Preliminary Assessment of Selected Local Plants for Their Phytochemicals and Antimalarial Properties

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CERTIFICATION

I hereby certify that the research and writing of this dissertation is entirely that of the student Ishmael A. Kamara and it has been carried out at the Faculty of Pharmaceutical Sciences, under my supervision, and endorse that this presentation is a true reflection of the experimental results obtained by him.

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DEDICATION

This dissertation work is dedicated to the Almighty Allah and my parents, especially my father (Sheik Abdulrahman Kamara) who have helped me greatly in diverse ways so far. I would also like to dedicate this work to Mr. Peter F.B. Yilla and wife, I am thankful and forever grateful to you for what you have done for me.

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DEFINITION OF ABBREVIATIONS

ACT – Artemisinin-based Combination Therapy.

CAM – Complementary and Alternative Medicines.

COMAHS – College of Medicine and Allied Health Sciences.

FBC – Fourah Bay College.

RDT – Rapid Diagnostic Test.

RF – Retention Factor.

TLC – Thin Layer Chromatography.

USL – University of Sierra Leone.

WHO – World Health Organization.

ABSTRACT

Malaria remains a major health concern despite significant progress in both preventive and therapeutic measures during the 20th century. Globally malaria cause 229 million cases and 409,000 fatalities in 2019, while in 2021 it was responsible for 247 million cases and 619,000 fatalities. This study verified the antiplasmodial properties of *Cassia sieberiana, Moringa oleifera, Senna siamea* and *Nuclea latifolia* plants.

The leaves, stem bark and roots of *Cassia sieberiana, Moringa oleifera, Senna siamea* and *Nuclea latifolia* in methanol extract showed alkaloids, glycosides, terpenoids, tannins, and saponins, carbohydrate and flavonoids. Antiplasmodial activity was also observed for the methanol extracts of *Cassia sieberiana, Moringa oleifera, Senna siamea* and *Nuclea latifolia.*

From the results, these plants can search for free radicals and contains bioactive chemicals that can kill the malaria parasite. The methanol extract was shown to be the most effective of the two extracts tested, implying that the plants should be exploited in the development of novel antimalarial medicines.

Keywords*: *Cassia Sieberiana, Moringa Oleifera, Senna Siamea* and *Nuclea Latifolia.

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CHAPTER ONE
INTRODUCTION

➢ *Background of the Study*

Malaria remains a major health concern despite significant progress in both preventive and therapeutic measures during the 20th century. Globally malaria caused an estimated 229 million cases and 409,000 fatalities in 2019 (Organization, 2019), while in 2021 it was responsible for 247 million cases and 619,000 fatalities. In 2021, the World Health Organization reported that the African region was home to 95% of malaria cases and 96% of worldwide deaths because of malaria. About 80% of these deaths were recorded from children under the age of five (Organization, 2022). This confirms that malaria is a severe issue for global public health, particularly in tropical and subtropical areas. Malaria is the ninth most significant cause of death with over a million victim sickness in terms of years of life lost due to incapacity (Annan et al., 2012).

Malaria is still a significant public health issue affecting many people in tropical and subtropical countries despite the tremendous progress made in recent years to reduce the disease burden (Murray et al., 2012).

Malaria cases in Sierra Leone fell from 473 cases per 100,000 people in 2001 to 328.2 cases per 100,000 people in 2020 (Organization, 2019).

The entire population of Sierra Leone is at risk from malaria, which is one of the leading diseases and causes of mortality there. Hospital consultations attest to this. The diagnosis of malaria affects four out of ten people (Barber et al., 2017). In Sierra Leone, malaria poses a major threat to young infants. The risk of malaria infection, illness, and death is greatest in young children. 20% of children in Sierra Leone are believed to have died as a result of malaria (Organization, 2016).

Malaria continues to grow despite significant attempts to eradicate or control it, because drug-resistant *Plasmodium falciparum* strains have emerged, notably in Southeast Asia. These strains are now known to be resistant to all existing antimalarial medications (White, 1992). Programs to eradicate malaria in endemic regions have been severely hindered by the rise of treatment resistance in *Plasmodium falciparum* (Price and Nosten, 2001). The World Health Organization (WHO) has advised using artemisinin combination therapy (ACT) to assure high cure rates and to counteract this threat, while discussion over the best combination and how ACTs should be used and supported is still ongoing, that is rapid diagnostic test (RDT) before treatment. The initial infectious biomass is rapidly reduced by artemisinin's, and the remaining parasites are eradicated by a more slowly eliminated component that is typically used in combination therapy. The use of ACT is justified by the increase of antimalarial effectiveness, facilitation of adherence to a complete course of therapy, and minimizing of selection of drug-resistant parasites (Price et al., 2006).

For the creation of new drugs with potential chemotherapeutic advantages, herbal remedies offer a fascinating yet mostly unexplored source. Given that a higher proportion of the population uses medicinal plant-based therapy, its validation is crucial for the emerging African nations (Koroma and Ita, 2009). This research was culled from a survey conducted to assess the use of herbal medicines for treatment of neglected tropical diseases among herbalists in three districts (Kambia, Kono and Kenema) of Sierra Leone. The herbal plants used for treatment of malaria were identified for their local names and scientific names by the Botany department at Fourah Bay College. Numerous plants have been discovered, but the antiplasmodial activity of the most widely used herbs for the treatment of malaria by local indigenous groups in the provinces and some areas of the Western Area in Sierra Leone are ***Moringa oleifera*** (Moringa), ***Senna siamea*** (Shekutoure), ***Cassia Sieberiana*** (gbangba), and ***Nauclea latifolia*** (yumbuyamba).

This study will investigate and analyze the phytochemistry, and antiplasmodial activity of the bark, leaves, and roots of these plants to inform policy makers, researchers, and the general population about the scientific investigations of those traditional herbs since this type of study has never been done in Sierra Leone. This study, therefore, will verify the antiplasmodial properties of these plants to justify their use as malaria remedies.

➢ *Problem Statement*

Since the beginning of civilization, humans have recognized the significance of medicinal plants, and now both urban and rural communities have made extensive use of them to cure a wide range of illnesses. To ensure their use in complementary and alternative medicine, their efficacy, proper use, substances used to treat a specific condition, curative dose, and safety profile must be thoroughly established. Sierra Leone is a region where malaria is endemic, and there are several plants that have been utilized by local people to manage and treat malaria. Thorough investigations utilizing scientific techniques must be carried out to ascertain the efficacy and toxicity of these plants that are regularly used by traditional medical practitioners. Majority of the population in Sierra Leone uses herbal medicines without proper knowledge of the dose, frequency, effects, and their interactions when combined with other medicines. The safety, potency, medicinally active and inactive components, and therapeutic profile of these plants have not been thoroughly studied despite the effects that have been recorded as their therapeutic benefit.

➢ *Significance of the Study*

Many people use herbal remedies as the first line of treatment in malaria-endemic areas, particularly in Africa. The cost of conventional medications, accessibility, perceived efficacy, lack of adverse side effects, and faith in conventional medicine are

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among the typical justifications for them to use herbal medicines. These plants have been the subject of numerous claims that have not been supported by science. There is a need to include scientific evidence to support these claims because it has been suggested that certain plants have medicinal effects but there is no clear scientific proof to support these claims. In this study, the phytochemicals in the various plant components (leaves, bark, and roots) will be examined, along with their composition in aqueous, petroleum ether, and methanol extraction. Their effectiveness in treating malaria will also be investigated, providing the framework for standardization and inclusion in medical procedures.

In many underdeveloped nations, including Sierra Leone, where majority of the population living in rural areas relies on plants as an easily accessible, first line, and affordable treatment for malaria. Many of these herbs, meanwhile, have not been thoroughly researched as antimalarials(Annan et al., 2012).

- *Main Aim of the Study*

The aim of this study is to test for preliminary assessment of selected plants for their phytochemicals and antiplasmodial activities.

- *Specific Objectives of the Study*

- To identify the phytochemicals, present in selected herbal plants.
- To identify the antiplasmodial activity in selected herbal plants.

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CHAPTER TWO
LITERATURE REVIEW

➢ *Importance of Herbal Medicines*

Medical plants provide as vital starting points for novel natural chemicals that provide chemical diversity. Since they are the foundation of so many complementary and alternative medications, food supplements, cosmetics, they are vital to many pharmaceutical enterprises. The complexity of the herbs and extracts that are distributed to such a diverse variety of markets and monitoring systems presents fundamental classification and quality difficulties. There is currently a greater requirement for suitable analytical techniques for their identification, standardization, and detection of pollutants and adulterants (Muyumba et al., 2021). Man has relied on plants to provide all his requirements, including those for food, clothing, medicine, flavour and fragrance, and shelter. Some significant medications that are still in use today were developed by these medical systems. Ayurvedic, Unani, and Chinese traditional medicine, among others, are highly developed systems of medicine that have their roots in plants. The African, Australian, Central and South American, among other less well-known medical systems. Today's search for new molecules has gone a slightly different path, with the chemist using the fields of ethnobotany and ethnopharmacognosy as guides to point them in the direction of various sources and classes of compounds. Because of its diversity, tropical flora plays a crucial role in this context in terms of its ability to generate fresh leads. However, the Convention on Biological Diversity should also be considered when addressing the issues of sovereignty and property rights (Gurib-Fakim, 2006).

Around 90% of the people in Africa and 70% of the population in India used traditional medicine to assist meet their healthcare needs. More than 90% of general hospitals in China have departments dedicated to conventional medicine, which accounts for about 40% of all healthcare services provided there. In comparison to Western medicine, 40% of the respondents to a poll performed in Hong Kong in 2003 exhibited a notable faith in TCM. 12.8% of the 21,923 adults in a research conducted in the US who took supplements used herbs (Wachtel-Galor and Benzie, 2011).

About 70% of anticancer medications were made from natural ingredients or plants. There are roughly 22,755 plant species in Southern Africa, and 3000 of those are said to be employed as traditional remedies. Over 27 million people in South Africa depend on traditional medicine for their healthcare. This revealed even more about the therapeutic value of herbal remedies (Twilley et al., 2020). Based on the data above, individuals are becoming more dependent on herbal remedies. The use of plants as food and medicine in different countries has also been mentioned, and this is a drawback. Numerous bioactive substances are advantageous to humans, it is a fact (Noila, 2020).

➢ *Traditional Uses and Safety of Herbal Medicines*

A number of supporters of herbal remedies contend that when taken appropriately and in accordance with standard therapeutic doses, products that have a long history of widespread use are generally safe (Fong, 2002). The extent to which the absence of toxicological evidence could be interpreted as proof of the safety or absence of toxicity of herbal medications is a critical question behind this assertion. It relies on the sort of toxic impact and the likelihood of finding such a negative consequence under the conditions present in the traditional usage to determine whether the absence of records of adverse effects is a sign of lack of toxicity. Herbal medicine is likely to be detected and linked to acute symptoms and short-term harmful consequences, such as gastrointestinal problems and dermatological abnormalities. Therefore, the absence of such findings offers some proof that these specific endpoints are safe. However, unless a properly designed epidemiology study (preferably a prospective cohort study) is carried out, it is unlikely that long-term adverse outcomes, such as cancer, liver and kidney damage, reproductive dysfunctions, birth defects, and several morbidities that are more difficult to detect, will be associated with the widespread use of a medicine. Therefore, the lack of proof of these negative effects in the context of traditional consumption of herbal medicines does not rule out the possibility that they could still occur. Drug safety is only presumed when the null hypothesis that there is no toxicity has not been disproven by a carefully planned and thorough collection of pre-clinical and clinical trials with the statistical power to reject it if it were wrong (Moreira et al., 2014).

➢ *Drug Safety and Efficacy*

When referring to pharmaceuticals, efficacy is the ability to produce a clinical benefit while safety refers to the possibility that the medication won't harm anyone when used as intended. The therapeutic indication for the medicine determines both safety and efficacy; in theory, a substance has no clinical utility if it is "safe" but ineffective or if it is active on a pertinent therapeutic target, but its use is hazardous. Even though these are equally important characteristics of any therapy, safety has historically been prioritized over evidence of efficacy in drug regulation. Pre-marketing testing was mandated by the Federal Food, Drug, and Cosmetic Act of 1938 in the US, for example, although Kefauver-Harris Amendments of 1962 imposed a comparable obligation to verify drug efficacy just 25 years later (Moreira et al., 2014).

The famous Latin phrase "primum non nocere" ("first, not do harm"), whose origin is unknown, conveys the idea that safety should come first in therapeutic interventions (Smith, 2005). Like this, doctors are expected to "keep [patients] from harm" in the traditional version of the Hippocratic Oath they swear before beginning their careers as doctors.

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Although the "non-maleficence" axiom has emerged as a cornerstone of therapeutics, it is ethically impossible to separate it from the beneficence principle. If utilizing currently accessible therapeutic interventions a clinical benefit greater than that provided by a placebo is achievable, then failing to prescribe the most potent treatment alternative available has a detrimental effect on the patient's health.

The hierarchy of sources of evidence for the safety and efficacy of therapeutic interventions, according to the widely accepted concept of evidence-based medicine (EBM; "...use of current best evidence in making decisions about the care of individual patients") (Sackett et al., 1996), is headed by controlled and randomized clinical trials and an unbiased systematic review with and without a meta-analysis. Clinical efficacy cannot be inferred solely from medical professionals' or experts' judgments or from pharmacological activities described in animal and/or in vitro research. It must be proven through well planned and carried out phase III research, or, in exceptional cases, phase II trials.

Randomized clinical trials using placebo controls have identified several potential side effects of medication therapy. The incidences of alleged side effects are compared between the reference and test drug groups if patients assigned to the control group are given the reference medication instead of a placebo. Controlled and randomized clinical trials, in general, offer the best available proof of safety prior to marketing. Clinical trial protocols do not, however, fully address all aspects of drug safety, necessitating non-clinical assays like long-term carcinogenicity tests (cancer-inducing potential), reproductive and developmental toxicity studies (teratogenic potential), and others (Moreira et al., 2014).

Herbs continue to be useful as a treatment for various illnesses that affect humans and animals, as well as useful beginning points for the discovery of bioactive molecules for drug development, thus care should be taken to keep impurities and pollutants to a minimum.

➢ *Adulteration of Medicinal Plants*

Making anything impure or changing its original form by adding materials or parts that are not typically a part of it, especially inferior ones, is known as adulteration. To increase the weight, the potency, or to lower the price of a pure therapeutic product, it is defined as the deliberate substitution or addition of a different or nearly related plant species or foreign ingredient. Most of the medicinal compounds used in complementary and alternative medicine (CAM) come from plants. However, species adulteration has affected the validity and security of complementary and alternative medicine (Nithaniyal et al., 2017).

In poor nations, complementary and alternative medicine (CAM) is extensively practiced and meets up to 80% of the population's healthcare needs. Due to the negative side effects and expensive cost of allopathic medications, it is also becoming more common in wealthy nations. According to the World Health Organization, between 38 and 75 percent of people in the USA, Australia, Belgium, Canada, and France have used complementary and alternative medicine at least once in their lifetime (WHO, 2002). By the year 2050, it is anticipated that rising global demand for CAM will increase the trade in medicinal plant raw drugs from about 120 billion USD to 7 trillion USD (Marichamy et al., 2014). The efficacy and safety of CAM, however, are seriously threatened by the frequent reports of species adulteration in marketed raw pharmaceuticals (Stoeckle et al., 2011, Liu et al., 2018).

Most raw drugs are traded in the form of dried, broken, or powdered leaves, flowers, seeds, stems, bark, roots, and other plant parts that lack the intact diagnostic characters needed for morphological identification by Linnaean taxonomy, making species authentication a difficult task. Alternative techniques used for this aim, including chemotaxonomy, chromatography, and microscopy, have sporadic success in identifying species. The use of intricate chemistries, the absence of novel chemicals, the influence of environmental conditions, the age of the plant, and geographic variations are some of the major drawbacks of these techniques (Techen et al., 2014).

➢ *Antiplasmodial Effects of Medicinal Plants*

Plasmodium falciparum is the malaria parasite that causes the most severe symptoms. The alleviation from malaria has long come in the form of herbal remedies, such as cinchona bark and Qing Hao leaves, which provided quinine and artemisinin respectively. However, malaria has long affected both the economic and emotional elements of humanity. Both the quinoline-based quinine and chloroquine later proved to be excellent treatments for malaria until quinoline resistance first appeared and quickly spread throughout much of the world (Kaushik et al., 2015). In this situation, artemisinin proved to be a clever, quick-acting, and effective medication for treating chloroquine-resistant malaria. However, artemisinin resistance in the form of sluggish parasite clearance is already on the horizon (White, 2012), raising fears that humanity may one day be without an efficient malaria treatment. This necessitates a thorough search for new anti-malarial drugs.

The utilization of conventional herbal remedies is a promising source for innovative, affordable malaria treatments. Natural goods, especially medicinal plants, have remained an essential source of novel pharmaceuticals despite pharmaceutical corporations' recent advances in synthetic chemistry and rational drug design (Kaushik et al., 2013, Lombardino and Lowe III, 2004). The extensive ethnopharmacological history of traditional knowledge and application connected with medicinal herbs is unquestionably a plus. It's highly conceivable that humans are already receiving a significant amount of protection from malaria because of their consumption as foods or spices. However, if the general principles of traditional knowledge can be supported by empirical research,

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reliable and economical treatments for the dreaded drug-resistant types of malaria may be discovered. The identification of novel malaria pharmacophores that can be chemically produced and fine-tuned as potential future medications can be facilitated by further such exploratory endeavours (Kaushik et al., 2015).

For research into the development of new anti-malarial medications, reviews of the antiplasmodial and anti-malarial properties of medicinal plants are required. In the scientific literature, very few reviews of the antiplasmodial or antimalarial properties of medicinal plants have been written (Ibrahim et al., 2012, Kaur and Kaur, 2017, Lemma et al., 2017, Lawal et al., 2015).

➢ *Description of Selected Medicinal Plants*

- *Cassia Sieberiana*

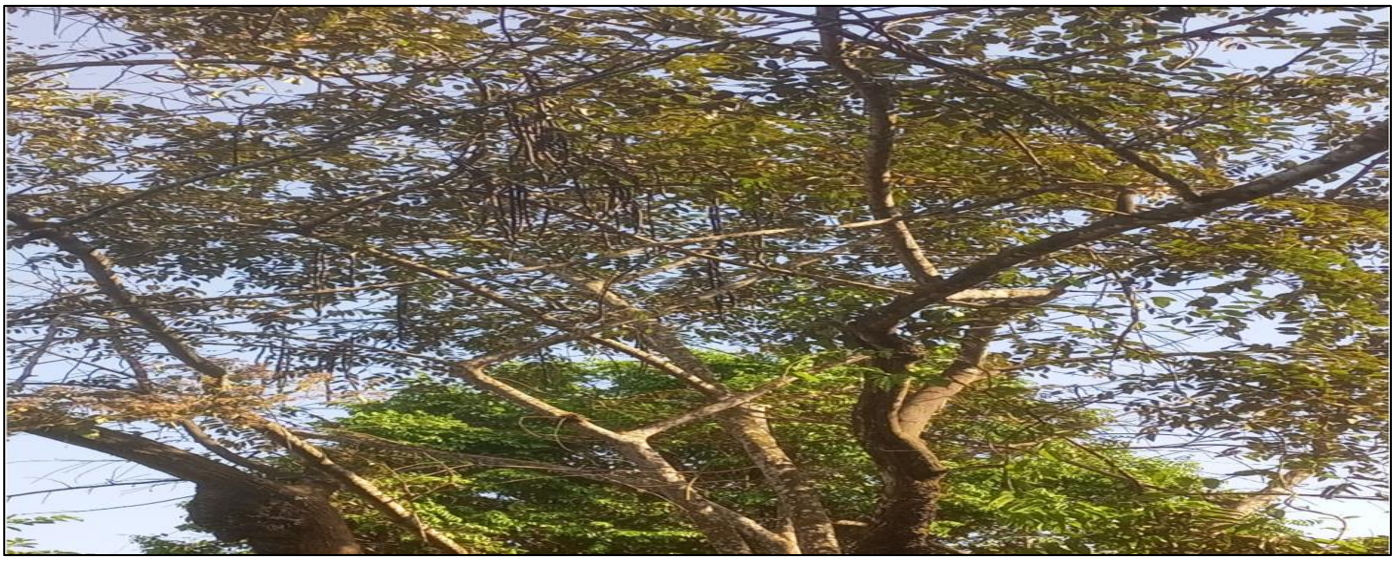

Fig 1 Cassia Sieberiana Plant

Cassia sieberiana is a deciduous tree that is native to Africa and can grow up to 20 metres tall. It is commonly found in savannas, riverbanks, and woodland areas. The tree has a straight trunk and a rounded crown with branches that droop downwards. The leaves are pinnate and can grow up 30 cm long, with several pairs of leaflets. The tree produces yellow flowers that are clustered together in panicles, which are followed by fruits that are long and narrow in shape. The bark, leaves, and roots of *Cassia sieberiana* have been used in traditional medicine for various purposes. For example, the bark and roots have been used to treat fever, diarrhoea, and respiratory infections, while the leaves been used to treat wounds, skin infections and eye problems.

African laburnum, also known as *Cassia sieberiana* (D.C.), is a widespread plant in sub-Saharan and Saharan Africa and is a member of the Caesalpiniaceae Leguminosae family. It is a savannah tree that grows in thickets and dry regions of the forest (GEARY et al., 1985). It is used in north-eastern and north-western Nigeria to treat malaria, inflammatory disorders, rheumatism, jaundice, diarrhoea, deworming, and as an aphrodisiac. It is also frequently included in African veterinary and human medicines. Most people can typically find, afford, and use the herb (Abdulrazak et al., 2015).

- *Moringa Oleifera*

Fig 2 Moringa Oleifera Plant

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Moringa oleifera Lam (syn. M. ptreygosperma Gaertn.) is one of the best known and most widely distributed and naturalized species of a monogeneric family Moringaceae. The tree is between 5 and 10 meters tall. It grows well in the tropical insular environment and is widespread close to the sand bottoms of rivers and streams. It can be found both wild and cultivated across the plains, notably in hedges and home yards. It thrives in arid or hot climates, can endure poor soils, and is not greatly impacted by drought. With a pH range of 5.0 to 9.0 and minimal annual rainfall requirements estimated at 250 mm and maximum at over 3000 mm, it can withstand a wide variety of precipitation. According to scientific reports, moringa leaves are a good source of natural antioxidants and are a rich source of beta-carotene, protein, vitamin C, calcium, and potassium. These antioxidant compounds, which include ascorbic acid, flavonoids, phenolics, and carotenoids, extend the shelf life of foods that contain fat (Toma and Deyno, 2014). Almost every part of this plant, including the root, bark, gum, leaf, fruit (pods), flowers, seeds, and seed oil, has been used in traditional South Asian and African medicine to treat a variety of conditions, including the management of inflammatory and infectious diseases as well as gastrointestinal, haematological, cardiovascular, and hepatocellular disorders. *M. oleifera, M. arborea, M. drouhardii, M. ovalifolia, M. longituba, M. rivae, M. borziana, M. corcanensis, M. hildebrandtii, M. ruspoliana, M. stenopetala, M. peregrine, and M. pygmaea* are some of the other most important species (Paikra and Gidwani, 2017).

- *Senna Siamea*

Fig 3 Cassia Sieberiana Plant

Cassia siamea (syn. *Senna siamea*) is an angiosperm native of Southeast Asia (Burma, Ceylon, India, Japan, Malaysia, Sri-Lanka and Thailand) and widely distributed in Africa (Cote d'Ivoire, Eritrea, Ethiopia, Ghana, Kenya, Malaysia, Nigeria, Sierra Leone, South of Africa, Tanzania, Togo, Uganda and Zambia), in Latin America (Cuba, Chile, Antigua and Barbuda, St Lucia, St Vincent and Grenadines and Trinidad and Tobago), and in Oceania (Australia and Fiji). *C. siamea* was once categorized under the Caesalpiniaceae family, then under the Leguminosae, and is currently categorized under the Fabaceae. This plant is a shrub that grows to a medium height of 10 to 12 meters, occasionally rising to 20 meters. The bole is brief, and the crown first appears compact and spherical before becoming uneven and spreading. The young bark is smooth and grey, later developing longitudinal cracks. The compound, alternating leaves measure 15–30 cm long and have 6–14 leaflets, each of which has a small bristle at the end. Bright yellow blooms grow in big, erect panicles that can reach 60 cm in length. The fruits are flat, 5 to 30 cm long, and constricted between the seeds. They have an indehiscent pod (Kamagaté et al., 2014).

- *Nuclea Latifolia*

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Fig 4 Nuclea Latifolia Plant

Nuclea latifolia is a member of the Rubiaceae family, which has about 630 genera and over 13000 plant species. It is a multi-stemmed, evergreen shrub or small tree that spreads. It has a unique flower as well as huge red balls of fruit with protruding stamens. It can reach a height of 200 m. It rarely grows taller than 20 feet, with a crooked bole, or larger trees in closed forests exceeding 100 feet tall and 8 feet in diameter. The plant's leaves are 4-5 inches, and its bark is tough. The opposing, rounded-ovate, glossy green leaves have tufts of hair and are glabrous. Usually pink, edible, sweet-sour pulp (a syncarp up to 3 inches in diameter) is enclosed by a fleshy, shallow-pitched fruit with many embedded seeds. Though undesirable, the fruits are nevertheless edible. The seeds typically have a nice flavour and are small, oval, abundant, and brownish. However, if consumed in excess, they can be emetic. The stem has a fibrous reddish slash and dark grey cracks. It is multi-stemmed and has an open canopy of flowers with terminal cymes of tiny, pale flowers with spherical heads. The flower head can be up to 2 mm in diameter and has a fragrant aroma that attracts bees. The calyces of the blooms are linked together (ME et al., 2016).

It is also referred to as the "African peach" and is used in the East and West African sub-regions of continental Africa where different extracts of the plant are used for the therapeutic management of malaria, hypertension, prolonged menstrual flow, cough, gonorrhoea, stomach disorders, dysentery, ulcers, and liver disorders (ME et al., 2016).

- *Botanical Classification of Selected Plants*

Table 1 Botanical Classification of Selected Plants

Kingdom	**Plantae**	**Plantae**	**Plantae**	**Plantae**
Phylum	Angiospermophyta	Tracheophyte	Tracheophyte	Spermatophyte
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida	Magnoliopsida
Subclass	Rosidae	Dileniidae	Caesalpinioideae	Angiosperm
Order	Fabales	Brassicales	Fabales	Gentianales
Family	Fabaceae	Moringaceae	Fabaceae	Rubiaceae
Genus	Cassia	Moringa	Senna	Nulcea
Species	C. sieberiana	M. oliefera	S. siamea	N. latifolia
Botanical name	Cassia sieberiana	Moringa oliefera	Senna siamea	Nuclea latifolia Smith

Table 2 Vernacular (Local) Names of Selected Plants

TRIBES	**Cassia sieberiana**	**Moringa oleifera**	**Nuclea latifolia**	**Senna siamea**
Mende	gBAnGBA		YUMBUYAMBA	
Temne	Ka-GBAnGBA	Ka – BAKeLAILAI	A – MALeKɛ	
Kono	KULUKAI		nDUNDU	
Kissi	PEBALE		KOLONDO	
Loko	KAnGAI		mBUNDU-nJAMBA	

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Gola	GAnA		YUMBUYAMBA	
Via	GAnA		LUNDUJAMBA	
Fula	SINJA		DUNDUKε	
Mandingo	SINJA		DUNDU	
Yalunka	GIRIGIRIN-NA	TEGITELE-NA	DUNDUKHA-NA	
Tonko Limba		KUBAnGBA	GBεLεGεDε	
Susu		MoReKoNTE	DUNDARE	
Creole		PAINBOD	IGBεSI	
Sharbro			GBILGBIL-Lε	

➢ *Ethnobotanical Uses of Selected Plants*

- *Ethnobotanical Uses of Cassia Sieberiana*

In traditional medicine, the leaves, roots, and pods are frequently employed. The twigs are used to cure insomnia. Use the liquid that is obtained from soaking the roots in water to massage your body and take a bath to relieve fatigue. Dysentery, diarrhoea, and vomiting are treated with a decoction of the bark, leaves, or root; the twigs are also used to cure trypanosomiasis. The encapsulated root bark is utilized at the Centre for Plant Medicine Research to treat dysmenorrhea and pain brought on by gastric ulcers. The root, leaf, and flower extracts are taken in combination as an expectorant and for dyspepsia. Conjunctivitis can be treated with the plant's root. Leaf is used as an antipyretic and for heartburn. To treat blotches on the skin caused by menstruation disorders, leaf and flower extracts are used (Usman et al., 2014).

- *Ethnobotanical Uses of Nuclea Latifolia*

Throughout recorded history, *N. latifolia* herbal treatments have been widely employed in numerous civilizations and continue to be the primary therapeutic medical treatment. The plant is frequently used in many African nations as a treatment for diabetes, dental cavities, infected mouth, diarrhoea, and discomfort. Treatment for sleeping sickness, malaria, leprosy, debility, hypertension, gastrointestinal diseases, and leprosy is among its additional uses (Adamu et al., 2012).

The plant's stem, bark, and roots are used in traditional African medicine to treat diabetes, hypertension, jaundice, malaria, diarrhoea, and dysentery (ME et al., 2016). The fruits are occasionally used to cure dysentery and piles. The herb is also used to treat delayed menstrual flow and sleeping sickness. Due to the plant's purported anti-malarial properties, it is also known as "Africa cinchona" or "Africa quinine."

- *Medicinal Uses of Nuclea Latifolia*

✓ *Roots*

It is used as a tonic and medicine for fever, toothaches, dental cavities, septic mouth, cough, stomach problems, diarrhoea, dysentery, treatment of gonorrhoea, and wounds in addition to treating malaria. It is used as an antidiabetic, an antiparasitic, to treat bronchitis, to cure pain in the legs and arms caused by pyrexia, and to treat cold extremities. Additionally, it functions as an aphrodisiac, analgesic, stimulant, and restorative. It is used to treat diabetes, hypertension, rheumatism, hepatitis, appetite loss, and other conditions. Additionally, it is used to treat premature labour in pregnant women, as a purgative, an antidepressant, and to induce abortion. It is also used to treat respiratory disorders like tuberculosis and asthma (Adamu et al., 2012, Jiofack et al., 2010).

✓ *Bark*

It is used to treat gonorrhoea, cough, wounds, and malaria. It works as a tonic and cure for stomach issues like diarrhoea and dysentery as well as for fever, toothaches, dental cavities, and infected mouth. Along with treating wounds and gonorrhoea, it is also used to treat premature labour in pregnant women (Jiofack et al., 2010).

✓ *Leaves*

It functions as an antipyretic, antidiabetic, and antimalarial. Stomach aches, constipation, fever, diarrhoea, sores, anxiety, depression, and epilepsy are all treated with it (Jiofack et al., 2010).

- *Ethnobotanical Uses of Moringa Oleifera*

M. oleifera is like nature's pharmacy. Its roots, leaves, bark, flowers, fruit of immature pods, seeds, and flowers have a variety of medicinal qualities, including the potential to be diuretic, antipyretic, antimicrobial, hepatoprotective, lower cholesterol, antispasmodic, antiulcer, and antitumor. Moringa is very helpful for depression, malnutrition, general weakness, and osteoporosis. Moringa was widely used in traditional medicines for the treatment of hysteria, pimples, shortness of breath, scouring, dementia, sore throat, sprains, tuberculosis, pregnancy, diabetes, lactation, semen deficiency, scurvy, psoriasis, hysteria, abnormal blood pressure, fever, eye and ear infections, cough, conjunctivitis, glandular issues, blood impurities, catarrh, bronchitis, anxiety, blackheads, skin infections and for anaemia (Kamran et al., 2020). According to studies, ancient cultures used moringa for its medicinal benefits. In addition to treating these conditions, *M. oleifera* is also used to treat cancer, cholera, chest congestion, asthma, headache, swellings, coughing, diarrhoea, painful joints, pimples, respiratory disorders, seizures, intestinal worms, breastfeeding,

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diabetes, and hypertension. As a defence mechanism against the microbial invasion, plants can produce flavonoids. Consuming flavonoids has been linked to protection against cancer, cardiovascular disease, and other chronic diseases linked to oxidative stress. The plant's leaf is the finest source of flavonoids. Moringa's antibacterial, antioxidant, anti-inflammatory, antispasmodic, and anticancer properties are what give it its medical significance (Kamran et al., 2020).

- *Ethnobotanical uses of Senna Siamea*

Regardless of the subspecies, the leaves, stems, roots, flowers, and seeds of *S. siamea* have been used to cure a variety of ailments, most notably malaria, a tropical endemic disease with a high morbidity rate (Kamagaté et al., 2014).

✓ *Leaves*

The plant's leaves are the most utilized component, particularly among Asian and African populations who prepare herbal treatments. In Burkina Faso, fresh and dried leaf decoction (boiled for 20 minutes in 1L of water) is used as a body wash or to cure liver diseases and malaria. The decoction of leaves is given orally (0.5 L, twice daily) in Côte d'Ivoire to treat malaria, stomach aches, and cough. Additionally, the leaves' decoction is used against malaria and utilized as an antibiotic in Sierra Leone and Togo. In Nigeria, the dried leaves are combined with lime (Citrus lemonum), pawpaw (Carica papaya), and lemon (Cymbopogon citratus) leaves before being boiled for an hour. The mixture's "tea" is consumed to ward off malaria. To alleviate abdominal pain, Ugandans pluck, clean, and chew the leaves before ingesting a drink. The leaves are properly cleansed and cooked in India. Honey is added to the decoction after it has been strained. Three times a day, a quarter glass (150 mL) of this remedy is consumed to treat fever and anaemia. To lessen the bitterness, fresh and dried leaves are cooked in Laos at a ratio of 1:3 for an hour or so, 2-3 times, and then pulverized to make a paste to which pork bones are then added. Before breakfast, people consume this "chi om leck" meal, a vegetable with sedative and euphoric properties (Kamagaté et al., 2014).

✓ *Roots*

Fever, constipation, hypertension, and sleeplessness are all treated using root decoction in Benin. In Kenya, a blend of roots from *S. siamea* and Zanthoxylum chalybeum is infused, decocted, or macerated as a remedy for snakebites. Herbalists in Southeast and Sub-Saharan Africa use the root decoction to treat diabetes mellitus. To heal sore throats, the roots are crushed and combined in these places before the aqueous extract is consumed. Small, repeated doses of the bark from macerated or decocted roots are consumed in Côte d'Ivoire to treat angina and malaria, respectively (Kamagaté et al., 2014).

✓ *Stem*

The decoction of the entire stem or the stem's bark is drunk or used as a body wash to combat malaria and liver diseases in Burkina Faso, Ghana, and Nigeria. In Malaysia, the same purposes were mentioned. As a laxative, crushed dried stems of *S. siamea* are combined with the fruit of *Xylopia aethiopica*. To combat diabetes, drink the stem bark decoction. In Japan, this decoction is used as a gentle, palatable, secure purgative (Kamagaté et al., 2014).

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CHAPTER THREE
METHODOLOGY

- *Type of Study*

This is an experimental study to test for preliminary assessment of selected local plants for their phytochemicals and antimalarial properties.

- *Sample Collection*

The leaves, stem bark and roots of ***Moringa oleifera, Senna siamea, Cassia Sieberiana*** *and* ***Nauclea latifolia*** were collected at Mount Aureol and Gloucester in the Western Area. The plants were identified and confirmed at the Botany department at Fourah Bay College, University of Sierra Leone (USL).

- *Study Duration*

This study took approximately 8 months starting 10th December – 31st July 2023.

- *Study Site*

This study was conducted at the department of Pharmaceutical Sciences laboratory, College of Medicine and Allied Health Sciences, University of Sierra Leone (USL) and Connaught Microbiology Laboratory, Connaught Teaching Hospital Complex.

- *Reagents and Instruments*

The reagents and chemical used: Petroleum ether, Methanol, 5% $FeCl_3$ solution, distilled water, Benzene, 10% (v/v) NH_3, 1% HCl, Alpha – naphthol 10%, Fehling`s reagent (A & B), Molisch`s reagent, acetic acid, Benedict`s reagent, dilute and concentrated sulphuric acid, Dragendorff's reagent, Mayer's reagent, Chloroform, Ammonia solution, ethyl acetate, Alcoholic solution, Ethanol (95% & 50%), Vanillin hydrochloric acid solution, Staining reagents.

- *Instruments*

Soxhlet apparatus, Soxhlet condenser, Thimble, Dropper pipette, Digital balance, funnel, water bath, Measuring cylinder, Beakers, Boiling tubes, Test tubes, Test tube rack, Test tube holders, EDTA tubes, round bottom flask, Lab coat, Ruler, Knife, Scissor, Hand gloves, Sieve, Filter papers, TLC paper, Heating mantle, Mortar and pestle, Column Chromatography and Thin Layer Chromatography, Slides, Light Microscope.

- *Test Methods*

- *Plant Extraction and Preparation*

The harvested plants were washed for dirty, microorganism and organic matter and air dried for 21 days at room temperature (25 to 30°C). In accordance with the increasing polarity of these solvents, petroleum ether and methanol were used consecutively to extract the pulverized plant samples from each plant. Each solvent was used to extract the four plants. The hot Soxhlet method was used to extract the solvent. Each container containing 20g of the plant material received 500ml of methanol for the methanol extract. For the petroleum ether extraction, the same amount of powder and solvent were used for the methanol extraction. The extracts for the various solvents were stored in amber bottles in preparation for thin layer chromatographic analysis, antimalarial activity testing, and phytochemical screening.

- *Sample Weighing*

Each sample was weighed using electronic balance.

Table 3 Shows the Weighing of Plant Samples for Aqueous Extraction (Methanol):

	Cassia sieberiana	**Moringa oleifera**	**Senna siamea**	**Nuclea latifolia**
Leaves	28.15g	23.59g	21.31g	24.95g
Barks	34.51g	24.10g	24.24g	23.73g
Roots	23.00g	19.85g	21.49g	19.58g

Table 4 Shows the Weighing of Plant Samples for Non – Aqueous Extraction (Petroleum Ether):

	Cassia sieberiana	**Moringa oleifera**	**Senna siamea**	**Nuclea latifolia**
Leaves	26.54g	24.41g	23.73g	28.06g
Barks	25.90g	24.38g	24.89g	22.63g
Roots	21.89g	21.90g	20.59g	21.01g

- *Phytochemical Analysis***:**

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Chemical tests were carried out on the extracts to identify the presence of the following phytochemicals: carbohydrate, glycosides, steroids, terpenoids tannins, saponins, flavonoids, anthraquinones and alkaloids as described by Sofowora, Trease and Evans Harborne (Sambo et al., 2015).

✓ *Test for Carbohydrates*

Molisch's test: 2ml of the prepared filtrate were mixed with 0.2ml alcoholic solution of alpha-naphthol 10% in addition to 2ml of sulphuric acid, a bluish violet zone formed indicate the presence of carbohydrate/glycosides.

Benedict's test: To 1ml of the filtrate, 5ml of Benedict's reagent were added. The mixture was heated, appearance of red precipitate indicates the presence of reducing sugar.

Fehling's test: In this test 5ml of the filtrate were treated with 5ml Fehling's solutions (A&B) and heated, the appearance of a red precipitate indicates the presence of reducing sugar.

✓ *Test for glycosides*

To 1ml of the extract was added 2 ml of acetic acid and then cooled in an ice bath at 40°C. To this mixture 1ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was added dropwise. The formation of an oil layer on top of solution indicated the presence of glycosides.

✓ *Test for alkaloids*

To 3ml of the extract was added 1ml of 1% HCl. This resulting mixture was then treated with few drops of Mayer's reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloid.

✓ *Test for Saponins*

Five drops of olive oil were added to 2ml of the plant extract and the mixture shaken vigorously. The formation of a stable emulsion indicated the presence of saponins.

✓ *Test for Tannins*

Two drops of 5% $FeCl_3$ were added to 1ml of the plant extract. The appearance of a dirty-green precipitate indicated the presence of tannins.

✓ *Test for Flavonoids*

To 1ml of the extract were added 3 drops of ammonia solution (NH_3) followed by 0.5ml of concentrated HCl. The resultant pale brown coloration of the entire mixture indicated the presence of flavonoids.

✓ *Test for Anthraquinones*

A Borntrager's test was performed whereby one millilitre of the plant filtrate was shaken with 10ml of benzene; the mixture was filtered and 5ml of 10% (v/v) ammonia was added, then shaken and observed. A pinkish solution indicates a positive test.

✓ *Test for Steroids*

1ml solution of the extract was added to 1ml of sulphuric acid. The appearance of red colour indicates the presence of steroids.

✓ *Test for Terpenoids*

To 5ml. of extract, 2ml. of chloroform was added followed by carefully addition of concentrated sulphuric acid and observed. The formation of reddish-brown colour layer at the junction confirms the presence of terpenoids.

- *Thin Layer Chromatography*:

TLC was used to examine the various methanol and Petroleum ether extracts on analytical plates with silica gel. The extracts were moved over the silica gel (stationary phase) using one solvent (moving phase). To match the containers and the quantity of extract samples, silica gels were made. 24 Silica gels, each measuring 10 cm in width and 10.0 cm in length, were created. On the top and bottom of the silica gel, a faint 1cm line was drawn. The various silica gels were spotted using capillary tubes. The three systems of solvents employed were Methanol, Ethanol and Acetic acid = 3:1:1.

- *Preparation of Sterile Extracts*

A stock solution of 100 µg/ml was prepared from the crude ethanolic extract of each plant material and filtered with millipore filter of size 0.2 µg under sterile conditions. Four-fold serial dilutions was prepared from each stock solution using Complete Parasite Medium, resulting in concentrations ranging from 100 - 0.0976562µg/ml. Artemether-Lumefantrine, a known standard anti-malarial drug, was used as a positive control (Annan et al., 2012).

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- *Preparation and Examination of Blood Smear*

Blood samples was collected from patients that are visiting the laboratory for malaria test. Each sample was carefully labelled and kept safe in a non-freezing compartment of the refrigerator. Specific volume (millilitre) of the blood was collected and a thin smear was prepared on a clean grease free dried slide. Thin blood smears were prepared on properly labelled slides. The blood smears were air-dried and fixed in methanol. The dried slides was Giemsa stained and observed with 100x microscopic lens in immersion oil using a light microscope (Annan et al., 2012). For samples that confirmed positive for the Plasmodium parasite, was tested against the aqueous and non-aqueous extracts of the plants.

- *Antiplasmodial Activities of the Extracts of Moringa Oleifera, Senna Siamea, Cassia Sieberiana and Nuclea Latifolia.*

Prepare three clean test tubes for each plant extracts. Take small volume of blood (1ml) that has been confirmed positive of malaria. Pipette 1ml of blood into the test tube. Take 100µl of each extract and pour on the blood sample in the test tube. The test tube was shaken thoroughly for 5 minutes and allow to stand for 24 hours.

- *Parasite Count Calculation*

Parasite count was calculated to ascertain the number of parasite load left in parasite per microliter ($p/\mu l$) after the extract has been introduced and left to stand for 24 hours by using the relation below:

For parasite above 5

$$P = \frac{No.of\ parasite\ left\ \times 8000}{200}$$

For parasite below 5

$$P = \frac{No.of\ parasite\ left\ \times 8000}{400}$$

For examples:

$$20P = \frac{20 \times 8000}{200} = 800\ p/\mu l \qquad 5P = \frac{5 \times 8000}{400} = 100\ p/\mu l$$

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CHAPTER FOUR
RESULTS

- *Phytochemical Screening of the Leaves, Bark, and Roots of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia.*

Methanol and Petroleum ether extracts of the leaves, bark, and roots of *Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia* showed the following observations of the various tests. The results proved the presence of phytochemicals.

Table 5 Phytochemical Screening Results.

Phytochemical tests	**Observations**
Carbohydrate	
i. Molisch's test	Bluish violet zone was seen
ii. Fehling`s test	Red precipitate seen
iii. Benedict test	Red precipitate
Alkaloids	Creamy white precipitate seen
Glycosides	Oily layer on top of solution seen
Steroids	No visible reaction seen
Terpenoids	Reddish brown colour seen
Tannins	Dirty- green precipitate seen
Saponins	Stable foam was seen
Flavonoids	Pale brown colour seen
Anthraquinones	A pinkish solution seen

- *Phytochemical Screening of the Leaves, Bark, And Roots of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia with Methanol Extracts.*

The screening of the aqueous extracts of *Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia* shows the following phytochemical composition in the different plant parts. The tables below show the presence of phytochemical components in both the leaves, bark, and the roots.

Table 6 Phytochemical Screening of the Leaves, Bark, and Roots of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia with Methanol Extracts.

Components	**A**			**B**			**C**			**D**		
	L	B	R	L	B	R	L	B	R	L	B	R
Tannins	++	+++	++	++	++	_	++	++	+	+	++	++
Saponins	+	+	++	+	_	+	++	++	+	+	++	+++
Flavonoids	+	++	_	_	_	_	+	+	++	+++	+	++
Anthraquinones	_	_	+	_	_	_	_	_	_	_	+	+
Terpenoids	_	+	++	_	++	++	_	++	++	_	++	+++
Steroids	++	+++	++	_	+	+	+	+	+	_	+	+++
Alkaloids	_	+	_	_	_	_	+	+	_	+	+	+
Glycosides	+++	+	+	++	_	++	++	+	_	+	++	+++
Carbohydrate												
Molisch's	+	+++	_	_	+	++	_	+	++	_	++	_
Fehling's	+++	++	++	+	+	+	+	+	+	+++	++	+
Benedict's	++	_	++	+	_	+	+	_	+	+	++	+

Key: A – Cassia sieberiana; B – Moringa oleifera; C – Senna siamea; D – Nuclea latifolia; L – Leaves; B – Bark; R – Roots; +++ Abundance, ++ Moderate, + Trace and – Absent.

- *Phytochemical Analysis of the Leaves, Bark, and Roots of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia with Petroleum Ether Extracts.*

The screening of the non - aqueous extracts of *Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia* shows the following phytochemical composition in the different plant parts. The tables below show the presence of phytochemical components in both the leaves, bark, and the roots.

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Table 7 Phytochemical Screening of the Leaves, Bark, and Roots of *Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia* with Petroleum Ether Extracts.

Components	**A**			**B**			**C**			**D**		
	L	B	R	L	B	R	L	B	R	L	B	R
Tannins	+	–	–	–	–	–	+	–	–	+	–	+
Saponins	+	+	+	++	+	+	+++	+	+	++	+	++
Flavonoids	+	+	–	–	–	–	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–	–	–	–	–	–	–
Terpenoids	+	–	–	++	–	–	–	–	–	–	–	–
Steroids	+	–	–	–	–	–	–	++	++	–	+	+
Alkaloids	+	–	–	–	–	–	–	–	–	–	–	–
Glycosides	+++	++	++	+++	++	++	+	++	++	+	++	+++
Carbohydrate												
Molisch's	+	–	–	+	–	+	++	–	–	++	–	–
Fehling's	++	++	+	++	+	++	++	–	–	–	–	–
Benedict's	–	–	–	–	–	–	–	–	–	–	–	–

Key: A – Cassia sieberiana; B – Moringa oleifera; C – Senna siamea; D – Nuclea latifolia; L – Leaves; B – Bark; R – Roots; +++ Abundance, ++ Moderate, + Trace and – Absent.

- *Thin Layer Chromatography Analysis of Methanol and Petroleum Ether Extracts of the Leaves, Bark, and Roots of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia.*

Thin layer chromatography result below indicates that many non-polar compounds were present in the leaves of *Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia* as compared to bark and roots.

Table 8 Thin Layer Chromatography of the Leaves, Bark, and Roots of *Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia* with Methanol and Petroleum Ether Extracts.

Plants/ Sample	**Sample extract**	**Number of layers**	**Methanol Rf value M: E: A (3:1:1)**	**Petroleum ether Rf value M: E: A (3:1:1)**
Cassia sieberiana	Leaves	A	0.89	0.68
		B	0.81	0.63
	Bark	A	0.93	–
		B	0.90	–
	Roots	A	0.97	–
Moringa oleifera	Leaves	A	0.88	0.71
		B	0.82	0.69
	Bark	A	0.90	–
		B	0.85	–
	Roots	A	0.93	–
Senna siamea	Leaves	A	0.99	0.77
		B	0.96	0.73
	Bark	A	0.95	0.95
		B	0.91	0.91
	Roots	A	0.92	0.95
Nuclea latifolia	Leaves	A	0.93	0.65
		B	0.90	0.60
	Bark	A	0.88	–
		B	0.84	–
	Roots	A	0.95	–

(–) no Visible Movement Seen.

- *Antiplasmodial Tests Results of the Leaves, Bark, and Roots of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia with Methanol Extract.*

The antiplasmodial testing of *Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia* methanol extracts showed that the leaves of *Senna siamea*, roots of *Cassia sieberiana*, roots of *Nuclea latifolia*, bark of *Senna siamea* and bark of *Cassia sieberiana* eradicate the parasite from the blood whiles the others only reduced the parasite count in the blood.

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Table 9 Antiplasmodial Tests Results Produced by Methanol Extract of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia.

Plant extract	**Leaves p/µl (p count)**	**Bark p/µl (p count)**	**Roots p/µl (p count)**	**ControlA/L**
Cassia sieberiana	800 p/µl (20 P)	NMPS	NMPS	Negative
Moringa oleifera	100 p/µl (5 P)	160 p/µl (8P)	1400p/µl (35P)	Negative
Senna siamea	NMPS	NMPS	1200p/µl (30P)	Negative
Nuclea latifolia	440 p/µl (11 P)	1000p/µl (25P)	NMPS	Negative

Initial Parasite count $= 3{,}600\ p/\mu l\ (90\ P)$

Key: NMPS – No malaria parasite seen A/L – Artemether – Lumefantrine P – Parasite

- *Antiplasmodial Tests Results of the Leaves, Bark, and Roots of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia with Petroleum Ether Extract.*

The antiplasmodial testing of *Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia* petroleum ether extracts showed no eradication of the parasite but reduces the parasite from the blood sample.

Table 10 Antiplasmodial Tests Results Produced by Petroleum Ether Extract of Cassia Sieberiana, Moringa Oleifera, Senna Siamea and Nuclea Latifolia.

Plant extract	**Leaves p/µl (p count)**	**Bark p/µl (p count)**	**Roots p/µl (p count)**	**Control A/L**
Cassia sieberiana	1200 p/µl (30 P)	1200 p/µl (30P)	1400 p/µl (35P)	Negative
Moringa oleifera	1200 p/µl (30 P)	3200 p/µl (80P)	1400p/µl (35P)	Negative
Senna siamea	1200 p/µl (30 P)	3200 p/µl (80P)	800p/µl (20P)	Negative
Nuclea latifolia	1200 p/µl (30 P)	3200p/µl (80P)	1600 p/µl (40P)	Negative

Initial Parasite count = 3,600 p/µl (90 P)

Key: NMPS – No malaria parasite seen A/L – Artemether – Lumefantrine P – Parasite

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CHAPTER FIVE
DISCUSSION

➢ *Phytochemical Screening of Cassia Sieberiana*

Screening of the methanol extract of the leaves, bark, and roots of Cassia sieberiana showed the presence of Tannins, Saponins, Flavonoids, Anthraquinone, Terpenoids, Steroids, Alkaloids, Glycosides and Carbohydrates. Tannins, steroids, and carbohydrates were found to be present in the leaves, bark, and roots while saponins was present in the roots with traces in both leaves and bark. Flavonoids were present in leaves and bark but absent in the roots. The roots contained traces of anthraquinones. Anthraquinones was found to be absent in the leaves and bark. Terpenoids were absent in the leaves but present in the bark and roots. Glycosides were present in the leaves but absent in the bark and roots. Alkaloids were present in the bark but absent in the leaves and roots of Cassia sieberiana. From a study done in Nigeria by Abdulrazak, et al. for the ethanolic extract of the roots, stem bark of Cassia sieberiana show the presence of tannins, steroids, and carbohydrate seen in the bark, this might clearly justified why the stem bark of this plant is used in the treatment of malaria (Abdulrazak et al., 2015).

➢ *Phytochemical Screening of Moringa Oleifera*

The phytochemical screening of the methanol extract of the leaves, bark, and roots of Moringa oleifera showed the presence of Tannins, Saponins, Terpenoids, Steroids, Glycosides and Carbohydrates and absence of Flavonoids, Anthraquinones, Alkaloids. Tannins were present in the leaves, bark, and absent in the roots while saponins and Glycosides were present in the leaves and roots and absent in the bark. Anthraquinones, Flavonoids, Alkaloids were absent in the leaves, bark, and roots of Moringa oleifera. Steroids and Terpenoids were present in the bark and roots and absent in the leaves. Carbohydrates were present in the leaves, bark, and roots of Moringa oleifera. Patel et al. conducted a study on phytochemical analysis and antifungal activity of Moringa oleifera and note the importance of the following phytochemicals such as alkaloids, flavonoids, and terpenoids. Alkaloids are organic chemical substances with simple nitrogen atoms that are found in nature. Alkaloids utilized as pharmaceuticals and recreational substances, and they frequently have pharmacological effects. Flavonoids serve as antioxidants and improve the effects of vitamin C. They have also been shown to have biological activity against cancers, viruses, and other microorganisms, as well as liver toxins. Terpenoids found in plants are widely used for their fragrant properties. They have a place in conventional herbal remedies and are being researched for their potential to have antibacterial, antineoplastic, and other pharmaceutical effects (Patel et al., 2014).

➢ *Phytochemical Screening of Senna Siamea*

The phytochemical screening of the methanol extract of the leaves, bark, and roots of Senna siamea showed the presence of Tannins, Saponins, Flavonoids, Terpenoids, Steroids, Alkaloids, Glycosides and Carbohydrates and absent of Anthraquinone. Tannins, Glycosides, Alkaloids and saponins were present in the leaves, bark but in absent in the roots. Flavonoids were present in the roots and absent in the leaves and bark. Anthraquinones was found to be absent in the leaves, bark, and roots of Senna siamea. Terpenoids were absent in the leaves but present in the bark and roots. Carbohydrates and Steroids were present in the leaves, bark, and roots of Senna siamea.

➢ *Phytochemical Screening of Nuclea Latifolia*

The phytochemical screening of the methanol extract of the leaves, bark, and roots of Nuclea latifolia showed the presence of Tannins, Saponins, Flavonoids, Anthraquinone, Terpenoids, Steroids, Alkaloids, Glycosides and Carbohydrate. Terpenoids were present in the bark and roots but absent in the leaves. Saponins and Flavonoids were present in the roots, bark, and leaves. Anthraquinones was found to be absent in the leaves. Terpenoids and steroids were absent in the leaves but present in the bark and roots. Glycosides, Alkaloids and Carbohydrates were found in the leaves, bark, and roots of Nuclea latifolia.

➢ *Phytochemical Screening of Cassia Sieberiana*

Screening of the petroleum ether extract of the leaves, bark, and roots of Cassia sieberiana showed the presence of Tannins, Saponins, Flavonoids, Terpenoids, Steroids, Alkaloids, Glycosides and Carbohydrates and absent of Anthraquinone. Tannins, Alkaloids and Terpenoids were present in the leaves and absent in the bark and roots. Saponins and Glycosides were present in the leaves, bark, and roots. Flavonoids were present in the leaves and bark but absent in the roots. Anthraquinones was found to be absent in the leaves, bark, and roots of Cassia sieberiana. Steroids were present in the leaves and absent in the bark and roots. Carbohydrates were present in the leaves but absent in the bark, and roots of Cassia sieberiana.

➢ *Phytochemical Screening of Moringa Oleifera*

The phytochemical screening of the petroleum ether extract of the leaves, bark, and roots of Moringa oleifera showed the presence of Saponins, Terpenoids, Glycosides and Carbohydrates and absence of Tannins, Flavonoids, Steroids, Alkaloids and Anthraquinone. Tannins were absent in the leaves, bark, and roots. Flavonoids, Anthraquinones, Steroids and Alkaloids were absent in the leaves, bark, and roots of Moringa oleifera. Terpenoids were present in the leaves and absent in the bark and roots. Glycosides and Saponins were present in the leaves, bark, and roots of Moringa oleifera. Carbohydrates were present in the leaves and roots but absent in the bark of Moringa oleifera.

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- *Phytochemical Screening of Senna Siamea*

The phytochemical screening of the petroleum ether extract of the leaves, bark, and roots of Senna siamea showed the presence of Tannins, Saponins, Steroids, Glycosides and Carbohydrates and absence of Terpenoids, Flavonoids, Alkaloids and Anthraquinone. Saponins were present in the leaves, bark, and roots. Flavonoids, Anthraquinones, Terpenoids and Alkaloids were absent in the leaves, bark, and roots of Senna siamea. Steroids were present in the bark and roots and absent in the leaves. Glycosides and Tannins were present in the bark, roots, and leaves of Senna siamea. Carbohydrates were present in the leaves but absent in the bark and roots of Senna siamea.

- *Phytochemical Screening of Nuclea Latifolia*

The phytochemical screening of the petroleum ether extract of the leaves, bark, and roots of Nuclea latifolia showed the presence of Tannins, Saponins, Steroids, Glycosides, and absence of Terpenoids, Flavonoids, Alkaloids, Anthraquinone and Carbohydrates. Tannins were present in the leaves and roots but absent in the bark. Flavonoids, Anthraquinones, Terpenoids, Carbohydrates and Alkaloids were absent in the leaves, bark, and roots of Nuclea latifolia. Steroids were present in the bark and roots and absent in the leaves. Glycosides and Saponins were present in the roots, bark, and leaves of Nuclea latifolia.

- *Thin Layer Chromatography Results*

Thin layer chromatography result in table 4.4 indicated that compounds were present in the leaves and bark of Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia as compared to the roots. Two layers of spots were noticed in the methanol extracts of the leaves and bark of Cassia sieberiana (A and B), and their highest retardation factor (Rf) value ranges from A (0.89 cm) to B (0.81 cm) for the leaves and A (0.93 cm) to B (0.90 cm) for the bark. Two layers of spots were also noticed in the methanol extracts of the leaves and bark of Moringa oleifera, Senna siamea and Nuclea latifolia (A to B). The extracts of methanol were selective with the moving and the stationary phase of the Silica gel.

For the petroleum ether extracts, only Senna siamea shows complete retardation factor value in all the parts (leaves, bark, and roots), the other plants that is Cassia sieberiana, Moringa oleifera and Nuclea latifolia only shows layers of spots in the leaves whiles the bark and roots shows no layer of spot. The leaves of the methanol extracts of Senna siamea showed the highest retardation factor value with a value of 0.99 cm, and the leaves of Nuclea latifolia showed the lowest retardation factor value, with a value of 0.60 cm. Methanol, ethanol, and acetic acid (3:1:1) displayed more compounds when used as a moving phase as compared to the other solvents. The study confirmed that there are more nonpolar compounds in the leaves Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia than the bark, and roots.

- *Ant plasmodial Test Results*

Ant plasmodial tests results produced by methanol extract of Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia. The methanol extract of the leaves, bark, and roots of Cassia sieberiana, Moringa oleifera, Senna siamea and Nuclea latifolia on positive blood samples showed a complete eradication in some plant parts and reduction of the number of parasites in other parts more than the petroleum ether extract which showed smaller reduction in the parasites count on the sample. In the methanol extracts, the bark, and roots of Cassia sieberiana, leaves and bark of Senna siamea and roots of Nuclea latifolia showed complete eradication of the malaria parasites from the positive blood sample followed by drastic decrease in parasite count by leaves (5 parasites) and bark (8 parasites) of Moringa oleifera and leaves (11 parasites) of Nuclea latifolia while the leaves (20 parasites) of Cassia sieberiana, roots (30 parasites) of Senna siamea, bark (25 parasites) of Nuclea latifolia and the roots (35 parasites) of Moringa oleifera have moderate effect on the parasite. However, artemether-lumefantrine, a known anti-malarial used as a positive control in this experiment, exhibited a much higher activity than the plant extracts under review. From the above results, this could possibly be responsible for the use of this plants by local indigenous people in Sierra Leone and Sub-Saharan Africa for the treatment malaria and other diseases. The use of herbal plants for the treatment of malaria has also been emphasized in other studies like Abdulrazak et al., 2015.

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CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

- *Conclusion*

The leaves, stem bark and roots of *Cassia sieberiana, Moringa oleifera, Senna siamea* and *Nuclea latifolia* in methanol extract showed alkaloids, glycosides, terpenoids, tannins, and saponins, carbohydrate and flavonoids. Antiplasmodial activity was also observed for the methanol extracts of *Cassia sieberiana, Moringa oleifera, Senna siamea* and *Nuclea latifolia*.

From the results obtained from this research, the selected plants can scavenge for free radicals and contains bioactive chemicals that can eradicate the malaria parasite. The methanol extract was shown to be the most effective of the two extracts tested, which implies that the plants should be exploited in the development of novel antimalarial medicines.

- *Recommendation*

This research paves the way for more attention and research into the active chemicals responsible for the selected local plants for their antimalarial effect. More research is needed to determine the precise mechanism of action by which extracts exert their antiplasmodial activity. Finally, efforts should be made to conduct in vivo studies with the extracts to confirm the current in vitro findings about the antiplasmodial effect of the plants. This is why in vivo findings such as toxicity of the plant to humans at different concentrations are very important in the use of the plant for treatment.

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ANNEXES

Appendix 1 Picture Showing the Air Drying of Selected Plants

Appendix 2 Picture Showing Soxhlet Extraction

Appendix 3 Picture Showing the Pulvirized Plants and Phytochemical Screening of Selected Plants

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Appendix 4 Picture Showing Thin Layer Chromatography of Selected Plants

Appendix 5 Picture Showing Microscopic Examination and Antiplasmodial Detection