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QUALITATIVE AND QUANTITATIVE EVALUATION OF CRYPTOLEPIS- BASED HERBAL FORMULATIONS IN GHANA

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSORPHY (MPHIL) DEGREE IN PHARMACEUTICS

BY

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DECLARATION

I declare that this thesis is my own work and idea and that it does not contain materials published by any other investigator except where due references and acknowledgement have been given. I further declare that this work has not been submitted to any institution or body for the award of any degree or certificate.

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ABSTRACT

Background: Cryptolepine is the first and main alkaloid isolated from the West African shrub *Cryptolepis Sanguinolenta*. Cryptolepine has been extensively studied and scientifically proven to exhibit antimuscarinic, antithrombotic, anti-inflammatory, anti-microbial, hypoglycaemic and antimalarial activities. It is also reported to be cytotoxic, being a DNA intercalator and a topoisomerase II inhibitor. In Ghana, *Cryptolepis*-based preparations which are mainly aqueous extracts of whole roots of *Cryptolepis sanguinolenta* are used as antimalarials. Currently, there are a number of branded *Cryptolepis Sanguinolenta* herbal formulations on the Ghanaian market. The proliferation of these brands calls for pharmacovigilance to ensure that spurious/falsely-labelled/falsified/counterfeit (SFFC) brands are identified to ensure the safety and quality of these formulations and hence improve malaria treatment outcomes.

Objectives: To qualitatively and quantitatively assess available commercialized brands of *Cryptolepis*-based formulations on the Ghanaian market using visual inspection and physicochemical analysis.

Method: Fourteen different brands of *Cryptolepis*-based herbal formulations were purchased from licensed wholesale and retail pharmacies and herbal shops within two cities (Accra and Kumasi) of Ghana. The brands were coded A to N. One to three different batches of each brand were used for the study. All the brands and batches were aqueous formulations and had FDB registration numbers. Physicochemical properties as well as packaging and labelling assessment were performed and compared to British Pharmacopoeia standards. Pour plate method was used to investigate the level of microbial contamination of the products. Evaluations of the brands were carried out to detect and quantify the active compound cryptolepine using a combination of solvent-solvent extraction and reverse phase high

performance liquid chromatography (HPLC). The cryptolepine contents of multiple batches were compared using one way ANOVA analysis. Samples were also screened for possible antimalarial adulterants such as artemether, lumifantrine, artesunate and amodiaquine using validated HPLC methods.

Results: All the brands did not meet one or more of the packaging and labelling requirements. All samples passed seal integrity test. Samples B, G and M passed the microbial contamination limit test whilst the rest failed. All samples contained glycosides, tannins, saponins, sterols and alkaloids. Cryptolepine was detected in all brands except E and F. The cryptolepine content quantified from the samples ranged from 0.0162 mg/ml to 0.3235 mg/ml. Batch 1 of sample M had the highest (0.324 mg/ml) cryptolepine content (n=3). Using one-way ANOVA analysis followed by Bonferroni *post hoc* multiple comparison test, there were significant variations (t values ≤ 0.05) in the cryptolepine content of the different batches of samples B, I, M and N. There were no detectable amounts of artemether, lumifantrine and amodiaquine in all the samples. Eleven of the fourteen samples gave positive test for artesunate.

Conclusion: All the fourteen brands of cryptolepis- based formulations were defective in one or more of the basic requirement of pharmaceutical formulations. Twelve samples contained cryptolepine in concentrations greater than the IC_{50} value of 0.134±0.037 µM but lower than LD_{50} value of 300mg/kg body weight. Eleven out of the fourteen brands may have been adulterated with Artesunate.

DEDICATION

This work is dedicated to God Almighty and my family.

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CHAPTER ONE

1.1 General Introduction

The use of traditional medicines (TM) / herbal medicines (HM) and traditional medical practices have become a common practice among people of different race, age, colour, occupation, educational ,financial and social status around the world (Calixto, 2000; Mensah and Gyasi, 2012; WHO, 2005).

These herbal medicines are marketed in different dosage forms to manage, prevent and treat a wide range of diseases around the world (Brautigam et al., 1998; Calixto, 2000; Harkey, Henderson, and Gershwin, 2001; Peter, 2001; Willcox and Bodeker, 2004). Today, the use of herbal medicines plays an increasingly vital role in health care globally (WHO, 2005), however, there are challenges in the harmonization of regulatory standards for these medicines.

Malaria is a mosquito borne infectious disease of public health concern in the world. The disease is a scourge in the health care of the tropical and subtropical domains of the world. The disease is reported to claim lives of all age groups in less transmission areas whereas in highly endemic regions, the lives of young children are least spared (WHO, 2014). Majority of malaria cases are reported in Sub-Saharan Africa, and the rest from around the world. Extensive research has been carried out on the cause of the disease, the presentation of the illness, the diagnosis and the treatment (Alfandari et al., 1996; Kengeya-Kayondo et al., 1994; Makler, Palmer, and Ager, 1998; Mirdha, Samantaray, and Mishra, 1997; WHO, 2014)

Chemotherapy is the centrepiece in malaria treatment. In malaria chemotherapy, orthodox medicines are mostly used in the developed world whereas those in the malaria-endemic less developed world use more of herbal remedies than the orthodox.

All over the world, medicinal plants have served as the main sources of lead compounds in discovery and development of new drug compounds as well as in the combat against diseases including malaria. As a result, anti-malarial medications such as quinine, chloroquine, amodiaquine, artemether, dihydroartemisinine and artesunate, have emerged in the fight against malaria (Osorio, Robledo, and Bastida, 2008; WHO, 2011, 2014). Close to 80% of antimalarial medications are obtained from plant origin. For example, quinine and cinchonine, from *Cinchona spp,* the artemisinins from *Artemisia annua* (Osorio et al., 2008).

Cryptolepis sanguinolenta (Asclepiadaceae) is a popular antimalarial plant in Sub-Saharan Africa. It has been used for decades by traditional healers and is claimed to be effective in the treatment of various infectious diseases such as amoebiasis, enteric fever and malaria (Boye and Ampofo, 1983). The plant has been studied over the years to prove its antimalarial, antihypertensive, hypoglycaemic, antimicrobial, antifungal, and anticancer activities (Ansah and Gooderham, 2002; Boakye-Yiadom, 1979; Cimanga, De Bruyne, Pieters, Vlietinck, and Turger, 1997; Kirby, Paine, Warhust, Noamese, and Phillipson, 1995; Kumar, Etukala, and Ablordeppey, 2008; Wright et al., 1996). Attempts have been made to develop the active metabolites of this plant into suitable pharmaceutical dosage forms (Bugyei, Boye, and Addy, 2010; Kuntworbe, 2012). However only aqueous formulations are currently available on the Ghanaian market and other countries (Bugyei et al., 2010; Kirby et al., 1995; Kumar et al., 2008).

In the management of malaria infections, the availability, affordability of antimalarial drugs and evidence of their efficacy are the priorities to most consumers around the world. In the tropics and sub-tropics where malaria is endemic and poverty is on the increase, people resort to herbal decoctions, tablets, powders, and infusions in treating malaria because of obvious reasons. In such instances, factors that contribute to the holistic safety of the medication such

as contamination, adulteration, directions for storage, dose and dosage regimen among others are often over looked (Renu, 2007).

The primary findings in this research have shown that there is the need for further improvement in the quality of Cryptolepis-based formulations currently in circulation. The presence of Artesunate in some brands raises doubt about their label claim of being herbal formulations. Also disturbing is the fact that some brands contain microbial contaminants beyond acceptable limits.

1.1.2 Justification

In spite of measures in place to curb it, malaria continues to be a disease of public health concern globally due to alarming mortality rates especially in children(WHO, 2014) .

Herbal formulations have long been trendy among malarious regions of the world. This is due to the perception that they are cheap, readily available, of fewer or no side effects(Mensah and Gyasi, 2012). The demand for herbal formulations in such regions continues to remain high and as such manufacturers of such formulations are likely to produce substandard medications to meet the demands of their consumers and maximize their profits(Osorio et al., 2008).

The profound use of *Cryptolepis sanguinolenta* as alternative treatment for uncomplicated malaria demands the necessity of the safety and quality assessment of commercialized formulations containing the plant extract.

Cryptolepine is the main alkaloid contained in *Cryptolepis sanguinolenta.* Its antiplasmodial activity has been intensively researched and proven (Bamgbose and Noamesi, 1981; Cimanga et al., 1997; Grellier et al., 1996; Kirby et al., 1995; Wright et al., 1996), This therefore makes the alkaloid a potential lead compound in antimalarial drug discovery.

There is however no scientific data on the safety and quality profile of commercialized herbal liquid formulations of the plant.

The ever increasing antimalarial drug resistance (especially due to substandard medications) and subsequent decline in antimalarial medication efficacy, calls for the need to evaluate commercialized herbal formulation containing *Cryptolepis sanguinolenta,* to aid counteract any threat of resistance of plasmodium parasites to this potential lead compound and to enhance malaria treatment outcomes.

1.1.3 Aim

The aim of this research is to assess the quality and safety of Cryptolepis-based herbal formulations on the Ghanaian market.

1.1.4 Specific objectives

To achieve the above aim, the following objectives were set:

- Assess the packaging and labelling of the formulations
- Conduct phytochemical screening on the formulations as well as the root powder of *Cryptolepis sanguinolenta.*
- Determine the level of microbial contamination of the formulations
- Develop a reverse phase high pressure liquid chromatography (HPLC) method with UV detection and validation to identify and quantify cryptolepine in the samples.
- Analyse the samples for the presence or otherwise of adulterants.

CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1 Malaria

2.1.1.1 Overview

Malaria originates from the words '*mal'* meaning poisoned or bad and '*aria'* meaning air. The disease was believed to arise from marshy areas hence the name MAL-ARIA. It was later recognized as an infectious disease caused by various species of the *Plasmodium* parasite.

The statistics for malaria according to the World Health Organization (W.H.O) are staggering. The statistics released in December, 2014 (WHO, 2014) revealed that, in the year 2013, there were about 198 million cases of malaria (with uncertainty range of 124 to 28 million) with estimated death of 584,000 (with an uncertainty range of 367.000 to 755,000). Malaria is clustered in some areas of China, Afghanistan, Sri Lanka, Brazil, Vietnam, India, Indonesia and Thailand. The disease is broadly distributed in Sub-Saharan Africa where majority of the world's malaria cases originate from (Renu, 2007) . In Africa, where a child dies every minute from malaria, the mortality rate is high in children under 5 years of age (Amexo, Tolhurst, Barnish, and Bates, 2004; WHO, 2014).

2.1.1.2 Transmission of malaria

The plasmodium parasites are hosted in and spread from man to man by the malaria vector known as the *Anopheles* mosquito. The anopheles mosquito often bites between dusk and dawn to spread the disease. In malaria endemic regions, there are four popular malaria causing species are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae.* Among these species, *Plasmodium falciparum* is the most lethal.

Plasmodium knowlesi, a species known to cause malaria in monkey and commonly found in south East Asia is reported to have cause malaria in humans(WHO, 2014) .

Malaria transmission is dependent on four main factors;

- The vector
- Environmental factors
- Type of parasite and
- The host

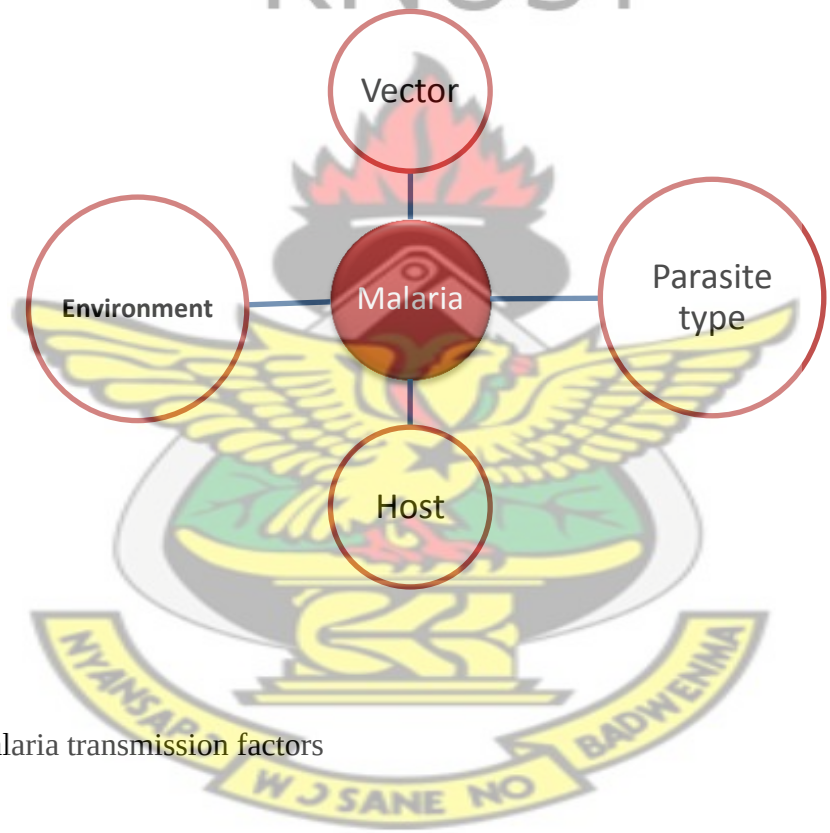

Figure 2.1: Malaria transmission factors

2.1.1.2.1 The vector

Malaria is transmitted solely through the bite of an infected female Anopheles mosquito. In the world, there are over 350 species of Anopheles mosquitoes of which approximately 60 are malaria vectors with 20 being of relevance in malaria transmission in man and animals.(WHO, 2014)The Anopheles mosquito resides and breeds in waterlogged areas such as puddles, ponds, rice farms, stagnant drainage systems and hoof prints. Transmission of

malaria depends on the breeding preference of the vector. Some vectors prefer deep collection of waters whereas others prefer a shallow collection of fresh water bodies.in both cases, transmission is intense in areas where mosquitoes are able to live long enough to allow the parasite to complete its development stages in the mosquito(Renu, 2007).

2.1.1.2.2 Environmental Factors

Temperature, humidity and rainfall patterns are essential factors that contribute to the survival of the mosquitoes around the globe. As the climate changes, throughout the year, reported cases of malaria also vary, this is due to the survival and number of mosquitoes during each climate change. Climatic conditions also play a role in the duration of the life cycle of the parasite in the vector(WHO, 2010a) . Mosquitoes thrive best in ponds and poodles, hence are usually found in such areas.

The map in figure 2.2 shows the current global malaria distribution which is represented by the yellow hatched lines. This distribution was done using modern climate variables i.e. 0.5 degree special resolution and is based on climatic suitability for malaria transmission(Rogers et al., 1993)

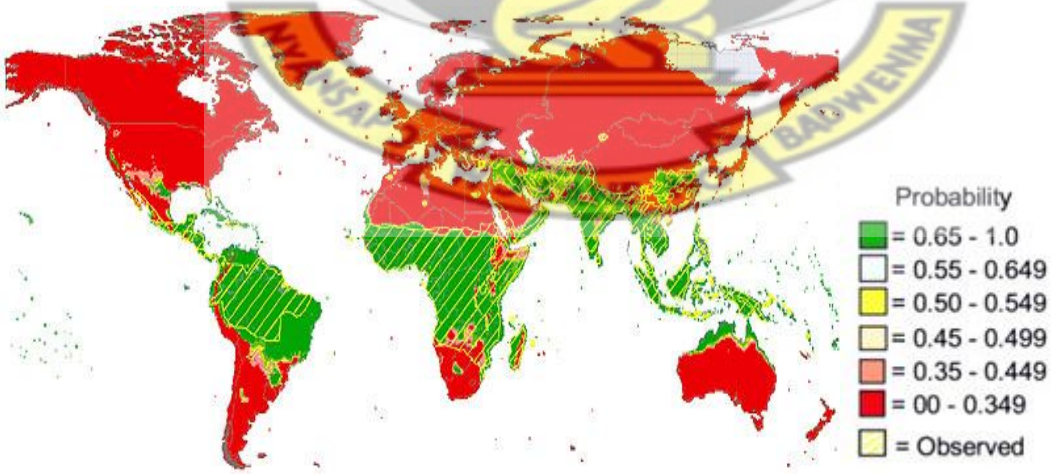

Figure 2.2: Observed and predicted global malaria distribution, using 1960-90 climate data according to the TALA research group.

The map in figure 2.3 shows the predicted distribution of malaria 35-40 years to come as compared to present day malaria transmission distribution

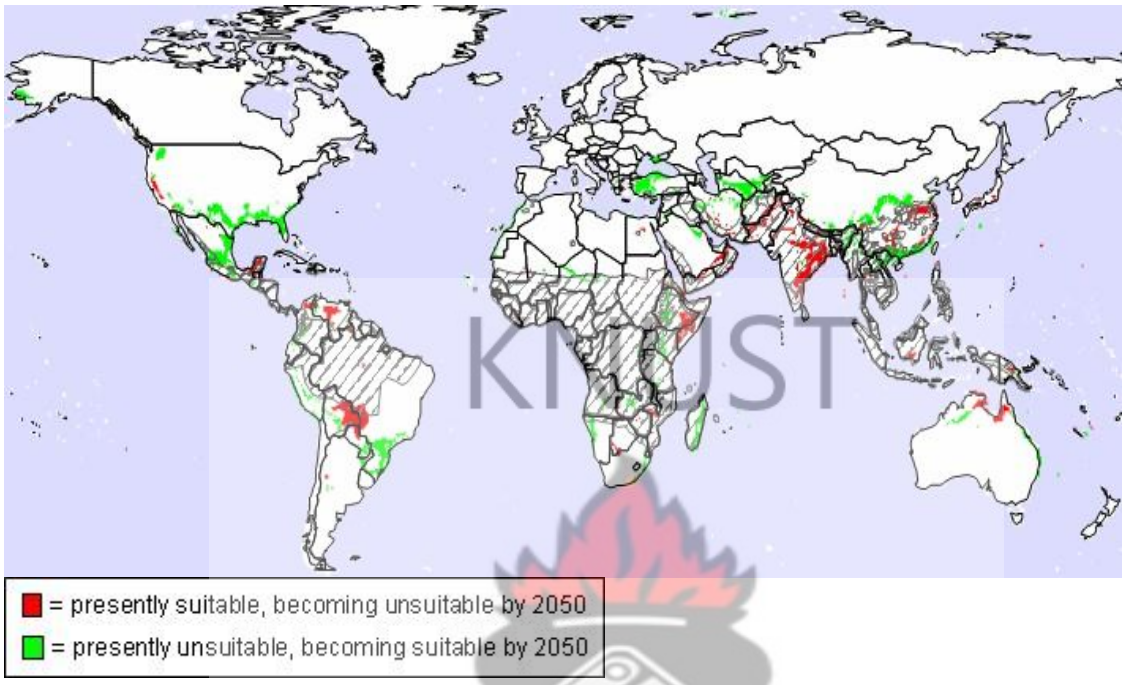

Figure 2.3: Predicted shift in global malaria distribution by the year 2050. credit to TALA research group (Rogers et al., 1993)

2.1.1.2.3 Parasite Type

There are five main types of malaria causing parasites in man namely; *Plasmodium ovale, Plasmodium falciparum, Plasmodium malariae, Plasmodium knowlesi* and *plasmodium vivax. Plasmodium falciparum* is dreadful because of its inability to be selective on old and young red cells. In species other than humans, *Plasmodium gallinaceum, Plasmodium berghei, Plasmodium chabaudi* and *Plasmodium vaughani*, cause malaria.

2.1.1.2.4 The Host

The immunity of the host plays an important role in the transmission of malaria from man to man. Years of exposure provides some partial immunity but does not guarantee complete immunity further years to come. This partial immunity however reduces the possibility of

severe malarial in individuals. This therefore explains the increased death in children (under 5 years) with malaria in the tropics. These children have no or very low immunity to the disease therefore sever malaria is common among them hence they die. Adults in low transmission zones around the world also have no or low immunity hence are not spared from severe malaria and death from the disease (WHO, 2014).

2.1.1.3 Life cycle of malaria parasite

The malaria parasites that affect humans have a complex life cycle which consists of the sexual and asexual stages. The sexual life cycle of the parasite begins when the female *Anopheles* mosquito bites an infected person and sucks the infected blood. The microgametocytes (male malaria parasite) in the ingested blood then produces about six flagella which separates from the parent body and moves through the coagulating blood to find, enter and fertilize a macrogamete (female malaria parasite). A gamete is formed after fertilization, which then travels to the walls of the stomach of the mosquito where it encysts under the outer lining of the stomach wall. Within this oocyte, there is multiplication of the parasites until thousands of them are formed. This causes a rapture of the oocyte to release spindle-shaped *sporozoites* which travel to the salivary gland of the mosquito. The whole process, that is from the ingestion of infected blood meal to the production of sporozoites for another infection can take between 7 to 21 days depending on the specie of the *Plasmodium*, the humidity and ambient temperature.

The asexual phase of the malaria parasite begins when an infected female *Anopheles* mosquito bites a non-infected human. During the bite, the mosquito introduces saliva, which serves as an anticoagulant to prevent the blood meal from clotting in the mosquito's tiny proboscis. The saliva introduced, contains the sporozoites from the sexual stage the of the life cycle, these sporozoites then move straight to the liver cells with the sole aim of invading the

cells. Within a liver cell, one parasite is able to generate thousands of parasites within 7-21 days. The division and generation of the parasite cause the liver cell to enlarge, at this stage the parasite in the liver cell is known as liver schizont. The liver cell burst to release the thousands of merozoites into the human blood stream where they quickly attach to and enter the red blood cells and start feeding on their contents. At this stage the parasites are known as trophozoites(WHO, 2010a, 2010b) . These stages are typical of *Plasmodium falciparum* and *Plasmodium malariae*. In the life cycle of *Plasmodium ovale* and *Plasmodium vivax* there is a slight variation. That is, when the sporozoites get into the liver cells, they do not immediately become schizonts; rather they go into a dormant phase and are called hypnozoites. These hypnozoites are responsible for the relapse of malaria at certain intervals after a malaria attack(Renu, 2007; WHO, 2010a, 2010b).

2.1.1.4 Malaria presentation/clinical signs/symptoms

The symptoms of malaria are similar to that of other medical conditions. However, in people who have never had malaria, it is reasonably simple to identify the disease (WHO, 2010a).

The symptoms of malaria are usually developed six to fifteen days after the bite of an infected mosquito or months after departure from malaria endemic regions. The very common symptoms of malaria include; headache, high fever, rigors (severe chills), general body pains, profuse sweating. Less common symptoms include; cough, nausea, vomiting, diarrhoea, anaemia. For patients with recurrent malaria, these symptoms are not clearly manifested to aid in diagnosis. Hence a laboratory test is the best way to diagnose malaria(Källandera, Nsungwa-Sabiitib, and Peterson, 2004; O'Dempsey et al., 1993; Renu, 2007; WHO, 2010a, 2010b).

Malaria can be classified as uncomplicated or severe depending on the symptoms presented by the patients. Uncomplicated malaria can be associated with all the human plasmodium

parasites but infection with *P. falciparum* is easily progressed into lethal form of malaria. The clinical manifestation of severe malaria in children include convulsions, hypoglycaemia (25% of children with severe malaria),and severe form of anaemia.in adults severe malaria is manifested by jaundice, pulmonary oedema and acute renal failure in some cases. Respiratory arrest, cerebral malaria, acidosis and shock cannot be left out of the symptoms of severe malaria since they can occur in both young and old(Idro, 2008; White, 1996)

2.1.1.5 Diagnosis of malaria

The symptoms of malaria makes it difficult to make a presumptive diagnosis of the disease(Amexo et al., 2004; Källandera et al., 2004). There are two main methods of diagnosing malaria. These methods are the clinical diagnosis and biological diagnosis.

Clinical diagnosis is by far the cheapest way of diagnosing malaria and it is solely based on the symptoms of malaria (Wongsrichanalai, Barcus, Muth, Sutamihardja, and Wernsdorfer, 2007) .however due to the fact that symptoms of malaria overlap with that of other diseases (O'Dempsey et al., 1993), malaria is often misdiagnosed and this leads to indiscriminate use of antimalarial.

Biological diagnosis of malaria goes a step further from clinical diagnosis. it involves the use of biological fluids from the patient involved. Microscopy is a typical example of biological diagnosis of malaria(Wongsrichanalai et al., 2007)

Malaria in a patient is confirmed using a parasite-based diagnostic tool or procedure. Microscopic examinations of a patient blood film is considered the gold standard to diagnose malaria.in this method of diagnosis, the microscopist is able to identify the parasites if they are present in the blood. Also, the stage of the life cycle of the parasite can also be determined to assist in the selection of appropriate antimalarial.

This method has its own set of limitations, in that; in Microscopy is a skilled exercise that requires an expert with precise differential and visual skills to produce accurate results.The methods also require a laboratory setting to aid in producing good results. The method is relatively expensive to run in most malaria endemic communities where health care centres a sparingly distributed (WHO, 2010a)

Rapid test (RT) for the detection of malaria parasite is preferred to the microscopy this is to reduce the patient waiting time at health care facilities and to provide a more convenient diagnosis outside the laboratory. Rapid test for malaria comes in two forms, these are the Rapid Diagnostic Test kit (RDT) and the Quantitative Buffy Coat (QBC)(Anthony, Bangs, Anthony, and Purnomo, 1992; Palmer et al., 1998a; Wongsrichanalai et al., 2007).

The use of rapid diagnostic test (RDT) kit facilitates the diagnosis of malaria in patients both at home or health centres (Palmer et al., 1998b). This is due to the fact that the kit is portable, the test is easy to perform and it does not require the services of a trained personal or any special laboratory setting or sophisticated equipment (Renu, 2007). The rapid diagnostic test kit detects the malaria antigen in blood sample from a finger prick of the patient. This takes about fifteen to thirty minutes and a result is obtained.

This diagnostic procedure albeit convenient, has its set of limitations. With the RDT, *Plasmodium falciparum* can be diagnosed but *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium malariae* cannot be distinguished out rightly (Wongsrichanalai et al., 2007).

The Quantitative Buffy Coat involves the use addition of the dye Fluorochrome acridine orange to blood specimen to stain the parasites (Anthony et al., 1992).Other techniques such as the Ligase Detection Reaction-Fluorescence Microsphere Assay LDR-FMA and the use of Laser Desorption Mass Spectrometry have also been suggested as methods for malaria

parasite detection in blood samples (Carnevale et al., 2007; Demirev et al., 2002; Scholl et al., 2004). None of these techniques has provided excellent results in malaria diagnosis due to the limitations associated with each method. For health workers in deprived communities and for people living in poor countries where health centres are scarce, clinical diagnosis which is based on the symptoms of malaria is the only option.

False positives from the diagnosis of malaria result in unnecessary treatment of malaria which usually comes at a cost. Also, false negatives which are likely to occur in the diagnosis of malaria leaves patients untreated or treated for other conditions and this comes with its financial burden and deterioration of patients' health, prolong treatment period and finally death. this results in negative socio-economic impact on the individual, society and the world at large (Amexo et al., 2004; Ohrt, Purnomo, Sutamihardja, Tang, and Kain, 2002)

2.1.1.6 Treatment of malaria

Malaria cases are curable if treated promptly with adequate medications. The WHO in its fight against malaria, recommends that all suspected malaria cases should be confirmed with either RDT or microscopy before treatment is initiated (WHO, 2014).

In malaria treatment the goal of therapy is to clear the malaria parasites and to inhibit the development of the parasite at any stage possible. To achieve the above goal, chemotherapy remains the mainstay in malaria treatment. Before the development of prescription drugs, herbal/plant medicine was used in the treatment of malaria.

Home treatment of malaria in malaria endemic regions has been done with folkloric antimalarials which include decoctions and infusions of *Cinchona sp.*, *Artemisia annua* , , *Ocimum canum, Azadirachta indica, Parkia biglobosa, Mangifera indica ,Nauclea latifolia, Ozoroa insignis* (Asase, Oteng-Yeboah, Odamtten, and Simmonds, 2005; Mueller et al.,

2004). *Cryptolepis sanguinolenta* is one of the plants used as an antimalarial agent and has acquired lots of attention by researchers around the world (Kirby et al., 1995; Onyeibor et al., 2005).

In malaria endemic regions ,where accessibility to health facilities is far-fetched, patients rely on these plant remedies from their back-yard gardens, neighbours and shops within their vicinity (Goodman et al., 2007).

Interventions have been made by the pharmaceutical industries in the treatment of malaria. A number of antimalarials have been developed from active phytoconstituents and others synthetically developed over the years.

These antimalarials can be classified based on their site of drug action (parasite life cycle classification) or their chemical composition (Egan, 2001; Egan et al., 2000).

2.1.1.6.1 Classificaton of antimalarial drugs based on parasite life cycle

1. Blood schizonticides; these are also known as suppressive agents examples include amodiaquine, chloroquine, doxycycline, tetracycline, mefloquine, quinine, halofantrine, artemisinine, sulphadoxine and pyrimethamine.
2. Tissue shizonticides; these are able to interrupt with the developmental stages in hepatic forms of the parasite. They are most useful in the treatment of tertian fever to prevent the relapse of infections of *Plasmodium ovale* and *Plasmodium vivax*. Examples are pyrimethamine and primaquine.
3. Gametocytes; these eliminate the sexual forms of the parasite. Examples include chloroquine, quinine and primaquine.
4. Sporonticides ; These include proguanil and pyrimethamine

2.1.1.6.2 Classification of antimalarial drugs based on chemical composition

1. 4-aminoquinolines; These include quinine, mefloquine, chloroquine, quinacrine
2. Dihyrofolate reductase inhibitors. examples are pyrimethamine, chlooquanide, trimethoprim
3. 8-aminoquinoline. Primaquine is an example of this group.
4. Sulphones/sulphonamides, dapsone and sulfadiazine are examples
5. Antibiotics .tetracycline, doxycycline are examples (Jambou, Le Bras, and Randrianarivelojosia, 2011).

Chloroquine was hailed the magic drug in the treatment of malaria (Cooper and Magwere, 2008), it was the ideal drug for the treatment of all forms of malaria. In spite of the fact that it causes nausea, transient neuropsychiatric syndrome and most commonly pruritis (in dark-skinned patients), it was generally well tolerated worldwide (White, 1996). Later on, resistance of *Plasmodium falciparum* to chloroquine was however detected (WHO, 2003).

In recent times the WHO has recommended the use of artemisinin-based combination therapy (ACT i.e. artemeter/lumifanrine,artesunate/amodiaquine,dihydroartemisinine-piperaquine) in the treatment of malaria following the resistance to and failure in monotherapies of artesunate, artemether, chloroquine over the years(Longo et al., 2006; WHO, 2003, 2011, 2014). Quinine is still being used in the treatment of malaria in pregnant women where ACT is not recommended. Sulphadoxine-pyromrthamine is also used as a single dose as intermittent presumptive treatment (IPT), in the case, the glucose 6 phosphate dehydrogenase G6PD status is considered first.

Antimalarials are often administered through the oral route. examples include ACT's, sulphadoxine-pyrimethamine, quinine, halofantrine, mefloquine, chloroquine.in severe conditions or when oral medication cannot be tolerated, the rectal route or parenteral route

are considered until orals can be administered. Artesunate suppositories are examples of rectal antimalarials. Parenteral formulations include artesunate, artemether and quinine.

Choice of medication in the treatment of malaria is dependent the severity of the malaria, patients age and weight, trimester of pregnancy, the causative parasite specie, the possible pattern of susceptibility of the parasite to antimalarials, availability and the cost of the medication (White, 1996; WHO, 2011).

2.1.1.7 Malaria control/prevention

In an attempt to control malaria in recent years, prompt diagnosis, appropriate medication the use of insecticide treated nets (ITN's), indoor residual spraying (IRS) and outdoor spraying are the main measures taken which have produced significant reduction in the spread of malaria around the world (WHO, 2010a, 2014).

2.2 Background of *Cryptolepis sanguinolenta*

There are in all twenty species of the genus Cryptolepis found in Africa,Asia,Papau New Guinea, Madagasca, Australia and Asia. In Africa, there are seven main species of Cryptolepis. Considering the distribution of these seven species,most of them are found close to the east coast of the continent with the exception of *Cryptolepis sanguinolenta* that is extensively distributed in the coastal region of west Africa(Paulo and Houghton, 2003)

Cryptolepis sanguinolenta (Lindl.) Schltr. is a typical tropical rain forest plant. It is a climbing shrub with slender thin stem. It belongs to the family Asclepiadaceae and subfamily Periplocaceae (Paulo and Houghton, 2003; PROTA, 2009).

Figure 2.4 Roots of *C. sanguinolenta*

Figure 2.5 Cut roots of *C. sanguinolenta*(x20)

In folkloric medicine, the bitter alcoholic or aqueous extract of the roots of the plant is popularly used. In Ghana, the plant is locally known as Kadze or nibima or gangamau or yellow-dye root or Ghana quinine(Tempesta, 2010). it is used by the people of the eastern region of Ghana to treat different forms of fever, some bacterial infections and malaria (Boye and Ampofo, 1983).

2.3 Bioactive constituent of *Cryptolepis sanguinolenta*

The first and main alkaloid isolated from the roots and leaves of Cryptolepis sanguinolenta is cryptolepine. The roots of the plant also contain other alkaloids that are structurally related to cryptolepine. these alkaloids are 11-hydroxycryptolepine, cryptotakieine, cryptospirolepine, isoneocryptolepine, neocryptine, isocryptolepine and quindoline (Bierer, M.Diana, Christopher, Jian, and Patricia, 1998; Parvatkar, Parameswaran, and Tilve, 2011; Wright et al., 2001)

2.4 Cryptolepine

Cryptolepine is one of the alkaloids that was chemically synthesized first before its isolation from its natural source. Among the species of Cryptolepis, *Cryptolepis triangularis* was the first source of cryptolepine. The isolation of cryptolepine from the *triangularis* species was first done by Cinquart in 1929 followed by Delvaux (Clinquart, 1929).It has also been

isolated from Cida acuta and Microphilis guianensis as well by other researchers(Gunatilaka, Sothrrswaran, Balasubramanian, Chandrasekara, and Sriyani, 1980; Lavrado, Paulo, Gut, Rosenthal, and Moreira, 2008). Cryptolepine was later isolated from the root of *Cryptolepis sanguinolenta*. Root samples of *Cryptolepis sanguinolenta* from Ghana were also investigated by Dwuma-Badu and his co-workers to isolate cryptolepine (Bierer et al., 1998).

Cryptolepine and its isomeric indoloquinolines exist as tetracyclic compounds in their salt form in acidic medium and give a characteristic yellow colouration. These tetracyclic compounds in their salt form exhibit several remarkable biological activities due to the N-5 atom in its structure. however, in basic medium, they exist as a free base and thus results in a characteristic purple colouration (Bierer et al., 1998). The base form has a molecular weight of 232 g/mole and a melting point range of 175-179^{0}C whereas the hydrochloride salt has a higher molecular weight of 268.5g/mol with a corresponding melting point of 268^{0}C(Bierer et al., 1998).

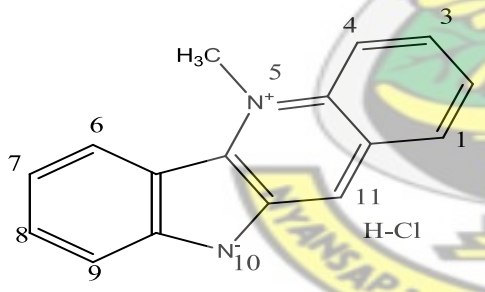

Figure 2.6: Chemical structure of cryptolepine hydrochloride

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2.5 Biological Evaluation of Cryptolepis sanguinolenta/Cryptolepine

2.5.1 Antiplasmodial Activity

Antiplasmodial activity of cryptolepine has been attributed to the N-5 methyl group on the compound as well as its DNA intercalation (Arzel et al., 2001; Bierer et al., 1998; Kirby et

al., 1995; Kumar et al., 2008; Onyeibor et al., 2005; Singh, Singh, and Ablordeppey, 1996; Wright et al., 2001). When a series of derivatives of cryptolepine was synthesized the IC50 values obtained against chloroquine resistant strains of *Plasmodium falciparum* (K1) were less than 0.1µM which in turn is about ten times lower than that cryptolepine. In vivo and in vitro antimalarial activity of cryptolepine confirmed the alkaloid has activity against chloroquine-resist strains of *Plasmodium falciparum* with IC50 value of 0.134±0±.037 µM, however, in an in vivo study using mice infected with *Plasmodium berghei*, a subcutaneous injection of 7-113 µM cryptolepine did not cause any detectable chemosuppression in the parasite. In the same study, to buttress the animalarial activity of cryptolepine, Kirby and his co-workers discovered an interaction between herring testes DNA and the alkaloid cryptolepine ,hence this DNA intercalation could augment the antiplasmodial activity in the in-vitro study (Kirby et al., 1995).

In another work to investigate antiplasmodial activity of cryptolepine against two chloroquine resistant Plasmodium falciparum strains K1 and W2, the IC50 values obtained were 33±0.1 ng/ml and 41±0.5 ng/ml respectively. From the experiment, cryptolepine was relatively potent against the test species compared to quinine, artemisinin and chloroquine as seen exhibited in the table below. These findings rank cryptolepine as a very important source of next generation antimalarial.

Table 1.1: IC_{50} values of quinine, artemisinin, chloroquine and cryptolepine against K1 and W2 chloroquine resistant strains of *Plasmodium falciparum*(Kirby et al., 1995)

Compound	IC50 values ng/ml	
	K1	W2
Quinine	35.2±2.0	102±1.1
Chloroquine	72±0.1	68±0.1
Artemisinin	3.3±0.1	2.7±0.1
Cryptolepine	33±0.1	41±0.5

Antimalarial activity of different extracts of cryptolepine (ethanol, methanol, aqueous) was studied to ascertain the their effects.it was observed that, methanolic was the most potent against the test organisms.in this work it was observed that quindoline(a demethylated cryptolepine at N-5 position)was inactive against the plasmodium species used hence a confirmation that the antiplasmodial activity of cryptolepine is linked to the N-5 methyl group(Cimanga et al., 1997).

2.5.2 Anticancer properties of Cryptolepis sanguinolenta/cryptolepine

Taxol, etoposide and vincristine are plant derived anticancer agents and have contributed immensely to cancer chemotherapy and this point to the fact that plants plants are significant avenues for new anticancer agents.

Cryptolepine has been researched to possess anticancer activity (IC50 1.3µM)(Kumar et al., 2008). It has reported to induce apoptosis in the HL-60 leukemia cells and also inhibit topoisomerase II and cytotoxicity activity(Ansah and Gooderham, 2002).

In a research by Ansah and Gooderham, in 2002, the in vitro toxicity of synthetic cryptolepine was investigated and compared with a traditionally used clinical aqueous

formulation of *Cryptolepine sanguinolenta* (TUC). It was established that both synthetic cryptolepine and the TUC formulation (10%w/v) have cytotoxic activity on Chinese hamster lung fibroblast cell line V79 cells which are mammalian cells.

The concentration of cryptolepine that exhibit substancial cytotoxic activity did not produce any mutagenicity. However, cryptolepine and TUC are potential leads in cancer chemotherapy due to the fact that they were able to exhibit low genotoxicity and cause apoptotic cell death of the mammalian cell lines(Ansah and Gooderham, 2002).

2.5.3 Antimicrobial activity of Cryptolepis sanguinolenta

The anti-fungal activities of ethanolic extracts, cold and hot aqueous extract were compared. The observations made were that, 85% of the test organisms (*Candida albicans*) were inhibited by the ethanolic extract and 75% of the test microbes were inhibited by the cold and hot aqueous extracts. This is agreeable to the fact that the alkaloids possess anti-fungal activity.

In a study that was carried out on sixteen (16) pathogenic bacteria that are resistant to a number of commercial and affordable antibiotics, interesting findings were made. The aqueous extract of *Cryptolepis sanguinolenta* produced MIC values ranging from 8.0-32.0mg/ml in twelve (12) test organisms(Boakye-Yiadom, 1979; Mills-Robertson, Tay, Duker-Eshun, Walana, and Badu, 2012).

there reports that cryptolepine has anxiolytic,hypoglycaemic,anti-hypertensive,anti-mauscarinic,anti- inflammatory effects (Bamgbose and Noamesi, 1981; Bierer et al., 1998; Onyeibor et al., 2005; Oyekan, Botting, and Noamesi, 1988)

2.6 Cryptolepine Formulation

Chemosuppressive and repository activity of two brands of herbal decoctions containing Cryptolepis sanguinolenta were studied using *Plasmodium berghei* (ANKA) in mice.

Acute toxicity test provided LD_{50} of the brands to be above 300mg/kg body weight. The study revealed that at a dose of 5ml/kg and 3ml/kg of the two brands used, significant chemosuppression of parasitemia of 61.9% and 58.2% were obtained respectively and when the doses of each brand was increased by a factor of ten (10), the initial concentration increased to 67% and 66.4%. This points to the fact that the herbal decoctions have marked antimalarial activity specifically, schizontocidal activity against *plasmodium berghei*. This chemosuppression is however dosing dependent(Tay, Dankwa, Gbedema, and Archibald, 2011). In another study, a teabag formulation (2.5g) of the powdered roots of C*ryptolepis sanguinolenta* were administered to 44 individuals with uncomplicated *falciparum* malaria at a dose of 7.5g (to be infused) daily in divided doses for seven days. This study was the first recorded clinical trial of the *Cryptolepis sanguinolenta* formulation. This was carried out in two popular polyclinics and one of the few teaching hospitals in Ghana, West Africa. In the clinical trial, twenty two of the patients were relieved of their malaria by the third day and all of the patients were cleared of Plasmodium falciparum malaria by the seventh day. The findings of this study confirmed the antiplasmodial activity of *Cryptolepis sanguinolenta* root powder and this activity was attributed specifically to the main alkaloid cryptolepine (Bugyei et al., 2010).

2.7 Pharmaceutical packaging

A pharmacticeutical container (with its closure) is a substance that is intended to or contains a medicinal preparation or substance and it maybe in direct contact with it. the packaging of pharmaceuticals is a means of economically providing identification, information,

presentation, protection , compliance and convenience to a product during transportation, storage, exhibition and it use(BP, 2007; Winfield, Rees, and Smith, 2009).

Pharmaceutical containers can be described as multidose, single dose, well closed. air tight, temper evident, and sealed containers. Packs can be classified as either primary or secondary.

Primary packs-these are the types that are in direct contact with the substance and have the ultimate role in maintaining the quality of the product example is a bottle.

Secondary packs-these contain the primary pack with the product. Their function is more of a protective and presentational one, in effect they improve the appearance of the product.

2.7.1 Challenges Associated with Good Packaging and Labelling

The concept that underlies packaging and labelling in the pharmaceutical industry is termed Trade Dress. Pharmaceutical companies incur a lot of cost in manufacturing medications of various classes.in addition to the cost of production, packaging of the product is an aspect that is well invested in because not only does it contain the product but also makes it more appealing to consumers.

One major challenge with packaging and labelling of these product is that manufactures always will have to comply with the regulations/protocols of the authorities responsible for the registration of their products to gain approval on the market.in doing so, they miss out on some details necessary for the patients as stated by various compendia. Human factors are not entirely considered.

Challenge number two is that manufactures are more concerned with commercial considerations than cutting down cost on the packages of their products. The point is the product must fit on shelves in pharmacies, they must be portable, hence must come in small size and single use hence there is little to no space for adequate labelling.

Change is an essential tool that is difficult to embark on. “Do not change what works” is a marketing cliché that keeps companies in business. If a pharmaceutical product is accepted and partronized by consumers and has wrong or inappropriate package and label, it is very difficult to correct these errors and this is a third challenge with packaging and labelling of medicines. The more successful a company is the more resistant it is to change.

Packaging and labelling of pharmaceuticals can be misleading if done the wrong way or in an ambiguous manner and this can lead to a cascade of medical errors ranging from wrong medication for the wrong patient, over dose and under dose of medications, wrong route of administration and wrong reconstitution (Willing and Stoker, 1992).

CHAPTER THREE

3.1 Materials and equipment

Mannitol salt agar, nutrient agar, Maconkey agar, saboroud agar and bismuth sulphite agar were obtained from the central stores of the Department of Pharmaceutics, KNUST, Ghana and Department of Microbiology, Central university College Ghana.

Dilute ammonia, chloroform, H_2SO_4, Dragendorffs reagent, Fehling's solution A and B, Lead acetate solution and all phytochemical screening reagents were obtained from the central store of the Department of Pharmacognosy KNUST, Ghana.

Formic acid, methanol, acetonitrile, and diethylamine were donated by Ghana standard Authority central store and were all HPLC grade. Cryptolepine powder and *Cryptolepis sanguinolenta* root powder were kindly donated by Professor Colin Wright of University of Bradford and Dr Noble Kuntworbe of Kwame Nkrumah University of Science and Technology (K.N.U.S.T), Kumasi, Ghana, respectively.

The equipment used are as follows;

Laboratory Incubator (Gallenkamp), oven (Gallenkamp), electrical balance (Mettler Toledo), pH meter (Mettler Toledo), HPLC system (THERMO FINNIGAN SpectraSYSTEM), UV detector (Thermo Finnigan) and general laboratory glass wares.

3.2 Methodology

3.2.1 Sampling and Profiling of Cryptolepis-based formulations

The samples used in the study were purchased from wholesale and retail Herbal shops and pharmacies within Accra and Kumasi Metropolises in the Greater Accra and Ashanti region, respectively; within a period of three months (from March to May,2015). Five brands were

obtained (purchased) from Accra and nine from Kumasi. For each brand, one to three different batches were obtained. In all, a total 92 samples were used for the study. The samples were coded A to N (identity of samples are withheld for ethical purpose).

3.2.2 Packaging and Labelling assessment

The samples collected were assessed based on the following parameters; type of packaging, closure type, type of container, active ingredient(s),Food and Drug Authority registration number, food and drug board registration number, medicine spoon/measuring cup/oral syringe and information on package i.e. name of product, quantity of medicine, batch number, storage instruction(s), expiry date, requirement for handling, manufacturing company, strength of active ingredient, instruction/direction for use and legibility of information (Wang, Cornelius and Gygax, 1996).

3.2.3 Phytochemical screening

The powdered roots of *Cryptolepis sanguinolenta* as well as the samples were screened for the presence or otherwise of major phytoconstituents such as alkaloids, tannins, glycosides, steroids and flavonoids.

3.2.3.1 General test for the presence of alkaloids

Samples were tested for the presence of alkaloids in line with the procedure described by Saha and co-workers (Saha, Mitra, and Kamala Gupta, 2012). Each sample (2ml) was rendered alkaline in a test tube with 1ml of dilute ammonia, and the alkaloids extracted with 3ml chloroform in a separating funnel. The chloroformic layer was separated and chloroform was evaporated off. The residue was dissolved in the 1% H_2SO_4. A drop of Dragendorff's reagent was added to the 1% H_2SO_4 extract. The presence of a reddish-orange (orange-red preferred) precipitate in the test tube indicates the presence of alkaloids.

3.2.3.2 Detection of Cryptolepine in samples

A specific volume of the sample in a 20 ml test tube was rendered distinctly alkaline (pH ≥10) with 2 ml of dilute ammonia, and shaken with 5 ml of chloroform and observed visually. Deep purple colouration of the chloroformic layer indicates the presence of the alkaloid cryptolepine (Saha et al., 2012). The observations were made relative to a reference powdered roots of Cryptolepis sanguinolenta which was similarly treated.

3.2.3.3 Glycosides

A specified volume of the sample was boiled with 5 ml dilute HCl for 5 minutes, cooled and rendered distinctly alkaline by adding 5 drops of 20% KOH. 1 ml each of Fehling's solution A and B was added and the mixture heated over a water bath. Brick-red precipitation indicates the presence of reducing sugars

3.2.3.4 Tannins

1 ml of the sample was diluted to 10 ml with distilled water followed by 5 drops of 1% lead acetate solution. The formation of precipitate indicates the presence of tannins in the sample.

3.2.3.5 Steroids

The sample was extracted with chloroform into a test tube. Concentrated H_2SO_4 was added carefully down the side of the test tube to form a layer below. The reddish-brown/cherry red ring formed at the interface of the two layers indicates the presence of triterpenoid nucleus (sterols).

3.2.4 Level of Microbial contamination

Different dilutions of the sample was prepared (1in 10, 1 in 100, 1 in 1000, and 1in 10,000). Using the pour plate method, 1ml of each dilution was placed in 5 different petri dishes and 20ml of nutrient agar, MacConkey agar, bismuth agar, mannitol salt agar and saboroud agar was added to the samples respectively. The dishes were incubated at 37^{o}C for 48 hours (saboraud at 25 ^{o} C for 72 hours) (BP, 2007). Triplicate determinations were done for each

media for each of the samples. The colony forming units were counted using a colony counter.

3.2.5 Development and validation of HPLC method

3.2.5.1 Instrumentation

A THERMO FINNIGAN SpectraSYSTEM HPLC system equipped with a quaternary gradient pump P400 SN112/12439-5, vacuum membrane degasser SCM1000 SN112/202380, variable-loop Autosampler AS300 with column oven and sample cooling system. The chromatographic column used is a Microsorb™ S1 89-100-D5E61002 equipped with a column guard. Data acquisition analysis and reporting were performed by ChromQuest (version 4.1) chromatographic software. A Thermo Finnigan dual wavelength UV/Vis programmable detector (UV 2000) was used to monitor the eluent.

3.2.5.2 Selection of mobile phase

A number of mobile phases were attempted, In each of these methods, the chromatographic conditions were varied, but this yielded peaks with poor resolutions and significant tailing and shouldering. After several screening of potential mobile phases, Acetonitrile (solution A) and water containing 0.66% formic acid (solution B) with pH 2.53 produced peaks with excellent resolutions when an isocratic approach was used. The mobile phase consisted of 30 % of solution A and 70 % of solution B.

3.2.5.3 Chromatographic conditions

The identification and quantification of cryptolepine from the samples was achieved using an isocratic elution at a flow rate of 1.5 mL/min using mobile phase composition of phase A (acetonitrile) and phase B(water containing 0.66% formic acid) at a temperature of 25 ^{o}C and a pH of 2.53. The sample injection volume was 10µL and UV detection was at 280 nm.

The total chromatographic run time was 15 minutes followed by a wash and re-equilibration of the column to initial mobile phase conditions. The flush volume for the experiment was 400 µL and the needle height from the bottom of HPLC vial was 2 mm. All solvents were HPLC grade.

The pump was purged at a flow rate of 5 ml/min when fresh mobile phase is prepared.

3.2.5.4 Sample preparation

The samples were thoroughly mixed to ensure uniform distribution of fine solute particles.4 ml of each sample was rendered alkaline using dilute ammonia and extracted with 5 (5ml) portions of chloroform using a separating funnel. The organic layer was evaporated to dryness. To each dry sample, 10 ml of mobile phase B was added and allowed to dissolve the sample completely. The solutions were sonicated, filtered with syringe filters and transferred into HPLC vials for analysis.

3.2.6 Validation of HPLC Method

3.2.6.1 Linearity

Different concentrations of the standard cryptolepine powder were prepared in the range 0.0005% w/v to 0.01% w/v. Each concentration was injected to produce a reproducible retention times and pea, the peak areas and retention times were noted. A calibration curve was obtained by plotting a graph of the increasing concentrations against the peak area. The slope of the graph and the coefficient of the regression line were noted.

3.2.6.2 Precision

A solution (0.067%w/v) of the pure sample was prepared using mobile phase solution. The solution was injected at three different times within a day and the peak area recorded. The concentrations were then calculated using the calibration curve and the percentage recovery

also deduced. The RSD of the recovered concentrations of cryptolepine were calculated and recorded.

3.2.6.3 Reproducibility

A 0.067%w/v solution of the pure sample was prepared using mobile phase solution. The solution was injected three times on three consecutive days and the peak area recorded. The concentrations, the percentage recovery were deduced. The RSD of the recovered concentrations were calculated and recorded.

3.2.6.4 Robustness

3.2.6.4.1 pH versus Retention Time/pH versus concentration

Keeping all chromatographic conditions constant, the pH was deliberately varied from 2.41 to 2.64 and the retention times for the pure sample (injected three times for each pH reading) was recorded. The corresponding concentrations were then calculated. The relationship between pH and retention time as well as pH and concentration was compared.

3.2.6.4.2 Flow rate versus Retention time

The flow rate was deliberately varied from 1.46 ml/min to 1.54 ml/min and the retention time noted. The effect of the flow rate on the retention time was studied.

3.2.7 Determination of Adulterants

The presence of artemether, lumifantrine, artesunate and amodiaquine were investigated using HPLC methods(Arun and Smith, 2011; César, Andrade Nogueira, and Antônio Pianetti, 2008; Le Vaillant et al., 2012)

CHAPTER FOUR

RESULTS

4.1 Profile of samples (Brands)

Profiles of samples for the study are shown in Table 4.1 below. All the samples used were within their shelf-life as indicated by the manufacturing and expiry dates on their labels

Table 4.1: Profile of samples for study

Sample Code	Batch Number	Manufacturing Date	Expiry Date
A1	DMP 006	06/ 2013	06/ 2015
A2	DMP OO3	-	-
B1	FM1/10/12	10/ 2012	10/ 2015
B2	FM2/02/13	02/ 2013	02/ 2015
C1	MM1/2014	01/ 2014	01/ 2016
C2	MM2/2014	05/ 2014	01/ 2016
D1	GM 002	11/ 2012	11/ 2014
D2	GM 001	06/ 2014	06/ 2015
E1	1073	07/ 2013	07/ 2015
E2	1074	10/ 2013	10/ 2015
F1	003-SF-12-CP	03/ 2014	03/ 2016

Table 4.1 Continuation

G1	2227635	12/ 2013	11/ 2014
G2	2276506	02/ 2014	01/ 2015
G3	2227667	08/ 2014	07/ 2016
H1	MR2/13	08/ 2013	08/ 2016
H2	MR3/14	03/ 2013	03/ 2016
I1	-	06/ 2013	06/ 2016
I2	-	06/ 2013	06/ 2016
I3	004	06/ 2014	06/ 2017
J1	EF 23-M06-13	06/ 2013	06/ 2015
K1	Mal50-Aug013wk3	08/ 2013	08/ 2015
K2	MLK50-May014wk4	05/ 2014	03/ 2017
K3	Mal50-Jan13wk2	01/ 2013	01/ 2015
L	KKC 04	05/ 2013	05/ 2016
M1	23-06-14	06/ 2014	07/ 2016
M2	14-05-14	05/ 2015	07/ 2016
N1	00179	04/ 2014	04/ 2016
N2	00175	10/ 2013	10/ 2015
N3	00176	11/ 2013	11/ 2015

Note: 1, 2, 3 represent different batches of each brand.

In all, fourteen different brands of *Cryptolepis sanguinolenta* containing herbal liquid formulations were sampled. Two samples lacked batch numbers, expiry date and manufacturing dates. Based on this, the packaging and labels of the samples were further investigated.

4.2 Packaging assessment

The samples were all packaged in plastic amber coloured medicine bottles with temper-evident seals.

All samples had both primary and secondary packages except samples E, M and N that has only primary packages.

Samples G and K were the only ones with medicine measuring cups for patients use.

Only sample G had medication literature in the form of a leaflet as part of the package.

4.3 Labelling assessment

Table 4.2: Information provided on primary (p) and secondary(s) labels of samples

PARAMETERS	SAMPLES													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Name of Product	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Quantity of medicine (volume)	+	+	S only	+	+	+	+	+	+	+	+	+	+	+
Batch Number	p≠s	+	+	+	+	+	P only	+	+	+	+	+	+	+
Storage Instructions	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Expiry Date	+	+	+	+	+	+	P only	+	+	+	+	+	+	+
Manufacturer	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Strength of active ingredient(s)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Direction for use	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Active ingredients	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FDB registration number	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Legibility of information	+	+	+	+	poor	+	+	+	+	+	+	+	+	+

Note: p≠s, information on p is different from that on s; +, Parameter is present on sample;
- Parameter is absent on sample.

There were inconsistencies observed in the labels of the primary and secondary packages of the samples for the study. Samples A, C, E and G exhibited such inconsistencies.

4.4 Phytochemical screening

Table 4.3: Phytoconstituents of the samples and the pure cryptolepine powder.

Phytochemicals	SAMPLES														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Root powder
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Flavonoids	-	+	+	-	+	+	+	+	+	+	-	-	+	+	-
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cryptolepine	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Sterols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: +, indicates the presence of phytochemical; -, indicates the absence of the phytoconstituent.

Samples E and F tested negative for the main phytochemical of interest and this provided a good basis for developing an analytical method for the identification and quantification of cryptolepine in the samples. A further step was taking my developing a reverse phase HPLC method for this purpose.

Table 4.4: Density, pH and colour of samples

Samples	pH	Density (g/ml)	Colour
A1	4.7133	1.4415	Yellow
A2	4.74	1.45845	Yellow
B1	4.14	1.38255	yellowish brown
B2	4.15	1.29015	yellowish brown
C1	5.7567	1.45845	Yellow
C2	5.76	1.4721	Yellow
D1	4.4533	1.4079	yellowish brown
D2	4.63	1.3983	yellowish brown
E1	5.8267	1.49715	Brown
E2	5.94	1.43535	Brown
F	5.6867	1.4064	Brown
G1	5.1833	1.38075	yellowish brown
G2	5.19	1.38195	yellowish brown
G3	5.1846	1.3878	yellowish brown
H1	4.2267	1.1703	Brown
H2	4.24	1.1574	Brown
I1	6.27	1.37145	Brown
I2	6.2613	1.4031	Brown
I3	6.2773	1.3608	Brown
J1	4.3667	1.58115	yellowish brown
K1	4.8167	1.41765	Brown
K2	4.82	1.4547	Brown
K3	4.82	1.386	Brown
L	5.2167	1.6815	yellowish brown
M1	4.8633	1.47045	Yellow
M2	4.87	1.48065	Yellow
N1	5.0367	1.47975	yellowish brown
N2	5.04	1.48905	yellowish brown
N3	5.0436	1.503	yellowish brown

Sample pH was within the range 4.14-6.2773 and density ranged from 1.29015-1.6815

4.5 Microbial contamination

Table 4.5: Microbial load of the different batches of samples

	Colony forming units/mL				
Microorganisms Sample code	Non-fastidious Aerobic Organisms (x 100)	Fungi (x 100)	*Staphylococcus aureus* (x 100)	*Escherichia coli* (x 100)	*Salmonella typhi* (x 100)
A1	4500 ± 3.97	0 ± 0.00	**300 ± 0.50**	0 ± 0.00	0 ± 0.00
A2	25400 ± 42.35	0 ± 0.00	0 ± 0.00	**2340000 ± 139.23**	**2300 ± 2.00**
B1	92100 ± 13	1600 ± 4.63	0 ± 0.00	0 ± 0.00	0 ± 0.00
B2	11900 ± 5.17	1300 ± 2.67	0 ± 0.00	0 ± 0.00	0 ± 0.00
C1	43400 ± 7.40	**24600 ± 4.00**	**28300 ± 4.50**	0 ± 0.00	0 ± 0.00
C2	51500 ± 7.09	0 ± 0.00	0 ± 0.00	**3200 ± 3.13**	0 ± 0.00
D1	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
D2	14310 ± 376.56	4500 ± 5.84	0 ± 0.00	**5400 ± 7.76**	**21500 ± 6.75**
E1	**1210000 ± 904.77**	**65700 ± 6.06**	**4200 ± 1.66**	**2310000 ± 4.50**	**6800 ± 3.38**
E2	**1340000 ± 573.97**	**15200 ± 4.64**	**143300 ± 4.09**	**8710000 ± 6.84**	**2000 ± 2.4**
F	12900±43.59	**28500 ± 5.89**	**10200 ± 2.47**	**23800 ± 4.36**	**4600 ± 1.79**
G1	22100 ± 4.93	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
G2	14000 ± 3.80	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
H1	76000 ± 4.77	1700 ± 3.81	**2300 ± 1.88**	**4000 ± 1.22**	0 ± 0.00
H2	76000 ± 4.76	3600 ± 4.29	**1300 ± 2.24**	**1300 ± 3.03**	0 ± 0.00
I1	5500 ± 7.69	6100 ± 7.18	**4700 ± 6.69**	**19100 ± 4.24**	**15700 ± 7.83**
I2	10400 ± 318.00	9700 ± 2.13	**8900 ± 4.92**	**21400 ± 5.38**	0 ± 0.00
I3	11700 ± 6.04	9600 ± 5.39	**3200 ± 2.50**	**1300 ± 3.77**	**200 ± 0.71**
J	75200 ± 4.42	600 ± 1.58	**7100 ± 2.30**	0 ± 0.00	**5100 ± 3.24**
K1	92000 ± 5.34	4500 ± 6.65	**8400 ± 2.26**	**42600 ± 5.10**	**10200 ± 4.00**
K2	11600 ± 5	4800 ± 2.96	**3500 ± 4.30**	**39400 ± 7.91**	**24700 ± 6.44**
K3	12500 ± 6.84	**136200 ± 6.04**	**18000 ± 3.43**	**25300 ± 3.81**	**0 ± 0.00**
L	76600 ± 15.62	376 ± 5.94	**63100 ± 7.33**	0 ± 0.00	**20800 ± 3.71**
M1	46900 ± 4.87	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
M2	43400 ± 4.82	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
N1	56900 ± 3.24	**5700 ± 2.70**	0 ± 0.00	0 ± 0.00	0 ± 0.00
N2	80400 ± 5.25	**1100 ± 2.39**	**1600 ± 3.71**	**43 ± 3.50**	0 ± 0.00
N3	75200 ± 4.28	**21400 ± 6.4**	**2100 ± 4.39**	**35 ± 3.21**	0 ± 0.00

NOTE: Bold figures indicate samples that failed a particular organism's range of values. Reference number of acceptable microorganism for the samples are as follows ;,Non-fastidious Aerobic Organisms less than (100,000)10^5,Fungi less than (10,000)10^4, *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* should be completely absent

All samples but B, G and M were contaminated beyond the standard permissible range,this is to say that samples that failed the test contained *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* (which are supposed to be completely absent) and also contained more than 100,000/ml of aerobic organisms and more than 10,000/ml of fungi,. This renders them unsafe for use and gives more than enough reason to conduct further investigations to confirm their quality for use. The reverse HPLC analytical method was next.

4.6 Development of HPLC Method

4.6.1 Selection of mobile phase

Table 4.6: Mobile phase composition and nature of peak produced

Mobile phase composition	**Peak nature**
Methanol	Broad apex
Methanol(solution A),Water(solution B)	Broad apex with shouldering
Acetonitrile(solution A),0.1%Formic acid in water(solution B)	Tailing of pointed peak
Acetonitrile(solution A),0.66%Formic acid in water(solution B)	Relatively resolved peak

The mobile phases above were tested and it was observed that a constant temperature of 25^0C, Acetonitrile (solution A), 0.66%Formic acid in water (solution B) provided comparatively resolved peaks. This mobile phase composition was selected, different combinations of the phases were tested and pH was adjusted to obtain sharp and well resolved peaks.

Selection of flow rate

Table 4.7: Flow rate versus retention time of pure cryptolepine (n=3)

Flow rate (ml/min)	Retention time (min)	Comments
4.0	0.836 ± 0.00473	Front tailing of peak observed
3.5	1.814 ± 0.02991	Peak shouldering observed
3.0	2.041 ± 0.05424	Peak shouldering observed
2.0	2.412 ± 0.09623	Peak shouldering observed
1.5	2.709 ± 0.10631	Symmetrical peak observed
1.0	3.961 ± 0.14199	Peak tailing observed
0.5	4.106 ± 0.27125	Peak tailing observed

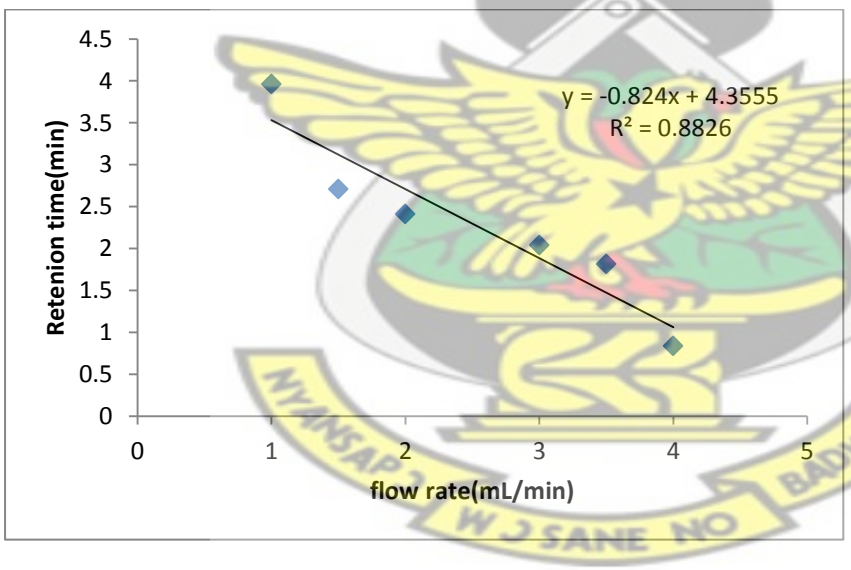

Figure 4. 1: Flow rate (ml/min) against retention time (min)

It was observed that as the flow rate increases, the retention time decreases, however peak tailing and shouldering was observed for the different flow rates chosen with the exception of a flow rate of 1.5mL/min. this therefore made the 1.5mL/min the flow rate of choice.

Table 4.8: Mobile phase composition ratio and the nature of peak produced

Phase A(Acetonitrile)	**Phase B(0.66% formic acid in water)**	**comments**
20%	80%	Peak tailing observed
30%	70%	Well resolved peak observed
50%	50%	Peak tailing observed
25%	75%	Peak tailing observed

Upon attempting different combination of the two solutions, 30% solution A and 70% solution B produced the best peaks for analysis.

4.6.2 Linear Regression Analysis

Table 4.9: Concentrations of standard and the peak area produced (n=3).

Concentration (%w/v)	Peak area
0.01	2137361
0.005	996559
0.002	339943
0.001	92038
0.0005	25914

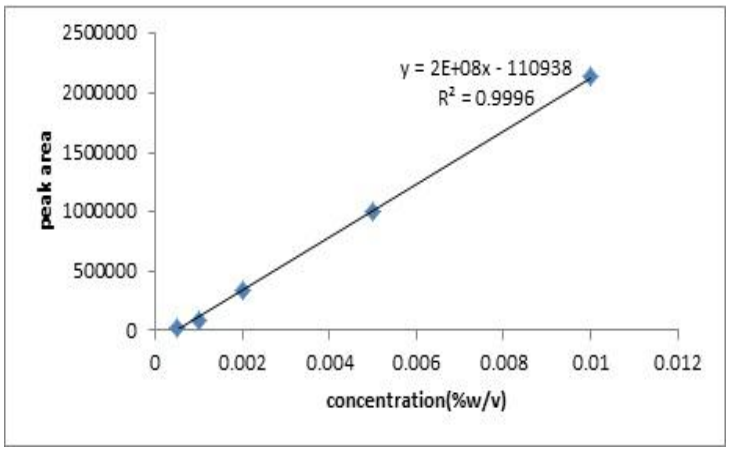

Figure 4. 2 calibration curve of concentration (%w/v) against peak area(concentration range 0.0005%w/v – 0.01%w/v, coefficient of correlation R^2=0.9996)

4.7 HPLC method validation

4.7.1 Limit of Detection (LOD)

Based on the standard deviation of the response and the slope (ICH Guidelines(ICH, 2005))

LOD=3.3σ/S

Where σ is the standard deviation of the response=20740 and S is the slope of the calibration curve=2x10^8

$LOD = \frac{3.3\times20740}{2X10^8} = 3.4221\mu g/\text{ml}$

4.7.2 Limit of Quantification (LOQ) =10 σ/S

Therefore, $LOQ = 10 \times \frac{20740}{2X10^8}$ =10.37 µg/mL

Table 4.10: Effect of flow rate on retention time (robustness)

Flow rate (ml/min)	Mean Retention time (min) (n=3)	RSD%
1.54	2.719±0.001528	0.06
1.52	2.714±0.001155	0.04
1.50	2.708±0.001528	0.06
1.48	2.702±0.001155	0.04
1.46	2.695±0.002000	0.07

Deliberate alteration of flow rate within the range of 1.46ml/min to 1.54ml/min, had no significant effect on the retention time.

Table 4.11: Effect of pH on Retention Time (robustness)

pH	Mean Retention time (min) (n=3)	RSD%
2.64	3.099 ± 0.01124	0.36
2.61	2.874 ± 0.01124	0.39
2.58	2.784 ± 0.003000	0.11
2.53	2.706 ± 0.002517	0.09
2.51	2.656 ± 0.006245	0.23
2.43	2.555 ± 0.003606	0.14
2.41	2.508 ± 0.004042	0.16

Deliberate alteration of pH within the range of 2.41 to 2.64, had no significant effect on the retention time.

Table 4.12: Effect of pH on concentration (robustness)

pH	Mean concentration (%w/v) (n=3)	RDS%
2.64	0.06609 ± 0.0005005	0.76
2.61	0.06414 ± 0.002704	4.22
2.58	0.06464 ± 0.002823	4.37
2.53	0.06663 ± 0.0003853	0.58
2.51	0.06565 ± 0.0003009	0.46
2.43	0.06420 ± 0.0008050	1.25
2.41	0.05696 ± 0.005557	9.76

Table 4.13: Intraday retention time (precision)

Determination	Mean retention time (min) (n=3)	RDS%
1	2.706 ± 0.002517	0.09
2	2.706 ± 0.002517	0.09
3	2.708 ± 0.001528	0.06

Table 4.14: Inter-day concentrations (reproducibility, repetability)

Days	Mean concentrations (n=3)	RDS%
1	98.31 ± 0.6637	0.68
2	99.31 ± 0.5784	0.58
3	90.94 ± 0.8701	0.96

4.8 Concentration of cryptolepine in samples established by using HPLC analysis

Table 4.15: Concentration of cryptolepine in different doses of sample

Samples	Concentration of Cryptolepine mg/ml (n=6)	Concentration Cryptolepine (mg) per dose of 30ml	Concentration of Cryptolepine (mg) in samples per daily dose
A1	0.0805042 ± 4.794	2.415126	7.245378
A2	0.048521 ± 19.18	1.45563	4.36689
B1	0.1660793 ± 2.914	4.982379	14.947137
B2	0.0529203 ± 10.26	1.587609	4.762827
C1	0.2252767 ± 2.684	6.758301	20.274903
C2	0.241922 ± 7.274	7.25766	21.77298
D1	0.0377903 ± 0.7157	1.133709	3.401127
D2	0.0162077 ± 0.1050	0.486231	1.458693
E1	0	0	0
E2	0	0	0
F	0	0	0
G1	0.0604116 ± 9.363	1.812348	5.437044
G2	0.055883 ± 8.453	1.67649	5.02947
H1	0.016246 ± 0.06894	0.48738	1.46214
H2	0.0199363 ± 0.3002	0.598089	1.794267
I1	0.0502867 ± 15.15	1.508601	4.525803
I2	0.0936777 ± 34.71	2.810331	8.430993
I3	0.0201787 ± 3.811	0.605361	1.816083
J	0.0238943 ± 0.2254	0.716829	2.150487
K1	0.0234433 ± 12.14	0.703299	2.109897
K2	0.064758 ± 20.48	1.94274	5.82822
K3	0.038588 ± 0.3396	1.15764	3.47292
L	0.0172557 ± 2.021	0.517671	1.553013
M1	0.3235137 ± 42.94	9.705411	29.116233
M2	0.2266583 ± 75.56	6.799749	20.399247
N1	0.094847 ± 0.7067	2.84541	8.53623
N2	0.0414903 ± 2.163	1.244709	3.734127
N3	0.0249247 ± 11.82	0.747741	2.243223

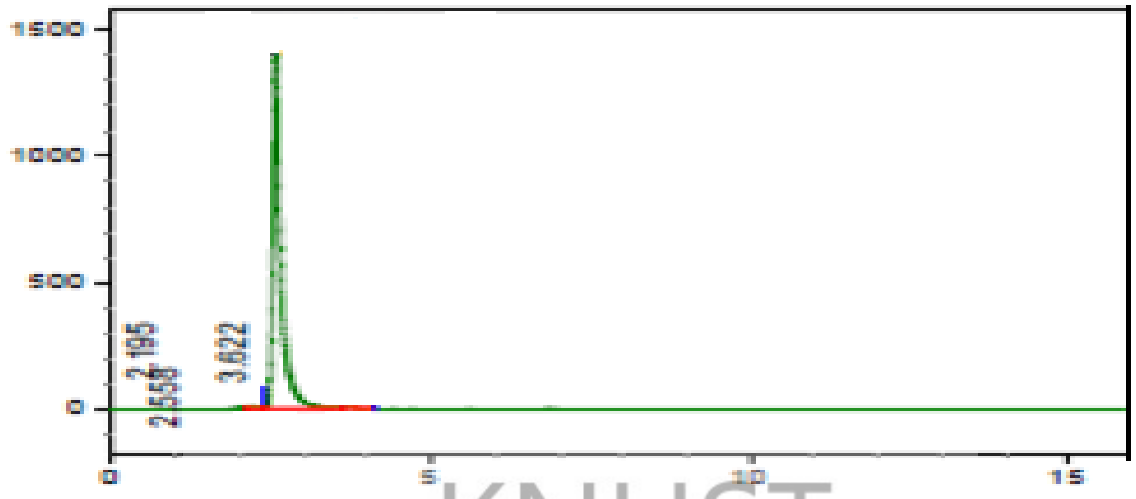

Figure 4.3: Chromatogram of Pure Cryptolepine

Figure 4.4: Chromatogram of sample containing cryptolepine

Comparison of concentrations of cryptolepine within the various batches of the brands

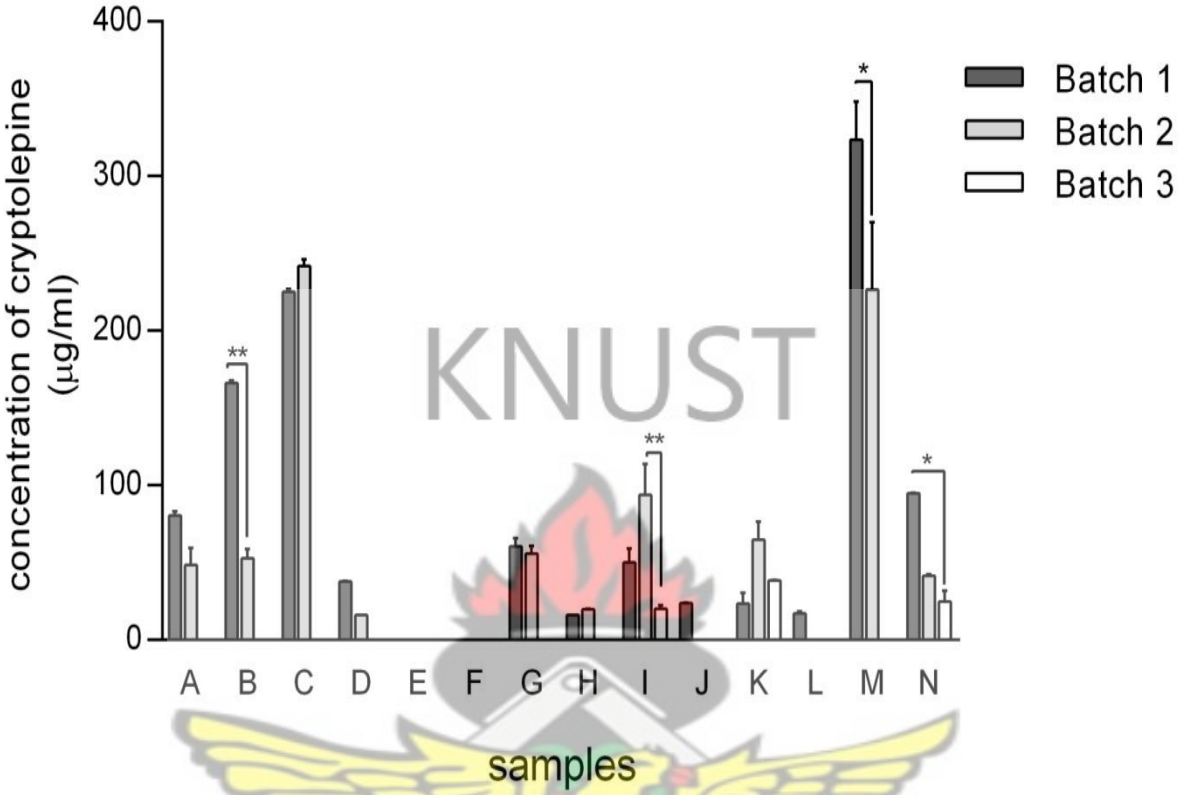

Figure 4.5: Concentration (µg/ml) of cryptolepine in different batches of all 14 brands.

Data is expressed as mean±SEM (n=3) *p<0.05, * * p<0.01(One-way ANOVA followed by Bonferroni p*ost hoc* test.

4.9 Determination of adulterants

Table 4.16: Identification of adulteration of samples with artemether, lumifantrine, amodiaquine and artesunate.

	Aduletrants			
Sample	Artemether	Lumifantrine	Amodiaquine	Artesunate
A	-	-	-	+
B	-	-	-	-
C	-	-	-	+
D	-	-	-	+
E	-	-	-	+
F	-	-	-	+
G	-	-	-	+
H	-	-	-	+
I	-	-	-	+
J	-	-	-	+
K	-	-	-	+
L	-	-	-	+
M	-	-	-	-
N	-	-	-	-

Note: +, presence of adulterant; -, Absence of adulterant

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

The upsurge in the use of herbal products in the management of a number of health conditions particularly malaria, has prompted the need to ascertain claims of efficacy, quality and safety of such products. A typical example is herbal formulations containing *Cryptolepis sanguinolenta* popularly indicated for the treatment of malaria.

Fourteen brands of commercialized herbal formulations containing *Cryptolepis sanguinolenta* were sampled for the study. These brands were sourced from popular wholesale and retail pharmacies and herbal shops in Accra and Kumasi which are two main cities in Ghana, West Africa. The samples were easily identified because the plants constituents were clearly written on their packages. They were all stored in plastic amber coloured medicine bottles with temper evident seals which had good integrity. It was observed that there were no leakages from all the containers and no fizzy sounds upon opening, that is to say, a good indication that it was void of external contamination on handling.

Thorough assessment on their packaging and labelling was carried out to investigate the Name of Product, Quantity of medicine (volume), Batch Number, Storage Instructions, Expiry Date, Manufacturer, Strength of active ingredient(s), Direction for use, Active ingredients and Legibility of information as seen in table 4.1, 4.2 and 4.3 .The findings were that all samples passed for all the parameters above with the exception of quantity of medicine, batch number and strength of active ingredient. There was no batch number on sample I1 and 12. Sample A2 had no expiry and manufacturing date. There were different batch numbers on the primary and secondary packages of sample A1. These inconsistencies are misleading and place the medications in an unsafe group as far as medication information

is concerned. There was no medication literature(in the form of a leaflet) in all samples with the exception of sample Therefore no further information about the medicine is available for reading.an interesting finding is that with the exception of samples G and K, none of the medications had measuring devices to take a dose of the medication. Consumers would have to rely on household cups and spoons (which are usually inaccurate) to take their medication (Bayor, Kipo, and Ofori-Kwakye, 2010). This could lead to over and under dosing of the medicines. Another interesting revelation was that, none of the samples had the strength of active ingredients stated anywhere on the container of the medicine. this is a very critical omission that raises the suspicion of spurious/fasely-labelled/fasified/counterfeit(SFFC) medicines (WHO, 2012).this could be mainly due to the universal challenges faced with quantifying active ingredients of herbal formulations which is the essence of this research.

Phytoconstituents screening revealed that samples contain tannins, glycoside, flavonoids, saponins and sterols. These secondary metabolites have reputable antimicrobial properties (Cimanga et al., 1997) hence their presence in the samples is expected to inhibit the proliferation of microorganisms in the products.

The samples for the study were yellowish, yellowish-brown or brown in colour. Confirms that, there is likelihood that they contain aqueous extract of *Cryptolepis sanguinolenta* roots. The mass to volume ratios (density) of the fourteen samples were between 1.29-1.68g/ml which points to the samples being aqueous formulations with relative high solute concentrations. The presence of the solutes (increases the mass) resulted in densities that were greater than that of water. The samples were acidic in nature as pH were observed to fall between 4.14-6.27. A low pH allows ionization of cryptolepine hence it can be said that the samples pH is appropriate for the ionization of the alkaloid; however the same cannot be said for sample I with a pH of 6.27.

Level of microbial contamination study revealed unacceptable contamination of samples (except B, G and M) with non-fastidious organisms, fungi, *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.* Even though cryptolepine and the secondary metabolites from the phytochemical screening are known antimicrobial agent, their presence in the sample could not surmount the microorganisms. The microbial contamination might be attributed to a higher bioburden of the samples which outwitted cryptolepine in the samples. Possible sources of these contaminants could be a summation of poor manufacturing practices.

The presence of these microorganisms in the samples could be a source of infections that is likely to bring about a cascade of socio-economic burden on the consumer, the country and the world at large especially in recent times that resistant strains of microorganisms is rampant.

As a step to establish the safety and quality of the samples, a simple , rapid, isocratic reverse-phase High Performance Liquid Chromatography (HPLC) has been developed to identify and quantify cryptolepine content in aqueous herbal formulations of Cryptolepis sanguinolenta on the Ghanaian market. This simple method employed the use of readily available mobile phase solution of a mixture of Acetonitrile (30%) and 0.66% Formic acid in water (70%).

The correlation coefficient R^2 of 0.9996 was produced when concentrations of pure cryptolepine of the range 0.0005%w/v – 0.01%w/v was plotted against peak areas produces. This value indicates that the concentrations used produced corresponding linear response and this agrees with standard analytical method validation (ICH, 2005). The limit of detection LOD which is the amount of analyte that can be detected by the method but not necessarily quantified was 3.4221µg/mL, which was less than the lowest concentration for the calibration curve. Limit of Quantification LOQ which is the lowest quantifiable amount by the method

was 10.37µg/mL which fell within the concentration range of the calibration curve details. These finding attest to the HPLC method being accurate. The capacity of the method to remain unaffected by intentional but small changes in chromatographic conditions was tested. Here, a deliberate alteration of pH and flow rate was done and their effect on retention time was noted. The relative standard deviation that was yielded at the end of the experiment fell below 2%.hence an indication that the method is robust and reliable during normal usage. The method was tested for its precision within the same day and different days to know whether laboratory variations will alter the method. The relative standard deviations for this work fell below 2% indicating that the method is precise.

The validated HPLC method was used to quantify the amount of cryptolepine in the samples. This was achieved by using the calibration curve equation. Cryptolepine was detected in all samples with the exception of E and F. no detectable amount of cryptolepine was found in E and F and this was forecasted by the initial determination of cryptolepine in the samples during the phytochemical screening. This is intriguing in the sense that the samples were clearly stated to contain the plant *Cryptolepis sanguinolenta*, the main source of cryptolepine but not even an amount as low as 3.4221µg/mL was detected. Reasons that can account for this finding are that;

1. The samples could have been wrongly labelled to contain the plant
2. The plant could be present as stated but the source, plant part, time and method for collection of the plant could have influence the content of cryptolepine.
3. Possible interaction of other constituents of the formulation with the cryptolepine in the samples.
4. Microbial degradation of the cryptolepine present since samples showed considerably high bioburden.

The concentration of Cryptolepine in the samples ranged from 0.485-9.706mg/ml with significant batch variations within a brand as well as variations among the brands ($p<0.05$) as seen in fig 4.9.2 .This is evident of little or no consensus among manufactures on the collection of plant, standard procedure, storage conditions.

The concentrations exceeds the IC_{50} values established for antimalarial properties,these are 0.134 ± 0.037 µg/mL(Kirby et al., 1995) and 0.033± 0.0001µg/mL (Cimanga et al., 1997). The variations in the concentrations of cryptolepine poses a threat in the consuming such product because different amounts of the active alkaloids are consumed with each batch and this could lead to an over or underdose of the medication. A serious effect will be seen when these concentrations get to values close to lethal doses that have been established already in other researches.

These concentrations when extrapolated can be used to determine the amount of crude root powder needed to yield a particular amount of cryptolepine needed for further analysis and serves as a milestone in standardizing herbal formulations containing Cryptolepine.

An acute toxicity study by Tay *et al* (2011) established that two of the brands understudy in this work had LD_{50} of 300 mg/kg body weight. The brand with highest content of cryptolepine per dose was 9.705 mg which will achieve a maximum daily concentration of 29.126 mg (3 times daily dosing as stated by manufactures). This is therefore too low to cause any acute toxicity in consumers.

The cytotoxicity of cryptolepine was pegged at 100mg/ml on Chinese hamster lung fibroblast cell line V79 which are mammalian cells (Ansah and Gooderham, 2002). This value compared to concentrations of cryptolepine in the samples confirms that the samples are safe as far as cytotoxicity is concerned.

It is tempting to adulterate herbal formulations with orthodox medicines known to be potent in the treatment of the indication for that particular herbal formulation. Usually the adulterants are relatively cheaper, readily available in desired quantities and have the tendency to alleviate ailment within a shorter time frame. In view of this, the presence of artemisinin-based combination therapies ACT's such as artemether, lumifantrine, artesunate and amodiaquine were investigated in the samples. These four were chosen because they are composites of malaria chemotherapy in Ghana. They are readily available over the counter medicines that can be found in the country. The samples for the study did not contain artemether, lumifantrin and amodiaquine. However, all the samples with the exception of samples B, M and N produced peaks that are comparable to that of pure Artesunate powder. This finding adds up to the above that the safety of these herbal medications is questionable. Artesunate which is highly water soluble was could have been deliberately added to the samples to enhance the antimalarial activity of the medication. It could potentiate the effect of the cryptolepine present, or in the case of samples E and F be the actual source of antimalarial agent in the sample. If so, then the assiduous attempts made by the World health organization to rule out monotherapy in the treatment of malarial have come to no avail. This could result in more complex antimalarial resistant strains of the human Plasmodium species.

5.2 Conclusions

The fourteen (14) brands of herbal formulations studied did not meet one or more of the standard quality assessment parameters. Investigations on the presence of adulterants revealed that about 79 % of the samples may have been adulterated with artesunate. Also, 21% of the study samples could be passed for microbial load safety.

This study developed a simple, robust, specific and precise HPLC protocol for identification and quantification of the cryptolepine alkaloid in herbal formulations. Two (2) of the fourteen

(14) samples had no detectable amounts of the cryptolepine. Quantitatively, there was batch-to-batch variation in cryptolepine content of the brands under study (P< 0.05, 0.01).For samples that contained Cryptolepine, the amounts quantified ranged from 0.0162 mg/ml to 0.3235 mg/ml which is in line with acceptable value of the alkaloid to elicit its antiplasmodial effect.

5.3 Recommendations

1. *In vivo* studies should be carried out on the brands (brand names available on request) to establish their antimalarial activity.
2. Further studies should be done to ascertain the toxicity profile of the samples.
3. Manufactures should establish standard manufacturing protocols to prevent inter batch variations

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