COMPARATIVE STUDY ON THE β-HEMATIN INHIBITORY POTENTIAL OF METHANOL STEMBARK EXTRACTS OF *ANNICKIA AFFINIS* AND *ANNICKIA CHLORANTHA*

BY

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DEDICATION

This report is dedicated to God Almighty who has seen me through this journey and also to my parents who have been nothing short of amazing.

CERTIFICATION

This is to certify that the project work titled **Comparative Study on the β-hematin Inhibitory Potential of Methanol Stembark Extracts of *Annickia affinis* and *Annickia chlorantha*** was carried out by BENEDICT OCHANYA ELIZABETH-JANE with Matric. No: LSC2103720 a student of the department of Biochemistry, Faculty of life science, University of Benin with Matric. No: LSC2103720.

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ABSTRACT

Malaria caused by *Plasmodium falciparum* is still a global challenge to date. The major process for malaria parasite survival within red blood cells is the detoxification of heme, a toxic byproduct released from hemoglobin digestion, into a crystalline pigment called hemozoin. Agents which inhibit this process can be used to curb the parasitic development. The discovery of such agents can be done using the β-hematin inhibition assay, in-vitro studies using hemin, otherwise known as synthetic heme. The ability for hemin to polymerize into β-hematin provides the assay the characteristic capacity to be used as a means to study the inhibition of hemozoin formation (β-hematin). This study seeks to compare the β-hematin inhibitory potential of the methanol stembarks of *Annickia affinis* and *Annickia chlorantha*. *Annickia affinis* and *Annickia chlorantha* stembark extracts were observed to possess notable capability in inhibiting β-hematin formation with *A. chlorantha* performing better at inhibiting β-hematin formation than *A. affinis* .

CHAPTER ONE

1.0. INTRODUCTION

Malaria has been a global health problem for millennia, affecting a gross number of people worldwide. Based off of estimates, there are roughly 290 million observed cases worldwide each year, with over 400,000 deaths . Malaria is caused by protozoa from the Plasmodium genus , with four species responsible for the human infection*: P. falciparum , P. vivax , P. ovale* , and *P. malariae*. Other plasmodia species can infect reptiles , birds, as well as mammals . (James and Stephen, 1996). Malaria can bear moderate symptoms - such as fever ,chills , and headache - and can also be very severe including fatigue, convulsions ,and difficulty breathing . Infants, children under the age of five, travelers, expectant m/others/women, and those living with HIV and AIDS are among those at high risk and can be very vulnerable to the infection.

Five known *Plasmodium* species causes malaria in humans with two of them - *P. falciparum* and *P. vivax* - posing tangible threat at the rate where *P. falciparum* has become the most common in Africa and *P. vivax* being the most prevalent in most countries outside of Sub-Saharan Africa.(WHO ,2025). Some malaria parasites, which often cause low-risk versions of the disease can live for years and occasionally induces relapses (Mayo Clinic, 2025).

Malaria being very much connected to marshy areas was termed "mal' aria" which means "bad air" under Italian nomenclature . The life cycle of the malaria parasite consists of intricate mechanisms that occur in both vertebrate hosts and mosquito vectors. One of the major setbacks in the advancement of modern medicine is due to the fact that the parasite has both sexual and asexual reproductive mechanisms. Malaria therapy dates as far back as the second century , around the period when

China applied Qinghai which is *Artemisia annua* in Latin to manage fever and chills and also during the invasion of the Peruvians, when they notably identified the now-popular Cinchona remedies from the bark of the Cinchona tree in the 16th century .

Scientists have done a great load of work in identifying valuable components of potent today's medicine such as in the case of the discovery of the key constituent of the *Cinchona succirubra* which had long been utilized in chemoprophylaxis and malaria treatment. Significant was the discovery of the *Artemisinin* from the plant *Artemisia annua* , by a group of Chinese students led by Dr. Youyou and it has proven it's antimalarial activity over time as it has been used in combination therapies around the world . However , considering the emergence in resistance to anti malarial agents in some areas, discovery of new anti-malarial agents with high therapeutic value, low toxicity, and low cost is necessary and of uttermost importance to the medical field (Muluemebet and Ephrem , 2023) .

1.1 LITERATURE REVIEW

Among the reported malaria cases , the African region bore the bulk of the global malaria burden accounting for 94% (246 million) of malaria incidence and 95% of malaria mortality (569,000) in the scenario of an estimated 263 million and 252 million cases recorded from 83 countries in 2023 and 2022 respectively. It was observed that children under the age of 5 , that came down with the infection accounted for over 76% of all malaria mortality in the African region (WHO, 2024).

The discovery and comprehension of how malaria was transmitted was first dicovered in the 1800s , when Laveran ,a scientist from Algeria identified the

etiology of malaria and the *Plasmodium* parasite around the year 1880-1882 (CDC, 2024). Many successful findings were engineered around 1900 and recorded at local levels in diverse areas of the world where the infection was prevalent . In time , the use of chemicals such as dichloro diphenyl trichloroethane tagged DDT was brought to light and applied, as its prospects of been used as an insecticide was discovered in 1939. The use of DDT was profound during the World War II, becoming the primary effective tool in the malaria eradication effort which started in 1955 under the League of Nations supervision. However, DDT was abandoned or rather, phased out in the year 1978 and in following years , the impact of long lasting impregnated nets also known as LLINs was hampered too, by a considerate amount of factors including the rise in resistance to insecticide in mosquito populations , Anopheles diversity , and changes in the behaviourial patterns of he Anopheles vector as some species became exophagic/ exophilic (Li et al., 2025).

1.1.1 EPIDEMIOLOGY OF MALARIA

From records and observations , the malaria parasite *Plasmodium falciparum* was the most dominating specie with an estimation of 69.2% (523/763) during the survey of the Hubei province between 2013 to 2018 with the highest peak occurring in 2014 where 105 imported cases of the infection were reported (Xia et al., 2022). The changes in the malaria parasite's distribution sequence in diverse regions is a major factor in malaria study . Such changes were observed in the nineteenth century when malaria was found more in Europe , parts of Australia , and North America compared to current times where it has been confined to

tropical areas such as Mexico, South and Central America, the Indian subcontinent , and Southeast Asia (Fisher, 2021).

Based on malaria stratification data , researchers can tell the decline or up-climb of malaria incidence in various sub-district levels . A classic study of the Harari region showed a declining trend in malaria cases from 2017 to 2019 providing a considerable decrease in the malaria transmission map and about 70% of the sub districts achieved elimination targets (Esayas et al., 2020). Most disease cases in countries such as Ethiopa, has been due to the burden of malaria resulting to a high level of recorded mortality with *P. falciparum* as well as *P. vivax* bearing the greater responsibility for malaria incidences. From reports , over 60% of the global population inhabits malaria risky areas and ever since the 2000s there has been an appreciated increase in investment to promote malaria interventions in Africa and Ethiopia ,both being countries with higher malaria burden (Fikadu & Ashenafi, 2023).On the stance of this progress, Countries like Ethiopia has strategized to achieve nationwide malaria elimination by 2030 via sub-national elimination in districts that has low malaria transmission. Deliberate and constant epidemiology evaluation of malaria changes are implemented in the control of the infection and thus the mortality rate , as well as investigation of casual relationships between risk factors and outcomes (Esayas et al., 2020).

1.1.2. ETIOLOGY OF MALARIA

The life cycle of Malaria starts with the conventional biting andsucking of an infected host during the gametocyte stage ,and this activity is usually carried out by the malaria vector . The sexual stage of reproduction of the malaria parasite occurs

in the vector whereby the male and female gametes mate in the gut , and inhabits it for the period of 10-12 days before maturing into sporozoites .The matured sporozoites migrates to the salivary gland of the vector , guaranteeing transmission

to a host during blood meals because upon feeding on a person the parasites in the saliva is released into the bloodstream and then travels to the liver where it inhabits for 10-14 days before attacking the erythrocytes resulting in typical symptoms such as a climbing fever and chills (CDC, 2024). For some persons , especially those who reside in endemic areas like the border regions of Thailand , Myanmar , and

Cambodia , may not manifest acute symptoms from malaria .The most severe type of malaria , *Plasmodium* falciparum , has a 7-14 day incubation period, resulting in brain malaria and even death in chronic stages. The *Plasmodium vivax* which can be a less serious form of malaria having an incubation period of 8-14 days and can lie latent in the liver for many years ,might result in a severe case when treatment is not received on time . With an incubation period of 18-40 days , the *Plasmodium malaria* species of malaria can induce chronic infection while *Plasmodium knowlesi* is a type of malaria that advances quickly but can remain dormant for many years following infection (Medpark Hospital,2025). Notably, the introduction of a non-native vector can pose a major threat to public health as it is certain to cause catastrophic outbreaks (CDC, 2024).

1.1.3. LIFE CYCLE OF MALARIA PARASITE

Two hosts are primarily involved in the life cycle of the malaria parasite. During a blood meal , a female Anopheles mosquito carrying the malaria virus injects sporozoites into the human host . However , infection of blood circulation which can cause relapse can still occur weeks or even years later due to the persistent

actions of the dormant stage in *P. vivax* and *P. ovale* species called hypnozoites. Here, the sporozoites attack the liver cells and develop into schizonts which ruptures to release merozoites, multiplying asexually in the erythrocytes, a process referred to as erythrocytic schizogony , following their preceding replication in the liver (non-erythrocytic schizogony). The ring stage trophozoites develop into schizonts which bursts upon release ,while the merozoites infects the erythrocytes and during a blood meal the Anopheles mosquito consumes the sexual erythrocytic stages of some parasites. It is worthy to note that the disease's clinical symptoms are caused by blood stage parasites . The sporogonic cycle is the term for the replication of the parasite in mosquitoes , where the microgametes break through the macrogametes in the mosquito's stomach to produce zygotes and in turn the zygotes grow elongated and dynamic (ookinetes), which infiltrate the mosquito's midgut wall and mature into oocysts. The oocysts develop , burst, and release sporozoites , which travel to the salivary glands of the mosquito. The malaria life cycle can be prolonged by injecting the sporozoites into a fresh human host (DPDx,2024).

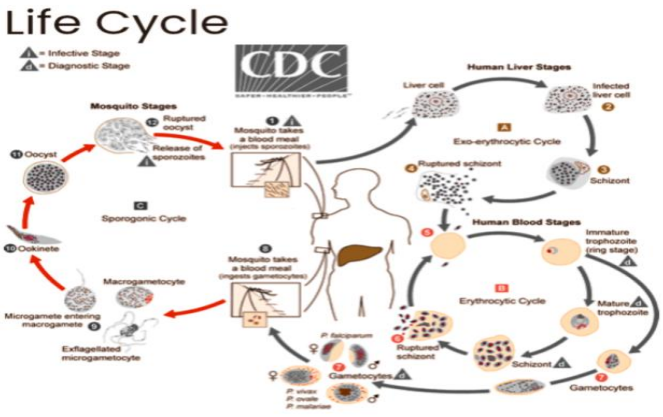

Figure 1: Diagram showing the life cycle of *Plasmodium* falciparum (Source: CDC DPDx, 2024)

1.1.4. CLINICAL SYMPTOMS OF MALARIA

Although the host's prior exposure or immunity to the malaria infection influences the symptomatology and period of incubation , patients with malaria begins to exhibit symptoms a few weeks after infection . One of the first symptoms among majority of malaria patients are headaches followed by clinical signs such as outbreaks of sweat, malaise , cough, fatigue , chills , arthralgia , myalgia , which occurs every 48 or 72 hours and depending on the species , the symptoms may progress to high fevers , shivering chills that lasts for one to two hours , convulsions eventually leading to diaphoresis , profuse perspiration and a fall in body temperature to normal or below normal (Bennett & Herchline, 2025). A large number of patients , typically in the early stages of infection may have multiple minor fever spikes each day but do not exhibit the typical fits . Due to many broods forming in the bloodstream , the periodicity of fever associated with each species , such as 48 hours for *P. falciparum* , *P. vivax* , and *P. ovale* also known as tertian fever, does not emerge during the initial infection .Patients with persistent concurrent infections are likely to have typical fever patterns (William et al.,) and atypical malarial symptoms which constitutes anorexia, sluggishness ,as well as vomitting , diarrhea , jaundice (Bennett &Herchline, 2025).

1.1.5 DIAGNOSIS OF MALARIA

Accurate diagnosis of malaria is essential for treating infected patients appropriately and preventing the infection from spreading across the community . Malaria dignosis can be challenging since medical professionals may have become unfamiliar with the disease in areas where it is no longer prevalent , which could cause them to ignore or overlook malaria as a potential diagnosis . In the end , they

can decide not to request a malaria test. Also , in certain areas where the disease is endemic , it can get so severe that a significant section of the populace is infected yet not sickened by the parasites (CDC, 2024).

CLINICAL DIAGNOSIS

As an addition to the malaria-specific diagnosis, the medical professional may ask for a routine chemical panel and a complete blood count . The aforementioned assessment will be useful in determining if the patient has low-risk or severe symptoms of the malaria infection in the event that the patient's test results does come out positive. Specifically , these tests can identify hyperbilirubinemia , severe anemia , hypoglycemia , renal failure and acid-base abnormalities (CDC, 2024).

MICROSCOPIC DIAGNOSIS

A drop of the patient's blood spread out as a blood smear on a microscope slide can be examined under the microscope to identify the malaria parasite .In order to give the parasite a unique appearance for easy identification , the specimen is typically stained with Giemsa stain prior to analysis . Before the diagnosis of malaria can be ruled out , the blood smear procedures should be carried out three times consecutively at a minimum , every 12 to 24 hours (CDC, 2024).

ANTIGEN DETECTION

Different test kits are available in the global context to identify antigens obtained from malaria parasites . These immunologic tests yield results in 12 to 15 minutes

with the application of dip sticks and cassette format . When accurate microscopic diagnosis is unavailable , these Rapid Diagnostic Tests (RDTs) provide a helpful alternative to microscopy (CDC, 2024).

MOLECULAR DIAGNOSIS

The polymerase chain reaction method is used to identify parasite nucleic acids and may be more sensitive than blood smear microscopy. In the typical hospital scenario , it is not very useful for diagnosing critically ill patients (CDC, 2024).

1.1.6 MANAGEMENT OF MALARIA

Because untreated uncomplicated malaria can progress to chronic stages and requires early identification as well as efficient and sensible therapy to curb the infection, treating malaria infection is both preventive and curative . WHO counsels the administration of combined therapy for all cases of malaria , with the application of at least two potent antimalarial agents that function through distinct pathways , in order to prevent or totally eliminate the spread of resistance to antimalarial agents(WHO,2016).

1.1.6.1 PHARMACOLOGICAL MANAGEMENT OF MALARIA USING ORTHODOX MEDICINES

Quinoline dérivatives offer multiple modes of action for treating malaria infection, according to a review of antimalarial pharmacology and an analysis of the findings of recent malaria research in Vietnam (Hunsicker, 1969).

However , in recent malaria trials conducted in Vietnam , no single antimalarial drug has produced sufficient cure rates .While *pyrimethamine* and a sulfonamide combination have manifested synergistic effects that resulted in improved outcomes , quinine and *pyrimethamine* has shown additive benefits (Hunsicker and Lawrence, 1969). The use of *Artemisinin* -based combination therapy (ACT) has become an important therapy strategy in recent years , but with the emergence of resistance to both past and present antimalarial agents , emphasis have become thorough on the need for further and more advanced research (Tse et al., 2019).

Since the isolation in 1820 of *quinine* from the bark of the *cinchona* tree, numerous natural and synthetic compounds have been developed to combat malaria including:

- *Mepacrine*: *Mepacrine* also referred to as *quinacrine*, was marketed under the trade name *Atabrine* and was primarily utilized as a preventative during World War II (Tse et al., 2019).

- *Chloroquine*: For the treatment of *P. vivax* in areas where resistance has not emerged , *Chloroquine* is listed on the WHO's Model List of Essential Medicines (MLEM) (WHO, 2024).

- *Mefloquine*: *Mefloquine* was developed in the 1970s by the United States Army and has been utilized as both a curative and a preventive medication. Being one of the medications on the MLEM,it was first applied in the management of chloroquine -resistant malaria (Edwin et al .,2019).

- *Pyronaridine* : The 2022 WHO guidelines update strongly recommended *Pyronaridine-artesunate* as the newest *arteminisin* –based combination therapy (ACT) for both uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* malaria. This combination is currently regarded as a safe and effective ACT for the

treatment of uncomplicated malaria in adults and children weighing 5kg and above in malaria-prevalent areas. *Pyronaridine* a blood schizonticide, exerts it's antimalarial activity by inhibiting haemozoin pigment formation in the parasite digestive vacuole, similar to other *quinine* –type antimalarial drugs. (Wan et al ., 2023).

1.1.6.2 HERBAL MANAGEMENT FOR MALARIA

Malaria treatment is increasingly challenged by malaria parasites resistance to *chloroquine*, a commonly used antimalarial drug in Nigeria. *Artemisinin* , derived from the Chinese medicinal plant, based on active principle of *Artemisia annua* has been used as an alternative to antimalarial agents that has seemingly lost potency due to resistance-developed parasite. Studies have shown that medicinal plants have been found useful in malaria therapy. Their parts such as barks, roots, leaves, or whole plants are valuable sources for developing new antimalarial drugs and have been effective in malaria therapy (Odiegbemi et al., 2024).

Scientific Name (Species)	Family Names	Local Names
Sphenocentrum jollyanum	*Menispermaceae*	Akerejupon
Rauvolfia vomitoria	*Apocynaceae*	Asofeyeje
Enantia chlorantia	*Annonaceae*	Osopa Awopa Dokita igbo
Khaya grandifoliola	*Meliaceae*	Oganwo
Melicia excelsa	*Moraceae*	Iroko
Senna siamea	*Caesalpiniaceae*	Kasia
Senna podocarpa	*Caesalpiniaceae*	Asunwonibile
Azadirachta indica	*Meliaceae*	Dogonyaro
Mangifera indica	*Anacardiaceae*	Mangoro
Physalis angulata	*Solanaceae*	Koropo

Table 1: Potential medicinal herbs for antimalarial therapy (Source: Adapted from Odiegbemi et al., 2024)

S/No.	Botanical Names	Parts Used	Method of Extraction
1.	*Nauclea latifolia*	Bark, roots	Tincture, Decoction
2.	*Morinda lucida*	Roots, leaves	Tincture, infusion
3.	*Enantia chlorantha*	Bark	Decoction, tincture, infusion
4.	*Alstonia boonei*	Bark	Infusion, tincture, decoction
5.	*Curcuma longa*	Rhizome	Tincture, Decoction
6.	*Allium sativum*	Bulb	Concoction tincture
7.	*Carica papaya*	Fruit, leaves	Infusion
8.	*Tithonia diversifolia*	Leaves	Infusion
9.	*Azadirachta indica*	Bark, leaves	Decoction

Table 2 : Parts of medicinal herbs used in malaria treatment (Source: Adapted from Odiegbemi et al., 2024)

1.1.6.3 EVOLUTION OF MALARIA RESEARCH

A number of significant discoveries and turning points have defined the development of malaria research , each of which has expanded our knowledge of the illness and its management. The discovery of *Plasmodium* parasites in the blood of infected patients by the French military physician Charles Loius Alphonse Laveran in 1880 marked the beginning of the current research of malaria ,earning him the Nobel Prize in the year 1907 in Physiology or Medicine. This proved that

malaria is a parasitic illness , laying the groundwork for additional research and analysis. The WHO established the Global Technical Strategy for Malaria 2016-2030 in 2000 with the goal of reducing malaria cases globally (Li et al., 2025).

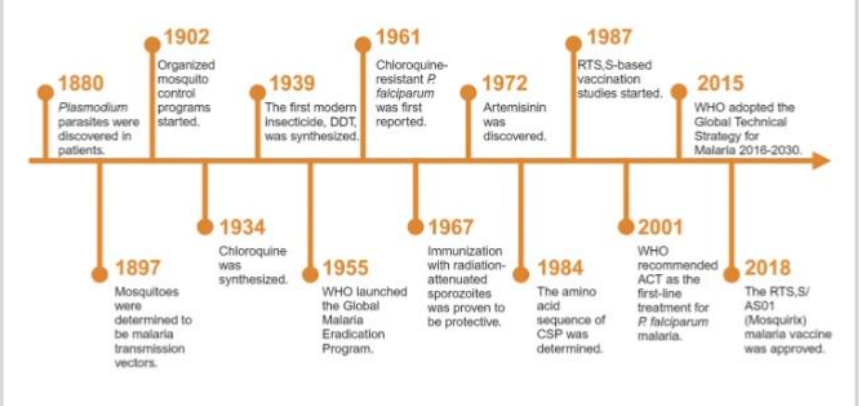

Figure 3: The key milestones in the history of malaria are depicted in this timeline (Source: Adapted from Li et al., 2025)

1.1.7 *ANNICKIA AFFINIS* AND *ANNICKIA CHLORANTHA*

In Western and Central Africa, the multipurpose medicinal plant specie *Annickia chlorantha* , which is a member of the *Annonaceae* family , has been widely used as a traditional therapy for malaria .Often referred to as yellow wood , it is also identified with several indigenous names,including Awopa, Osu pupa or Doikitaigbo in Yoruba, Osomolu in Ikale, Eruemeru in Nigeria , Kakerim in Boku, Erenba-vbogo in Benin, Mfo in Boulou , Mpouley in Mabea , Njie in Douala, Yellow moambi in English, and Moambi jaune in French .*Chlorantha* is a wide-spread decorative dense forest tree found in lowland forests throughout sub-Saharan Africa . It can be found in the Eastern and Western forests of Cameroon, the southern region of Nigeria, Gabon, Guinea ,and Ivory Coast, Liberia, Angola (Cabinda), and DR Congo (Province Bas-Congo) (Anyutoulou et al., 2021).

Analgesic, antioxidant, anti-convulsive, anti-diabetic, anti-inflammatory,anti-microbial, anti-mycobacterial, anti-plasmodial, antipyretic, antisickling, antitumor, antiulcer, antiviral, hepatoprotective, hemostatic, testiculoprotective, and uterine stimulation properties are just a few of the pharmacological characteristics of the *A. chlorantha* plant (Sarbadhikary & George, 2002). Anemia, bacterial infections, fever, infected wounds, infective hepatitis, jaundice, leprosy spots, malaria, rickettsia fever, stomach aches, tuberculosis, typhoid fever, urinary tract infections, and yellow fever are among the many human ailments that have traditionally been treated with these plant parts , its bark, roots and stems inclusive (Anyutoulou et al., 2021). Traditionally, brew and extracts from stem bark of *A. chlorantha* either alone or in combination with extracts from diverse therapeutic drugs such as Rauvolfia vomitaria and Fagara macrophylla and/or Nauclea latifolia have been mainly used to treat aches, boils, chills, fever, hepatitis, malaria symptoms, sore, vomiting. Hepatitis, intestinal worms, intestinal spasms, jaundice, sexual asthenia, typhoid fever, and urinary tract infections have all been found to be effectively managed by oral stem bark extract administration. Stem bark, however, has been shown to be useful as a hemostatic agent, uterine stimulant, as well as treating leprosy spots, stomach issues, and certain forms of cutaneous, gastric, and duodenal ulcers. Dried stem bark can be used to treat conditions like hepatic disorders, malaria, tuberculosis, and ulcers, and while its root extract has been utilized for antimalarial, anti-jaundice, and antipyretic reasons, the bark infusion has demonstrated efficacy in treating wounds and cough (Sarbadhikary & George, 2002).

Figure 4: *Annickia chlorantha* (Oliv.) (Source: Sarbadhikary & George, 2002)

- ***Annickia affinis***

Known as the African yellow wood ,the plant species *Annickia affinis* belongs to the Annonaceae family and is used extensively in Central Africa and some regions of West Africa to treat a variety of illnesses. It has a straight , cylindrical trunk that is about 80cm in diameter , smooth, grey-brown to blackish bark, and leaves that are narrowly elliptic to obovate, acuminate or acute at the apex and cuneate at the base , with a petiole that is between 8 and 12 mm long and a leaf blade that is 3.5 × 27 cm long and 1.5 × 9cm wide .From observations , the lower part of the leaf's pubescent surface is short and pale green when fresh, having its bifid or trifid hairs pointing in the direction of the apex. Its glabrous upper surface is glossy dark green when fresh to grey/brown to black when dry (Erhunse et al., 2024).

Figure 5: *Annickia affinis* (Source: Erhunse et al., 2024)

1.1.7.1 TAXONOMIC CLASSIFICATION OF *ANNICKIA AFFINIS* AND ANNICKIA CHLORANTHA

ANNICKIA AFFINIS

Kingdom: Plantae

Division: Tracheophytes

Clade: Angiosperms

Clade: Magnoliids

Order: Magnoliales

Family: Annonaceae

Genus: Annickia

Species: *A. affinis*

Binomial name: *Annickia affinis*

ANNICKIA CHLORANTHA

Kingdom: Plantae

Division: Tracheophytes

Clade: Angiosperms

Clade: Magnoliids

Order: Magnoliales

Family: Annonaceae

Genus: Annickia

Species: *A. chlorantha*

Binomial name : *Annickia chlorantha* (Oliv.) Setten & Maas

Synonyms Enantia chlorantha Oliv.

(Source: Sarbadhikary & George, 2002; Erhunse et al., 2024)

1.1.7.2. ANTI MALARIAL PROPERTIES OF *ANNICKIA AFFINIS* AND ANNICKIA CHLORANTHA

Annickia affinis and *Annickia cholorantha* share similar bioactive compounds, including flavonoids and phenolic compounds, alkaloids (such as isoquinoline and protoberberine), terpenes (with sesquiterpenes) are found in both plants. These plants also contain acetogenins which are known for their anticancer properties and

are highly therapeutical in antimalarial treatments. The presence of these bioactive compounds in both *Annickia affinis* and *Annickia chlorantha* contributes to their anti-malarial effects (Sarbadhikary & George, 2002; Erhunse et al., 2024).

1.1.7.3 ANTIMICROBIAL PROPERTIES OF *ANNICKIA AFFINIS* AND ANNICKIA CHLORANTHA

Annickia affinis and *Annickia chlorantha* are valued for their broad-spectrum antimicrobial effects, particularly against bacterial and protozoan pathogens. Traditionally, both species have been used in the treatment of diseases such as malaria, typhoid, and jaundice due to their ample phytochemical composition (Anyutoulou et al., 2021). *Annickia chlorantha* demonstrates antimicrobial efficacy, with activity observed against several bacterial strains, including *Klebsiella pneumoniae* and is vastly used in ethnomedicine for managing infectious diseases such as malaria, hepatitis, and jaundice. Its antipyretic potential also helps it to alleviate fever (Sarbadhikary & George, 2002). *Annickia affinis* has shown potent in vitro activity against *Plasmodium* falciparum, aiding its application in traditional antimalarial therapies (Erhunse et al., 2024). Extracts from these plants, particularly those obtained with methanol, have demonstrated notable antibacterial effects, specifically against pathogens such as *Salmonella typhi* and *Neisseria gonorrhoeae*, but with limited actions against fungal activity in the case of Candida albicans (Anyutoulou et al., 2021).

AIM OF STUDY

This study compared the β-hematin (synthetic hemozoin) inhibitory potential of methanol stem bark extracts of *Annickia affinis* and *Annickia chlorantha* , in order to assess their antimalarial mechanism of action.

CHAPTER TWO

MATERIALS AND METHOD

Various materials and methods were employed during the course of this study to ensure a successful and effective analysis.

2.1 MATERIALS

The following materials were used for the analysis

1. PLANT SAMPLES

The plant used for this analysis were *Annickia affinis* and *Annickia chlorantha* stembark extracts.

2. REAGENTS

- Methanol
- Distilled water
- 0.5 M Sodium acetate buffer
- 0.1M Sodium Hydroxide
- Dimethyl Sulfoxide
- Hemin Chloride

3. APPARATUS

- Eppendorf tubes

- Eppendorf racks

- Micropipette and tips

- Gloves

- Weighing balance

- pH meter

- Beakers

- Stainless steel spatula

- Petri dish

- Plastic bottles to contain samples

- Foil paper

- Spectrophotometer

- Centrifuge

2.2 METHODOLOGY

PROCEDURES FOR CARRYING OUT IN VITRO HEMOZOIN STUDY

Hemin chloride (250 µL, 0.5 mg/mL) was freshly prepared in DMSO. To this, 500 µL of 0.5 M sodium acetate buffer at pH 4.4 and 250 µL of the test extracts (*Annickia affinis* and *Annickia chlorantha*) (0-500µg/mL), positive control (*Quinine*), was then added in Eppendorf tubes.

This mixture was kept at 37℃ for 24 hours. Thereafter, the mixture in the Eppendorf tubes were centrifuged for 10mins at 4000 rpm. The supernatant was then removed following which the tubes were washed again with 1000µL DMSO per tube so as to remove unreacted hemin. This was followed by centrifugation for another 10 mins and then the supernatant was removed.

The β-hematin remaining was then dissolved in 1000µL of 0.1M NaOH, forming an alkaline hematin. This was measured at 405nm using a spectrophotometer.

CHAPTER THREE

RESULTS

3.1. β-HEMATIN INHIBITORY POTENTIAL OF METHANOL STEMBARK EXTRACTS OF *ANNICKIA AFFINIS* AND *ANNICKIA CHLORANTHA*

The malaria parasite *Plasmodium* can only survive in the human host using the mechanism of hemozoin formation which involves the polymerization of heme into an inert crystal called hemozoin .This is because upon digestion of the host's hemoglobin inside the red blood cell, free heme is released which can generate reactive oxygen species, disrupting cell membranes and can be very toxic to the parasite , hence the action of hemozoin formation takes place.

Scientist can observe the parasite's acidic digestive vacuole conditions in the lab where it naturally crystallizes into β-hematin (synthetic hemozoin) and then in vitro hemozoin inhibition studies is conducted to test how drugs or compounds can block this crystallization process outside the body. During the course of this study *Annickia affinis* and *Annickia chlorantha* were plant species used to test for the inhibition of hemozoin formation. Spectrophotometric readings were taken to determine the concentration of the dissolved hemozoin still present in the sample.

The chart below shows the comparative evaluation of the Inhibitory concentration 50 (IC_{50}) values of *Annickia affinis, Annickia chlorantha* and a standard antimalarial drug; *quinine*. Lower IC_{50} values indicate stronger inhibition and potential antimalarial efficacy, thus *Annickia chlorantha* shows a significant potential to be studied as an anti-malarial agent in comparison with *Annickia affinis* (Figure 6).

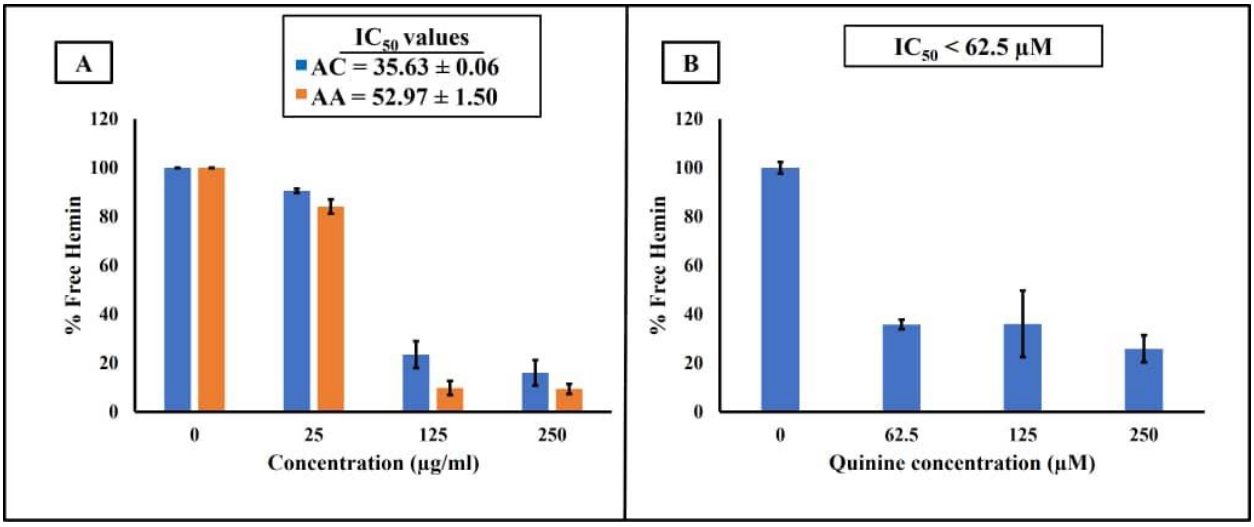

Figure 6: In vitro β-hematin inhibitory potential of methanol stembark extracts of *Annickia affinis* and *Annickia chlorantha* (A) and a standard antimalarial drug known to possess this property (B).

CHAPTER FOUR

DISCUSSION AND CONCLUSION

Annickia affinis and *Annickia chlorantha* , both referred to as Yellow wood in western and central Africa, are closely related species and have been constantly identified as same plant. However, studies have proven that they are separate with individual characteristic properties peculiar to each one. Some of such distinctive properties are listed in the table below :

Annickia chlorantha	*Annickia affinis*
i. It possesses bright green leaves that are brown to grey-green at the upper surface when dry, and a rough grey bark.	i. It possesses dark green leaves which are grey-brown to almost black at the upper surface when dry, and a smooth greyish-brown bark.
ii. It is rich in flavonoids	ii. It is rich in berberine
iii. Hairs on lower leaf surface can be manifested as single, bifid, trifid ,or stellate that points in all	iii. It is characterized by simple hairs found under the leaf on the midrib that points

direction. iv. It has a good amount of antiplasmodial activity	towards the apex. iv . It has shown a greater likelyhood to contain more antiplasmodial activity.

Table 3 : Differences between the plant specie of *Annickia chlorantha* and *Annickia affinis* (Source: Sarbadhikary & George, 2002; Erhunse et al., 2024)

The comparative analysis of the methanolic stem bark extracts of *Annickia chlorantha* and *Annickia affinis* revealed notable differences in their β-hematin inhibitory activities. This is because the IC_{50} value -which is an abbreviate for inhibitory potential 50- refers to the concentration of the sample or drug needed to inhibit 50% of β-hematin formation and therefore can be used to determine the potency of the plant samples or synthetic drug under appropriate experimental conditions. Lower IC_{50} values indicates higher inhibitory activity, hence in hemozoin formation research, compounds that inhibit hemozoin formation at a low IC_{50} value are considered as potential antimalarial candidates capable to manage malaria at non-toxic levels. Therefore, the comparative study of the β-hematin inhibitory potential of both *Annickia chlorantha* and *Annickia affinis* plant species showed that *Annickia chlorantha* had more potency to be employed in antimalaria therapy, as a result of its lower IC_{50} value, than *Annickia affinis* . But this does not mean *Annickia affinis* cannot be further studied or investigated in order to be applied in malaria management. From this study, using *quinine* as a

standard with an IC_{50} value of <62.5 µM thus, validating the protocol used for assessing both plants.

The result of the study goes further to prove that *Annickia chlorantha* and *Annickia affinis* are two separate species with differing capacity but belonging to the same family and thus should not be identified as similar or as one. More studies on both plants are required for simple means of differentiating both plant samples.

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