailed protocols not only facilitate consistency and reproducibility in

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phytochemical investigations but also enable researchers to accurately interpret the results, leading to informed conclusions about the potential health benefits and pharmacological properties of the studied plants.

Phytochemical analysis plays a significant role in the fields of ethnobotany, pharmaceutical research, and natural product development. Understanding the phytochemical profile of plants can guide the discovery of novel compounds for drug development and promote the use of herbal remedies in traditional medicine. Moreover, as the global interest in natural products continues to rise, phytochemical analysis stands as a cornerstone of research that seeks to bridge traditional knowledge with modern scientific inquiry.

In conclusion, the identification of phytochemicals through systematic analysis not only enhances our understanding of plant biology but also opens avenues for potential applications in medicine and nutrition. The chemical tests performed according to the established procedures of Trease and Evans and Sofowora are instrumental in this endeavor, underscoring the importance of rigorous scientific investigation in uncovering the wealth of bioactive constituents in the plant kingdom (*Guidelines for the Use and Interpretation of Assays for Monitoring Autophagy (3rd Edition) - PubMed*, n.d.).

➢ *Phytochemical Screening Procedures*

- *Test for Glycosides*

One milliliter (1 mL) of the plant extract was combined with 2 mL of acetic acid and the mixture was cooled in an ice bath maintained at 4°C. Subsequently, 1 mL of concentrated sulfuric acid (H_2SO_4) was added dropwise. The formation of an oily layer on the surface of the solution was indicative of the presence of glycosides.

- *Test for Alkaloids*

Three milliliters (3 mL) of the extract were mixed with 1 mL of 1% hydrochloric acid (HCl). To this solution, a few drops of Mayer's reagent were added. The formation of a creamy white precipitation signified the presence of alkaloids.

- *Test for Saponins*

Five drops of olive oil were added to 2 mL of the plant extract. The mixture was then vigorously shaken. The development of a stable emulsion was taken as evidence for the presence of saponins.

- *Test for Tannins*

Two drops of 5% ferric chloride ($FeCl_3$) solution were added to 1 mL of the plant extract. The appearance of a dirty-green precipitate confirmed the presence of tannins.

- *Test for Flavonoids*

To 1 mL of the extract, 3 drops of ammonia solution (NH_3) were added, followed by the addition of 0.5 mL concentrated hydrochloric acid (HCl). The pale brown coloration of the mixture was considered a positive indication of flavonoids.

- *Test for Anthraquinones (Bornträger's Test)*

One milliliter (1 mL) of the plant filtrate was shaken with 10 mL of benzene, and the mixture was subsequently filtered. To the filtrate, 5 mL of 10% (v/v) ammonia solution was added and the mixture was shaken again. The appearance of a pinkish coloration indicated the presence of anthraquinones.

- *Test for Steroids*

One milliliter (1 mL) of the extract was treated with 1 mL of concentrated sulfuric acid. The development of red coloration was interpreted as a positive result for steroids.

- *Test for Terpenoids*

Five milliliters (5 mL) of the extract were mixed with 2 mL of chloroform. Concentrated sulfuric acid was then carefully added along the side of the test tube. The formation of a reddish-brown layer at the interface confirmed the presence of terpenoids.

- *Test for Phenolic Compounds (Lead Acetate Test)*

A portion of the plant extract was dissolved in distilled water to form a solution. A few drops of lead acetate solution were then added to this mixture. The formation of a white precipitate was taken as a positive indication of the presence of phenolic compounds, due to the reaction between phenols and lead ions resulting in the formation of insoluble lead-phenolate complexes.

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Table 7 Phytochemical Constituents of *Cassia siamea* Leaf and Stem Bark

Compound	Leaf	Stem
Alkaloids	–	–
Anthraquinones	–	–
Flavonoids	+	+
Glycosides	++	+
Saponins	+	++
Tannins	++	++
Steroids	+	+
Terpenoids	–	–
Phenols	+	–

Legend: "+" = Present; "++" = Strongly Present; "–" = Absent

➢ *Sterility Test of Plant Extracts*

The assessment of sterility in plant extracts, particularly aqueous and methanolic extracts, is a critical preliminary step before evaluating their potential antimicrobial properties. This process ensures that any observed antimicrobial activity during subsequent tests is attributable solely to the active compounds within the extracts and not to contaminants that may interfere with the results.

In this study, sterilization efficacy was evaluated by inoculating 1 ml samples of both the aqueous and methanolic extracts onto nutrient agar plates. These plates were incubated at a controlled temperature of 37°C for 24 hours. The choice of nutrient agar provides an enriched medium conducive to the growth of a wide array of microbial organisms, thereby making it an effective medium for sterility testing.

After the incubation period, the plates were inspected for signs of microbial growth. The absence of growth in both the aqueous and methanolic extracts post-incubation indicated that the extracts were indeed sterile. This finding is significant, as it validates the integrity of the extracts and confirms that no extraneous microbial contaminants were present during the antimicrobial activity assessment.

Following the successful sterility test, the next phase involved evaluating the antimicrobial activity of the extracts. This step is crucial for determining the potential therapeutic applications of the plant extracts. By ensuring that the extracts are free from contamination, researchers can accurately attribute any antimicrobial effects observed to the phytochemicals present within the extracts.

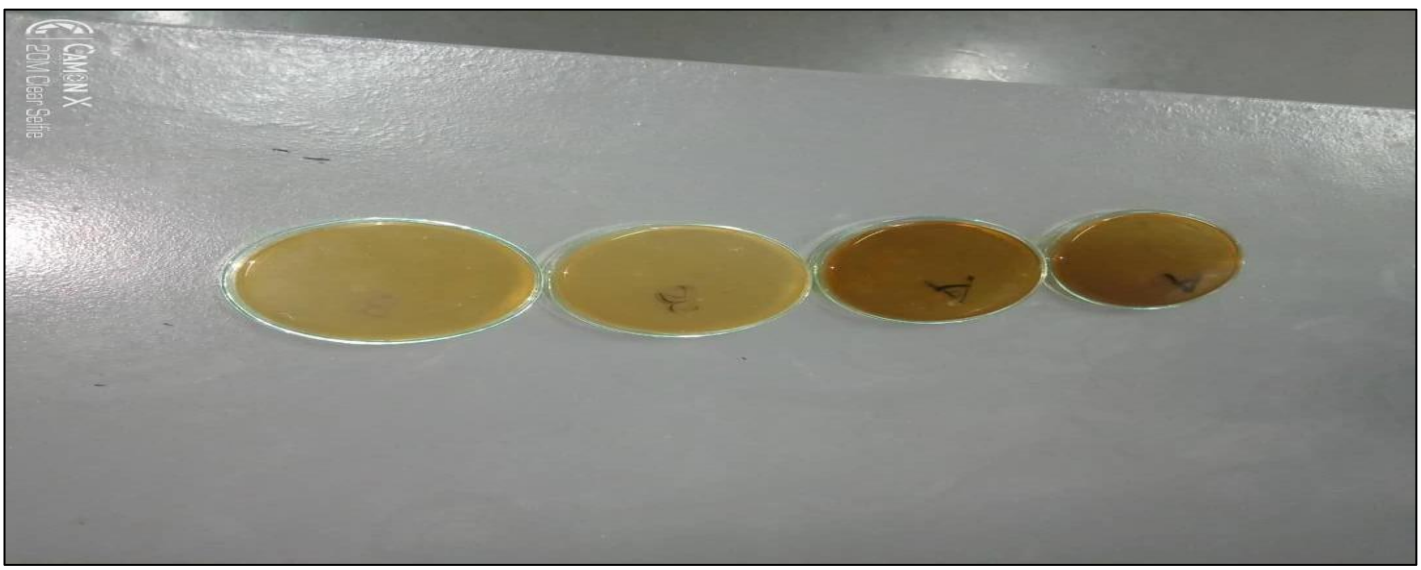

Fig 3 Filtrate and Crude Extracts of *Cassia siamea* Leaf and Stem Bark in Petri Dishes

- *Standardization of Inoculums for Microbiological Testing*

In microbiological analysis, the standardization of inoculums is a critical step that ensures the reliability and reproducibility of test results. This process is particularly vital when working with bacterial isolates, as variations in inoculum size can significantly affect the outcomes of assays, including antimicrobial susceptibility tests. In this study, bacterial isolates of test organisms were obtained from stock cultures maintained at the Department of Microbiology Laboratory, Connaught Teaching Complex Hospital, Freetown.

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The initial phase of standardization involved the resuscitation of the bacterial cultures. Test organisms were inoculated onto nutrient agar plates and incubated at 37°C for 24 hours to ensure robust growth. Post-incubation, a loopful of each bacterial isolate was carefully picked using a sterile wire loop to prevent contamination. The selected isolates were then emulsified in 3 mL of sterile physiological saline, creating a suspension suitable for further analysis.

To achieve accurate standardization, the turbidity of the bacterial suspension was compared to that of a 0.5 McFarland standard. The McFarland standard is a widely accepted reference in microbiology that provides a visual baseline for the turbidity associated with a specific bacterial concentration, typically around 1.5 x 10^8 CFU/mL for the 0.5 McFarland standard. Matching the turbidity ensures that the inoculums used in subsequent testing are within a consistent range, thereby enhancing the reliability of the experimental findings.

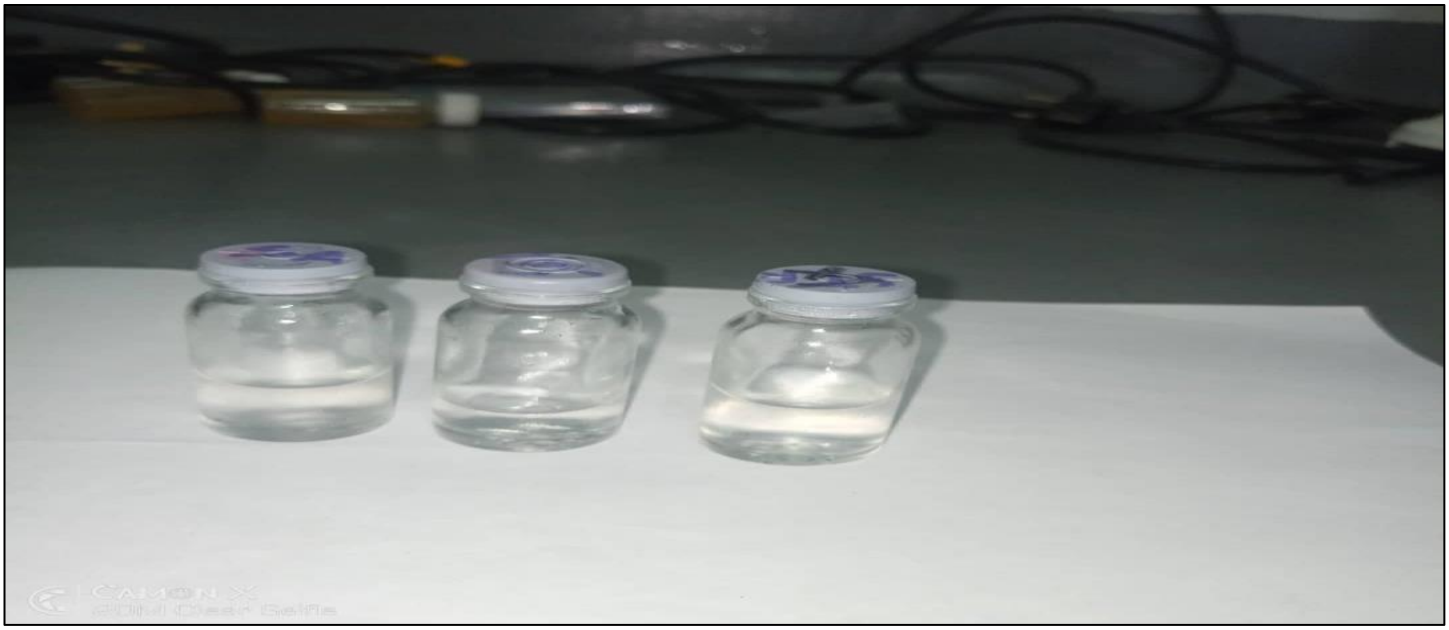

Fig 4 Bacterial Isolates Emulsified in Sodium Chloride (NaCl) Solution

- *Antimicrobial Activity Testing of Cassia siamea Crude Extracts*

Antimicrobial activity testing is a crucial aspect of evaluating the therapeutic potential of natural products, particularly those derived from medicinal plants. This study investigates the antimicrobial efficacy of crude extracts from the leaf and stem bark of *Cassia siamea* using the well diffusion method.

To conduct sensitivity testing, bacterial isolates of *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Salmonella typhimurium* were used. These microorganisms were cultivated on Mueller Hinton agar, a standard medium that supports the growth of a wide variety of bacteria, making it an ideal choice for antimicrobial susceptibility testing. Following the preparation of the agar plates, the surfaces were uniformly swabbed with a cotton wool applicator moistened with bacterial suspensions standardized to a 0.5 McFarland turbidity standard, ensuring consistent inoculum density across the tests.

For each of the twelve agar plates, a sterile 3 mm cork borer was utilized to create wells, into which 0.5 ml of each crude extract was introduced aseptically. It is essential to allow the extracts to diffuse into the medium for approximately 30 minutes before incubation; this ensures optimal interaction between the antimicrobial compounds and the bacterial strains. Subsequently, the plates were incubated at 37°C for 24 hours to facilitate bacterial growth and possible inhibition.

The determination of antimicrobial activity was based on the presence and measurement of zones of inhibition surrounding the wells containing the crude extracts. A clear zone indicated effective antimicrobial action, with the diameter measured in millimeters to quantify the sensitivity of the bacterial isolates to the extracts.

This methodology provides a reliable assessment of the potential of *Cassia siamea* as an antimicrobial agent, contributing to the broader scope of research in natural product pharmacology. The results could pave the way for further exploration and development of plant-based antibiotics, which are crucial in the face of rising antibiotic resistance.

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Table 8 Antibacterial Activity of Cassia siamea Leaf Extracts against Selected Test Organisms

Test Organisms	Methanol Extract (mm)	Aqueous Extract (mm)
Streptococcus pyogenes	9.50	10.00
Salmonella typhimurium	14.00	14.00
Staphylococcus aureus	13.00	15.00

Table 9 Antibacterial Activity of Cassia siamea Stem Bark Extracts against Selected Test Organisms

Test Organisms	Methanol Extract (mm)	Aqueous Extract (mm)
Streptococcus pyogenes	14.00	4.00
Salmonella typhimurium	8.00	0.00
Staphylococcus aureus	16.00	4.50

- *Determination of Minimum Inhibitory Concentration (MIC) of Cassia siamea Extracts*

The determination of the Minimum Inhibitory Concentration (MIC) serves as a crucial parameter in assessing the antimicrobial efficacy of plant extracts. This study focuses on the MIC of the crude extracts derived from the leaf and stem bark of *Cassia siamea*; a plant recognized for its medicinal properties. The evaluation was conducted through a systematic dilution approach, generating various concentrations of the extracts specifically, 10, 5, 2.5, 1.25, and 0.65 mg/ml.

To perform the MIC determination, equal volumes of each diluted crude extract and nutrient broth were mixed in test tubes to create a conducive environment for bacterial growth. A standardized inoculum, specifically 0.1 ml at a concentration of 1.0 x 10^4 CFU/ml, was then introduced into each tube. The incubation of the tubes was conducted aerobically at a controlled temperature of 37°C for 24 hours. This incubation period is essential for allowing any potential antimicrobial activity to manifest.

For accuracy, control tubes containing the extract and growth media but lacking inoculum were utilized to ensure that any observed effects were solely attributable to the extract's antimicrobial properties. Following the incubation period, the evaluation of bacterial growth was performed through the observation of turbidity in each tube. The MIC was identified as the lowest concentration of the extract that resulted in no visible bacterial growth characterized by the absence of turbidity when compared to the control tubes.

Table 10 Minimum Inhibitory Concentration (MIC) of Aqueous Leaf Extract of *Cassia siamea* Against Test Organisms

Test Organisms	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.65 mg/mL
Streptococcus pyogenes	–	–	+	+	+
Salmonella typhimurium	–	–	–	+	+
Staphylococcus aureus	–	–	–	+	+

Table 11 Minimum Inhibitory Concentration (MIC) of Methanol Stem Bark Extract of *Cassia siamea* Against Test Organisms

Test Organisms	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.65 mg/mL
Streptococcus pyogenes	–	+	+	+	+
Salmonella typhimurium	+	+	+	+	+
Staphylococcus aureus	–	–	+	+	+

- *Legend:*

– = No growth (inhibition observed)
+ = Growth observed (no inhibition)

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CHAPTER FOUR
DISCUSSION

➢ *Discussion on the Phytochemical Analysis of Cassia siamea*

Phytochemical analysis plays a critical role in identifying the secondary metabolites present in plant compounds that not only serve as natural defenses against microbial pathogens and pests but also exhibit significant biological and pharmacological activities. These bioactive constituents have been widely recognized for their potential in the development of therapeutic agents and modern pharmaceuticals.

In this study, the phytochemical screening of the leaf and stem bark of *Cassia siamea* revealed the presence of flavonoids, saponins, tannins, steroids, and glycosides, while phenols were detected exclusively in the leaf. Notably, alkaloids, anthraquinones, and terpenoids were absent in both plant parts, as presented in Table 1. These findings are consistent with those reported by Bukar et al. (2009), who also observed the presence of tannins and steroids in *C. siamea* leaves.

The identified phytochemicals are known to contribute to the medicinal value of plants. Saponins and tannins, for instance, possess antimicrobial properties and are effective in inhibiting bacterial growth and protecting plants from fungal infections. Flavonoids are associated with anticancer and antithrombotic activities, while steroids and glycosides have been implicated in various therapeutic effects. The presence of these compounds in *Cassia siamea* may therefore underpin its traditional uses in the treatment of conditions such as constipation, microbial infections, and malaria.

Evaluation of the antimicrobial activity of the crude leaf extracts demonstrated that the aqueous extract exhibited superior inhibitory effects on the test organisms, with zones of inhibition ranging from 10.00 mm to 15.00 mm (Table 2), compared to the methanol extract, which produced zones between 9.50 mm and 14.00 mm. In contrast, the methanol extract of the stem bark showed higher antimicrobial activity than its aqueous counterpart, with inhibition zones ranging from 8.00 mm to

16.00 mm, while the aqueous extract had minimal to no activity, with zones between 0.00 mm and 4.50 mm (Table 3).

Minimum Inhibitory Concentration (MIC) assays further supported these findings. The aqueous leaf extract showed an MIC of 2.5 mg/ml against *Salmonella typhi* and *Staphylococcus aureus*, and 5.0 mg/ml against *Streptococcus pyogenes* (Table 4). For the methanol stem bark extract, the MIC was 5.0 mg/ml for *S. aureus* and 10.0 mg/ml for *S. pyogenes*, with no observed inhibitory effect on S. typhi (Table 5).

The antimicrobial efficacy of these extracts is attributed to their bioactive phytochemicals, which may act through various mechanisms. These include disruption of cellular processes such as metabolism, modulation of gene expression, and alteration of membrane permeability. Kotzekidou (2008) proposed that one such mechanism involves damage to the cytoplasmic membrane, thereby impairing electron transport and active transport systems. It is likely that the phytochemicals identified in Cassia siamea exert antimicrobial effects through similar pathways.

In summary, this study confirms the presence of several bioactive compounds in *Cassia siamea*, particularly flavonoids, saponins, tannins, and steroids, which possess significant therapeutic potential. These findings provide scientific support for the traditional medicinal applications of the plant and underscore the importance of solvent selection in maximizing the extraction and efficacy of phytochemicals.

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CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

➢ *Conclusion*

Based on the findings of this study, several key conclusions can be drawn:

The phytochemical analysis revealed a distinct variation in the composition and concentration of bioactive compounds between the leaf and stem bark of *Cassia siamea*. These differences suggest that certain phytochemicals may be either unique to specific plant parts or present in higher concentrations in one part than the other. Notably, the aqueous crude extract of the leaf demonstrated greater antimicrobial activity against the test organisms compared to the methanol extract, indicating that water may be a more effective solvent for extracting active constituents from the leaf. Conversely, for the stem bark, the methanol extract exhibited higher antimicrobial activity, implying that methanol is more efficient in extracting bioactive compounds from this part of the plant.

These observations underscore the significance of solvent selection in phytochemical extraction, as the same plant material can yield varying antimicrobial effects depending on the solvent used. This highlights the importance of optimizing extraction methods to maximize the therapeutic potential of medicinal plants.

The determination of Minimum Inhibitory Concentration (MIC), a critical parameter in antimicrobial studies, further confirmed the influence of extraction solvent on biological activity. MIC not only provides a measure of the potency of antimicrobial agents but also guides appropriate antibiotic selection and dosage, ultimately helping to reduce the risk of antimicrobial resistance. The differences observed in MIC values among the various crude extracts (refer to Tables 4 and 5) emphasize the pivotal role solvents play in dissolving and isolating the active principles of a plant.

Therefore, this study supports the potential application of *Cassia siamea* in the treatment of infections caused by the test organisms and highlights the need for further research.

➢ *Recommendations*

- Further studies should be conducted to isolate, identify, and characterize the specific antimicrobial compounds present in *Cassia siamea*.
- Toxicological evaluations of the plant's crude extracts are necessary to assess their safety profiles for potential therapeutic use.
- The antioxidant properties of both the leaf and stem bark should be investigated, given the reported diverse bioactivities of the plant.
- Efforts should be made to support and encourage local researchers to conduct phytochemical and bioactivity studies on plants traditionally used in their communities.
- Additional research is recommended to determine the Minimum Bactericidal Concentration (MBC) of the extracts to complement MIC data and better understand the plant's antimicrobial potential.

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