EFFICACY AND CHEMICAL CONSTITUENTS OF SOME HERBAL MEDICINES USED FOR TREATMENT OF ANAEMIA, IN BLANTYRE DISTRICT IN MALAWI

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A thesis submitted to the University of Malawi in partial fulfilment of the requirements for the degree of Master of Science in Applied Chemistry.

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Declaration

I declare that this thesis, submitted herein, is my original work, except where reference has duly been acknowledged. It has not been presented for any other award at this or any other university.

Signed: --- -----------------------

Doris Butao Date

This thesis has satisfied the requirements of the Master of Science degree in Applied Chemistry, and has been submitted with our approval as supervisors.

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This thesis has been submitted with the approval of the Department of Chemistry

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Head of Chemistry Department Date

Dedication

This thesis is dedicated to three important people in my life, my late father who never lived to see my advancement in his career, to one brave, strong and wonderful woman, my mother, Anabengo. Mother, I will always cherish your courage, sense of vision and determination. Lastly to my dearest son Blessings, who has been a source of inspiration to my life.

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List of abbreviations

AIDS	Acquired Immune-Deficiency Syndrome
CHSU	Community Health Sciences Unit
HCT	Haematocrit
Hb	Haemoglobin
HIV	Human Immune-deficiency Virus
MICAH	Micronutrients and Health
MOHP	Ministry of Health and Population
RDA	Recommended Dietary Allowance
TBA	Traditional Birth Attendant
TB	Tuberculosis
UNICEF	United Nations International Children Fund
WHO	World Health Organization
WVI	World Vision International

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Abstract

The thesis was designed to determine the efficacy and chemical composition of herbal medicines used for the treatment of anaemia in Malawi. The herbal medicines included in the study were established through a questionnaire survey of traditional healers in Blantyre district. The list of herbal medicine studied include *Toona ciliate, Dalbergia nitidula, Psidium guajava, Ensente livingstonianum, Pericopsis angolensis, Elephantorrhiza goetzei, Acalypha villicaulis,* and *Persia americana*.

Chemical composition of the herbal medicine was determined with respect to the contents of protein, iron, ascorbic acid, zinc and copper, these being the substances involved in blood production, or that facilitate absorption of iron. The herbal medicines were also screened for the presence of the following classes of phytochemical compounds: alkaloids, saponins, tannins, terpenoids, steroids, flavanoids, anthracyanins and anthraquinones. The herbal medicines were found to contain differing amounts of these chemical components within the following ranges: moisture 6.1 – 10.1 %, protein 3.2 – 29.3 %, iron 7.5 – 69.8 mg/100g, ascorbic acid 10.6 – 63.8 mg/100g, copper 0.3 – 3.7 mg/100g and zinc 3.1 – 6.5 mg/100g. *Ensente livingstonianum* was found to contain the highest amount of iron (69.8 mg/100g), ascorbic acid (63.8 mg/100g) and copper (3.7 mg/100g). Iron is a primary raw material of haemoglobin, ascorbic acid reduces ferric iron to ferrous iron which can be absorbed, and copper containing proteins are involved in the mobilization of iron from its storage sites to the plasma.

The efficacy of the herbal medicines for the treatment of anaemia was determined for avocado pear (*Persia americana*), through clinical trials at a herbal clinic (Chirimba). Forty four (44) patients who showed signs and symptoms of anaemia, confirmed by low haemoglobin levels upon testing by a qualified laboratory technician at the herbal clinic, were given to drink three times a day, 250 mls of a liquid herbal medicine prepared from a mixture of 700 g avocado leaves and 150 g avocado root bark which had been boiled with water for 3 hours to a final volume of 15 liters. After 7 and 14 days treatment, patients were checked for symptoms of anaemia, and had their haemoglobin levels measured. All the patients who returned for follow-up assessment, 33 out of 44, showed increased levels of haemoglobin. After the first week,

haemoglobin levels increased by 1.58 g/dl ±0.19 g/dl on average. After the second week, 14 out of the 33 patients returned for follow-up, and all showed an increase in haemoglobin levels of 1.20 g/dl ±0.33 g/dl on average. These increases in haemoglobin levels were comparable to those expected, 1.5 – 2.1 g/dl in a month, with iron supplementation by standard regime in ideal situations.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Problem statement

Anaemia is defined as a reduction in the total volume of red blood cells in the body below the normal reference values (Potter et al, 2006). In Malawi, anaemia accounts for a substantial proportion of hospital admissions, morbidity and mortality especially among pregnant women and young children (MOHP/NSO/UNICEF/CDC, 2003). In 2002, according to hospital records, in Malawi, anaemia was the third cause of mortality and the fifth cause of hospital admissions among the general population (Health Information Management System Report, 2002).

The prevalence of anaemia in children under five years is very high; it ranges from 71% to 90% with 50% to 64% being moderate to severe anaemia. On the other hand, as high as 54% to 92% of the antenatal women are anaemic. This is based on numerous population and hospital-based surveys that were conducted (Malawi Ministry of Health and Population, 1999, Mkanda et al, 2001, MOHP/NSO/UNICEF/CDC, 2003).

The problem of anaemia in Malawi affects over 50% of the population, including men, women and children, thereby causing reduced work capability, ill health, and increasing morbidity and mortality (Malawi Ministry of Health and Population, 1999, MOHP/NSO/UNICEF/CDC, 2003). The commonly used treatment for anaemia is ferrous sulphate/folate (iron) tablets, which contain 60 milligram (60 mg) iron. It is also used for preventive purposes among pregnant women. In 2001, the coverage of iron tablets among pregnant women was only 22% (Mkanda et al, 2001). The low coverage of iron tablets is associated with irregular supplies, non-availability at the clinics, non-compliance of users and high cost for the rural population (Mkanda et al, 2001). This shows that the iron tablet treatment is failing to meet the health demands of the country.

Traditional medicines have always supplemented the western medicine in Malawi and there is a large and active traditional health sector, more especially in the rural areas. If the traditional medicines were effective and safe, they could provide an alternative to ferrous sulphate that is within people's reach and affordable.

The choices that people make when seeking health care are due to their perception of the nature of their disease or illness, their cultural understanding of the cause of the illness, the efficacy of the various treatments and their previous experience with the various health care alternatives (WHO/EDM/TRM/2000.1). Many people in Malawi believe in traditional medicine. Traditional healers are well respected in their communities, they are readily accessible and usually found within short walking distances, they treat psychological as well as biological cause of an illness, and they provide more time for consultation and address social problems (e.g. theft and bad luck) which western medicine does not treat (Ndibwami et al, 1998).

In Malawi, there have been so many studies aimed at identifying the types of plant species that are used as traditional medicines for various ailments (Seyani and Chikuni (1997), Williamson (1974), Morris (1991), Carney (1999), Mwanyambo and Nihero (1998). There has never been any studies to evaluate the effectiveness of these traditional medicines. There is need to establish the efficacy of these medicines. A few studies have been conducted on chemical composition with emphasis on classes of phytochemical compounds only (Msonthi, 1994).

1.2 Traditional medicine versus western medicine in Malawi

Although the present health system is western oriented, there is a large and active traditional health sector in Malawi. Medicinal plants play a key role for basic health care especially in rural areas of many countries including Malawi (Ndibwami et al, 1998). Malawi has a very rich collection of medicinal flora as seen from several research reports (Seyani and Chikuni (1997), Williamson (1974), Morris (1991), Carney (1999), Mwanyambo and Nihero (1998). People have used plants as therapeutic agents since time immemorial and all human communities have a wealth of folk knowledge relating to the use of plants as medicine (Morris, 1991). However, despite the vast knowledge and practice, there is deficiency in information about the safety, efficacy and quality of the medicinal herbs and herbal preparations. Research and documentation in this subject is still very little. This study was undertaken to provide information on the herbal medicines that are being used to treat anaemia patients by a few selected traditional healers in Blantyre district.

1.3 Objectives of the study

The objective of this study was to examine the chemical composition of some herbal medicines that are being used to treat anaemia patients in Blantyre district and assess the efficacy of herbal medicine prepared from avocado pear leaves and root barks.

The specific objectives of the study were as follows:

- To draw up an inventory of herbal medicines that are being used to treat anaemia patients, their scientific and local names, plant part used, preparation and dosage from previous research as well as traditional healers.
- To analyse the herbal medicines used for treatment of anaemia for the presence of iron, vitamin C (ascorbic acid), protein, zinc, copper and classes of phytochemical compounds.
- To assess the efficacy of herbal medicine from avocado pear leaves and root barks, on treatment of anaemia using haemoglobin level as indicator.
- To assess the variation in chemical compsition of avocado pear due to variety, leaf position, and plant part.

1.4 Research questions

In order to achieve the objectives of the study, the results of the research must answer the following questions.

Inventory of herbal medicines for anaemia treatment

- What are the names of herbs that are used to treat anaemia?
- What is the plant part that is used to prepare the medicine?
- How is the medicine prepared?
- What amount is used in the preparation?
- What is the prescribed or recommended dosage?

- How does information on herbs for anaemia treatment from literature compare with information from traditional healers?

Chemical and phytochemical screening of the herbal medicines

- How much of the essential chemicals required for blood production (iron, ascorbic acid, protein, zinc, copper) are present?
- What classes of phytochemical compounds are present?
- How do the quantities of these essential chemicals compare with the International Recommended Dietary Allowances (RDA)?

Effectiveness of Avocado leaves and root barks in raising haemoglobin levels

- How much have the haemoglobin levels changed in relation to treatment period?
- How does the change in haemoglobin levels compare for clients with malaria and or stool parasites?

Variation in chemical composition

- How much of the variation in chemical composition is due to variety?
- How much of the variation in chemical composition is due to leaf position?
- How much of the variation in chemical composition is due to plant part?

1.5 The structure of the thesis

The rest of this thesis is laid out as follows:

Chapter Two: Detailed review of literature related to anaemia and traditional medicine.

Chapter Three: Information on materials and methods used during the study.

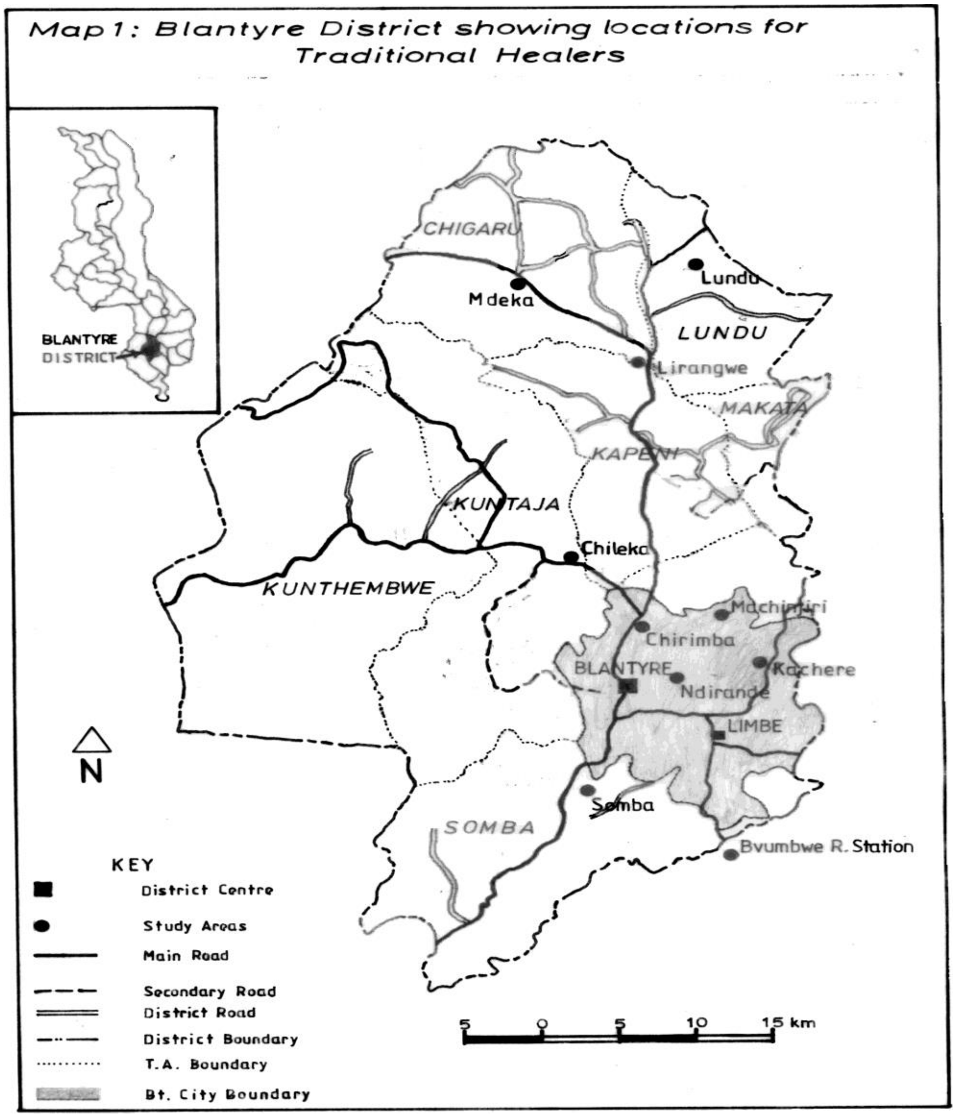
Chapter Four: Results and discussion.

Chapter Five: Conclusions and recommendations.

References and appendices are included after chapter five.

1.6 Study site

The study was conducted in Blantyre district. Map 1 shows locations of traditional healers who provided herbal medicines for the study (Lirangwi, Somba, Kachele, Lundu, Chirimba, Machinjiri, Chileka, mdeka and Ndirande). It also shows Chirimba township where efficacy study was conducted and Bvumbwe Research Station from where samples of avocado pear varieties were obtained.



Map 1: Blantyre district showing locations of traditional healers and study site

Blantyre district was chosen as study site because it is one of the most populous districts of Malawi, and the industrial hub of the country. It attracts people from all over the country including traditional healers. Hence an assumption could be made that the situation in the district could be a representative of the country.

Blantyre district is situated in the southern region of Malawi. It has a population of 809,397 people, and a growth rate of 2.9% (NSO, 2000). It comprises 9 traditional authorities and it is bordered by Chiradzulu, Chikwawa, Thyolo and Mwanza districts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Herbal medicine and anaemia

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients; parts of plants, or other plant materials, or combinations thereof (WHO/EDM/TRM/2000.1). In Africa, plant resources offer a variety of products used as food, medicine, timber, fodder, building materials, crafting, fuel wood etc. Good food plays an important role in prevention of illnesses such as malnutrition and anaemia. It has been shown that 70% to 80% of people in Africa consult traditional medical practitioners for their primary health care (Salomao, et al, 2001).

In Nigeria, the drinking of an aqueous extract of the bark of *Adansonia digitata* is used in traditional medicine as a treatment for sickle cell anaemia (Adesanya et al, 1988). Pregnant women use traditional black rings on their middle fingers as a prophylactic measure to prevent pregnancy anaemia (Elegbe et al, 1984). In Costa Rica and Nigeria, in 2004 and 2005, doctors Carmona and Sokoto respectively, had promising results with fruit extract of Carao (*Cassia grandis*), in treating anaemia patients. It increased haemoglobin levels and hematocrit drastically in both studies (Standish, 2005).

In Kenya, Omolo et al, analysed 8 plants, which are traditionally used in Eastern Africa to treat anaemia. Out of these 8 plants, he detected prominent amount of iron from root barks of two medicinal plants, *Bridelia cathartica* and *Lannea stuhlmanii.* He detected 35.69 and 35.21 milligram per 100 gram (mg/100g) dry material from root bark of *Bridelia cathartica* and *Lannea stuhlmanii* respectively (Omolo et al, 1997).

In Southern Africa, about 4000 species of plants are used as medicines, treating a wide range of diseases, including anaemia (Van Wyk and Gericke, 2000). In Mozambique up to approximately

550 plant species have been identified as being used in traditional medicine. Nearly all diseases in Mozambique have been handled by traditional medicine except illnesses requiring a major surgical intervention (Van Wyk and Gericke, 2000).

2.2 Herbal medicine in Malawi

In Malawi there is a large and active traditional health sector and by 1998 the Herbalist Association of Malawi had a membership of about 135,000 (Ndibwami et al, 1998). The MOHP estimated that there were 5,000 Traditional Birth Attendants (TBAs) in 1998 mostly in the rural areas, where 90.0% of the population lives.

Traditional healers use various plant species to treat anaemia. The information on the herbal medicines that are used to treat anaemia in Malawi, including the part of plant used, the mode of administration, mode of preparation and dose are included in the results (Tables 4 and 5).

Medicinal plants play a key role for basic health care, particularly in rural areas. Traditional healers handle most local health problems since the number of hospitals and health personnel is very small (Msonthi, 1994).

In 2002, according to the Poverty Reduction Strategy Paper, the population per physician ratio was estimated at 1:45,737. These ratios are very high as compared to the ratio of 1:138 traditional healers to patients (Chikuni, 1995). The paper also states that only 3% of the entire population live in a village with a health centre and that people seeking medical services avoid clinics due to poor quality infrastructure and the shortage of medical workers, drugs and medical supplies (GOM, 2002).

According to Morris (1991), every person in the rural areas of Malawi is essentially a practising herbalist, and knows a variety of herbs to treat common ailments

The WHO Regional director for Africa, during the Expert committee meeting on traditional medicine, in Zimbabwe, affirmed that there was evidence that traditional medicine was effective in the treatment of diseases such as malaria, diabetes, hypertension and sickle cell anaemia. He further stressed the need for research to evaluate the safety, efficacy and quality of all the traditional medicines and herbal preparations (WHO, 2001).

The Government of Malawi recognises the importance of traditional medicine and the role that herbalists play in providing primary health care services. Hence it inaugurated the "African Traditional Medicines Day" on 5th December 2003, facilitated a meeting to form the "Malawi Traditional Healers Umbrella Organisation" (MTHUO) in January 2004, and produced the "Traditional Medicine Policy" and the "Code of Ethics for Traditional Health Practitioners of Malawi" in July 2006.

2.2.1 Species of medicinal plants in Malawi

Although deforestation is threatening the survival of some medicinal plants, the vegetation of Malawi with its 5500-6000 identified species provides a wide range of plants to be used by traditional healers (Seyani and Chikuni, 1997). The actual number of plant species used in traditional medicine is not known because of incomplete documentation (Mwanyambo and Nihero, 1998), and also because traditional medicine is practised in secrecy (Ndibwami et al, 1998).

In 1974, Jesse Williamson listed 122 plant species used as medicine in Malawi. Morris in 1996 collected over 1500 species of plants during his research in the southern Malawi. Out of these, 516 had known medicinal uses (Williamsons, 1975 and Morris, 1996).

2.2.2 Conservation of medicinal plants

Efforts to conserve medicinal plants in Malawi date back to the 1980s when the Malawi Government encouraged traditional healers to form herbalist associations (Seyani and Chikuni, 1997). The best-organized and registered associations include Herbalist Association of Malawi, International Traditional Medicine Council of Malawi, International Traditional Health Practitioners and Research Council of Malawi and Chizgani Ethno medical Association of Malawi (Ndibwami et al, 1998). However 89% of traditional healers were registered with the

Herbalist Association of Malawi (Ndibwami et al, 1998). Members of these associations are encouraged to conserve, utilize and sustain the medicinal plants (Seyani and Chikuni, 1997). Other efforts include establishment of medicinal plant gardens such as the one in Mwanza district and establishment of nurseries for medicinal plants like those in Zomba and Mangochi districts (Mwanyambo and Nihero, 1998).

2.3 Anaemia

Anaemia is usually measured by the haemoglobin concentration (Hb) in red blood cells or haematocrit level (hct) (Potter et al, 2006). Thus anaemia means a deficiency in the quality or quantity of red blood cells. Haemoglobin is the oxygen-carrying component in the red blood cells and is expressed as grams of haemoglobin per decilitre of red blood cells (g/dl). Haematocrit is the volume of red blood cells expressed as a percentage of total blood volume (%).

The cut-offs for anaemia vary by population group. For example, a haemoglobin level of less than 11.0 g/dl is considered anaemic for children and pregnant women (Table 1).

Table 1: Normal haemoglobin and haematocrit ranges

Population	Haemoglobin (g/dl)	Haematocrit (%)
Infant (full term)	10.5 - 19.5	32 - 60
Children (1 - 9 years)	11.0 - 14.0	33 - 40
Children (10 -12 years)	11.5 - 15.0	35 - 45
Women (adult)	12.0 - 16.0	36 - 44
Pregnant Women	11.0 – 14.0	33 - 42
Men	13.0 – 18.0	40 - 50

Source: WHO/UNICEF/UN, Geneva, 1995.

Severe anaemia is defined as Hb less than 7.0 g/dl and very severe anaemia as Hb less than 4.0 g/dl (WHO, 1995).

Anaemia can present itself with one or more of the following signs and symptoms; tiredness, body weakness, dizziness, headaches, heart palpitations, breathlessness, pale palm creases, pale nail beds, pale gums, pale eyelid lining and swelling of the feet (Potter et al, 2006).

2.4 Causes of anaemia

Anaemia results from one or more of the following processes:

- Defective red blood cell production due to lack of essential nutrients in the diet, poor bioavailability of iron, or increased utilization of nutrients such as during pregnancy, lactation or rapid growth periods.
- Increased red cell destruction (haemolysis) due to parasitic diseases such as malaria or genetic conditions such as sickle cell anaemia or thalassemia.
- Blood loss resulting from intestinal worm infestation notably hookworm or from heavy menstrual flow (Malawi Ministry of Health and Population, 1998, Potter et al, 2006).

Although iron deficiency is the most common cause of anaemia, especially among younger children and women of childbearing age, a variety of other nutrients too are essential for production of red blood cells and their deficiencies may cause anaemia. The essential ones include folic acid, vitamin B_{12}, and iron. The others include ascorbic acid (vitamin C), protein, vitamin A, pyridoxine (vitamin B_6), copper, zinc and vitamin E. Anaemia is also influenced (caused or worsened) by parasitic diseases like malaria and worms, chronic illnesses like TB and HIV, haemoglobinopathies and climatic changes (Gillespie and Johnston, 1998 and Davidson et al, 1975).

2.4.1 Iron

Iron is a primary raw material of haemoglobin. It is needed by the body for bone marrow to make haemoglobin for new red blood cells and it is an essential component of the processes involved in the transfer of oxygen. Before iron can be absorbed in the gut, it must be soluble. Iron is absorbed effectively in the ferrous state (Fe^{2+}) than the ferric state (Fe^{3+}). Iron in food occurs in two forms: non-haem iron and haem iron (MOHP, 1998). Non-haem iron is mainly available as ferric hydroxide complexes bound to proteins, amino acids and organic acids. It is found in milk, egg and plant food. Before this iron is absorbed it has to be split from the complex and be reduced to the divalent state. Non-haem iron is greatly affected by both inhibitors and enhancers so that there can be a tenfold variation in absorption rates. Haem iron is found in food, which contain blood or muscle (animal origin). Haem complex is absorbed intact into the intestinal epithelial cells and only then is the iron split off (Gillespie and Johnston, 1998).

2.4.2 Folic acid

Folic acid (figure 1) is one of the water soluble B-vitamins (vitamin Bc) and is present in all foods of plant and animal origin particularly liver, leafy vegetables, fruit, pulses and yeast. It is needed for proper production of lymphocytes and is vital for red blood cells health and transports oxygen. Without folic acid and vitamin B12, stem cells cannot produce the blood cells. Folic acid deficiency occurs when absorbed folate does not meet requirements over time, and may lead to anaemia. Folate rich food can be depleted of folate by prolonged cooking (Gillespie and Johnston, 1998).

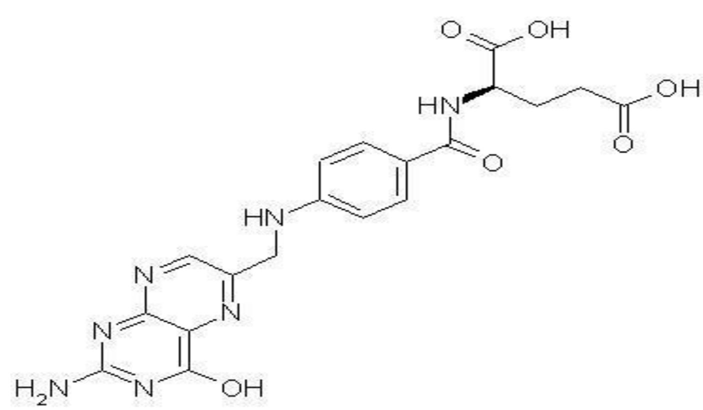


Figure 1: Chemical structure of folic acid

2.4.3 Vitamin B12

Vitamin B_{12} (figure 2) is present in food of animal origin, and it is not available from plants. It is needed for proper production of lymphocytes and is vital for red blood cells health and transports oxygen. Without vitamin B12 and folic acid, stem cells cannot produce the blood cells (Gillespie and Johnston, 1998).

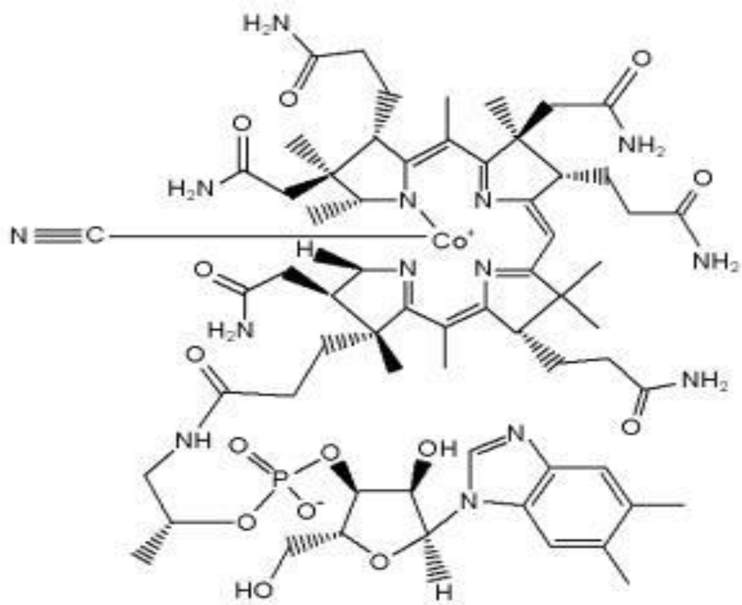


Figure 2: Chemical structure of vitamin B12

2.4.4 Ascorbic acid (vitamin C)

Ascorbic acid (figure 3) and dehydroascorbic acid are both biologically active. In fresh foods, the reduced form (ascorbic acid) is the major one present, but the amount of dehydroascorbic acid form increases during cooking and processing. Ascorbic acid is a white, water-soluble crystalline material that is stable in dry form. It is easily oxidised in solution, especially on exposure to heat. Oxidation can be accelerated by the presence of copper or iron and by an alkaline condition. It reduces ferric to ferrous iron in the intestinal tract to facilitate absorption. It is involved in the transfer of iron from plasma transferrin to liver ferritin. It also combines with iron to form a soluble chelate in the presence of low pH (Gillespie and Johnston, 1998).

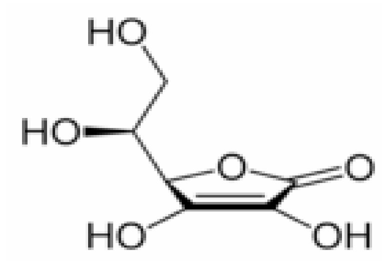


Figure 3: Chemical structure of ascorbic acid

2.4.5 Beta carotene (vitamin A)

Vitamin A, hydrolytic product of beta-carotene (figure 4), is involved in the mobilisation of stored iron. Poor vitamin A status has been reported to be associated with altered iron utilisation and metabolism. Hence, this results in iron deficiency anaemia (Gillespie and Johnston, 1998).

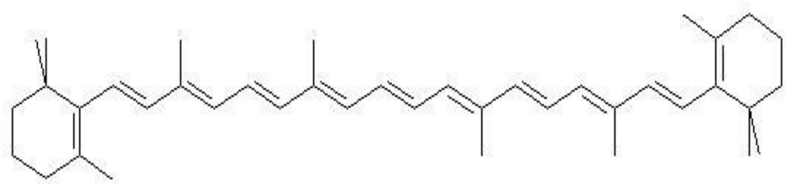


Figure 4: Chemical structure of beta carotene

2.4.6 Proteins

Proteins are important for the proper production of the protein portion of the framework of the red blood cells and the synthesis of globulin. If the intake of amino acids is diminished, the synthesis of globulin is affected and may result in nutritional anaemia.

2.4.7 Zinc and Copper

Zinc is present in all meat, legumes and unmealed cereals. Deficiency of zinc causes mild anaemia. Copper is essential for proper formation of haemoglobin. Copper containing protein is essential for normal mobilisation of iron from its storage sites to the plasma. If copper is deficient, iron cannot be released, and this leads to low serum iron and haemoglobin levels, even in the presence of normal iron stores (Mahan and Escott-Stump, 1996).

2.4.8 Haemoglobinopathies

The presence of abnormal haemoglobin in the red blood cells renders the red blood cells liable to haemolysis, hence anaemia may develop. This is seen in the case of sickle cell anaemia and thalassemia (Davidson et al, 1975).

2.4.9 Malaria and climatic changes

Of the four species of malaria parasites, *Plasmodium falciparum* is the most common cause of malaria infection in Malawi. An attack of malaria infection, especially when due to *Plasmodium falciparum*, is always accompanied by haemolysis (blood destruction) and in a severe or prolonged attack, severe anaemia may ensue. Malaria is thought to be the primary cause of severe anaemia in at least 50% of subjects living in malaria endemic areas (Gillespie and Johnston, 1998). The treatment of malaria in individuals and its eradication from areas of low or moderate endemicity are thus prerequisites for treatment and prevention of anaemia (Davidson et al, 1975).

Seasonality in malaria prevalence and seasonal changes in dietary intake of nutrients such as vitamin A and folate during the year may partially account for seasonal trends in anaemia. Hence seasonality is also an important issue with respect to anaemia. In Mali an increase in anaemia prevalence from 30% in April to 58% in October was observed in school children. During the same sampling periods, malaria prevalence also doubled (Gillespie and Johnston, 1998).

2.4.10 Intestinal parasites

Hookworm and schistosomiasis are intestinal helminths, which can cause anaemia aetiology where they are prevalent. Hookworms and *Schistosoma mansoni* cause intestinal blood loss by feeding on blood through the intestinal mucosa. *Schistosoma haematobium* causes urinary iron loss, which follows epithelial damage to the mesentery of the bladder. Deworming reduces iron loss through intestinal bleeding (Gillespie and Johnston, 1998).

2.4.11 HIV/AIDS

Anaemia is strongly associated with HIV infection particularly in Africa. The mechanisms include: anaemia of chronic disorders like TB, HIV action on the stem cells and other precursors, an imbalance of growth factors due to HIV actions on macrophages, fibroblasts and T-cells, uncontrolled provirus B19 infection, nutritional deficiencies from anorexia, auto immune haemolysis (Malawi Ministry of Health and Population, 1998).

2.5 Types of anaemia

There are basically three main types of anaemia, namely, nutritional deficiency anaemia, haemorrhagic anaemia and haemolytic anaemia (haemoglobinopathies). It is classified into these three categories based on the underlying cause (Gillespie and Johnston, 1998 and Potter et al, 2006).

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2.5.1 Nutritional deficiency anaemia

This is anaemia that is caused by a deficiency of one or more of the essential nutrients that are required for erythropoiesis (red blood cell formation). The most important nutrients needed are iron, folic acid and vitamin B_{12} and the others being protein, vitamin A, pyridoxine (vitamin B_6), ascorbic acid, vitamin E and the micronutrients copper and zinc. Deficiency results from one or more of the following factors:

- Poor bioavailability.
- Inadequate ingestion.
- Defective absorption.
- Imperfect utilization.
- Injury to the bone marrow.

- Increased requirements as in pregnancy, lactation or during rapid growth periods of adolescence (Davidson, et. al. 1975 and Potter et al, 2006).

2.5.2 Haemorrhagic anaemia

This is anaemia that results from haemorrhage or blood loss; blood loss may result from intestinal worm infestations, accidents, childbirth, menstrual loss etc.

2.5.3 Haemolytic anaemia

Haemolysis is the destruction of red blood cells, which may result from parasitic infections like malaria whereby the parasite goes inside the red blood cells. Haemolysis may also result from haemoglobinopathies.

Haemoglobinopathy is a type of anaemia, which is due to the presence of abnormal haemoglobin in the red blood cells, which renders the red blood cells liable to haemolysis, and hence anaemia may develop. This is seen in sickle cell anaemia and thalassaemia (Gillespie and Johnston, 1998 and Potter et al, 2006)

2.6 Consequences of anaemia

It has been shown that during pregnancy anaemia increases overall infant mortality, it increases the risk of death through haemorrhage, which is a largest cause of maternal death in developing countries, it increases maternal morbidity and the risk of pre-term delivery, it is associated with low birth weight and increased infant mortality and morbidity. Anaemia is directly responsible for at least 40% of maternal deaths. It decreases physical work capacity for any population group (Gillespie and Johnston, 1998 and Malawi Ministry of Health and Population, 1998).

Among children, anaemia impairs cognitive development and immune mechanisms; it is associated with increased mortality and morbidity; it causes a reduction in performance on psychomotor tests; and it causes a reduction in concentration, attention span and work capacity, hence reduction in productivity (Gillespie and Johnston, 1998 and Malawi Ministry of Health and Population, 1998)

2.7 Anaemia situation in Malawi

2.7.1 Anaemia in children

A baseline survey conducted by World Vision Micronutrient and Health (MICAH) project in 1996, which covered all the three regions of the country found an anaemia prevalence of 71.3% in children under the age of five, with a mean haemoglobin concentration level of 9.8 g/dl (Zamaere, 1997). In the Ekwendeni area, a study was conducted in 1997, on 577 children aged between 1 and 5 years. It was found that anaemia prevalence was 83.0% with 64.0% having haemoglobin level below 10.0g/dl (Young, et al, 1997A). In 1994 – 95 a survey conducted in Chikwawa area involving infants and young children found that 69% of infants 0 to 6 months of age and 90% of those aged 6 to 12 months were anaemic (Chimsuku, 1996). Shrestha in a 1991 survey of school-aged children conducted in Ntcheu found that 18.0% of 424 children aged 6 to 8 years were anaemic (Shrestha, 1994). During recent evaluation surveys by MICAH and MOHP, it was found that about 80% of the children surveyed were anaemic in both cases (Mkanda et al, 2001 and MOHP/NSO/UNICEF/CDC, 2003).

2.7.2 Anaemia in antenatal women

Most at risk groups of people with anaemia in Malawi are children under the age of 5 years and antenatal women. In 1997, the Ministry of Health conducted a national anaemia study, which covered 814 women. Among these 62.3% were anaemic compared to 56.2% in 1993 and 70% in 1991. Another survey conducted during the same year (1997) in Ekwendeni revealed an anaemia prevalence of 62% with 34.0% having their haemoglobin levels below 10.0 g/dl (Young et al, 1997B). The World Vision 1996 survey revealed anaemia rate of 83% with 58.5% of the women having Haemoglobin levels below 10.0 g/dl (Zamaere, 1997). Other surveys in the south of the country revealed anaemia rates of 54 – 65% in Mangochi district, 67% in Thyolo area (Williams, 1997) and 85 – 92% in Chikwawa area, depending on the season of testing (Chimsuku, 1996). In surveys which covered all the 3 regions of Malawi it was discovered that about 45% (Mkanda et al, 2001) and 22.1% (MOHP/NSO/UNICEF/CDC, 2003) of pregnant women surveyed were anaemic.

2.7.3 Anaemia in women of childbearing age

Anaemia is also a problem among women of childbearing age though to a smaller extent. This was revealed in a study conducted in 1997 at Ekwendeni girls' secondary school, which revealed that 10% of the girls aged between 14 and 20 years were anaemic. However the anaemia prevalence rates increased with increasing age (Young et al, 1997C). In Thyolo the anaemia rate among 220 women was 37% (Lavene et al, 1997). Another study in the same district (Thyolo) found anaemia rate of 38 % among women who had recently delivered (Williams, 1997). During a recent study by MOHP, it was found that 27% of the non-pregnant women surveyed were anaemic (MOHP/NSO/UNICEF/CDC, 2003).

2.7.4 Anaemia in men

As stated by Robbinett, 1996, all ages and both sexes are affected by anaemia, but prevalence varies by group. A similar scenario applies for Malawi. From data elaboration above, it has been observed that there are anaemia prevalence in excess of 70% among children under five years old, above 54% among pregnant women and between 10% and 38% among women of childbearing age. Anaemia is also a problem among men. This was revealed by a study conducted in Thyolo district by Project Hope which revealed anaemia prevalence of 19% among 315 men, using 12g/dl as cut off instead of 13g/dl as recommended by WHO (Williams, 1997). In a recent national survey by MOHP, the prevalence, using a cut-off of 12.0g/dl, was 17.4% (MOHP/NSO/UNICEF/CDC, 2003).

2.7.5 Anaemia among in-patients

Anaemia accounts for a large proportion of hospital admissions morbidity and mortality especially among pregnant women and young children. A hospital-based study at Kamuzu Central Hospital found that 23% of admissions in 1992 were anaemic with haemoglobin levels less than 9.0 g/dl (Nelson et al, 1992). In 1991, it was discovered that anaemia was the primary discharge diagnosis in 16.0% of the children at Kamuzu Central Hospital (Nelson et al, 1992).

Anaemia in Malawi has consistently been regarded as a major reason for health seeking behaviour. In 1992, anaemia was the third major cause of hospital admissions and the second most common among children under 5 years of age (CHSU, 1992– 1995).

The information above confirms the fact that anaemia is highly prevalent among Malawians affecting all ages and both sexes.

2.8 Anaemia intervention measures in Malawi

The Government together with organizations like World Vision International (WVI), United Nations Children's Fund (UNICEF), Project Hope, and Ekwendeni Mission Hospital have consolidated their efforts to prevent and control the problem of anaemia (Malawi Ministry of Health and Population, 1998). These intervention measures include:

- Supplementation of iron and folate capsules to pregnant women and children.
- Fortification for a few selected centrally processed foods like complementary food and maize meal.
- Dietary diversification through promotion of production of small livestock, iron and Vitamin C rich foods especially indigenous varieties of fruits and vegetables.
- Public health measures through provision of deworming drugs, antimalarial tablets, and insecticide treated nets and water and sanitation programmes.
- Nutrition education and communication.
- Formation of the National Consultative Group Task Force on Anaemia which produced a National Plan Of Action for the Control and Prevention of Anaemia (1999 – 2004), with specific objectives and strategies.

From the list of interventions listed above, it can be observed that the Government (MOHP) does not have any specific interventions that directly address the aspect of traditional remedies that are there in the communities, being used to alleviate the problem of anaemia. However, both the Government and NGOs acknowledge that anaemia is highly prevalent in Malawi (GOM, 2002).

Despite all these efforts by government and non-governmental organisations, the problem of anaemia is still prevalent in Malawi, affecting a large proportion of the population as seen from several reports above.

In the year 2000, according to a knowledge, altitude and practice (KAP) survey, it was found out that knowledge about anaemia as a disease among the communities was high, being over 90.0% and knowledge about causes of anaemia was 58.0%, whereas knowledge on how to prevent anaemia was only 25.0% (Mkanda et al, 2001).

The Government's recommendation currently is that each pregnant woman be given one or two tablets a day of ferrous sulphate (60 milligram elemental iron per tablet) plus folic acid (250 microgram) from mid pregnancy until term. However this is limited by problems in procurement, supply and distribution as well as potential problems on the side of the receiver related to adherence and compliance (Malawi Ministry of Health and Population, 1998).

In the year 2000, the coverage of iron supplementation was only about 22% and almost half (48%) of those not taking iron tablets reported that they were not taking the tablets because the Antenatal Clinic (ANC) and Traditional Birth Attendants (TBA) did not have the iron tablets regularly (Mkanda et al, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design.

The study was designed to have four phases as shown below:

Phase 1 involved literature review on previous research work which had been conducted in Malawi and interviews with some herbalists from Blantyre district in order to come up with an inventory of herbal medicine that are used for anaemia treatment in Malawi.

Phase 2 involved collections of herbal medicines from ten traditional healers based in some townships of Blantyre district. The herbal medicine were later analysed in the Laboratory at Chancellor College and Geological Survey.

Phase 3 involved clinical trials to assess the efficacy of herbal medicine from avocado leaves and root barks. This was chosen because it was the widely mentioned and used herbal medicine for anaemia treatment.

Phase 4 involved examinations of different avocado varieties for variations in chemical composition due to variety, and leaf position, and plant part.

3.2 Phase 1: Inventory of herbal medicines

3.2.1 Study site

An inventory list of herbal medicine used for treatment of anaemia was prepared in two parts. The first inventory was prepared from previous research reports (Table 4) and the second inventory was from interviews with ten traditional healers from Blantyre district (Table 5). These traditional healers are practising herbalists in Blantyre district townships of Chirimba, Lirangwi, Somba, Kachele, Lundu, Machinjiri, Chileka, Mdeka and Ndirande (Map 1).

3.2.2 Sampling and data collection

A convenience sampling technique which is sometimes known as grab or opportunity sapling was applied when collecting the herbal medicine from the traditional healers. This method uses the most readily available subjects (Varkevisser et al, 1991). It was difficult to apply any selective sampling technique because it is difficult for traditional healers to give information on the names and preparation of their herbs, since traditional medicine is practiced in secrecy (Ndibwami et al, 1998). Samples were collected from ten traditional healers that co-operated and gave consent.

3.2.3 Data analysis

The information that was obtained was tabulated (Tables 3 and 4). The information comprised local names of the herbal medicine, plant part used as medicine, mode of preparation, route of administration and dose. The National Herbarium and Botanical Gardens of Malawi in Zomba district provided the scientific names of the herbal medicines. These were derived from the local names and not the sample specimen because the traditional healers refused to provide us with unprocessed samples.

3.3 Phase 2: Chemical and phytochemical screening of herbal medicines

3.3.1 Sampling sites

Samples of herbal medicines for analysis of iron, ascorbic acid, protein, copper, zinc and classes of phytochemical compounds were collected from traditional healers, already processed, based in Blantyre district. These came from Chirimba, Lirangwi, Somba, Kachele, Lundu, Machinjiri, Chileka, Mdeka and Ndirande townships (Map1).

3.3.2 Sample collection

The samples were collected from ten traditional healers and the sample size was ten herbal medicines (one sample per traditional healer). These were from the following plants; *Toona* *ciliate, Dalbergia nitidula, Psidium guajava, Ensente livingstonianum, Pericopsis angolensis, Elephantorrhiza goetzei, Acalypha villicaulis,* and *Persia americana*. The herbal medicines comprised of pounded leaves, root barks, root stems and stem barks. They were all in pounded form, but comprised one type of herbal medicine. They were not mixed types.

3.3.3 Sample analysis

The herbal medicines, were collected and taken to Chancellor College for chemical analysis. The samples were analysed in triplicates and the mean was used. They were analysed for the presence and quantities of iron, copper, zinc, ascorbic acid, protein (essential nutrients for blood production) and important inorganic constituents of food like sodium, potassium, magnesium, calcium, and phosphorus. They were also analysed for the presence of classes of phytochemical compounds like alkaloids, tannins, saponins, anthraquinones, anthracyanins, steroids, flavanoids and terpenoids. These are important primary compounds used in drug production (Sofowora, 1993).

3.3.4 Analytical methods

3.3.4.1 Moisture content determination

Moisture content assisted in calculating results on dry matter basis. To determine the moisture content of each herbal medicine, 2 g of samples were each placed in three separate crucibles, which were previously dried in an oven at 105ºC, cooled in a dessicator and weighed until constant weight was obtained. The crucibles with the samples were covered. These were placed in an air-regulated oven at 105ºC for 2 hours. They were cooled in a dessicator and weighed. The heating, cooling and weighing process was repeated until constant weight was obtained (Egan et al, 1981).

Results calculation

The moisture content = % loss in weight. This was obtained using the formula:

$\{(W1 - W2) / M\} \times 100$ where

W1 = weight of crucible and cover + sample before heating.

W2 = weight of crucible and cover + sample after heating.

M = mass of sample before heating.

3.3.4.2 Minerals determination (Zinc, iron, copper, calcium, magnesium, manganese, chromium, potassium, sodium)

Preparation of samples:

Samples were air dried and ground. Moisture content was determined for each sample before analysis, which was later used in calculations. The minerals were extracted from approximately 1 g of sample (weight recorded) through wet-ashing using 70 % HNO_3 (Nitric acid) and 60 % $HClO_4$ (perchloric acid). HCl (hydrochloric acid) (1+1) was added and quantity transferred to 50 ml volumetric flask**.** The analyses were done using atomic absorption spectrophotometer (AAS), Buck scientific model number 200A (Helrich, 1990).

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Standard stock solutions:

Copper stock solution: (1000 µg Cu/ml).

1.000 g pure copper metal was dissolved in a minimum amount of HNO_3 (nitric acid), 5 ml 0.1 N HCl (hydrochloric acid) was added, evaporated to almost dryness and diluted to 1 litre with 0.1 N HCl.

Iron stock solution: (1000 µg Fe/ml).

1.000 g pure iron wire was dissolved in approximately 30 ml of 6 N HCl with boiling and diluted to 1 litre with de-ionised water.

Zinc stock solution: (1000 µg Zn/ml).

1.000 g pure zinc metal was dissolved in approximately 10 ml 6 N HCl and diluted to 1 litre with de-ionised water.

Calcium stock solution: (25 µg Ca/ml).

1.249 g $CaCO_3$ (calcium carbonate) was dissolved in a minimum amount of 3 N HCl, diluted to 1 litre with de-ionised water. 50 ml was further diluted to 1 litre with de-ionised water.

Magnesium stock solution: (1000 µg Mg/ml).

1.000 g pure magnesium metal was dissolved in 50ml water, 10ml 6 N HCl was slowly added and diluted to 1 litre with de-ionised water.

Manganese stock solution: (1000 µg Mn/ml).

1.582 g MnO_2 (manganese dioxide) was dissolved in approximately 30ml 6 N HCl, boiled to remove Cl and diluted to 1 litre with de-ionised water.

Sodium stock solution: (1000 µg Na/ml).

2.542 g of sodium chloride (NaCl) was dissolved in water and diluted to 1 litre with de-ionised water.

Potassium stock solution: (1000 µgK/ml).

1.9068 g-dried KCl (potassium chloride) was dissolved in water for 2 hours at 105 degrees and diluted to 1 litre with de-ionised water.

Chromium stock solution: (1000 µg Cr/ml).

3.735 g of potassium chromate was dissolved in water and diluted to 1 litre with water.

Lanthanum stock solution: (50 g La/L).

58.65 g La_2O_3 (lanthanum trioxide) was dissolved in 250 ml 0.5 N HCl was added slowly and diluted to 1 litre with de-ionised water.

Working standards

Aliquots of the stock solutions were diluted with 0.5 N HCl to make 4 or more standard solutions of each element within range of determination.

Determination

Table 2. AAS operating parameters

Element	Wavelength (nm)	Flame	Standard Range (mg/L)
Copper	324.7	Air – C_2H_2	0 – 5
Iron	248.3	Rich Air – C_2H_2	0 – 100
Zinc	213.8	Air – C_2H_2	0 – 5
Potassium	766.5	Air – C_2H_2	0 – 100
Calcium	422.7	Rich Air – C_2H_2	0 – 20
Magnesium	285.2	Rich Air – C_2H_2	0 – 15
Chromium	357.9	Air-Air rich/yellow	0 - 5
Sodium	589.0	Air – C_2H_2	0 - 40

Four or more standard solutions within analytical range were read before and after each group of 10 samples. Calibration curve was prepared from average of each standard before and after sample group. Necessary dilutions with 10% HCl was made to obtain solutions within range of instrument.

Data analysis

The concentrations of samples in mg/L were read from a plot of absorption values against concentration in Microsoft Excel. Since there was 1g in 50mls, all values in mg/L were divided by 20 (1000/50) to get the amount per gram, and multiplied by 100 to get the amount of mineral per 100gram of sample (mg/100g).

3.3.4.3 Crude Protein Determination

The method was based on the wet digestion of the sample by heating approximately 2 g of sample (mass recorded) with 20 ml concentrated H_2SO_4 (sulphuric acid) in the presence of copper sulphate and potassium sulphate to effect the reduction of organic nitrogen in the sample to ammonia, which is retained in solution as ammonium sulphate. The digest having been made alkaline is distilled to release ammonia, which is trapped and titrated with 0.05 N HCl until colour changed from green to pink (Helrich, 1990).

Calculation of results

The amount of crude protein in the sample in grams per 100 grams (%) was obtained using the formula below (Helrich, 1990).

Crude protein (g/100 g) = {(V1-V2) x df x [HCl] x 1.4 x 6.25}/G.

Where;	V1	=	Titrant volume for sample
	V2	=	Titrant volume for blank
	Df	=	Dilution factor
	[HCl]	=	Concentration of titrant ([HCl] = 0.014 N)
	1.4	=	Constant for Nitrogen
	6.25	=	General constant.
	G	=	Mass of sample

3.3.4.4 Determination of Ascorbic acid

Sample preparation

Ascorbic acid content of the herbal medicines was estimated by macerating 25 g of the sample in a blender, with extraction solution (H_3PO_4-HOAc) prepared by dissolving 15 g of phosphoric acid in 40 ml acetic acid and 200ml water, and diluted to 500 ml with deionised water. The extract was titrated with 2, 6-dichloroindophenol. Ascorbic acid reduced oxidation – reduction indicator dye, 2, 6-dichloroindophenol to colourless solution (Egan et al, 1981).

Working Reagents

Ascorbic acid standard solution (1 mg/ml).

Exactly 50 mg USP ascorbic acid reference standard that had been stored in dessicator away from direct sunlight was diluted to 50 ml immediately before use with extraction solution (H_3PO_4-HOAc).

Indophenol standard solution

Exactly 50 mg of 2, 6-dichloroindophenol-sodium salt that had been stored in dessicator over soda lime, was dissolved in 50 ml water to which has been added 42 mg $NaHCO_3$ (sodium bicarbonate). It was diluted to 200 ml with deionised water.

Titration

Standardisation of indophenol solution

Three 2.0 ml aliquots ascorbic acid standard solution were transferred to each of the three 50ml erlenmeyer flasks containing 5.0 ml H_3PO_4-HOAc solution (extraction solution) and was titrated rapidly with indophenol solution from 50 ml burette until light but distinct rose pink persists for 5 or more seconds. Each titration used about 17 ml of indophenol solution.

Blank

3 blanks were prepared composed of 7.0 ml H_3PO_4-HOAc solution plus volume of water approximately equal to volume of indophenol solution used in standard titrations (about 17 ml). These were titrated with indophenol solution. Each titration used about 0.1 ml of indophenol solution.

Calculation of results

After subtracting average blanks (approximately 0.1 ml) from standardisation titrations, concentration of indophenol solution as mg ascorbic acid equivalent to 1.0 ml indophenol reagent was calculated. This value was multiplied by the volume of titrant for samples to get the milligrams of ascorbic acid.

3.3.4.5 Phytochemical screening

The study was limited to eight (8) classes of phytochemical compounds, like alkaloids, terpenoids, steroids, saponins, flavanoids, tannins, anthraquinones and anthracyanins due to availability of testing materials. However all the classes of phytochemical compounds, which are secondary metabolites of plants belong to three major classes namely; alkaloids, phenolics (flavanoids, tannins, anthracyanins) and terpenoids (Morris and Robbins, 1997).

Screening for the presence of alkaloids

50 ml of 5 % (v/v) hydrochloric acid solution was added to 5 g of powdered plant material. This was let to stand for 24 hours, shaking mixture regularly. The mixture was filtered. To 1 ml

filtrate, 10 drops of Meyer’s reagent and to another 1ml filtrate, 10 drops Dragendorff reagent were added and mixed. The precipitate formed with each of the reagents was taken as preliminary evidence for the presence of alkaloids (Harborne, 1973).

Confirmation test for alkaloids

For those extracts that gave a positive preliminary test a confirmation test was performed as follows: 40 ml of sample filtrate was basified to pH 8-9 with a few drops of 10% ammonia (aq). Extraction was done twice with 40ml chloroform by shaking and separating the chloroform layer. The two chloroform layers were combined and chloroform evaporated to dryness on water bath. Residue was dissolved in 3 ml of 10 % HCl aqueous solution and divided into two equal portions. Treating one portion with Meyer’s reagent and the other with Draggendorff reagent the precipitate formed with each of the reagents was taken as evidence for the presence of alkaloids.

Screening for the presence of terpenoids and steroids

20 ml of diethyl ether was added to1g of powdered plant material in a 50 ml stopper conical flask. This was let to stand for 24 hours, shaking mixture regularly and filtered. Ten drops of filtrate was put in two separate porcelain crucibles and heated to dryness in a water bath. To one crucible, 10 drops of concentrated sulphuric acid was added, mixed and colour recorded. To the other crucible, 10 drops of acetic anhydride was added, followed by 10 drops of concentrated sulphuric acid, mixed and colour recorded (Harborne, 1973).

The appearance of green to blue colour indicated presence of sterols. The appearance of pink to purple colour indicated presence of terpenoids.

Screening for the presence of saponins

20 ml deionised water was added to 1 g of powdered plant material. This was let to stand for 24 hours, shaking mixture regularly, and filtered. 10 ml of the filtrate was transferred into a 16 mm x 16 mm test tube, shook vigorously for 10 seconds and let to stand for 20 minutes, after which presence of foam was checked and the length measured in millimetres. The foam that persisted for 20 minutes was taken as an evidence for the presence of saponins (Harborne, 1973).

Screening for the presence of flavanoids

50 ml of deionised water was added to 5 g of powdered plant material. This was let to stand for 24 hours, shaking mixture regularly, and filtered. To 2 ml of filtrate, 0.5 ml of a 1:1:1 mixture of hydrochloric acid, methanol and water was added and mixed. A few magnesium turnings were added and colour change to pink or red was taken as an evidence for the presence of flavanoids (Harborne, 1973).

Screening for the presence of tannins

20 ml of deionised water was added to 1 g of powdered plant material. This was let to stand for 24 hours and filtered. 10 drops salted gelatin reagent (10 g gelatin to 100ml water) was added 2 ml of filtrate, followed by 10 drops of 10 % Sodium chloride solution (10 g NaCl to 100ml water). Precipitate formed with salted gelatin reagent only was taken as an evidence of positive reaction. Whereas, a precipitate by both reagents was an indication of negative results (Sofowora 1993, and Harborne, 1973).

Confirmation test for tannins

To a fresh 2 ml filtrate, 10 drops of 0.5 M aqueous ferric chloride solutions were added. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins.

Screening for the presence of anthraquinones

2 g of powdered plant material was moistened with a little of 10 % hydrochloric acid solution (aqueous). 5 ml of chloroform-ether (25:75) solution was added and let to stand for 24 hours, shaking mixture regularly. This was filtered. To 1ml of filtrate, 1 ml of 10 % sodium hydroxide solution (aqueous) was added and mixture shaken. The presence of pink, red or violet colour in the lower phase (NaOH) indicated the presence of anthraquinones (Sofowora, 1993).

Screening for the presence of anthracyanins

20 ml of deionised water was added to 1 g of powdered plant material. This was let to stand for 24 hours, shaking mixture regularly, and filtered. To 5 ml of filtrate, 2 ml of 2 M hydrochloric acid aqueous solutions was added. Mixture was heated in a water bath at boiling temperature for

30 minutes. Colour change to pink or red was taken as evidence for the presence of anthracyanins (Harborne, 1973).

3.3.4.6 Determination of Phosphorus

Phosphorus was determined using a vanadomolybdophosphoric acid colorimetric method as follows: In a dilute orthophosphate solution, ammonium molybdate reacted under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid was formed. The intensity of the yellow colour was directly proportional to concentration of phosphorus. Hence concentrations of samples were obtained from a standard plot.

3.3.5 Data analysis

Data analysis was done using Microsoft Excel for standard calibration, and GenStat, Analysis of Variance, for least significant differences (LSD).

3.4 Phase 3: Efficacy of avocado on anaemia treatment

Ethical approval

The research proposal titled "*Preliminary investigation on the effectiveness of traditional herbs on treatment and control of anaemia*" (Ref: P.01/02/167) aimed at assessing the effecacy of herbal medicines (avocado pear leaves and root barks) was submitted to the College of Medicine Research Committee in October 2002. It was approved on 27th November 2002 (Ref: MC/COMREC/16), (Appendix VII).

3.4.1 Study site

A study to assess the efficacy of herbal medicine prepared from avocado leaves and root barks was conducted at a traditional healers clinic in Chirimba, in Blantyre district (Map 1).

3.4.2 Sampling technique

The correct procedure was to use a blind or double blind method using the substance of interest and placebo. However, in this study, a convenience sampling method was used where study units selected were those who happen to be available at the time of data collection and gave their consent (Varkevisser et al, 1991). This was due to a very low turn up of clients.

The participants to the study comprised the young and old, and they were of different age groups. Those that were sick at the time of the study and had come to the traditional healer's clinic for treatment, and at the same time happened to be anaemic. Participation in the study was voluntary.

Anaemia diagnosis was based on clinical assessment through observation of the presence of one or more of the following signs and symptoms: - pale palm creases, oedema, yellowish skin, tiredness, heart palpitations, pale gums, dizziness, pale nail beds, headache, pale eyelid lining and body weakness. A Senior Clinical Officer was involved in the clinical diagnosis of anaemia. The anaemia was later confirmed by checking their haemoglobin levels by the Chief Laboratory Technician at the herbal clinic. The Senior Clinical Officer and Chief Laboratory Technician were employees of Mlambe Mission Hospital, in Lunzu, temporarily employed by the researcher.

3.4.3 Sample size

The planned sample size was 100. However due to poor turn up of clients, voluntary participation and costs, the sample size obtained was only 44. Out of these, those who returned for first follow up assessment were only 33 (75 %). A total of 14 clients managed to come back for second follow up assessment (46 %).

3.4.4 Recruitment of clients

The recruitment procedure of survey participants was as follows: Verbal and written consent was obtained from clients to participate in the study after explaining the purpose of the study to them. For clients below the age of ten, guardian's consent was sought. After consent, a qualified

Clinical Officer at the herbalist's clinic identified anaemic individuals using clinical assessment. A qualified Laboratory Technician checked haemoglobin levels of those that gave consent and were anaemic clinically, using HemoCue equipment to confirm the clinical assessment. These haemoglobin levels served as baseline haemoglobin data. Individuals with any level of anaemia (i.e. haemoglobin level of less than 11.0 g/L) were enrolled into the study. On enrolment day, clients were also tested for malaria parasites using field stain method, intestinal parasites and schistosoma haematobia using concentration methods (Appendix IV). The clients who were very weak and febrile were not requested to enrol in the study.

HemoCue is a portable, accurate and battery operated haemoglobinometer with a sensitivity of 85 % in the field and specificity of 94 %. It requires only 10 ul (0.01 ml) of blood to do the test (Robbinett et, al. 1996).

3.4.5 Management of clients at the clinic

After the client had given consent, and was confirmed to be anaemic clinically and biochemically, a questionnaire was administered to him/her by the Clinical Officer. The client was given one week's treatment by the Traditional Healer and advised to come back after one week for review and a new supply of herbal medicine.

The treatment was made using an already prepared herbal medicine, in liquid form. It was prepared by the traditional healer, using four handfuls (about 700 g) of avocado leaves and one handful (about 150 g) of root barks. These were boiled in a pot of water for 3 hours and final volume of herbal medicine was 15 litres. The patient was expected to drink 5 liters of medicine for a week, at a dose of one cup (about 250 ml), three times a day. The preparation and dose of the medicine was decided upon by the traditional healer.

3.4.6 Data collection and collection period

Data collection started in December 2002 and ended in May 2003. The first client was enrolled on 21st December 2002 and the last was enrolled on 26th April 2003. Data was collected through observations, interviews and laboratory/biochemical analysis for haemoglobin levels, malaria parasites, and stool and urine parasites.

A questionnaire (Appendix I) was used to capture data on, age of participant, gender of participant, duration of anaemia, signs and symptoms of anaemia present, weekly haemoglobin levels, any observable improvement of signs and symptoms, presence of malaria, intestinal and urine parasites.

In addition to the questionnaire, a weekly monitoring sheet was also used to capture data on treatment performance (Appendix II).

3.4.7 Data analysis

Questionnaire: EPI- INFO6 version 6.04b was used for all data entry. Preliminary data analysis, and frequency distributions were done using the same package (EPI-INFO 6.4). Pearson's Chi-square test was employed to determine statistical significance or otherwise for any observed differences.

3.5 Phase 4: Variations in chemical composition

3.5.1 Study site, sample size and sample collection

The samples of different avocado varieties, which were used in this phase, were obtained from Bvumbwe Research Station, in Thyolo district with the assistance of a Horticulturist. This was the only place, which had identified avocado varieties.

There were five varieties available at the time of study and all of these were used. These are all *Persia* species from *Lauraceae* family. The varieties were Benik (Figure 5), Fuertte (Figure 6), Mayapan (Figure 7), Hass (Figure 8) and Ex-Chitedze (Figure 9). One local variety from the same place whose variety could not be identified was included among the samples (Figure 10). The samples were collected during the month of April 2003 and all the varieties had fruits on.

Figure 5: Picture of leaves and roots of avocado variety Benik

Figure 6: Picture of leaves, fruits and roots of avocado variety Fuertte

Figure 7: Picture of leaves, fruits and roots of avocado variety Mayapan

Figure 8: Picture of leaves, fruits and roots of avocado variety Hass

Figure 9: Picture of leaves, fruits and roots of avocado variety Ex-Chitedze

Figure 10: Picture of leaves, fruits and roots of avocado local variety

3.5.2 Sampling techniques and sample preparation

The samples that were analysed for each variety were as follows,

1. A mixture of leaves from the first to the fifth position (1 – 5) from the tip dried and pounded to fine powder.

2. A mixture of leaves from the sixth to the tenth position (6 – 10) from the tip dried and pounded to fine powder.
3. A mixture of leaves from the eleventh to the fifteenth position (11 – 15) from the tip dried and pounded to fine powder.
4. Root bark dried and pounded to fine powder.
5. Mixture of fresh leaves pounded.
6. Mixture of fresh root barks pounded.

According to Herbalists in Blantyre district, they believe that the trees with deep green and large leaves produce better medicine. They also said the tender and young leaves are mostly used than the tougher and older leaves (Verbal Communication with Blantyre Herbalists, 2003).
The different avocado varieties were given identity codes as follows:

A = Benik.
B = Fuertte.
C = Mayapan.
D = Hass.
E = Ex- Chitedze.
F = Local Breed.
G = Chirimba avocado (This is the one that was used in efficacy study and was identified as variety Hass by a Horticulturist).

3.5.3 Sample analysis

The samples were analysed for the presence and quantities of essential nutrients like protein, iron, ascorbic acid, copper, and zinc. The Atomic Absorption Spectrophotometer (AAS) was calibrated using standards of known concentrations and blank. The analytical methods and data analysis methods used were as outlined above under section 3.3.4.

3.5.4 Data analysis

Data analysis was done using Microsoft Excel for standard calibration, Genstat, ANOVA for least significant differences and coefficient of variations.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Findings for phase 1: Inventory of herbal medicine for anaemia treatment

4.1.1 List of anaemia herbal medicines from literature.

From literature search, it has been discovered that in Malawi, anaemia is being treated, among other things, with herbal medicine. Table 3 below summaries a list of 14 plants indicated by the National AIDS Control Program (1998), Msanangwe (1999), Catholic nuns verbal communication (2001), Mwanyambo and Nihero (1998), and Carney (1999), as herbal medicine for treatment of anaemia in Malawi. The Table presents the scientific and local names of the plant species, part of plant used, route of administration, and source of information. Additional information on mode of preparation, and prescribed dosage is contained in Appendix V.

Table 3: Summary of information on anaemia herbal medicines and source

Scientific Name	Local Name	Plant part used	Route	Source
Persia amaricana	Peyala	Leaves	Oral	(Poor Claras, 2001)
Persia americana	Peyala	Leaves	Oral	Msanagwe, 1999
Persia americana	Peyala	Leaves	Oral	NACP, 1998.
Persia americana	Peyala	Fruit	Oral	NACP, 1998.
Moringa ovalifolia	Chamwamba	Leaves	Oral	NACP, 1998.
Citrus limonium	Mandimu	Fruit	Oral	NACP, 1998.
Vinca rosea	Maluati	Flowers	Oral	NACP, 1998.
Musa sapientum	Nthochi	Dried flowers	Oral	NACP, 1998.
Droogmansia pteropus *Ximenia caffra*	Nlundelunde Mpinjipinji	Roots Roots	Oral Oral	M and N 1998.
Uapaca nitida	Udzu/masuku	Roots	Oral	M and N 1998.
Cucumis hirsutus	Nkukunyajila/ Bowa, mkuwikuwi	Roots	Oral	M and N 1998.
Azanza garckeana	Ntowo, matowo	Roots Roots and bark	Oral Oral	M and N 1998. Carney, 1999.
Bauhinia thonningii	Chitimbe	Stem bark	Oral	M and N 1998.
Vitex doniana	Tonongoli or Jumu	Bark	Oral	Carney, 1999.
Rhynchoma minima	Mukavanti or Chirambe chachikulu	Roots from small plant	Oral	Carney, 1999.
Commiphora massamicensis	Matondo	Bark	Oral	Carney, 1999.

Key: NACP = National AIDS Control Programme, Route = Route of administration, M and N = Mwanyambo and Nihero, Source = Source of information.

4.1.2 Anaemia herbal medicines from Blantyre district traditional healers

From interviews with some traditional healers from Blantyre district, it has been confirmed what literature claimed that in Malawi, anaemia is being treated with herbal medicines. Table 4 below summaries a list of plant species obtained from traditional healers from Blantyre district as herbal medicine which they use for treatment of anaemia. The Table presents the location of the traditional healer, scientific and local names of the plant species, part of plant used, and route of administration. Additional information on mode of preparation, and prescribed dosage is contained in Appendix VI.

Table 4: Summary of information on herbal medicines used by herbalists to treat anaemia from Blantyre district

ID	Herbalist location	Local name	Scientific name	Plant part used	Route of administration.
TH1	Lirangwi	Senderela	*Toona ciliata*	Stem bark	Oral
TH2	Somba	Mkulasenga	*Dalbergia nitidula*	Stem bark	Oral
TH3	Kachele	Gwawa	*Psidium guajava*	Leaves	Oral
TH4	Lundu	Chizuzu	*Ensente livingstonianum*	Root stem	Oral
TH5	Chirimba	Muwanga	*Pericopsis angolensis*	Stem bark	Oral
TH6	Machinjiri	Chiteta	*Elephantorrhiza goetzei*	Root stem	Oral
TH7	Chileka	Mkulasenga	*Dalbergia nitidula*	Root bark	Oral
TH8	Mdeka	Mwanawa mphepo	*Acalypha villicaulis*	Root bark & stem	Oral
TH9	Ndirande	Chizuzu	*Ensente livingstonianum*	Root stem	Oral
TH10	Chirimba	Peyala	*Persia americana*	Leaves	Oral

4.1.3 Discussion on anaemia herbal medicines inventory

It shows from Tables 3 and 4, that in Malawi, anaemia is being treated using a wide range of plant species.

The anaemia medicinal plants reported in the literature from previous research (Table 3) are not as those provided by Blantyre district traditional healers (Table 4), except for *Persia americana* (avocado pear) which seems to be widely used and known as treatment for anaemia. All the traditional healers who were interviewed in Blantyre district mentioned it. Carney (1999), National AIDS Control Programme (1998), Ndibwami et al (1998), and Msanangwe (1999), also mentioned it in their research reports.

Dalbergia nitidula and *Esente livingstonianum* were the second most commonly used since each was mentioned by two herbalists, from different places. Morris also indicated in his book 'Medicinal Plants of Malawi' that *D. nitidula* is used as medicine to increase blood (Morris, 1991).

Different parts of the plant species are used for preparation of the medicine. These parts include leaves, fruit, flowers, roots, root barks, root stem and stem bark. However, according to the table, it is the roots, which are mostly targeted.

4.2 Results for phase 2: Analysis of herbal medicine for protein, iron, ascorbic acid, zinc, copper and classes of phytochemical compounds

4.2.1 Chemical composition of the herbal medicines from Blantyre district herbalists

Chemical composition of the herbal medicines was determined with respect to content of protein, iron, ascorbic acid, zinc and copper (Table 5a), these being the substances that play a role in the production of red blood cells.

All values are reported as amount in milligrams per 100 grams (mg/100g) on dry weight basis, except crude protein and moisture, which are reported as percentages. The values are reported as mean ± sd, n = 3.

The herbal medicines were found to contain differing amounts of these chemical components within the following ranges: moisture 6.1 – 10.1 %, protein 3.2 – 29.3 %, iron 7.5 – 69.8 mg/100g, ascorbic acid 10.6 – 63.8 mg/100g, copper 0.3 – 3.7 mg/100g and zinc 3.1 – 6.5 mg/100g. *Ensente livingstonianum* (TH4) was found to contain the highest amount of iron (69.8 mg/100g), ascorbic acid (63.8 mg/100g) and copper (3.7 mg/100g), seconded by *Dalbergia nitidula*. Iron is a primary raw material of haemoglobin, ascorbic acid reduces ferric iron to ferrous iron which can be absorbed, and copper containing proteins are involved in the mobilization of iron from its storage sites to the plasma.

Table 5a. Chemical composition of the herbal medicines from Blantyre district herbalists

Test	Sample description and analysis results												
	TH1	TH2	TH3	TH4	TH5	TH6	TH7	TH8	TH9	TH10			
	Dried stem bark	Dried stem bark	Dried Leaves	Dried Root stem	Dried Stem bark	Dried Root stem	Dried Root bark	Dried root bark & stem	Dried Root stem	Dried leaves	Mean	LSD (0.05)	CV %
Moisture (%)	9.5 ±0.1	6.9 ±0.2	6.4 ±0.2	7.3 ±0.1	6.1 ±0.0	10.1 ±0.4	8.3 ±0.3	9.5 ±0.1	8.5 ±0.2	8.2 ±0.0	8.1 ±1.4	2.8	17.1
Protein (%)	8.0 ±0.2	4.2 ±0.3	16.0 ±0.1	6.8 ±0.4	29.3 ±0.2	8.9 ±0.1	3.2 ±0.2	7.0 ±0.1	8.0 ±0.3	13.8 ±0.1	10.5 ±7.7	15.3	72.8
Iron (mg/100g)	28.1 ± 0.0	20.5 ± 0.0	59.4 ± 3.9	69.8 ±1.3	7.5 ±0.0	15.8 ±0.3	59.4 ±1.3	56.8 ±3.9	11.9 ±0.1	45.6 ±2.4	37.5 ±23.2	46.4	61.9
Ascorbic acid (mg/100g)	14.2 ±0.1	31.9 ±0.0	24.8 ±0.2	63.8 ±0.1	31.9 ±0.1	42.7 ±0.0	60.3 ±0.2	10.6 ±0.0	35.5 ±0.2	31.9 ±0.1	34.8 ±17.3	34.6	49.7
Zinc (mg/100g)	6.5 ± 0.0	3.4 ±0.0	3.9 ±0.0	3.2 ± 0.2	2.8 ±0.0	4.2 ±0.1	7.3 ±0.2	3.1 ±0.1	5.2 ± 0.1	3.2 ±0.1	4.3 ±1.6	3.1	36.3
Copper (mg/100g)	1.7 ± 0.0	0.3 ± 0.0	0.9 ± 0.0	3.7 ± 0.1	0.3 ±0.0	0.6 ±0.0	2.7 ±0.0	1.1 ±0.1	0.3 ± 0.1	1.8 ± 0.0	1.3 ±1.1	2.3	85.5

Key: TH1= *Toona ciliate*, TH2= *Dalbergia nitidula*, TH3= *Psidium guajava*, TH4= *Ensente livingstonianum*, TH5= *Pericopsis angolensis*, TH6= *Elephantorrhiza goetzei*, TH7= *Dalbergia nitidula*, TH8= *Acalypha villicaulis*, TH9= *Ensente livingstonianum*, TH10= *Persia americana*.

Further chemical analysis was done on the herbal medicine with respect to the quantities of some important inorganic constituents of food from the nutritional point of view. These important inorganic constituents of food include; phosphorus, potassium, sodium, calcium, magnesium, and chromium (Table 5b).

All values are reported as amount in milligrams per 100 grams (mg/100g) on dry weight basis, except crude protein and moisture, which are reported as percentages. The values are reported as mean ± sd, n = 3.

The herbal medicines were found to contain differing amounts of these chemical components within the following ranges: phosphorus 72.1 – 320.1 mg/100g, potassium 233.4 – 2240.0 mg/100g, sodium 20.5 – 35.6 mg/100g, magnesium 36.4 – 414.6 mg/100g, calcium 340.5 – 1829.3 mg/100g and chromium 0.7 – 3.6 mg/100g. *Persia americana* (TH 10), which commonly used for anaemia treatment, was found to contain the highest amount of chromium (3.6 mg/100g). Chromium regulates gene expression, potentiates insulin action and influences carbohydrate, lipid, and protein metabolism (Davidson et al, 1975).

Table 5b. Chemical composition of the herbal medicines from Blantyre district herbalists

Test	Sample description and analysis results												
	TH1	TH2	TH3	TH4	TH5	TH6	TH7	TH8	TH9	TH10			
	Dried stem bark	Dried stem bark	Dried Leaves	Dried Root stem	Dried Stem bark	Dried Root stem	Dried Root bark	Dried Root bark & stem	Dried Root stem	Dried leaves	Mean	LSD (0.05)	CV %
Phosphorus (mg/100g)	320.1 ±3.9	106.0± 6.5	186.8 ±1.3	87.8 ±0.0	293.6 ±1.3	155.5± 0.0	80.0 ±0.0	72.1 ±2.6	137.3 ±1.3	182.2 ± 0.1	162.1 ±86.6	173.1	53.4
Potassium (mg/100g)	1736.8 ±23.6	573.3± 15.7	1060.7 ±7.9	746.3 ±7.9	824.9 ±0.0	2240.0 ±15.7	510.4 ±0.0	730.6±0.0	233.4 ±1.5	604.0± 0.7	926.0 ±611.3	1222.6	66.0
Sodium (mg/100g)	27.3 ±0.1	35.6 ±0.0	26.7 ±0.0	23.8 ±0.7	20.8 ±0.7	32.6 ±0.0	22.3 ±0.0	25.2 ±0.7	22.3 ±0.0	25.8 ±0.0	26.2 ±4.7	9.4	17.8
Magnesium (mg/100g)	155.0 ±1.8	147.3 ±0.4	164.2 ±0.7	36.4 ±0.0	414.6 ±0.7	134.6 ±1.1	318.6 ±0.0	191.1±2.1	119.0 ±1.1	268.3 ±1.1	194.9 ±109.7	219.4	56.3
Calcium (mg/100g)	957.9 ±2.0	1685.5 ±15.7	930.4 ±5.9	985.4 ±2.0	1742. 8±7.9	368.0 ±0.0	1829. 3±7.9	879.3±0.0	340.5 ±0.0	1489.1 ±0.0	1120.8 ±543.3	1087.0	48.5
Chromium (mg/100g)	0.7 ±0.1	0.7 ±0.1	1.7 ±.0.3	2.7 ±1.0	0.7 ±0.1	0.7 ±0.1	0.7 ±0.1	0.7 ±0.1	0.7 ±0.1	3.6 ±1.2	1.3 ±1.1	2.1	81.4

Key: TH1 = Toona ciliate, TH2 = Dalbergia nitidula, TH3 = Psidium guajava, TH4 = Ensente livingstonianum, TH5 = Pericopsis angolensis, TH6 = Elephantorrhiza goetzei, TH7 = Dalbergia nitidula, TH8 = Acalypha villicaulis, TH9 = Ensente livingstonianum, TH10 = Persia americana.

4.2.2 Summary of the doses prescribed by herbalist converted into masses

Table 6 below summarises the doses for the herbal medicine as prescribed by the herbalist. These doses were converted to mass in milligrams to find out the amount of herbs that one is supposed to take per day. These masses were further used to calculate the amount of essential nutrients that one takes in a day if one adheres to the prescribed dose. Samples TH3 (*Psidium guajava*) and TH10 (*Persia americana*) are not included in this analysis because the mode of preparation and administration is different

Table 6: Summary of the doses prescribed by herbalist converted into masses

ID	Local name	Scientific name	Plant part used	Dose/day	Amount taken per day (mg)
TH1	Sendelera	*Toona ciliata*	Stem bark	½ tsp x 2	422.2
TH2	Mkulasenga	*Dalbergia nitidula*	Stem bark	¼ tsp x 3	462.5
TH3	Gwawa	*Psidium guajava*	Leaves		
TH4	Chizuzu	*Ensente livingstonianum*	Root stem	¼ tsp x 2	194.6
TH5	Muwanga	*Pericopsis angolensis*	Stem bark	1/4tsp x 2	210.6
TH6	Chiteta	*Elephantorrhiza goetzei*	Root stem	¼ tsp x 2	240.1
TH7	Mkulasenga	*Dalbergia nitidula*	Root bark	½ tsp x 3	513.0
TH8	Mwanamphepo	*Acalypha villicaulis*	Root bark & stem	¼ tsp x 2	267.4
TH9	Chizuzu	*Ensente livingstonianum*	Root stem	½ tsp x 2	559.6
TH10	Peyala	*Persia americana*	Leaves		

4.2.3 Comparison between chemical content of prescribed dose of herbal medicine and Required Daily Allowances (RDA)

Data in Table 7 showed that the herbal medicines, if taken according to the herbalists' prescriptions, would provide very low amounts of nutrients in comparison with the required daily allowances (RDAs). They could provide iron ranging from 2.0 % to 30.5 % of the RDAs, and ascorbic acid ranging from 0.5 % to 6.0% of the RDAs. Treatment of anaemia using folate tablets supplies 4 to 6 times higher iron content (60 mg iron) than the RDAs (10 – 15 mg/day).

Among the herbal medicines, it was found that sample TH7 (*Dalbergia nitidula*) could provide the highest amount of iron and ascorbic acid. Thus iron, 30.5 % of RDA and ascorbic acid, 6.0 % of RDA respectively. *Esente livingstonianum* (TH4) came on third position in terms of iron proportion, and fourth position for ascorbic acid proportion.

Table 7: Comparison of herbal medicine chemical content per daily dose (table 7) to Required Daily Allowances (RDA)

RDA / ID	Protein g/day	P mg /day	Mn mg /day	Cr mg/day	Na mg /day	K mg/day	Mg mg /day	Ca mg /day	Cu mg /day	Zinc mg /day	Iron mg/day	Ascorbic acid mg/day
RDA	44	800-1200	2.0-5.0	0.05-0.2	500-3000	2000	280-250	800-1200	1.5-3.0	12-15	10-15	50-90
ID												
TH1	0.328	14.101	0.254	0.009	1.159	6.471	6.471	40.527	0.072	0.274	1.187 (12%)	0.599(1%)
TH2	0.180	4.601	0.090	0.137	1.646	6.795	6.795	77.228	0.001	0.159	0.950 (10%)	1.485 (3%)
TH3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TH4	0.140	1.708	0.111	0.085	0.448	0.708	0.708	19.215	0.074	0.062	1.359 (14%)	1.24(2.4%)
TH5	0.655	6.211	0.436	0.063	0.454	17.372	9.162	36.869	0.007	0.059	0.160 (2%)	0.67(1.3%)
TH6	0.211	3.734	0.238	0.071	0.784	3.2055	3.205	8.835	0.015	0.100	0.381(4%)	1.025(2%)
TH7	0.173	4.101	0.467	0.152	1.143	16.342	16.342	94.248	0.135	0.372	3.047(31%)	3.093(6%)
TH8	0.190	1.859	0.091	0.079	0.655	5.166	5.166	23.512	0.057	0.082	1.519(15%)	0.28(0.5%)
TH9	0.467	7.609	0.069	0.166	1.247	6.720	6.720	19.052	0.018	0.293	0.668(7%)	1.987(4%)
TH10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Key: ND means not done, P = Phosphorus, Mn = Manganese, Cr = Chromium, Na = Sodium, K= Potassium, Mg = Magnesium, Ca = Calcium, Cu = Copper.

4.2.4 Phytochemical screening for Blantyre district herbal medicine

The phytochemical compounds which were predominant in most of the herbal medicines screened, include tannins, saponins, flavonoids, anthraquinones and anthracyanins (Table 8). Alkaloids which have important clinical use in analgesics, antimalarias, intispasmotics treatment of hypertention, mental disorders, and tumours, were detected in one sample only, thus the stem bark of *Pericopsis angolensis* (TH5). The tuber of *Elephantorhiza goetzei*, (TH6) was also screened for phytochemical compounds, and it was found to contain steroids, terpenoids, saponins, flavonoids and anthraquinones. It was negative for alkaloids and tannins (Msonthi, 1994).

Herbal medicines TH4 (*Ensente livingstonianum*) and TH7 (*Dabergia nitidula*), contained similar classes of phytochemical compounds to *Percia americana*. These are the herbal medicine which also contained higher levels of iron, ascorbic acid, copper and zinc (Table 5a).

Tannins which are believed to bind to biological compounds such as proteins and carbohydrates hence inhibiting absorption of some of these compounds including iron was predominantly high in most of the herbal medicines screened, including *Persia americana*, (TH10), which was used in efficacy trial.

Table 8: Phytochemical screening results for Blantyre district herbal medicines

ID	Plant part	ALK	TN	ST	TP	SP	FV	AQ	AC
TH1	Stem bark	-	-	+	+	-	-	-	-
TH2	Stem bark	-	+++	+	+	++	-	+	++
TH3	Leaf	-	-	+	-	-	-	-	-
TH4	Root (bark + stem)	-	+++	-	-	+++	++	+	++
TH5	Stem bark	+++	-	-	-	+++	-	-	-
TH6	Root stem (no bark)	-	+++	-	-	+	++	++	++
TH7	Root bark	-	+++	-	-	+	++	++	++
TH8	Root (bark + stem)	-	-	-	-	+++	-	-	+
TH9	Root stem (no bark)	-	+++	+	-	+++	+++	+++	+++
TH10	Leaf	-	+++	-	-	+	++	++	++

Key: ALK = Alkaloids, TN = Tannins, ST = Steroids, TP = Terpenoids, SP = Saponins, FV = Flavanoids, AQ = Anthraquinones, AC = Anthracyanins

(-) = Negative

(+) = Weak positive

(++) = Moderate positive

(+++) = Strong positive

4.3 Results of phase 3: Efficacy of avocado pear on anaemia

4.3.1 Description of the herbalist

Ireen Mtonya, the herbalist at whose clinic efficacy study was conducted, opened a herbal clinic at her home in Chirimba township in Blantyre in 1984.

She sees an average of 10 anaemia patients per week. The signs and symptoms that guide her that a client is anaemic are tiredness, heart palpitation, dizziness, headache, and body weakness. However, according to her, most clients that visit the clinic complain of tiredness, heart palpitation and body weakness.

She uses four handfuls (700grams), and one handful (150grams) of root barks of avocado pear, fresh or dry to prepare 15 litres of medicine for anaemia treatment. The root barks are cut into small pieces, mixed with the leaves and boiled for about 3 hours. The treatment is 5 litres of medicine to be drunk for 1 week. Patients are advised to drink 250 millilitres of the medicine, three times a day. That is morning, noon and evenings after eating any food. During the course of treatment, they are advised not to eat pork, cat fish (mlamba), or soaked rice, not to drink beer or smoke tobacco.

She reported that, so far there has not been any complaint from clients on side effects or adverse reaction to treatment. Patients start feeling improvement, according to her observation, after, on average, 3 days of treatment. Other diseases, which she claims to cure, include HIV/AIDS, cancer, tuberculosis, and karposis (she calls it "kapusitsa"). She monitors progress of clients during weekly medicine collection days. If a patient is not responding to treatment, she sends them to a hospital.

4.3.2 Description of the clients enrolled into the study

Clients that were enrolled into the study were 44, but it was only 33 clients who managed to come back for follow-up assessment. The clients who did not return for follow-up assessment were dropped from the study. The study group comprised 12 males (36 %) and 21 females (64 %). These clients were all residing in Blantyre district, but coming from ten (10) different districts, out of the 27 districts of Malawi. Out of the 33, 11(33.3 %) were from Blantyre district, 5(15.2 %) from Thyolo district, 4(12.1 %) each from Zomba and Balaka districts, 3(9.1 %) from Ntcheu district, 2(6.1%), from Mulanje. There was a client each for Lilongwe, Chikwawa, Nkhata Bay and Nkhotakota districts respectively (Figure 11).

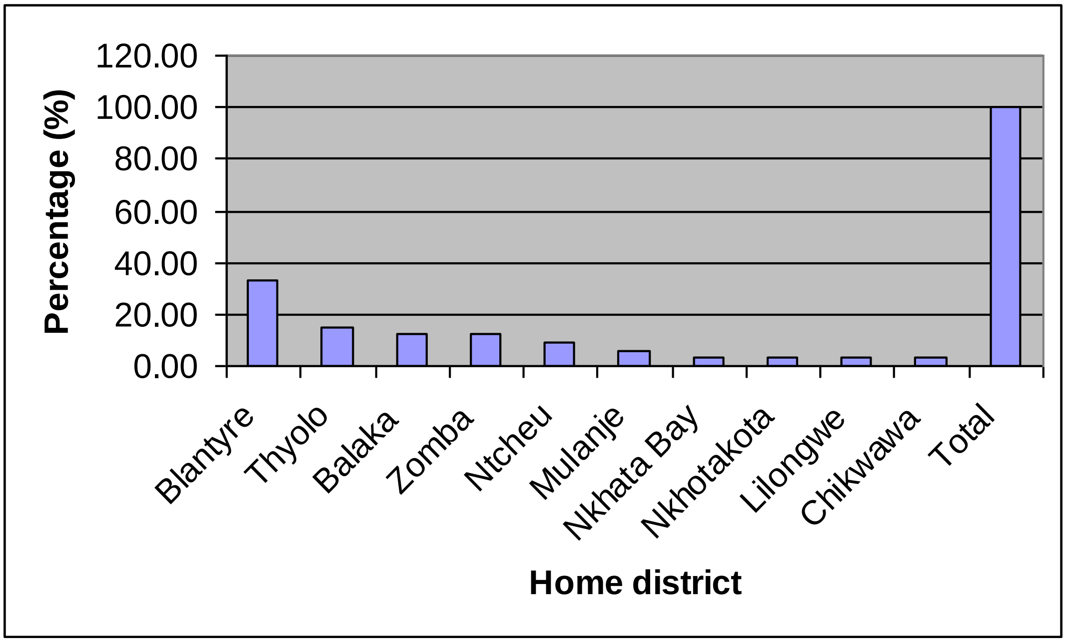

Figure 11: Home districts for the clients that were enrolled in the efficacy study

The clients belonged to a wide range of different denominations (Figure 12). There were a total of 10 denominations reported. These include; Roman Catholic (RC), Church of Central African Presbyterian (CCAP), Jehovah Witnesses (JW), Seventh Day Adventist (SDA), Anglican, Islam, Church of Christ (CC), African Abraham (AA), Pentecostal and Baptist.

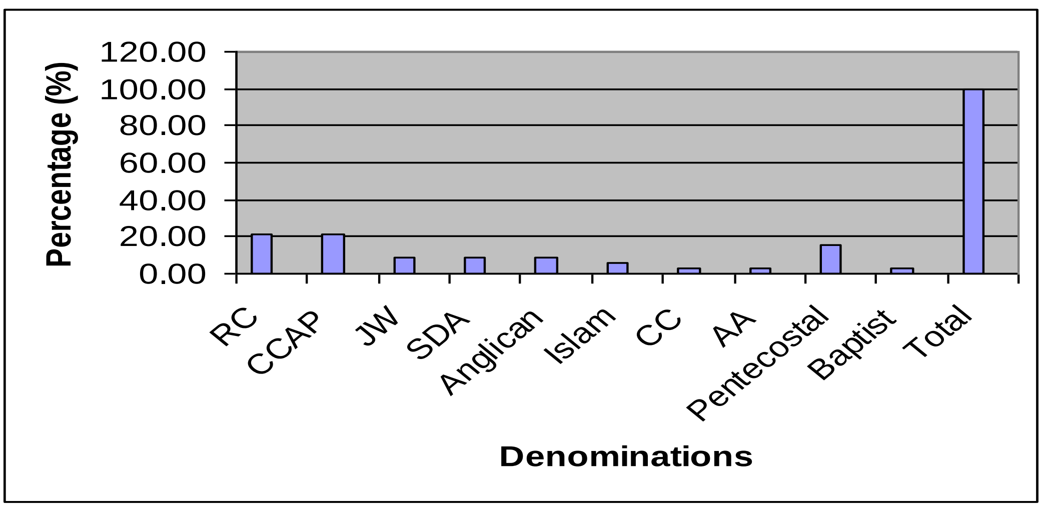

Figure 12: Denominations to which clients on efficacy study belonged

The study group comprised twenty two (22) clients who were aged over 20 years, 9 were aged between 1 and 10 years, with 1 client aged between 10 and 14. Twenty one (21) out the 33 clients (63.6%) had previously visited various hospitals and came to the traditional healer because they felt no improvement after being treated with iron tablets.

All clients who returned for follow up assessment and for more medicine (33 out of 41) felt that the herbal medicine was effective in treating anaemia and reported that they started feeling better from a range of 3 to 6th day after commencing treatment. According to the clinical assessment, most clients presented at the clinic with signs and symptoms which included body weaknesses, followed by headache and dizziness. Most clients had more than two signs and symptoms of anaemia (Figure 13).

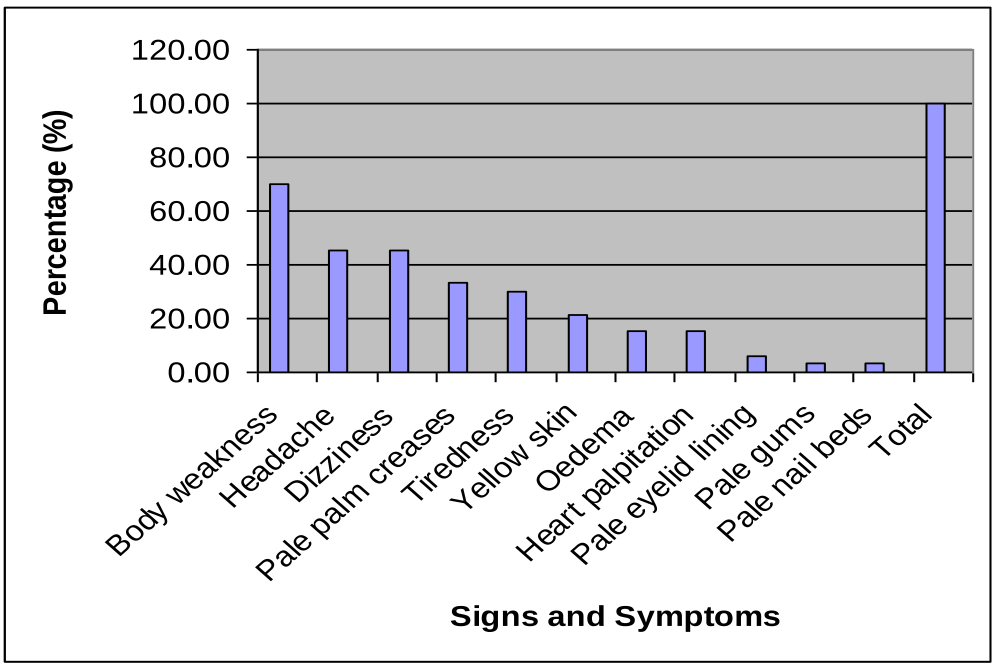

Figure 13: Signs and symptoms of anaemia presented by clients on efficacy study

4.3.3 Haemoglobin levels for study clients over a three week period

Tables 9a and 9b below, show changes in haemoglobin levels for each individual client. It also gives information on age, gender and whether the client had malaria and/or stool and urine parasitic infections.

Table 9a: Haemoglobin levels for study clients who were assessed three times

ID	Gender	AGE	HB1 g/dl	HB2 g/dl	HB3 g/dl	Blood film analysis	Urine analysis	Stool analysis
1	Female	20-24	10.6	12.3	13.3	--	--	Ascaris
2	Male	45-49	9.6	11.9	12.8	--	--	--
3	Male	40-44	8.6	9.8	11.8	--	--	--
4	Female	30-34	7.4	9.6	11.4	--	--	--
5	Female	25-29	10.5	11.4	13.3	--	WBC	--
6	Female	25-29	9.8	10.7	10.9	--	WBC	--
7	Male	1-10	8.4	9.5	9.8	--	--	--
8	Female	1-10	9.6	11.8	14.0	--	--	--
9	Female	30-34	5.6	6.6	6.6	--	--	--
10	Female	45-49	9.6	10.6	12.8	--	WBC	--
11	Male	45-49	9.8	10.8	13.4	--	WBC	--
12	Male	35-39	8.6	9.8	11.6	--	WBC	--
13	Female	30-34	9.4	10.6	11.8	--	WBC	--
14	Male	40-44	8.7	10.2	12.6	--	--	--

KEY: for table 9a

-- stands for ‘’parasites not present’’.

HB1 stands for haemoglobin level on enrolment day.

HB2 stands for haemoglobin level on 7th day of treatment.

HB3 stands for haemoglobin level on 14th day of treatment.

WBC stands for the presence of white blood cells.

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Table 9b: Haemoglobin levels for study clients who were assessed twice

ID	Gender	AGE	HB1 g/dl	HB2 g/dl	HB3 g/dl	Blood film analysis	Urine analysis	Stool analysis
1	Male	1-10	8.7	10.9	NF	Malaria	SH	--
2	Male	45-49	9.6	12.6	NF	--	--	Ascaris
3	Male	1-10	8.5	11.2	NF	Malaria	--	--
4	Male	1-10	6.0	10.6	NF	Malaria	--	--
5	Female	20-24	7.5	9.8	NF	--	--	HW
6	Female	25-29	9.3	10.0	NF	--	--	--
7	Female	1-10	9.5	12.3	NF	--	--	HW
8	Female	1-10	7.6	11.8	NF	--	--	--
9	Female	30-34	8.6	9.0	NF	--	SH	HW
10	Male	30-34	8.6	9.8	NF	--	--	--
11	Female	35-39	8.6	9.4	NF	--	--	--
12	Male	40-44	9.4	10.2	NF	--	--	--
13	Female	20-24	9.8	11.7	NF	--	--	HW
14	Male	1-10	9.1	10.2	NF	--	--	--
15	Female	1-10	8.7	9.0	NF	Malaria	--	--
16	Male	40-44	8.6	9.8	NF	--	--	--
17	Female	35-39	9.8	10.8	NF	--	--	--
18	Female	25-29	8.2	10.1	NF	--	WBC	--
19	Female	25-29	9.0	10.3	NF	--	--	Ascaris

KEY: for table 9b

-- stands for ‘’parasites not found’’

Malaria stands for the presence of malaria parasites.

No follow up (no data) is denoted **(NF).**

HB1 stands for haemoglobin level on enrolment day.

HB2 stands for haemoglobin level on 7th day of treatment.

HB3 stands for haemoglobin level on 14th day of treatment.

WBC stands for the presence of white blood cells.

HW stands for the presence of hookworms.

SH stands for the presence of schistosoma haematobium ova.

4.3.4 Chemical composition of the herbal medicine used during efficacy study

The herbal medicine that was used for treating anaemia patients, when analysed for various parameters (Table 10), was found to contain 0.09% protein, 7.00mg/100g (or 3.97mg/L) iron and 18.45mg/100g (32.56mg/L) ascorbic acid. The prescribed dosage for this medicine was 750 mls per day. This would provide about 2.98 mg of iron per day. The amount of iron in this medicine was very low if compared to required daily allowance of 10- 15 mg/day (Mahan, 1996). Besides, vegetable iron is poorly absorbed. The required iron would not come from the medicine alone, but also from food and other things which the clients ate.

However this herbal medicine from avocado contained higher amount of iron than the extract of Carao fruit (*Cassia grandis*), which contained 1.45mg iron per 100g dry powder, but was effective in treatment of anaemia in Nigeria and Costa-Rica (Standish, 2005). There was probably a factor in the medicine that favours the absorption of iron and its metabolism in the bone marrow.

Table 10: Chemical composition of the herbal medicine used in efficacy study

Parameter	**Amount (n=3)**
Crude Protein (%)	0.09 ± 0.03
Iron (mg/100g)	7.00 ± 0.18
Ascorbic acid (mg/100g)	18.45 ± 0.35
Zinc (mg/100g)	3.99 ± 0.01
Cupper (mg/100g)	0.05 ± 0.02
Chromium (mg/100g)	0.32 ± 0.01
Calcium (mg/100g)	7.28 ± 0.18
Magnesium (mg/100g)	9.61 ± 0.04

4.3.5 Discussion on efficacy study findings

More females (64%) were enrolled into the study than males (36%). All clients enrolled into the study experienced an increase in their haemoglobin levels. During the first week of treatment, haemoglobin levels, for all patients, increased by an average of 1.58g/dl ± 0.19g/dl. During the second week of treatment, haemoglobin levels increased by an average of 1.20g/dl ± 0.33g/dl. This is comparable with iron supplementation by standard regime in an ideal situation, which is supposed to increase the haemoglobin level by 1.5 to 2.1g/dl in a month (WHO/UNICEF/UN, 1995). However it is in line with what Reeve reported that some patients respond rapidly to iron (ferrous sulphate) orally with a rise of 1 to 2 g/dl in a week (Reeve, 1986).

Patients with malaria, stool and urine parasites registered an increase in haemoglobin levels. Malaria and intestinal worms were expected to influence anaemia through blood destruction (malaria) and blood loss (intestinal parasites) (Malawi Ministry of Health and Population, 1998).

The prevalence of malaria was 12.1% (4 clients). All these were aged 1 to 10 years. It comprised of 3 males and 1 female. The prevalence of intestinal worms was 21.2% (3 with ascaris and 4 with hookworm). This is less as compared to national figure of 50% (Verbal communication with National Bilharzia Program Officer, 2004). The clients who were found with malaria, interstinal worms and bilharzia were not put on a different treatment. Hence, they were not treated for any disease other than anaemia.

The herbal medicine from avocado contained higher amount of iron than the extract of Carao fruit (*Cassia grandis*), which contained 1.45mg iron per 100g dry powder, but was effective in treament of anaemia in Nigeria and Costa-Rica (Standish, 2005). There was probably a factor in the medicine that favours the absorption of iron and its metabolism in the bone marrow.

4.4 Results for phase 4: Effects of variety, leaf position, and plant part on chemical composition

For this purpose, emphasis was on essential substances for blood production like iron, ascorbic acid, protein, zinc, and copper.

4.4.1 Effect of variety, leaf position and plant part on iron distribution

Chirimba avocado had significantly higher ($P < 0.05$) levels of iron than varieties Mayapan, Hass and local for leaf powders from position 1-5 (Table 11). For powders of leaves from position 6-10, Chirimba avocado had significantly high levels of iron than varieties Benik, Hass and Ex-Chitedze. There were also higher levels of iron in leaves from position 11 -15 for Chirimba avocado than Hass, Ex-Chitedze and local. For the root bark powders, Chirimba avocado had higher iron content than Ex-Chitedze. Chirimba avocado and Fuertte were outstanding in terms of iron levels, seconded by Benik and Mayapan.

Comparing different plant parts, it was found that for all varieties, there were significantly higher levels of iron in root bark powders than the leaf powders.

Table 11: Distribution of iron, expressed as milligram iron per 100 gram sample, (mg/100g) according to variety, leaf position and plant part (n = 3)

Sample	**Varieties**							**MEAN**	**LSD (0.05)**	**CV %**
	A	**B**	**C**	**D**	**E**	**F**	**G**			
Dry powder of leaf 1-5	51.9 ±2.5	45.9 ±3.0	33.3 ±0.8	10.1 ±1.5	57.7 ±0.6	32.6 ±3.5	75.8 ±1.2	43.9 ±21.0	42.1	48.0
Dry powder of leaf 6-10	37.7 ±3.2	55.2 ±1.2	62.7 ±3.0	26.2 ±1.0	23.3 ±2.8	52.7 ±2.4	92.4 ±2.2	50.0 ±23.9	47.8	47.8
Dry powder of leaf 11-15	37.7 ±3.6	60.2 ±2.4	35.2 ±1.4	22.6 ±1.9	12.6 ±2.2	17.5 ±0.4	81.2 ±0.2	38.1 ±24.7	49.5	64.8
Mixed leaf powder	42.4 ±3.3	53.8 ±1.0	43.7 ±1.2	19.6 ±1.5	31.1 ±2.0	34.3 ±1.0	82.8 ±1.1	44.0 ±20.2	40.5	46.1
Root bark powder	208.1 ±1.5	203.1 ±3.5	218.2 ±3.0	158.0 ±2.4	92.8 ±3.1	193.1 ±3.5	229.1 ±0.8	186.1 ±46.9	93.8	25.2
Mixed fresh leaves	69.3 ±1.0	71.0 ±0.8	85.5 ±0.0	118.3 ±1.7	54.5 ±1.0	78.3 ±2.0	51.0 ±4.2	75.4 ±22.5	45.0	29.9
Fresh root barks	58.2 ±1.0	69.8 ±1.8	93.2 ±2.0	68.4 ±0.2	55.4 ±1.0	62.6 ±0.1	57.4 ±3.2	66.4 ±13.0	26.1	19.6
MEAN	72.2 ±56.5	79.7 ±51.0	81.69 ±59.9	60.5 ±53.1	46.8 ±24.9	67.3 ±54.7	95.7 ±56.1	77.9 ±55.3		
LSD(0.05)	113.0	101.9	119.7	106.1	49.7	109.4	112.2	110.5		
CV%	78.2	63.8	73.3	87.8	53.2	81.3	58.7	70.9		

Key:

A is Benik, B is Fuertte, C is Mayapan,

D is Hass, E is Ex-chitedze, F is Local breed

G is Chirimba avocado.

4.4.2 Effect of variety, leaf position and plant part on ascorbic acid (vitamin C) distribution

Variety Benik had significantly higher ($P < 0.05$) levels of ascorbic acid than varieties Mayapan, Hass, Ex-Chitedze and local for leaf powders from position 1-5 (Table 12). For powders of leaves from position 6-10, varieties Benik and Fuertte had significantly high levels of ascorbic acid than varieties Hass, Ex-Chitedze and Chirimba avocado. There were higher levels of ascorbic acid in leaves from position 11 -15 for variety Mayapan than Hass, Ex-Chitedze, local and Chirimba avocado. For the root bark powders, variety Mayapan had higher ascorbic acid content than all varieties except Benik. Benik, Fuertte and Mayapan were outstanding in terms of ascorbic acid levels.

Comparing different plant parts, it was found that for all varieties, there were no significant difference in the levels of ascorbic acid for root bark powders and the leaf powders. Hence no difference due to plant part.

Table 12: Distribution of ascorbic acid, expressed as milligram ascorbic acid per 100 gram sample (mg/100g) according to variety, leaf position and plant part (n = 3)

Sample	Varieties							MEAN	LSD (0.05)	CV %
	A	B	C	D	E	F	G			
Dry powder of leaf 1-5	28.8 ±0.1	21.6 ±0.0	14.4 ±1.2	18.0 ±0.0	18.0 ±0.1	14.4 ±0.2	19.5 ±1.2	19.2 ±4.9	9.9	25.7
Dry powder of leaf 6-10	28.8 ±0.2	28.8 ±0.1	25.2 ±1.1	18.0 ±0.1	18.0 ±0.1	21.6 ±0.2	19.2 ±2.1	22.8 ±4.8	9.6	21.1
Dry powder of leaf 11-15	28.8 ±0.1	28.0 ±1.1	46.8 ±1.1	14.4 ±0.1	10.8 ±0.1	10.8 ±0.1	16.0 ±1.0	22.2 ±13.2	26.4	59.3
Mixed leaf powder	28.8 ±0.0	24.8 ±0.1	28.8 ±1.1	16.7 ±0.2	15.6 ±1.2	16.6 ±1.1	18.0 ±0.8	21.3 ±5.9	11.9	27.8
Root bark powder	28.8 ±0.1	18.0 ±1.1	46.8 ±1.1	14.4 ±0.1	10.8 ±0.1	10.8 ±0.1	18.0 ±1.2	21.1 ±12.9	25.9	61.1
Mixed fresh leaves	22.0 ±0.0	23.0 ±0.1	29.5 ±0.2	26.5 ±0.0	29.5 ±1.1	23.5 ±1.1	28.5 ±0.4	26.1 ±3.2	6.4	12.4
Fresh root barks	21.7 ±0.3	20.0 ±1.2	31.2 ±0.2	15.6 ±2.3	28.4 ±3.2	15.5 ±0.1	26.6 ±2.2	22.7 ±6.2	12.4	27.3
MEAN	26.8 ±3.1	23.5 ±3.7	31.8 ±10.8	17.7 ±3.9	18.7 ±7.0	16.2 ±4.5	20.8 ±4.4	27.4 ±4.1		
LSD(0.05)	6.3	7.4	21.5	7.7	14.1	9.1	8.8	8.1		
CV%	11.7	15.8	33.8	21.9	37.5	28.1	21.2	14.8		

Key:

A is Benik, B is Fuertte, C is Mayapan,

D is Hass, E is Ex-chitedze, F is Local breed

G is Chirimba avocado (Identified as Hass).

4.4.3 Effect of variety, leaf position and plant part on crude protein distribution

Analysis of powders of leaves from position 1-5 from the tip showed that Chirimba avocado had significantly higher levels ($P<0.05$) of crude protein than Benik (Table 13). There were also higher levels of crude protein in Chirimba avocado than Benik and Hass, for powders of leaves from position 6 – 10. Variety Hass had higher crude protein levels in the powder of leaves from position 11-15 than Benik, Fuertte, Mayapan, and Ex-Chitedze. In dry root barks, it was Chirimba avocado, which had significantly higher levels of crude protein. Chirimba avocado was outstanding in crude protein content, seconded by the local variety.

Comparing the different plant parts, there were higher levels in leaves than root barks for all the other varieties except Chirimba avocado, which had its highest levels in the dried root barks.

Table 13: Distribution of Crude Protein, expressed as gram crude protein per 100 gram sample (g/100g), according to variety, leaf position and plant part (n = 3)

Sample	**Varieties**							**MEAN**	**LSD (0.05)**	**CV%**
	A	**B**	**C**	**D**	**E**	**F**	**G**			
Dry powder of leaf 1-5	10.2 ±1.1	17.9 ±0.2	13.7 ±0.1	16.4 ±0.3	15.9 ±0.3	14.4 ±1.1	18.8 ±1.2	15.3 ±2.9	5.8	18.8
Dry powder of leaf 6-10	11.4 ±0.1	17.9 ±1.1	14.0 ±1.0	9.2 ±0.2	13.5 ±1.1	17.3 ±1.1	19.2 ±1.3	14.6 ±3.7	7.3	25.0
Dry powder of leaf 11-15	13.4 ±0.1	10.0 ±0.0	12.5 ±0.1	21.0 ±0.1	14.2 ±0.3	14.5 ±0.1	16.0 ±2.1	14.5 ±3.4	6.8	23.5
Mixed leaf powder	11.7 ±0.1	15.3 ±0.1	13.4 ±1.1	15.5 ±1.2	15.5 ±1.0	15.4 ±1.2	18.5 ±1.1	15.0 ±2.1	4.2	14.0
Root bark powder	11.5 ±0.3	7.3 ±0.3	9.9 ±0.1	9.1 ±0.3	5.3 ±0.1	12.4 ±1.0	28.3 ±1.1	12.0 ±7.6	15.2	63.4
Mixed fresh leaves	13.5 ±1.0	15.8 ±1.0	8.4 ±0.4	10.1 ±0.1	13.6 ±0.1	12.6 ±1.0	18.7 ±1.3	13.2 ±3.4	6.8	25.8
Fresh root barks	3.0 ±0.1	3.1 ±0.1	4.8 ±0.2	3.2 ±0.1	5.1 ±0.6	4.1 ±0.4	5.7 ±0.1	4.1 ±1.1	2.2	26.2
MEAN	10.7 ±3.3	12.5 ±5.3	11.0 ±3.2	12.1 ±5.5	11.9 ±4.3	13.0 ±3.9	17.9 ±6.1	11.4 ±3.5		
LSD(0.05)	6.6	10.7	6.4	11.0	8.6	7.9	12.3	7.0		
CV%	31.0	42.7	29.0	45.4	36.2	30.3	34.3	30.7		

Key:

A is Benik, B is Fuertte, C is Mayapan,

D is Hass, E is Ex-chitedze, F is Local breed

G is Chirimba avocado (Identified as Hass).

4.4.4 Effect of variety, leaf position and plant part on zinc distribution

Analysis of powders of leaves from position 1-5 from the tip showed that variety Ex-Chitedze had significantly higher levels ($P<0.05$) of zinc than Benik, Fuertte, Mayapan and Local variety (Table 14). Chirimba avocado was found to be the one with higher zinc levels in the powder of leaves from position 6-10 than fuertte, Mayapan, Hass and Local variety. In powders of leaves from position 11-15 and dry root barks, it was variety Fuertte which had significantly higher levels of zinc than the Local variety. Ex- Chitedze was outstanding in zinc content.

There were no significant differences ($P<0.05$) in the levels of zinc for both powder of leaves and root bark powders for all varieties, except for Chilimba avocado which had high levels of zinc in leaf powder than root bark powder.

Table 14: Distribution of Zinc, expressed as milligram Zinc per 100 gram sample, (mg/100g), according to variety, leaf position and plant part (n = 3)

Sample	Varieties							MEAN	LSD (0.05)	CV %
	A	B	C	D	E	F	G			
Dry powder of leaf 1-5	11.5 ±0.1	9.9 ±0.2	8.5 ±0.1	13.8 ±1.1	19.0 ±0.2	8.5 ±0.1	11.8 ±1.2	11.9 ±3.7	7.4	31.0
Dry powder of leaf 6-10	9.5 ±0.1	8.0 ±0.1	8.0 ±0.2	8.2 ±0.1	14.8 ±0.1	8.7 ±0.1	15.2 ±1.0	10.3 ±3.2	6.5	31.2
Dry powder of leaf 11-15	13.3 ±0.2	15.9 ±1.1	7.9 ±0.1	8.0 ±0.1	11.6 ±0.3	5.0 ±0.2	15.0 ±1.1	11.0 ±4.1	8.2	37.2
Mixed leaf powder	11.4 ±1.2	11.3 ±1.0	8.1 ±0.2	10.0 ±0.4	11.8 ±1.1	7.4 ±0.1	14.4 ±1.1	10.6 ±2.4	4.8	22.3
Root bark powder	7.9 ±0.2	12.8 ±0.1	8.6 ±0.2	8.1 ±0.2	8.6 ±0.1	6.2 ±0.1	2.4 ±0.5	7.8 ±3.1	6.2	39.9
Mixed fresh leaves	3.2 ±0.3	1.4 ±0.3	1.7 ±0.1	3.5 ±0.1	4.6 ±0.2	1.9 ±0.2	0.7 ±0.1	2.4 ±1.4	2.7	56.5
Fresh root barks	0.7 ±0.1	0.9 ±0.2	1.5 ±1.0	1.4 ±0.4	3.2 ±1.0	1.9 ±0.1	0.9 ±0.2	1.5 ±0.9	1.7	57.2
MEAN	8.2 ±4.3	8.6 ±5.2	6.3 ±3.0	7.6 ±3.8	10.5 ±5.1	5.7 ±2.7	8.6 ±6.4	7.7 ±4.0		
LSD(0.05)	8.6	10.5	6.0	7.6	10.3	5.3	12.8	8.1		
CV%	52.5	60.8	47.4	50.0	48.9	46.9	74.4	52.4		

Key:

A is Benik, B is Fuertte, C is Mayapan,

D is Hass, E is Ex-chitedze, F is Local breed

G is Chirimba avocado (Identified as Hass).

4.4.5 Effect of variety, leaf position and plant part on copper distribution

Chirimba avocado had significantly higher (P <0.05) levels of copper than varieties Mayapan, and Hass for leaf powders from position 1-5 (Table 15). For powders of leaves from position 6-10, Chirimba avocado had significantly high levels of copper than varieties Benik, Hass and Ex-Chitedze. There were also higher levels of copper in leaves from position 11 -15 for Chirimba avocado than all other varieties. For the root bark powders, Benik had higher copper content than Mayapan, Hass, Ex-Chitedze and the Local variety. Chirimba avocado and Fuertte were outstanding in terms of copper levels

Comparing different plant parts, it was found that for all varieties, there were no significant differences in the levels of copper in root bark powders and the leaf powders.

Table 15: Distribution of Copper, expressed as milligram copper per 100 gram dry powder (mg/100g), according to variety, leaf position, variety and plant part (n = 3)

Sample	**Varieties**							**MEAN**	**LSD (0.05)**	**CV%**
	A	B	C	D	E	F	G			
Dry powder of leaf 1-5	0.9 ±0.1	1.7 ±0.1	0.0 ±0.0	0.0 ±0.0	1.1 ±0.1	1.6 ±0.1	2.1 ±0.2	1.1 ±0.8	1.6	77.8
Dry powder of leaf 6-10	0.0 ±0.0	1.7 ±0.1	0.3 ±0.1	0.1 ±0.0	0.0 ±0.0	1.2 ±0.1	1.9 ±0.1	0.7 ±0.9	1.8	112.3
Dry powder of leaf 11-15	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.7 ±0.1	0.1 ±0.3	0.5	264.6
Mixed leaf powder	0.3 ±0.1	1.2 ±0.2	0.1 ±0.1	0.1 ±0.1	0.4 ±0.1	0.9 ±0.2	2.0 ±0.1	0.7 ±0.6	1.4	98.1
Root bark powder	1.2 ±0.1	0.7 ±0.1	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.8 ±0.2	0.4 ±0.5	1.1	130.9
Mixed fresh leaves	1.3 ±0.2	0.4 ±0.1	0.4 ±0.2	2.5 ±0.2	1.5 ±0.1	1.3 ±0.1	1.5 ±0.2	1.3 ±0.7	1.5	56.9
Fresh root barks	0.6 ±0.1	0.8 ±0.2	0.7 ±0.1	1.3 ±0.1	1.9 ±0.1	1.8 ±0.1	1.3 ±0.1	1.2 ±0.5	1.1	43.4
MEAN	0.6 ±0.5	0.9 ±0.6	0.2 ±0.2	0.6 ±0.9	0.7 ±0.7	1.0 ±0.7	1.5 ±0.5	0.6 ±0.3		
LSD(0.05)	1.0	1.2	0.5	1.8	1.5	1.3	1.1	0.5		
CV%	81.4	64.0	115.5	155.8	105.3	68.9	35.7	43.8		

Key:

A is Benik, B is Fuertte, C is Mayapan,

D is Hass, E is Ex-chitedze, F is Local breed

G is Chirimba avocado (Identified as Hass).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The study has shown evidence that anaemia in Malawi is being treated with herbal medicine using a wide range of plant species. Avocado pear (*Persia americana)* has been found to be the widely used and known treatment for anaemia, followed by *Dalbergia nitidula* and *Esente livingstonianum*. Different parts of the plant species are used for preparation of the medicine. These parts include leaves, fruits, flowers, roots, root barks, root stems and stem barks. However, according to the findings, it is the roots, which are mostly targeted; this poses a threat to the survival of the plant species population.

Chemical composition of the herbal medicine showed that the herbal medicines were found to contain differing amounts of the chemical components within the following ranges: moisture 6.1 – 10.1 %, protein 3.2 – 29.3 %, iron 7.5 – 69.8 mg/100g, ascorbic acid 10.6 – 63.8 mg/100g, copper 0.3 – 3.7 mg/100g and zinc 3.1 – 6.5 mg/100g. *Ensente livingstonianum* was found to contain the highest amount of iron (69.8 mg/100g), ascorbic acid (63.8 mg/100g) and copper (3.7 mg/100g). It was seconded by *Dalbergia nitidula*. Iron is a primary raw material of haemoglobin, ascorbic acid reduces ferric iron to ferrous iron which can be absorbed, and copper containing proteins are involved in the mobilization of iron from its storage sites to the plasma. The herbal medicine *D. nitidula* in addition, also contained high levels of zinc. *D. nitidula* was also mentioned in the book 'Medicinal Plants of Malawi' as medicine to increase blood.

The Phytochemical screening of the herbal medicines revealed that Malawian herbal medicines which are used for anaemia treatment by herbalists in Blantyre district, contained classes of phytochemical compounds like alkaloids, saponins, tannins, terpenoids, steroids, anthracyanins and anthraquinones. *D. nitidula* and *E. livingstonianum* contained similar classes of phytochemical compounds with *Persia americana*, the widely used medicine for anaemia treatment.

All clients that were enrolled into the study and returned for follow up assessment (33 out of 41) had their haemoglobin levels increased during treatment time. During the first week of treatment, haemoglobin levels increased by an average of 1.58g/dl ± 0.19g/dl. During the second week of treatment, among the clients that came for second follow up assessment (15 out of 33), haemoglobin levels increased by an average of 1.20g/dl ± 0.33g/dl. This is comparable with iron supplementation by standard regime in an ideal situation, which is supposed to increase the haemoglobin level by 1.5 to 2.1g/dl in a month. It is also in line with what Reeve reported that some patients respond rapidly to iron orally with a rise of 1 to 2 g/dl in a week.

More females (64%) were enrolled than males. Age, gender and the presence of malaria parasites, stool and urine parasites did not have influence on the changes in haemoglobin levels in relation to treatment period. Malaria and intestinal worms were expected to influence anaemia through blood destruction (malaria) and blood loss (intestinal worms).

Therefore these findings showed that using haemoglobin levels as an indicator of effectiveness, the herbal medicine, which was used in this study, was effective in anaemia treatment.

The study on various avocado varieties to determine variations in chemical distribution according to variety, leaf position and plant part showed that Chirimba avocado (used in efficacy study) had high levels of iron, protein, and copper. Variety Fuerrte contained high levels of iron and copper, variety Benik had high ascorbic acid levels, whereas variety Ex-Chitedze, contained high zinc levels. For all the varieties, the root barks contained significantly higher ($P<0.05$) amounts of iron than the leaves.

In the first 15 leaves, there were no significant differences in the levels of iron, protein, ascorbic acid, copper and zinc. Therefore any avocado variety and any of the leaves within the first 15 leaves if used as herbal medicine a patient can have same benefits.

The commonly used treatment for anaemia in Malawi is folate (ferrous sulphate) tablets, which contain 60 milligram of iron. There was no herbal medicine among those analysed which could supply such high amount at the prescribed dose. However the herbal medicine from avocado pear was effective in raising haemoglobin levels of anaemic individuals significantly. This happened despite the low levels of iron, and besides, vegetable iron is poorly absorbed. The benefit of this herbal medicine appeared not to be directly related to its iron content, but rather to some factor, which is not known, that affected blood generation or iron metabolism.

In conlusion, *Persia americana Dalbergia nitidula* and *Esente livingstonianum* could be used as herbal medicines for anaemia treatment.

5.2 Recommendations

The study recommends that traditional medicine needs to be thoroughly evaluated, given due recognition and developed so as to improve its efficacy, safety, availability and wider application at low cost. It is already the peoples own health care system and is well accepted by them. It has certain advantages over imported systems of medicine in any setting because as an integral part of people's culture, it is particularly effective in sorting certain cultural health problems. It can and does freely contribute to scientific and universal medicine. Its recognition, promotion and development will secure due respect for people's culture and heritage.

The study also recommends that a further investigation be conducted on the medicine used during the efficacy study to find out what made the haemoglobin levels of patients enrolled to increase that fast. This to be coupled with a thorough follow up on peoples diets.

Another study on therapeutic activity should be conducted with a control group being put on ferrous sulphate treatment and over a longer period of time.

A study to be conducted to assess chemical composition of avocado leaves at various seasons.

This study also recommends that research be conducted on root barks of *Persia americana* and *Dalbergia nitidula,* and root stem of *Ensente livingstonianum*, which had high levels of iron, ascorbic acid, copper and zinc, for anaemia treatment. This research should investigate the possibility of using root barks for anaemia treatment, safety, and mode of administration.

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APPENDICES

Appendix I: Questionnaire For Patients on Herbal Treatment

(To be administered by Clinical Officer and/or Principal Investigator)

A. IDENTIFICATION

A1	Date of interview	----/----/2002	CODE
A2	Name of client		
A3	Home district		
A4	Home village		

B. SOCIO ECONOMIC INFORMATION

			CODE
B1	Gender of respondent	1 [ ] Male 2 [ ] Female	
B2	Age of respondent	[ ] <1-10 [ ] 10-14 [ ] 15-19 [ ] 20-24 [ ] 25-29 [ ] 30-34 [ ] 35-39 [ ] 40-44 [ ] 45-49	
B3	Highest education level of respondent	[ ] None [ ] std 1- 5 [ ] std 6-8 [ ] JCE [ ] MSCE [ ] Technical college [ ] University	

		[ ] Adult literacy [ ] Other (specify)	
B4	Denomination of respondent	[ ] Anglican [ ] Roman catholic [ ] C.C.A.P [ ] S.D.A [ ] Other (specify)	
B5	Occupation of respondent	[ ] None [ ] Farming [ ] Housewife [ ] Business [ ] Other (specify)	
B6	Marital status	[ ] Single [ ] Married [ ] Separated [ ] Widow [ ] Other (specify)	

C. INFORMATION ON THE DISEASE ANAEMIA, KNOWLEGDE, SIGNS AND SYMPTOMS

			CODE
C1	Are you aware that you are suffering from anaemia? (Kodi mukudziwa kuti muli ndi vuto lochepa magazi nthupi?)	[ ] Yes [ ] No	
C2	How long have you had this anaemia problem? (Mwakhala ndi matendawa kwa nthawi yayitali bwanji?)	[ ] Days. [ ] weeks [ ] months	

C3	Did you ever visit the hospital for anaemia treatment before coming to this herbal clinic? (Koma munakalandirako chithandizo cha matendawa ku chipatala?)	[ ] Yes [ ] No	
C4	What are the signs and symptoms of anaemia that you know? (Ndizizindikiro zanji zosonyeza kuti munthu ali ndi magazi ochepa nthupi zomwe inu mukuzidziwa?)	[ ] Pale palm creases [ ] Oedema [ ] Yellowish skin [ ] Tiredness [ ] Heart palpitations [ ] Pale gums [ ] Dizziness [ ] Pale nail beds [ ] Headache [ ] Pale eyelid lining. [ ] Body Weakness	
C5	What are the signs and symptoms of anaemia that you are experiencing now? (Ndizizindiro zanji zimene mulinazo pano?)	[ ] Pale palm creases [ ] Oedema [ ] Yellowish skin [ ] Tiredness [ ] Heart palpitations [ ] Pale gums [ ] Dizziness [ ] Pale nail beds [ ] Headache [ ] Pale eyelid lining. [ ] Body Weakness	

INFORMATION ABOUT TRADITIONAL MEDICINE IN USE

(To be filled 7 days after patient has started treatment)

			CODE
D1	What are the names of the herbs that you are using to prepare the anaemia medicine? (Dzina la mankhwala omwe mukumwa)		
D2	How is the medicine prepared? (Kodi mukumakonza bwanji?) - dry or fresh - amount of herbs - amount of water, fanta, phala		
D3	What is the plant part that is used to prepare the medicine? (Ndi gawo liti la mtengo komwe mankhwalawa akuchokera?)	[ ] Leaves [ ] Root bark [ ] Root with bark [ ] tuber [ ] stem with bark [ ] stem bark	
D4	What is the amount that is required to be taken per; (Mumamwa a mbili bwanji pa nthawi?) Per time…….(mls)(check drinking cup) Per Day…………. (frequency)		
D5	For How long is the medicine supposed to be taken? (Mankhwalawa mukuyenera kumwa nthawi yayitali bwanji?) (to capture dose and storage period) - Days - Weeks		

D6	How is the medicine taken in relation to food?(Kodi kamwedwe kake mukutani mogwirizana ndi chakudya?)	[ ] after food [ ] before food [ ] with food [ ] other (specify)	
D7	Are there any precaution measures which you are advised to observe during this time that you are on treatment?		
D8	a. When taking the medicine do you feel anything unusual? (side effects).(Pali zodabwitsa zomwe mukumva kapena kuona zomwe zayamba mutayamba kumwa mankhwalawa?) b. Mention the side effects	[ ] Yes [ ] No	
D9	Do you think the medicine is effective in treating anaemia? (Kodi inu mukuona kuti mankhwalawa akukuthandizani pa matenda anu?) If Yes, after how many days of taking the medicine did you start feeling better? ------------------------------days Describe the feeling--------------------------	[ ] Yes [ ] No	
D10	What other medical problems are you suffering from? (Pali matenda ena amene inu mukudwala panopa?) Are you receiving treatment for the mentioned diseases?	1. ----------------------- 2. ------------------------ [ ] Yes [ ] No	

E. OTHER INFORMATION

			CODE
E1	In the past month, did you ever lose blood through; (Kodi mwezi wathawu mwatayapo magazi kudzera munjira izi?) Accident (date ---/---/2002) Prolonged menstruation (date ---/---/2002) Any bleeding (specify)--------------------------- (date ---/---/2002)	[ ] Yes [ ] No [ ] Yes [ ] No	
E2	The laboratory analysis results. Haemoglobin level …………………mg/dl Malaria parasites ……………………… Urine test …………………………… Stool analysis ……………………		

GENERAL COMMENT---

Appendix II: Weekly Monitoring Sheet

NAME OF CLIENT ……………………………………………………………

VILLAGE…………………CLINIC………………………………………….

DATE	ACTIVITY	Hgb (g/dl)	SIGNS AND SYMPTOMS	COMMENTS
	Enrolment			
	1st Monitoring			
	2nd Monitoring			
	3rd Monitoring			
	4th Monitoring			

KEY OF SIGNS AND SYMPTOMS

A: Body Weakness

B: Pale palm creases

C: Oedema

D: Yellowish skin

E: Tiredness

F: Heart palpitations

G: Pale gums

H: Dizziness

I: Pale nail beds

J: Headache

K: Pale eyelid lining

Appendix III: Consent Form

CHICHEWA:

Ife tachokera ku Chancellor College. Tili pakafukufuku ofuna kuona momwe mankhwala a chikuda (azitsamba) amathandizira odwala matenda ochepa magazi nthupi. Vuto lochepa magazi nthupi limatha kuyamba kapena kupitilila ngati munthu ali ndi njoka za mmimba, likodzo kapena malungo, kuonjezera pa zina.

Choncho pa kafukufuku wathuyu timafunanso tione kuti kodi anthu amene ali ndimagazi ochepawa, magazi awo ndiambili bwanji? Nanga kodi ali ndi njoka za mmimba, likodzo kapena malungo? Izi tingadziwe poyesa mikodzo, chimbudzi ndi magazi a pachala.

Kodi inu mukufuna kutithandiza polowa nawo mukafukufuyu?
Eya ndikufuna kulowa.

Dzina------------------------- Tsiku---------------------

ENGLISH

We are coming from Chancellor College. We are here to conduct research on the effectiveness of traditional herbs on treatment and control of anaemia. The problem of anaemia can start or made worse if a person is infected with malaria parasites, is infested with intestinal worms and schostosomiasis among other things. In this research we also wish to investigate the haemoglobin level, presence of malaria parasites, worm infestation and schistosomiasis among anaemia patients. These will be achieved through analysis of urine, stool and finger prick blood film.

Would you like to participate in this research?

Yes I agree to take part in the research.

Name --------------------------------Date---------------------

Appendix IV: Laboratory analysis procedures for malaria, urine and stool.

List of Materials

The list of materials that were used for stool and urine analysis were as follow, dispersing plastic bottles, disposable plastic pipettes, diethyl ether, formaldehyde, strainer (400um mesh size), plastic screw capped centrifuge tubes, sodium chloride, test tube racks, stool and urine containers, applicator sticks, microscope slides and slides cover slip, microscope, generator, centrifuge, field stain A and B, blood collection lancerts, cotton wool, methylated spirit, drier and pails.

Analysis procedures

Stool analysis (Concentration method)

- 10ml of 10% formal saline was added to each test tube; about 2g of feacal matter was also added and emulsified completely.
- Emulsified material was sieved into another test tube through a 400um mesh and centrifuged at 1500rpm for 10 minutes.
- The supernatant was decanted and normal saline was added to the sediments to 10ml mark and mixed.
- 3 ml of diethyl ether was added and centrifuged at 2000rpm for two minutes.
- The ether layer was broken using an applicator stick and the supernatant decanted.
- Using a plastic pipette a drop of the sediments was transferred onto a slide, covered with cover slip and slide examined under low power objective for ova and results recorded.

Urine analysis

- 10ml of 10% urine was added to each test tube after mixing thoroughly. The urine was centrifuged at 1500rpm for 10 minutes. The supernatant was decanted.
- Using a plastic pipette a drop of the sediments was transferred onto a slide, covered with cover slip and slide examined under low power objective for ova and results recorded.

Appendix V: Summary of information on anaemia herbal medicines form literature and source

Scientific name	Local name	English name	Plant part used	Route.	Mode of preparation.	Dose (Adults)	Source.
Persia amaricana	Peyala	Avocado pear	Leaves	Oral	Cut 10 fresh leaves into small parts, soak in 3 litres of water, and boil until solution is reddish in colour. Remove solution, add sugar to taste. Prepare new everyday.	Drink all the 3litres within a day. Enough for 15 people.	Catholic Nuns (Poor Claras, 2001)
Persia americana	Peyala	Avocado pear	Leaves	Oral	Boil 2 handfuls of leaves in 2 teacups of water for 3 minutes. Allow to cool. Add salt and decant liquid.	Drink liquid, 200ml (1 cup), 3 times a day.	Msanagwe, 1999
Persia americana	Peyala	Avocado pear	Leaves	Oral	Boil a handful of chopped leaves in 4 cups of water for 15-30 minutes. Add sugar to taste.	Drink decoction within a day. Make fresh decoction every day	NACP, 1998.
Persia americana	Peyala	Avocado pear	Fruit	Oral	Take the ripe pulp of the fruit and add sugar or salt to taste	Eat as often as necessary	NACP, 1998.

Moringa ovalifolia or M/oleifera	Chamwamba	Phantom tree	Leaves	Oral	Mix in relish (ndiwo) or cook it as relish or soup.	Eat as relish two times a day.	NACP, 1998.
Citrus limonium	Mandimu	Lemon	Fruit	Oral	Puncture fruit with a nail, leave it for 30minutes. Remove nail and squeeze out the juice from the fruit.	Drink the juice once a week for three weeks.(Citrus acid dissolves the natural iron from the nails)	NACP, 1998.
Vinca rosea	Maluati (periwinkle)	Periwinkle	White/Pink Flowers	Oral	Soak one handful of flowers in a cup of cold water overnight	Drink one cup of the extract daily	NACP, 1998.
Musa sapientum	Nthochi	Banana / plantains	Dried flowers	Oral	Cook as relish with tomato and onions	Eat as often as available.	NACP, 1998.
Droogmansia pteropus *Ximenia caffra*	Nlundelunde Mpinjipinji	 Sourplum	Roots Roots	Oral Oral	Boil the roots and add salt Boil the roots and add salt.	Drink 3 times a day. Drink 3 times a day.	Mwanyambo and Nihero 1998.
Uapaca nitida (Grasses)	Udzu/ Nsechela or	Narrow leafed	Roots	Oral	Boil roots and inhale the vapour. Soak grassroots in water	Drink 4 times a day.	Mwanyambo and Nihero

	masuku	mahobohobo					1998.
Cucumis hirsutus	Nkukunyajila / Bowa, mkuwikuwi		Roots	Oral	Roots pounded into powder to use it in porridge. Mushroom cook and eat	Eat porridge from powder 2 times.	Mwanyambo and Nihero 1998.
Azanza garckeana	Ntowo, matowo	Snot apple	Roots Roots and bark	Oral Oral	Cook the roots and add salt. Remove the roots and mix the cooked liquid with fanta or any soft drink. Soak the roots and bark in water.	Drink as often as required. Drink solution morning and night.	Mwanyambo and Nihero 1998. Carney, 1999.
Bauhinia thonningii	Chitimbe	Camels foot	Stem bark	Oral	Boil the stem bark	Drink 4 times a day.	Mwanyambo and Nihero 1998.
Vitex doniana	Tonongoli or Jumu	Blackplum	Bark	Oral	Grind bark and soak in a pot, add salt and cook.	Drink half cup two times a day (morning and night) for 4 days	Carney, 1999.
Rhynchoma minima	Mukavanti or chirambe		Roots from	Oral	Grind roots and add to porridge.	Half cup. Two times a day, for 3	Carney, 1999.

	chachikulu		small plant			days.	
Commiphora massamicensis	Matondo	Pepper-leaf corkwood	Bark	Oral	Grind barks and dry. Boil in water with salt.	Two and half (2 ½) Tablespoons once a day for 4 to 8 days.	Carney, 1999.

Key: NACP = National AIDS Control Programme, Route = Route of administration.

Appendix VI: Summary of information on herbal medicines used by herbalists to treat anaemia from Blantyre district

ID	Herbalist Location	Herbal medicine used	Scientific Name	Plant part used	Route of administration.	Mode of preparation	Dose (Adults)
TH1	Lirangwi	Senderela	*Toona ciliata*	Stem bark	Oral	Dry the stem bark in open air, pound to fine powder and sieve. Use sieved powder.	Half teaspoon of powder in 100 mls water or in porridge. Morning and Evening
TH2	Somba	Mkulasenga	*Dallbergia nitidula*	Stem bark	Oral	Dry the stem bark in open air, pound to fine powder and sieve. Use sieved powder.	Quarter teaspoon of powder in 100 mls water. Morning, Noon and Evening
TH3	Kachele	Gwawa	*Psidium guajava*	Leaves	Oral	Pound to break to small pieces. 2 handfuls of Dry or fresh leaves, Soak in 300mls, add water daily and change leaves after 4 days.	Take 150mls of solution once, in the morning.
TH4	Lundu	Chizuzu	*Ensente livingstonianum*	Root stem	Oral	Remove stem bark and pound stem to small pieces. Dry in open air, pound to fine powder and sieve. Use the sieved powder.	Quarter teaspoon of powder in 100 mls Cocacola. Morning and Evening.
TH5	Chirimba	Muwanga	*Pericopsis angolensis*	Stem bark	Oral	Dry the stem bark in open air, pound to fine powder and sieve. Use the sieved powder.	Quarter teaspoon of powder in 150 mls water or porridge. Morning and Evening.

TH6	Machinjiri	Chiteta	*Elephantorrhiza goetzei*	Root stem	Oral	Remove root bark; cut the root stem to small pieces, dry, pound to fine powder and sieve. Use sieved powder.	Quarter teaspoon of powder in 100 mls water. Morning, and Evening
TH7	Chileka	Mkulasenga	*Dalbergia nitidula*	Root bark	Oral	Separate stem and bark of roots. Dry the root bark in open air, pound to fine powder and sieve. Use sieved powder.	Half teaspoon of powder in 100 mls water. Morning, Noon and Evening
TH8	Mdeka	Mwanawa mphepo	*Acalypha villicaulis*	Root bark & stem	Oral	Cut the root stem together with bark to small pieces, dry, pound to fine powder and sieve. Use sieved powder.	Quarter teaspoon of powder in 100 mls water. Morning, and Evening
TH9	Ndirande	Chizuzu	*Ensente livingstonianum*	Root stem	Oral	Remove root bark, cut root stem to small pieces and dry in open air. Pound the root stem pieces to fine powder sieve and use the sieved powder.	Half teaspoon of powder in 100 mls fanta or water. Morning, and Evening
TH 10	Chirimba	Peyala	*Persia americana*	Leaves	Oral	Cut fresh leaves in small pieces, 4 handfuls leaves boil for 1 hour in a pot of water.	One cup (250mls), Morning, and Evening.

Appendix VII: Research Approval Letter from Research Committee (College of Medicine)