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Use of Traditional Herbal Medicines to Cure Malaria

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ABSTRACT

The problem of resistance by malaria causing parasites to drugs in current usage, or of malaria vectors to pesticides has prompted nations to refocus attention on implementing research on new drug development and new pesticides development. The world appears to be rediscovering the importance of traditional medicines and people are resorting to use traditional medicines in different names such as Alternative Medicines, or Complementary and Alternative Medicine (CAM). The suffering and economic hardships due to malaria in endemic areas call for extensive research and development of effective and safe antimalaria drugs. Surveys carried out in some malaria endemic areas of Zimbabwe in this study revealed that people are confident in the efficacy of herbal medicines for malaria, for example A. amara, A. digitata, D. nitidula, L. discolour, V. infausta and many others. Samples of Aristolochia petersiana were collected from Chipinge and the other plant samples were collected from Gutu in 2009 and experimental work completed in 2009 at the University of Zimbabwe and the then Blaire Research laboratory. Efficacies of extracts of parts of these plants were invitro assayed against malaria parasites and aliquots were added to solutions of fansidar and invitro assayed to establish their effects on the efficacy of fansidar. Some of the extracts increased the zone of inhibition of fansidar, yet others decreased it. Thus the claim by ethnomedical practitioners that ethnomedicines and western medicines combine to make western medicines more effective should not be taken at face value. Behaviours of these, like any other chemicals, may go either way. They may be antagonistic or potentiate or may be synergistic, or may not have any effect at all. Actual behaviour needs be established by experimentation. However, some plant drugs may compete established drugs for use against malaria.

Key words: *Albizia amara, Adensonia digitata, antagonists, Dalbergia nitidula, Lannea discolour, Vangueria infausta, antagonists, antimalaria drugs, drug resistance, potentiate, synergistic, traditional medicines*

INTRODUCTION

Malaria has proved to be one of the most debilitating illnesses of all time and is the most common parasitic disease in sub-Saharan Africa [1,2]. Malaria is the single most important cause of ill health, death and poverty in Sub-Saharan Africa [3,4]. The disease is a major obstruction to social and economic development in Africa, causing enormous misery and suffering through the pain of fevers and the anguish of bereavement, with one African child dying every 40 seconds [4]. In 2004 the World Health Organization (WHO) estimated that 300 million acute cases of malaria occur worldwide each year, resulting in one million deaths. Ninety per cent of these deaths occur in Sub-Saharan Africa, most of victims being children aged less than five years. Aproximately 40% of the world's population, mostly in the world's poorest countries, is at risk of contracting malaria [5].

Malaria is a particular burden to pregnant women and children below 5 years of age. Pregnant women in areas of unstable malaria and non-immune pregnant mothers originating from non-endemic countries are susceptible to manifestations of severe malaria like anybody else. But they suffer more than those indigenous to the malaria endemic areas. Malaria makes them have an increased risk of abortion, stillbirth, premature delivery and low birth weight of their infants. This group of women have a malaria mortality rate which is 2-10 times higher than in non-pregnant women. Indigenous children below the age of 5 years, inhabiting highly endemic areas are highly susceptible to all the manifestations of *P. falciparum* malaria. Those who survive this critical period achieve a relative tolerance of the infection and become semi-immune to malaria [6].

Malaria accounts for 40% of public health expenditure. The disease results in loss of life, loss of productivity due to illness and premature deaths, and hinders children in their schooling and social development through absence from school and permanent neurological or other damage associated with severe episodes of malaria. Malaria, a major cause of infant mortality, is the only insect borne parasitic disease comparable in impact to the world's major transmittable diseases such as HIV/AIDS

and tuberculosis. In endemic areas, malaria is the most frequent cause of attendance at health facilities accounting for up to 40% of out-patient attendances, 20% of in-patient admissions and up to 14% of in-patient deaths. In children, besides leading to illness and death, it also has long term consequences such as low birth weight, chronic anaemia, retarded growth, and in some cases severe mental retardation. Even non-severe malaria has been found to compromise school performance of children, potentially contributing to the maintenance of underdevelopment in countries endemic to malaria [7]. In pregnancy, malaria may cause maternal anaemia, premature births, low-weight babies and still births. As many as 60% of miscarriages may be attributed to malaria and the disease also contributes to poverty due to loss of considerable productive time due to personal sickness or looking after sick relatives. Coupled with that, the cost of treatment or preventing of mosquito bites through use of mosquito repellents or mosquitocides or mosquito nets is high for the inhabitants of malaria endemic areas all over sub-Saharan Africa.

Four major problems are associated with the management of malaria. The most important one is that the parasites which cause malaria are resistant to or are developing resistance to the most widely available, affordable and safest first line treatments such as chloroquine and fansidar. In addition, the overall control of the malaria vectors, the mosquitoes, is made difficult by their resistance to a wide range of insecticides. Further to that, a new and rapidly developing problem is the widespread production of fake antimalarial drugs. Many of the countries in the malaria endemic areas of the world lack the necessary infrastructure and resources to manage and control malaria as well as to ward off fake curing agents [4,8].

Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs. Medicinal plants contain active principles which are responsible for their medicinal properties [9] and herbal preparations account for 30-50 % of the total medicine consumption. Gelfand [10] described how British explorers were cured of malaria by rural people in southern Africa using traditional medicines long before colonization.

The common malaria causing parasite in Zimbabwe, Plasmodium falciparum, has developed resistance to chloroquine, fansidar, and quinine, particularly when the drugs are used in monotherapy [11]. These drugs are the mainstay of malaria treatment in Africa and are imported, causing serious strains on the national fiscus [10]. Combination therapy has in some cases proved to be useful and the strains on the fiscus might be alleviated if home produced drugs are used in combination with the established drugs [12].

Research Questions

1. What are the views of people who live in malaria endemic areas say about herbal medicines?
2. What can be done to prove the efficacy of herbal medicines?
3. Are herbal medicines compatible with western medicines?

METHODOLOGY

Elderly people in malaria endemic areas of upper and middle Save catchment (Makoni southwest and Gutu south east) were interviewed between 2005 and 2009 with a view to establish their knowledge and views about values of traditional medicines used to treat malaria. The interviews were designed such that respondents would freely discuss and volunteer information about traditional medicines. The researcher aimed at collecting information about herbs used for malaria treatment. The respondents were people aged 50 years and above and traditional healers of all ages.

Views of Respondents

People talked about the widespread suffering and death caused by malaria, the failure of the safest and most affordable currently available antimalarial drugs to treat the disease because of drug resistance by the parasites or pesticides' resistance by the mosquitoes themselves. They felt that there was an urgent need to develop new drugs or vaccines for the treatment, management, prevention and control of malaria or mosquitocides to destroy the mosquitoes or repellents to ward off the vectors. Their views agreed with literature which indicated that the world appears to be re-discovering the importance of traditional medicine and people are resorting to use of traditional medicine in different names, such as Alternative Medicine or Complementary and Alternative Medicine (CAM). The number of medical doctors who had undergone special training in natural remedy in German almost doubled to

10800 between 1995 and 2000 [13].Three quarters of the people living with HIV/AIDS in San Fransisco, London, and South America use CAM. Nine out of ten people in the German population has used CAM at some point in their lives. Certain countries such as Sri Lanka, India, China, Bhutan, Nepal, DPRK, Mali and Vietnam have successfully integrated traditional medicine with modern medicine in their official healthcare, with the encouragement of WHO, because the western system of medicine can no longer cope with current morbidity and mortality rates [12].

Large percentages of western populations now use some form of alternative medicine (57 % in Australia, 46 % in Germany, and 49 % in France. Between 1991 and 1997 the use of herbal medicines in the United States grew by 380 %. People are turning to alternative medicine because it is safe and it works. Over 80 per cent of the world population use alternative medicine as the basis of the healthcare system [14].

Traditional healers lamented activities by phytochemists, pharmacists, and ethnobotanists from all over the world who use the knowledge of traditional healers to be able to select and test new medicinal plants and never return to say thank you to suppliers of information. They boasted about the efficacies of their medicines and in agreement with literature too. The efficacies of herbal medicines in the treatment of malaria have been recognized for a long time [11]. The Portuguese discovered that slaves in south America were using the bark of the stem of Cinchona plant to cure malaria and fever and quinine was subsequently commercially obtained from the bark and root of the plant [11] and many other drugs have since been commercially obtained from plants and it may be wise to assume that other plants not previously evaluated may yield chemicals of medicinal value. The strain on the fiscus would be alleviated if home produced drugs are used in combination with the established drugs [15].

According to specialists at a conference in Nairobi on 4 November 2009, Africa should be encouraged to use traditional herbal medicines to prevent some of the one million deaths that occur on the continent annually because of malaria. Many poor people die due to inaccessibility of treatment, particularly in the rural areas where people are so poor that they cannot afford modern malarial drugs. Thus, traditional medicines could be an important and sustainable source of treatment.

Africa could draw on experiences in India where medicinal plants have been successfully used in the control of malaria-related deaths, leading to a reduction of 5-10 times among people who use certain types of traditional medicinal plants, compared to those who do not

A study carried out in Uganda in 2006 revealed that many people preferred allopathic medicine to traditional medicine, the most common reason being ignorance of the traditional knowledge necessary to exploit plants for the treatment of malaria. A second reason was the belief that allopathic medicines were superior to herbal medicines in the treatment of malaria and that they were easily accessible and is administered in precise doses. There is also the problem of conflicts with religious beliefs. Some religious organizations preach against use of herbal medicine by their members.

Traditional healers and the elders concurred that community members prefer herbal medicines to allopathic medicine because herbal medicines are free and readily available and they believe that they are also more effective than allopathic medicines. Some do not even consult Traditional Medicine Practitioners for the treatment of malaria because they know how to prepare the necessary herbal medicines themselves and they share information on malaria treatment among themselves. This, they claim, is necessary and handy because on average a person may suffer from malaria 6 times per year. Traditional medicines are administered as water extracts in variable doses and over varied time periods.

Traditional healers complained about some religious leaders influenced the community members against herbal medicines. The survey revealed that women were more knowledgeable about herbal medicines than men. Women tended to know medicinal plants in general and plants used to treat malaria in particular, probably because women have to look for medicines to treat children as they spend more time with children than do men.

The plants they used for malaria treatment are shrubs or trees, leaves being the commonly used parts. The plant parts for use are collected as and when they are needed, and they may be collected any time with no rituals necessarily performed during the process of collecting or processing. Twigs are preferentially collected because of their ease of processing. Only a handful of the young leaves are collected, used, and the remainder discarded. Roots or bark are dug up or cut off the stem, usually respectfully, and at times accompanied with prayer. Prayers were commonly used by healers who were

spirit mediums, in agreement with reports by Kazembe [16] who reported that spirit mediums believed that massive influenced efficacies of herbal medicines.

Herbal medicines are usually prepared mainly as water extracts based on single plant species for oral intake. Some are prepared as decoctions. Some preparations consist of mixtures processed from different plants. The appropriate plant parts are cleaned of debris. The extracts are processed from leaves which are crushed and squeezed in a small amount of water until they form froth. Large leaf particles are removed and water added to the solution to make a volume of about 500 ml. At times a mixture of an appropriate amount of leaves, sometimes of bark or roots, from a variety of plant species are boiled in water and used as steam baths or drunk.

Doses are variable and are determined according to the age of the patient, varying from 1 to 3 tablespoons for children younger than 5 years to a cupful for adults, taken 1 to 3 times a day for a period of 1 to 3 days or until the patient's condition has improved. Prepared medicines are normally never kept and all that remains after use is discarded. There is no need to keep any because the plants from which they are produced are readily available.

Community Knowledge about Malaria

Thus, communities usually have good knowledge about malaria and can readily distinguish malaria from other types of fever on the basis of signs and symptoms. Villagers are aware of the malaria symptoms: fever, headache, vomiting and flu-like symptoms. They know that malaria is caused by mosquitoes, and that mosquitoes thrive in bushy surroundings and where there is stagnant water.

Herbal Medicines used to treat Malaria

The immense suffering and economic costs observed and described for most of the malaria endemic areas, especially in sub-Saharan Africa, calls for extensive research and development of effective and safe antimalarial drugs. The worsening antimalarial resistance coupled with the difficulties for households to access and afford effective antimalarial drugs, renders development and promotion of phytomedicines the most probable sustainable solution to malaria treatment. Besides, herbal medicines are widely believed to be safe and efficacious. Most drugs used in allopathic medicine have been derived from higher plants using leads from traditional knowledge. Examples of some of the most successful antimalarial agents developed from plants relying on traditional knowledge leads include quinolines and artemisinin derivatives.

The surveys carried revealed that rural people in these malaria endemic areas of Zimbabwe are confident in the efficacy of herbal medicines for malaria such as *Adansonia digitata, Albizia amara , Dalbergia nitidula, Lannea discolor, Vangueria infausta* and many others, as sources of antimalarial agents. These reports influenced the decision on studies to establish the effects of extracts of the plants on the efficacy of the antimalarial drug, fansidar. Similar studies have been published [12].

Promising herbal medicines should be subjected to phytochemical analysis and identification of active ingredients and clinical trials conducted to confirm their efficacy and safety, and also determine recommended doses in line with World Health Organization Guidelines [17].

Validation may help to identify safe and efficacious antimalarial, and may also increase confidence in the use of herbal medicines among users leading to wider acceptance. The validation of the efficacy and safety of herbal medicines can also help create a herbal medicine market, with possibilities of adding value to medicinal plants to the benefit of conservation of biodiversity. Some of the reasons why traditional knowledge of herbal medicines is considered reliable is that indigenous communities, through long periods of experimentation with herbal medicines, are likely to have discarded preparations with low efficacy or acute toxicity, and retained those that are effective and tolerably safe

Preservation of traditional knowledge

Many people fear that traditional medicinal knowledge might be lost with the death of present owners of the knowledge, that is, traditional medical practitioners. The situation in Zimbabwe is rather tricky. Most of the traditional medical practitioners are spirit mediums. The spirit mediums argue that knowledge of herbal medicines is not a property of the mediums, but of the spirits. Some of the traditional healers argue that the death of a medium will not necessarily mean the disappearance of the knowledge. The spirit will bestow the knowledge on the successor medium. The knowledge that needs preserving is that which is in the hands of non-spirit medium practitioners. Unlike the mediums, these learn both the medicinal plants information and the art of curing. Such practitioners are, however, few compared to spirit mediums and they usually keep records. Traditional healers are more concerned

with the fast disappearance of some medicinal plants. The muringa plant is a case in point. This plant used to be abundant in the forests, especially in the Chipinge area. One hardly finds it at present and the few plants that have survived are dying because people remove the bark around the trunk.

Justification for this type of research

Low lying areas of Zimbabwe (Zambezi, Limpopo and Save valleys) are endemic for Plasmodium falciparum malaria which accounts for the greater majority of all malaria cases seen at health centres in southern Africa [18]. The National Malaria Control Programme of Zimbabwe was put in place in 1947 to help keep the transmission of malaria under control through multi-disciplinary approaches which include chemotherapy and vector control, but malaria infections continue to cause serious health problems. Chemotherapy involving use of single drugs in prophylaxis or therapy, or combination therapy in which drug combinations are used and vector control involving measures aimed at eradication of the anopheles mosquito which transmits the disease causing parasites (Plasmodium malaria parasites) have been employed but the problem rages on. DDT has been the insecticide of choice for decades but it is now being discouraged because of its environmental effects. Personal control measures which include use of mosquito repellents and mosquito nets to deter mosquitoes are being actively encouraged [18]. WHO has recommended combination therapy, with dose monitoring therapy regimes, to ascertain the interactions of the drugs and their resultant therapeutic potency as well as evaluation of side effects. The United Nations concentration on Africa is justified by the fact that of the 300-500 million cases recorded annually worldwide, 80% occur in Africa, leading to serious illness and death in sub-Saharan Africa [19]. The progress being done in stamping out the disease is being hampered by the disease developing resistance to every treatment.

Governments in the Southern Africa Development Community region have responded to the parasite resistance by changing their antimalarial policies. Malawi adopted a new drug policy in 1993, which directly contributed to a 20% reduction in deaths of children by 1995 and Zambia modified its policies in 1996 and achieved a 15% reduction in children's deaths due to malaria by 1998 [20]. Kenya adopted fansidar as the first line malaria treatment drug in 1997. Initially the drug was effective but started showing resistance [21]. Uganda adopted artemisnin-based combination treatment to replace chloroquine and fansidar in 2006, and Zimbabwe has continuously adjusted its malaria policy in order to cope with the ever increasing resistance of the parasite towards drugs in use.

Regional standards for monitoring drug resistance were set up in collaboration with WHO [20] as a critical step for future efforts to evaluate the continued efficacy of existing drug treatment policies and combination therapy that includes artemisnin as the standard treatment in countries where malaria is resistant to older individual drugs was recommended. The low availability of artemisnin in Africa due to low production levels and comparatively high cost, dosing complexity and lack of clinical experience with artemisnin-based combinations renders it prudent for Africa to develop its own home grown alternative combination therapies.

Quinine has been the accepted remedy for malaria and the recognized prophylaxis, but the parasite has developed resistance to it [2]. Development of malarial vaccines in response to emergent resistant *P. falciparum* is in progress. Clinical trials of the vaccine SPF 66 was considered promising though not impressive. With the economic problems associated with artemisnin from China, Southern Africa had its hope on *Artemisia afra* Jacq (African wormwood) commonly used by traditional healers for the treatment of fevers and for malaria. Extracts from this plant were able to kill the parasite but did not contain artemisnin [22]. Thus, from other plants, other compounds can still be found to deal with the problem of malaria. Fansidar, a sulfadoxine-pyrimethane mixture, has been used to treat malaria in Africa for some time because of its effectiveness against P. falciparum [23]. It is readily available due to its low cost and has thus been used extensively despite its having some side effects, resulting in the parasite developing resistance. Recently it has been used in combination with other drugs in combination therapy to improve on its efficacy as in:

(a) fansider-artesunate combination, an effective drug at clearing malaria parasites from blood at a particularly infectious stage of parasite development [9] and

(b) fansidar-amodiaquine combination, a useful stop-gap treatment in areas without access to artemisnin drugs and areas where malaria resistance to fansisdar and amodiaquine are still low.

Combination malaria therapies have also been tried in South East Asia, where malaria is also a major public health problem [20]. Collaborative researches between academics and traditional healers have

resulted in the identification of herbal remedies for different ailments including malaria, broadening the horizons of knowledge in the area of Natural Products Chemistry as well as benefiting people immensely by lowering the cost of treatment of ailments [21]. Bioassay-guided fractionation of crude plant extracts leading to rapid isolation of the active constituents will be used in the search for new herbal antimalarial remedies. Almost 120 plant species were screened for antimalarial activity in the decade 1985-1995 leading to the identification of the root bark of the *Uvaria* species as containing the most active compounds against malaria [9].

Researchers on the efficacy of herbal medicines have not reported serious adverse effects. Some effects on biochemical variables (most commonly liver function tests) have been reported, but no cases of toxicity have been reported. Minor side effects which have been reported include diarrhoea and bitter taste. Some herbal antimalarials can be so bitter as to deter children from taking them. The other complaint is about doses which often need to be taken repeatedly, and the volume may be large and inconvenient.

Some of the information about the plants used for malaria cure and assayed in this study.

Albizia amara

Subspecies: Sericocephala; Family: Fabaceae – Mimosodeae. Common name: Bitter albizia (English); Muchangiza (Shona); Mugarahanga (Shona); Mugunduzi (Shona); Muora (Shona); Umbola (Ndebele).

Photographed in Sundar Nursary, Dehli

Botanical name: *Albizia amara subsp. amara* **Family:** *Mimosaceae* (Touch-me-not family) **Synonyms:** Mimosa amara. By *Reinhard Fichtl*

Functional uses of *A. amara* include deriving tannin or dyestuff from the bark. However, it is alleged that the seeds are poisonous. In traditional medicine the roots are chewed and applied to an eye infection of cattle. Fruits are also used as an emetic and for treating coughs and malaria. Saponins are extracted from the roots and leaves. Tannins and gums are extracted from the bark and the gum is used against ulcers; fruits are said to cure malaria and coughs. *A. amara* is attractive and can be planted in urban areas as an ornamental and avenue tree. It could also be more widely grown as boundary marker. Many streets of Harare are lined with *A. amara* plants.

Dalbergia nitidula

D. nitidula, a leguminous plant, is also known as glossy flat-bean or purplewood dalbergia English), Mudima or Murima (Shona).

Photo: Bart Wursten

Uses

In Tanzania chopped and cooked young leaves are used as a vegetable mixed with pounded groundnut. Leaves, roots and bark are used in traditional medicine in East and southern Africa. Chewed leaves are

applied to snakebites and leaves are rubbed on abscesses. Warm water in which pounded roots have been soaked is gargled to treat toothache; root decoctions and infusions are taken for treatment of malaria and cough. The roots are pounded and administered in soup and used to treat epilepsy or to be used as an emetic. In Zimbabwe the roots are taken as an aphrodisiac and the bark is used to treat different types of wounds and ulcers. Caution is needed because the roots are highly toxic. In Tanzania the Haya people in Bugabo use more than one plant species in the preparation of herbal remedies for the treatment of different ailments. For example to treat malaria, one of the herbal remedies involves mixing the roots of *Vernonia amygdalina* with the stem bark of *Sapium ellipticum* and the leaves of *Dalbergia nitidula*, *Desmodium salicifolium* and *Eriosema psoraleoides* then boiling them together and drinking the decoction. The use of more than one plant in preparing herbal preparations is normally attributed to the synergistic effect that extracts from the different plants are thought to have during treatment. Extracts of *Dalbergia nitidula* have been shown to have an (LC_{50} 0.87 µg/ml) on the Brine shrimp, indicating the possibility that the plant extracts may be toxic or contain useful cytotoxic compounds not reported by traditional healers.

The foliage is commonly browsed by livestock.

Properties

The heartwood is purplish brown. The density of the wood is about 780 kg/m^3 at 0% moisture content. The wood is very durable and resistant to termite attack. The roots are toxic. Several isoflavonoids, isoflavonoid-neoflavonoid dimers, pterocarpan-neoflavonoid oligomers and rotenoid glycosides have been isolated from the bark and heartwood.

Isoflavonoids form a large and very distinctive subclass of the flavonoid family with a wide variety of structural variations encountered in nature, although their distribution among plants is relatively sparse. Continued interest in the isolation and characterization of new isoflavonoids arises due to the diverse range of biological activities including antimicrobial, oestrogenic and insecticidal activities which they possess. The pharmacological properties of isoflavonoids have been reported, especially papers detailing the relationship between consumption of isoflavonoids and reduction in the incidence of cardiovascular disease, cancer, and their implication in contraception both in humans and livestock.

Dalbergia is a large pantropical genus comprising about 250 species. Tropical Asia and tropical America have about 70 species each, continental Africa has about 50 and Madagascar slightly over 40.

Prospects

Trees of *Dalbergia nitidula* are too small sized to be of economic importance for their timber, but locally the wood is highly valued for small items because of its durability. Several interesting medicinal applications are known for *Dalbergia nitidula*, but little pharmacological research has been done and more research is warranted, especially concerning external applications on wounds, ulcers and abscesses. The safe use of the leaves as a vegetable and forage should be confirmed by phytochemical investigations and toxicity tests.

Vangueria infausta

Synonym: *Vangueria tomentosa*

Vernacula names: Munjiro or Munzviro or Munzvirwa (Shona); Umthofu or Umviyo (Ndebele); Velvet wild medlar (English). The generic name *Vangueria* was derived from the Madagascan name for *Vangueria edulis*: *voa vanguer*. The word *infausta* (Latin) means bad luck, referring to the magical properties it is believed to have.

Vangueria infausta is a popular wild fruit. In South Africa the tree is believed to possess evil powers. Even the wood should not be used for making fire. It is believed that it could cause cattle to bear only male offspring. Despite this, the plant is used extensively.

Uses

Different parts of this plant have been used traditionally for treatment of malaria, wounds, menstrual and uterine problems, and genital swellings among others. Recent pharmacological reports have shown that extracts from leaves and roots of this plant exhibited significant antiplasmodial activity. For example, an infusion of the roots and leaves is used to treat malaria, chest ailments like pneumonia, as a

Photo: Bart Wursten

purgative and to treat ringworms, and an infusion of the leaves is used for the relief of toothache. A decoction of the pounded leaves and small twigs is used for the treatment of swelling of the limbs by bathing the affected parts, especially in children.

Nundkumar and Ojewole [24] reported the leaf extracts of *Vangueria infausta* revealed antimalarial activity with IC_{50} values of 10-20mcg/ml and de Boer et al [25] reported on the activity against *Candida albicans*, *Aspergillus fumigatus*, *Fusarium culmorum*, *Staphylococcus aureus*, *Pseudomonas syringae*, and *Erwinia amylovora*. Phytochemical analysis revealed the presence of anthraquinones, flavonoids, seccoirridoids and terpenoids.

Vangueria infausta is usually administered as decoction or an infusion in water or as a suspension of the finely ground powder in water and fed to cattle suffering from East Coast Fever, and to people to cure various infections. *Vangueria infausta* has anthelmintic action and antiplasmodial activity. The plant is used against malaria, chickenpox, parasitic worm infections, peptic ulcers and stomachache, prevention of contagious diseases, and to cure cattle suffering from East Coast Fever [24].

Adansonia digitata.

Family: Malvaceae

Common names: baobab, Cream of Tartar tree, monkey-bread tree, lemonade tree (Eng.); kremetartboom(Afr.); isimuku, umShimulu, isiMuhu (Zulu); *ximuwu* (Tsonga (Tswana); muvhuyu (Venda), muwuyu (Shona). The name *Adansonia* was given to this tree to commemorate the French surgeon *Michel Adanson* (1727-1806); *digitata* meaning hand-like, referring to the shape of the leaves. The African baobab tree is one of the plant kingdom's strangest (if not most grotesque) wonders. The baobab tree is regarded as the largest succulent plant in the world. It is steeped in a wealth of mystique, legend and superstition wherever it occurs in Africa, a tree that can provide, food, water, shelter and relief from sickness.

The various parts of the baobab are used to treat a large number of ailments, nearly every part of the tree having some medicinal value. For example, powered bark is mixed with porridge to treat malaria; the pulp of the fruit is mixed with honey and used for coughing; the leaves are used for diarrhoea, fever, inflammation, kidney and bladder diseases, blood clearing, and asthma; the leaves also serve as emollients and are used to help extract guinea worm; the fruits and seeds are used for dysentery, fever,

haemoptysis and diarrhoea; dry powered roots are prepared as a mash for malaria; and gum from the bark is used for cleaning sores (Westman Draft).

Baobab flower

Baobab fruit

Nigerians drink aqueous extracts of the bark of *A. digitata* as a treatment for sickle cell anaemia. In a study to check the efficacy of this preparation, the aqueous and methanolic extracts of the bark, as well as their ether fractions, were incubated with 2% sodium metabisulphite sickled washed HbSS blood samples. The results showed that the extracts possess reversal antisickling properties. However, no inhibitory antisickling activity was observed for any of the extracts when they were incubated with the HbSS blood samples for 6 h prior to deoxygenation by sodium metabisulphite. The authors concluded that the low level of reversal activity compared to p-hydroxybenzoic acid and the absence of inhibitory activity in vitro do not justify the use of *A. digitata* for the prevention of sickling crises [26].

Adansonia digitata extracts showed anti-fungal activity and was found to inhibit the classical pathway of complement suggesting that an immunological basis for its in vivo activity was identified. This study has confirmed some of the ethnobotanical reports of Hawaiian medicinal plants having curative properties against infections using biological assays *in vitro* [27].

Lannea discolour

Live-long (E); dikbas (A); Baumtraube (G); musamba (L); musinga (Rk); mushama (T); Chizhenje (S); Mugan'acha (S); Muhumbukumbu (S); Mumbumbu (S); Mupuri (S); Mushamba (S); Tree grape (E); Morula-mopsane.

An infusion of the bark and roots of *Lannea discolor* is used for treating fevers and constipation in children. The fruit and various parts of the plant are used in traditional medicine. For example an infusion of the bark and roots is used for treating fevers, malaria and constipation [28].

Lannea edulis has been prescribed extensively in the traditional medical practice of Zimbabwe and other parts of Africa. Mutagenicity testing using *Salmonella typhimurium* strain TA97a indicated that the Aqueous extracts displayed marginal mutagenicity but the extract of *Lannea discolor* was nonmutagenic [29].

Aristolochia species (Aristolochiaceae) are widely used in many regions of the world. Seven species – *Aristolochia indica* L. (Asia), *Aristolochia serpentaria* L. (North America), *Aristolochia debilis* Sieb & Zucch. (China), *Aristolochia acuminata* Lam (India), *Aristolochia trilobata* L. (Central/South America, Caribbean), *Aristolochia clematitis* L. (Europe) and *Aristolochia bracteolata* Lam. (Africa) – are reported widely as being used medicinally. The medical uses vary, but of particular interest are uses in case of gastrointestinal problems, which is likely to result in repeated exposure to the botanical drugs by an individual. In China and Europe species of *Aristolochia* have been associated with nephropathy and the causative agent is believed to be *Aristolochic acids.* Use of species of *Aristolochia* in traditional medicine is reported to be common in India and Central America. Anecdotal evidence highlights the widespread use of Aristolochia species (Aristolochiaceae) in many regions of the world. Seven species – Aristolochia indica L. (Asia), Aristolochia serpentaria L. (North America), Aristolochia debilis Sieb & Zucch. (China), *Aristolochia acuminata* Lam (India), *Aristolochia trilobata* L. (Central/South America, Caribbean), Aristolochia clematitis L. (Europe) and *Aristolochia bracteolata* Lam. (Africa) – are reported widely as being used medicinally.

Effects of plants extracts on the efficacy of fansidar

Fansidar is an antimalarial drug which has been used in southern Africa for some time. It contains two medicines: sulfadoxine and premethamine, a combination which may be used to treat malaria by killing malaria parasites in the red blood cells. Fansidar is taken by mouth, with food or milk and plenty of fluids. The drug should be taken for the length of time prescribed to avoid inadequate treatment and reinfection. Fansidar may also be used for prevention of malaria, but it is important to know that prophylaxis does not necessarily mean that one will not contract the disease. Preventive steps such as use of protective clothing, insect repellents, and mosquito netting around the bed to further prevent mosquito bites that could cause malaria should be taken.

Fansidar may cause serious, even fatal, side effects such as severe peeling skin, blood disorders or liver damage. The patient should stop using fansidar and notify the doctor as soon as complications are noticed. Fansidar is also used to treat other conditions, including prevention of pneumonia in AIDS sufferers. It also treats toxoplasmosis, a parasite-caused disease. The drug should be taken after a meal, with plenty of water or other fluid. The whole tablet should be swallowed and not chewed or broken. Plenty of water should be taken to prevent kidney stones whilst taking the medication.

Premethamine is an antiparasitic drug which prevents growth and reproduction of parasites. Sulfadoxine is a sulfa drug that fights bacteria in the body. Hence the combination of premethamine and sulfadoxine is used to treat malaria, a disease caused by parasites. Parasites that cause malaria typically enter the body through the bite of a mosquito. The premethamine-sulfadoxine combination is given when other antimalarial medications may not be as effective in treatment or prevention [30].

The development of resistance to this drug and other complications demand that steps be taken to improve the efficacy of this drug for example by adding small amounts of other compounds which might act synergistically with the active components of the drug. One way to do that is trying the effect of added extracts of plant antimalarials.

MATERIALS AND METHODS

(i) *Sample collection*

Samples of the bark of *Aristolochia petersiana*, Malundu in vernacular, and a small intact branch were collected from Chipinge in 2005, following the advice of traditional healers. Samples of roots and shoots of *Vangueria infausta*, *Adansonia digitata, Lannea discolor* and samples of fruits of *Albizia amara* were collected from around Mazambara Secondary School in Zimuto, in January 2007, following the advice of villagers. Samples of the bark and shoots of *Dalbergia nitudula* were collected from Centenary District, Mashonaland Central Province, Zimbabwe in December 2007. All samples were identified at the Botanical Gardens, Ministry of Agriculture, Harare, Zimbabwe, in January 2007.

(ii) *Sample processing*

The roots of *Vangueria infausta, Adansonia digitata, Lannea discolour* and the barks of *Dalbergia nitudula* and *Aristolochia petersiana* were chopped to small pieces, dried at room temperature in the shade and ground to powder using a mill in the Chemistry Department, at the University of Zimbabwe. The fruits of *Albizia amara* were opened up to release the seeds. The seeds were dried at room temperature in the shade and ground to powder also using the mill. The powders from the mill were exhaustively extracted with ethyl acetate at room temperature, then with methanol. The extracts were decanted and filtered under gravity and evaporated to dryness using rotary evaporator, repeating the extraction two more times, and finally combining the extracts. Samples of the ethyl acetate and the methanol extracts were dissolved in water and used in the anti-malarial evaluations. Ascertaining the effectiveness of combination therapy has been done by use of antimicrobial susceptibility tests [Cheesebrough and Monica, 2006] aimed at determining the inhibition of growth of a microbial culture by drugs, and was used to determine the effectiveness of fansidar in various combinations with plant extracts.

(iii) *Standardization of test drugs*

Fansidar (100 mg) was dissolved in distilled water (100 ml). The resultant concentration was then halved, and the volume made up with distilled water to produce weaker dilutions of the original concentrations. For esample, from 1mg/ml, a solution of 0.5mg/ml was prepared, then 0.025, then 0.0125mg/ml. A similar procedure was used to produce the dilutions of the methanolic extracts of *Vangueria infausta* (0.026 g in 26 ml of distilled water), *Adansonia digitata* (0.029 g in 29 ml of distilled

water), *Dalbergia nitudula* (0.027 g in 27 ml of distilled water), *Lannea discolor* (0.036 g in 36 ml of distilled water), and *Albizia amara* (0.022 g in 22 ml of distilled water). The ethyl acetate extracts of *Vangueria infausta* (0.029 g), *Adansonia digitata* (0.025 g), *Aristolochia petersiana* (0.028g), *Dalbergia nitudula* (0.029 g), *Lannea discolor* (0.026 g) and *Albizia amara* (0.018 g) were each dissolved in a minimum of ethanol to assist dissolution in water and then dissolved in 18 ml of distilled water. Dilutions of the ethyl acetate extracts were then made similarly to the methanol extracts, above. Fansidar and the plant extracts were subjected to standardization procedures to ascertain the efficacy of each of them on the growth of the malaria parasites.

Six disc tests were performed on the protozoal culture for each of the test drugs according to Bauer-Kirby test system in which a culture is inoculated onto the surface of Mueller-Hinton agar, followed by addition of drug impregnated discs to the agar, establishing a concentration gradient. Inhibition of protozoa growth is indicated by a clear zone around the drug discs (zone of inhibition). The diameter of the zone of inhibition reflects the solubility properties of the particular drug and the sensitivity of the microorganism to the specific drug. Paper discs impregnated with the test drug were placed onto the medium which was uniformly inoculated with the test organism. The zones of inhibition were recorded and averages calculated as follows:

Mean inhibition diameter = Sum of all inhibition diameters/ Number of discs used

The ability of the drug to inhibit protozoa growth was estimated using the average diameter of the zone of inhibition. In each experiment involving the combination of fansidar and the test extract, the volume of the mixture was kept constant at 50ml to ensure that the concentration of the plant extract was the one that was varied. Fansidar was initially in a large excess, hence it would still be present in reasonable amounts to cause inhibition within the standardized range. The parameter of interest will be the amount of extract added and the inhibition it causes. The efficiency of the drugs and drug combinations was estimated from inhibition of growth of protozoa. The greater the zone of inhibition will be assumed to mean the greater the therapeutic potency.

Fansidar and each plant extract were subjected to standardization procedures to ascertain the efficacy of each of them on the growth of the malaria parasites. Then fansidar and the different plant extracts were then mixed in different proportions and the standardization procedure repeated to establish the effect of the mixture on the inhibition of growth.

Table 1. Standardization of Fancidar

Diameter of zone of inhibition (mm)

No. Disc Number

	Fansidar (mg/ml)	1	2	3	4	5	6	average
1	3.13	9	11	8	10	9	7	9
2	6.25	10	8	14	12	13	15	12
3	12.50	16	13	14	13	18	16	15
4	25.00	17	14	14	13	16	16	15

Table 2. Effects of EtOAc extract of *Aristolochia petersiana* on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	A petersiana (mg/ml)	1	2	3	4	5	6	average
1	3.57	9	10	10	11	11	9	10
2	7.13	12	13	12	12	10	13	12
3	14.25	13	14	13	13	13	14	13
4	28.50	14	13	13	13	14	13	13

Table 3. Effects of MeOH extract of *Aristolochia petersiana* on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	A petersiana (mg/ml)	1	2	3	4	5	6	average
6	3.44	13	14	13	13	14	13	13
7	6.875	14	15	14	15	15	15	15
8	13.75	16	17	16	16	16	17	16
9	27.50	17	16	16	17	16	16	16

The standardization procedure did not take into consideration the resistance of the parasite to either fansidar or *Aristolochia petersiana*. If the average zone of inhibition increased in the presence of the A.

petersiana extract, then it was taken to mean that the two drugs complemented each other in inhibiting growth and the relationship was assumed to be synergistic.

Tests were carried out with extracts of *Adansonia digitata, Albizia amara, Aristolochia petersiana, Dalbegia nitudula, Lanea discolour and Vangueria infausta.*

Six disc tests for each sample were performed per protozoal culture. Six paper discs impregnated with the test drug were put onto each inoculated medium and the degree of inhibition measured as the diameter of the zone of inhibition.

Average inhibition diameter = sum of all zones of inhibition (mm)/ number of discs used

Effects of combinations of fansidar and extracts on the growth of the malaria parasites

The combined action of the drugs with fansidar was investigated using an initial volume of 50 ml of fansidar. This volume was progressively reduced by one ml at a time to 45 ml while adding the herbal extract to maintain the total volume at 50 ml. The fansidar and the plant extracts mixtures were then standardized as above to establish the effect of the extracts on the inhibition of protozoa growth by fansidar, thus investigating the synergistic or antagonistic effects of drugs by applying mixtures of serial dilutions of fansidar and each of the individual extracts so that their zones of inhibition overlapped. A blank sample of ethanol was used as a negative control, while fansidar was used as a positive control. Results were read after 72 hours incubation at 36^{o}C, to ensure that the growth of the cultures of the protozoa were comparable in all the incubated cultures. The test compounds diffused together during incubation, and the extent of the inhibition areas within the area of overlap indicated whether stimulation or antagonism had taken place. An increase in the zone of inhibition indicated potentiation or synergism, while a decrease in the zone of inhibition diameter indicated antagonism. Some assays were rejected in cases of contamination and discontinuity, the densities of the inocula being unable to support a semi-confluent growth, or when overlapping inhibition zones were detected. The diameters of inhibition zones exhibited a bimodal distribution where one group with a small inhibition zone could be categorized as resistant, whereas the other group with bigger zones was considered sensitive.

Table 4. Effects of the EtOAc Extract of *A. petersiana* on the Efficacy of fansidar on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	9	10	9	10	10	10	10
2	48 ml fansidar + 2ml	11	10	11	11	11	12	11
3	47 ml fansidar + 3ml	12	13	12	12	13	12	12
4	46 ml fansidar + 4ml	14	14	14	13	14	14	14
5	45 ml fansidar + 5ml	14	15	14	14	14	14	14

The efficacy of the EtOAc-fansidar mixture (14mm) was slightly lower than that of fansidar alone which was 15 mm. Hence the extract may be considered to have been antagonistic to fansidar.

Table 5. Effects of the MeOH Extract of *A. petersiana* on the Efficacy of fansidar on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	13	14	13	13	13	13	13
2	48 ml fansidar + 2ml	15	16	16	16	16	16	16
3	47 ml fansidar + 3ml	1 7	17	17	16	17	18	17
4	46 ml fansidar + 4ml	18	18	19	18	18	18	18
5	45 ml fansidar + 5ml	18	18	18	18	19	18	18

The efficacy of the MeOH extract, with the maximum zone of inhibition of 18mm was higher than that of fansidar alone which was 15mm. Hence the extract may be considered to be synergistic with fansidar

Table 6. Effects of EtOAc Extract of *A. digitata* on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	EtOAc extract mg/ml	1	2	3	4	5	6	average
6	3.19	4	6	5	5	5	-	5
7	6.37	8	5	7	9	10	9	8
8	12.74	12	12	13	=	12	12	12
9	25.48	12	13	13	12	12	12	12

Table 7. Effects of the EtOAc Extract of *Adansonia digitata* on the Efficacy of fansidar on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	10	11	9	10	-	-	10
2	48 ml fansidar + 2ml	11	12	10	11	-	-	11
3	47 ml fansidar + 3ml	12	13	12	11	12	12	12
4	46 ml fansidar + 4ml	12	12	11	11	12	-	12
5	45 ml fansidar + 5ml	12	12	12	13	12	-	12

The EtOAc extract of *Adansonia digitata* did appear to be antagonistic to the antimalarial activity of fansidar. The diameter of zone of inhibition for the mixture (12mm) was lower than that for fansidar(15mm).

Table 8. Effects of extract MeOH extract of *A. digitata* on Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	3.63	12	11	13	12	12	12	12
7	7.26	13	15	12	17	14	13	14
8	14.52	18	18	16	17	16	-	17
9	29.04	18	18	16	17	16	17	17

The MeOH extract of *Adansonia digitata* (maximum diameter of 17mm) appeared more active than fansidar (maximum diameter of 15mm).

Table 9. Effects of the MeOH Extract of *Adansonia digitata* on the Efficacy of fansidar on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	12	13	11	12	12	-	12
2	48 ml fansidar + 2ml	13	13	14	16	14	14	14
3	47 ml fansidar + 3ml	15	15	14	15	-	15	15
4	46 ml fansidar + 4ml	19	19	20	18	19	19	19
5	45 ml fansidar + 5ml	19	19	19	19	19	-	19

There appeared to be synergism between fansidar and the MeOH extract of *Adansonia digitata*, giving a maximum diameter of zone of inhibition of 19mm compared to fansidar (15mm) and the MeOH extract (17mm).

Table 10. Effects of extract EtOAC extract of *Albizia amara* on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	2.37	9	9	10	8	9	-	9
7	4.74	13	11	12	17	14	9	12
8	9.48	13	14	15	14	14	14	14
9	18.96	14	14	14	14	14	13	14

Table 11. Effects of the EtOAc Extract of *Albizia amara* on the Efficacy of fansidar on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	9	9	10	9	9	9	9
2	48 ml fansidar + 2ml	13	11	12	15	14	9	12
3	47 ml fansidar + 3ml	13	14	15	14	14	-	14
4	46 ml fansidar + 4ml	14	14	14	-	14	14	14
5	45 ml fansidar + 5ml	14	14	14	15	14	14	14

There was no difference between the diameter of zone of inhibition for the EtOAc extract (14mm) and for the EtOAc/fansidar mixture (14mm). However these were both slightly lower than the diameter of zone of inhibition for fansidar (15mm).

Table 12. Effects of extract MeOH extract of *Albizia amara* on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	2.77	11	9	10	10	10	10	10
7	5.53	12	14	15	10	16	11	13
8	11.06	16	17	15	16	16	-	16
9	22.12	16	16	16	16	-	-	16

Table 13. Effects of the MeOH Extract of *Albizia amara* on the Growth of the Efficacy of fansidar on the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	9	10	10	11	10	10	10
2	48 ml fansidar + 2ml	11	11	11	11	10	12	11
3	47 ml fansidar + 3ml	11	11	11	12	10	10	11
4	46 ml fansidar + 4ml	12	12	11	10	12	10	12
5	45 ml fansidar + 5ml	12	11	13	12	12	-	12

The MeOH extract of *Albizia amara* had a diameter of zone of inhibition with a maximum of 16mm compared to fansidar (15mm) and extract/fansidar mixture (12mm). It would appear that the MeOH extract was antagonistic to fansidar, leading to lowering of the activity of fansidar.

Table 14. Effects of EtOAc extract of *Dalbergia nitidula* on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	3.67	8	8	8	-	-	-	8
7	7.33	10	9	13	14	12	8	11
8	14.66	13	14	15	14	-	-	14
9	29.32	14	13	15	14	-	-	14

Table 15. Effects of the EtOAc Extract of *Dalbergia nitidula* on the Efficacy of fansidar on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Drug combination	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	12	13	11	12	12	-	12
2	48 ml fansidar + 2ml	13	13	13	13	13	-	13
3	47 ml fansidar + 3ml	14	16	12	14	14	-	14
4	46 ml fansidar + 4ml	15	14	15	15	15	-	15
5	45 ml fansidar + 5ml	15	15	15	15	-	-	15

The EtOAc extract, maximum diameter of zone of inhibition (14mm) was slightly lower than fansidar, maximum diameter of zone of inhibition (15mm) and fansidar/EtOAc extract, maximum diameter of zone of inhibition (15mm). It would appear that there was interaction between the active constituents in fansidar and those in the extract.

Table 16. Effects of extract MeOH extract of *Dalbergia nitidula* on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	3.44	11	11	13	13	12	12	12
7	6.875	12	14	16	17	15	16	15
8	13.75	18	19	16	15	17	-	17
9	27.50	17	18	17	17	-	17	17

Table 17. Effects of the MeOH Extract of *Dalbergia nitidula* on the Efficacy of fansidar on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	13	14	14	13	11	11	13
2	48 ml fansidar + 2ml	15	16	14	15	15	14	15
3	47 ml fansidar + 3ml	17	18	16	18	18	16	17
4	46 ml fansidar + 4ml	18	18	18	18	18	17	18
5	45 ml fansidar + 5ml	18	18	18	18	18	17	18

The maximum diameter of the zone of inhibition for the MeOH extract (17mm) was higher than that for fansidar (15mm), but lower than that for the MeOH extract/fansidar mixture (18mm). It can, thus be concluded that the extract had synergistic relationship with fansidar.

Table 18. Effects of the EtOAC extract of *Lannea discolor* on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	2.399	6	3	3	4	4	-	4
7	6.598	5	4	6	8	9	4	6
8	13.20	9	9	8	9	9	8	9
9	26.40	9	9	9	-	-	9	9

Table 19. Effects of the EtOAc of Extract of *Lannea discolor* on the Efficacy of fansidar on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	10	11	10	9	-	-	10
2	48 ml fansidar + 2ml	11	13	9	11	11	11	11
3	47 ml fansidar + 3ml	11	11	12	11	-	-	11
4	46 ml fansidar + 4ml	12	12	13	10	12	-	12
5	45 ml fansidar + 5ml	12	12	12	-	-	-	12

The antimalarial activity of the EtOAc extract of *Lannea discolour* was low, maximum diameter of zone of inhibition (12mm), compared to fansidar (15 mm). It also lowered the zone for fansidar in the mixture to 12 mm. It was, thus, antagonistic to fansidar.

Table 20. Effects of MeOH extract of *Lannea discolor* on the Growth of the Malaria Parasite
Diameter of zone of inhibition (mm)
No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	4.59	14	14	12	12	13	-	13
7	9.19	16	14	18	13	15	14	15
8	18.37	18	19	17	18	18	18	18
9	36.74	19	19	17	18	18	-	18

Table 21. Effects of the MeOH of Extract of *Lannea discolor* on the Efficacy of fansidar on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	13	13	13	13	-	-	13
2	48 ml fansidar + 2ml	14	13	12	15	16	13	14
3	47 ml fansidar + 3ml	15	15	15	15	14	16	15
4	46 ml fansidar + 4ml	19	20	20	18	19	18	19
5	45 ml fansidar + 5ml	19	19	18	20	19	-	19

The maximum diameter of the zone of inhibition for the MeOH extract of *Lannea discolor* (18mm) was higher than that of fansidar (15mm) and lower than that of fansidar/MeOH extract (19mm). This was probably resulting from a synergistic relationship with fansidar.

Table 22. Effects of EtOAc extract of *V. infausta* on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	3.63	6	10	8	8	8	-	8
7	7.255	8	10	7	12	14	9	10
8	14.53	13	14	15	14	14	14	14
9	29.06	13	15	14	14	15	13	14

Table 23. Effects of the EtOAc Extract of *Vangueria infausta* on the Efficacy of fansidar on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No Disc number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar +1ml_	11	9	10	10	-	-	10
2	48 ml fansidar +2ml	12	11	11	-	10	-	11
3	47 ml fansidar +3ml	12	13	13	12	-	10	12
4	46 ml fansidar +4ml	12	12	13	11	12	-	12
5	45 ml fansidar +5ml	12	12	13	12	-	-	12

The maximum diameter of zone of inhibition EtOAc extract of *Vangueria infausta* (14mm) was comparable with fansidar (15mm) and higher than that for the EtOAc extract/fansidar mixture (12mm). It appears to be antagonistic to fansidar.

Table 24. Effects of MeOH extract of *Vangueria infausta* on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No. Disc Number

	Extract (mg/ml)	1	2	3	4	5	6	average
6	3.32	10	11	11	9	9	-	10
7	6.64	10	16	12	13	14	19	14
8	13.28	19	-	19	19	-	19	19
9	26.56	19	20	18	19	19	-	19

Table 25. Effects of the MeOH of Extract of *Vangueria infausta* on the Efficacy of fansidar on the Growth of the Malaria Parasite

Diameter of zone of inhibition (mm)

No Disc number

	Drug combinations	1	2	3	4	5	6	average
1	49 ml fansidar + 1ml	11	12	11	11	11	-	11
2	48 ml fansidar + 2ml	12	12	12	12	11	13	12
3	47 ml fansidar + 3ml	14	14	12	13	13	13	13
4	46 ml fansidar + 4ml	14	14	15	15	13	13	14
5	45 ml fansidar + 5ml	14	14	15	13	15	14	14

The maximum diameter of zone of inhibition for the MeOH extract of *Vangueria infausta* was(19mm) compared to that of fansidar (15mm), and higher than for the MeOH extract of Vangueria

infausta/fansidar mixture (14mm). Thus, like the case for the EtOAc extract, the MeOH extract was antagonistic to fansidar.

CONCLUSION

The diameter of inhibition of growth of plasmodial parasites on agar increased with increase in the concentration of the antiplasmodial drug up to a maximum point, then remained constant when no further increase in the concentration of the drug had effect. The maximum diameter of the zone of inhibition of growth of plasmodial parasites on agar by fansidar was 15 mm (Table 1). Some of the extracts increased the diameter of the zone for fansidar, yet others decreased it. This work should be repeated using other standard drugs to develop a data base on the interaction of standard drugs with plant extracts.

Discussion of evaluation of effect of extracts on the inhibition of microbial growth by fansidar

The variation in the diameters of the inhibition zones was considerable. Unavoidable difficulties were encountered in determining the edges of the zones of inhibition because of hazy limits of growth, largely due to two methodological factors: (a) the medium or inoculum density and (b) the timing of disc application. Moreover, some reports have raised concern over the use of agar in general.

The use of too dense inocula may, in part explain the great variability in zone diameters. The timing of disc application is also a serious factor. All discs should be applied at the same time onto the medium upon which the medium is growing. The greater the residence times of the discs on the agar medium, the greater the extent of diffusion. Diffusion should be made to occur to comparable extents such that the extent of inhibition of the growth of protozoa or lack of it will be directly attributable to the susceptibility or resistance of the protozoa to the drug or drug combinations. The protozoal culture will suffer a retardation of growth with time. In some cases the prevailing conditions, such as nutrients for metabolism and accumulating waste products, will not favour the growth of protozoa. There is, thus, need for a trade-off between the residence time and the protozoal life cycle.

Disc diffusion methods are widely used for susceptibility estimations predominantly because they are simple to perform. They are reasonably accurate when performed to the stipulated procedure and require little expense or sophisticated equipment. They are, however, not unsuitable for erythrocytic, and most fatal, phase of the parasite. Hence their use for clinical purposes is limited.

The disc diffusion theory may be subject to the following errors:

(a) The theory holds up to a limiting concentration.
(b) The sample may undergo changes such as association or dissociation.
(c) The solvent used for dissolving the sample may also affect the protozoal culture.

Interpretative criteria and schemes for susceptibility tests have been developed by a number of scientific committees such as the National Committee for Clinical Laboratory Standards (NCCLS) [31], the Norwegian Working Group on Antibiotics [32], de I'Antibiogramme de in Societe Francaise de Nicrobiologie [33], and the British Society for Antimicrobial Chemotherapy (BSAC) [34]. These groups have not come up with recommendations which are consistent between groups. Existing interpretative schemes are constructed for human, rapidly growing bacteria. *Plasmodium falciparum* is predominantly erythrocytic in its life cycle, a fastidious organism requiring specific growth conditions. Results of these assays were read after 72 hours incubation at 37^{o}C to ensure that the growth of the cultures of the protozoa was comparable in all incubated cultures. The temperature of 37^{o} was used because it is the temperature at which the parasite finds itself in the human body.

Some assays were rejected in cases of contamination and discontinuity, the densities of inocula being unable to support a semi-confluent growth, or when overlapping inhibition zones were detected. The diameter of inhibition zone sizes exhibited a bimodal distribution where one group with a small inhibition zone could be categorized as resistant, whereas the other group with bigger zones was considered sensitive.

The extracts of *Adansonia digitata*

The EtOAc extract of *Adansonia digitata* gave maximum zone of inhibition diameter of 10mm at a concentration of 12.74 mg/ml. The MeOH extract was more effective, giving a maximum diameter of the zone of inhibition of 17mm at a concentration of 14.52 mg/ml. Thus, the two extracts of *A. digitata* were capable of inhibiting the growth of the protozoa, the MeOh extract being the more effective.

The inhition of the MeOH extract was greater than that of fansidar which had a maximum of 15mm at a concentration of 12.5 mg/ml. That the crude MeOH extract could perform better than fansidar was a pleasant surprise.

Combination of extracts with fansidar

The EtOAc extract appeared to be antagonistic to fansidar. The mixture gave a maximum diameter of the zone of inhibition of 12mm, compared to the extract on its own (10mm and fansidar on its own (15mm). The MeOH extract gave a different picture. It appeared to synergistic with fansidar, giving a maximum diameter of the zone of inhibition of 19mm, compared to 17mm on its own and fansidar (15mm). There aqppears to be a case for the further investigation of the MeOH extract, even for replacement of fansidar. . If *in vivo* investigation results could be similar to those obtained in the *in vitro* studies, the methanol extract may be developed for use as an anti-malarial agent on its own or in combination therapy.

The extracts of *Albizia amara*

The EtOAc extract of *Albizia amara* gave a maximum diameter of the zone of inhibition of 14mm at a concentration of 9.46 mg/ml, very close to that of fansidar (15mm). The MeOH extract of *Albizia amara* gave the maximum of 16mm at a concentration of 11.06 mg/ml, better than fansidar.

The combination of fansidar with the EtOAc extract of *Albizia amara* gave a maximum at 13mm, thus 2mm lower than fansidar. The MeOH extract gave a maximum of 12mm, thus lower than the EtOAc extract. It would appear that the compounds in these two extracts are antagonistic to fansidar. Thus a person taking fansidar should not take *Albizia amara* to cure malaria. In the absence of better curing agents, the extracts might still be useful for malaria cure. However, the extracts might be investigated to reverse overdose or poisoning by famsidar or related substances. The EtOAc extract is comparable with fansidar whilst the MeOH extract is a better performer than fansidar.

The lowering of the efficacy of fansidar is an observation that is contrary to the popular belief among ethnomedical practitioners that combination of ethnomedicines with western medicines raises the efficacy of western medicines. There is need to carry out tests to be able to establish whether the ethnomedicine is antagonistic, or it potentiates, or whether it is synergistic in its effects.

The extract of *Dalbergia nitidula*

The EtOAc extract of *Dalbergia nitidula* gave a maximum diameter for the zone of inhibition of 14 mm at a concentration of 14.66 mg/ml, compared to fansidar which gave 15mm. The MeOH extract of *Dalbergia nitidula* gave 17mm at a concentration of 13.75. This was greater than what was given by fansidar (15mm). Thus the EtOAc extract is comparable with fansidar whilst the MeOH extract is a better performer than fansidar.

The combination of fansidar and the EtOAc extract gave a maximum at 15mm, very much the same as fansidar. Fansidar in combination with the MeOH extract gave the maximum at 18mm, very much better than fansidar. A case for synergism is thus suggested and appears attractive to investigate the plant further for combination therapy with fansidar. It could even be investigated for malaria cure on its own.

The extracts of *Aristolochia petersiana*

The efficacy of the EtOAc extract of A. petersiana was slightly lower (max. zone of inhibition:12mm) than that of fansidar (15mm). For the MeOH extract the max. zone of inhibition was 16mm compared to that of fansidar which was 15mm. The efficacy of the EtOAc extract-fansidar mixture was lower than that of fansidar (14mm) compared to the MeOH extract-fansidar mixture which was 18mm. Thus, whereas the EtOAc extract appeared antagomistic to fansidar, the MeOH extract was synergistic.

The extracts of *Lannea discolour*

The EtOAc extract of *Lannea discolour* gave a maximum at 9mm, at a concentration of 13.20 mg/ml. The MeOH extract gave a maximum of 18mm, at 18.37 mg/ml. It was better than fansidar (15mm), at a concentration of 12.5 mg/ml. The combination of *Lannea discolour* and fansidar was not effective, and gave lower values than fansidar. The extract appeared antagonistic to fansidar. The MeOH extract gave a maximum of 19mm compared to fansidar. It was possibly synergistic and could be investigated to replace fansidar. Mixing fansidar with the ethyl acetate extract of *Lannea discolor* gave a maximum inhibition zone of 12 mm after addition of 4 ml extract to 46 ml fansidar compared to fansidar on its own (15 mm). Thus the extract had the effect of lowering the effect of fansidar. The methanol extract gave a maximum inhibition zone of 19 mm after addition of 4 ml extract to 46 ml fansidar, raising the

effect of fansidar from a maximum of 15 mm to 19mm. Thus, whilst the ethyl acetate extract might not be useful in the cure of malaria, the methanol extract is likely to be effective on its own or in combination in raising the efficacy of fansidar. The methanol extract could thus be used on its own or in combination with fansidar to cure malaria.

The extracts of *Vangueria infausta*

The EtOAc extract of *Vangueria infausta* gave a maximum of 14mm at a concentration of 14.51 mg/ml. The MeOH extract was well above fansidar, at a maximum of 19mm, at a concentration of 13.28 mg/ml. The combination of the EtOAc extract with fansidar gave a maximum at 12mm compared to fansidar which gave a maximum at 15mm and the MeOH extract in combination with fansidar gave a maximum at 14mm, thus lowering that for fansidar. Hence both the EtOAC and the MeOH extracts might have been antagonistic to fansidar, and should not be used in combination with fansidar and people taking fansidar should not take them to cure malaria. They might, however, be useful in cases of fansidar overdose.

Thus the methanol extract of *V. infausta* on its own was more effective than fansidar, but the ethyl acetate extract is slightly less effective than fansidar. Assuming that this trend in the in *vitro* studies will be repeated in the in *vivo*, the methanol extract could be developed into a formidable antimalarial agent. It should, however, not be taken at the same time with fansidar as it will make fansidar less effective. This observation is contrary to claims by traditional healers that traditional medicines could safely be taken together with western medicines (personal observations). The *in vitro* effect of the ethyl acetate extract of *V. infausta* on the inhibition of the growth of protozoa is comparable, though slightly lower than that of fansidar. The extract could be used in place of fansidar. In combination with fansidar, the extract is antagonistic, lowering the effect of fansidar to undesirable levels. Hence, like the case for the methanol extract, the ethyl acetate extract may not be used in combination with fansidar as its effects are antagonistic to those of fansidar.

SUMMARY

All of the EtOAc extracts gave diameters of the zones of inhibition which were lower than that of fansidar and all of the MeOH extracts gave diameters which were larger than that of fansidar. The EtOAc extract of all the plants studied in this project, except that of A. nitidula, lower the efficacy of fansidar. The EtOAc extract of A. nitidula does not appear to have effect on the efficacy of fansidar. All the MeOH extract, except that of *Albizia amara* raise the effects of fansidar.

In the event that a malaria cure is to be developed from any of the plants, the MeOH extract would be the one to target, based on these in vitro assays. EtOAc extracts less polar compounds than MeOH does. It so happens that the effective antiprotozoal compounds in these plants are polar, hence extractable by polar solvents like MeOH. Thus, there is nothing magical about MeOH in these observations.

Similarly, there is nothing sinister about some compounds of plant origin being antagonistic to synthetic compounds. Compounds in general, synthetic or natural, may affect the performance of each other or may not affect the performance of each other at all. They may be antagonistic or synergistic of indifferent. Synergism or antagonism are properties of compounds and not their origins.

CONCLUSION

The results of this project raise two important points: (1) that the claim by ethnomedical practitioners that ethnomedicines combine to make western medicines more effective should not be taken at face value. The ethnomedicines must be considered like any other chemicals. Their behaviour in combination with other chemicals may go either way. They may be antagonistic, or they may potentiate, or they may be synergistic, or they may not have effect on one another. The actual behaviour of the drugs must be established by experimentation; and (2) that there exist some drugs in plants that may compete existing western drugs such as fansidar for use as antimalaria agents. Drugs from *Adansonia digitata* and *Vangueria infausta* methanol extracts are such potential competitors. The plants could be subjected to *in vivo* trials as antimalaria agents, both as individual drugs or in combination therapy.

Herbal cure of malaria

Indigenous people of malaria endemic areas have lived with malaria transmitting mosquitoes since time immemorial. With time they became immune to malaria although they occasionally succumbed to

it. They invariably knew what to do about the disease before the advent of modern medicines. They even cured some of the European explorers long before colonization [11]. So malaria cure did not come with colonization. Just as Central Americans used Chincona, everybody else had their answer to malaria. Whoever suggested that the chincona plant was the only one that could cure malaria? The forests are abound with plants known to successfully cure malaria. Helping locals to develop and enrich their local plant medicinal cures is bound to go a long way towards taming the disease.

Quinine and fansidar have been the mainstay of malaria cure in sub-Saharan Africa for a long time. The advent of parasite resistance to these two drugs has ushered in the use of artemisnin drugs. Thus the malaria cure regimes in the modern era started off with quinine, then fansidar, and now we have artemisnin and combination drugs. What would the story of malaria resistance have been had a number of drugs been developed and used instead of everybody using one drug? What would the story have been had local drugs been developed to cure malaria?

Fansidar has been a reliable anti-malaria drug in sub-Saharan Africa. *In vitro* assays have been carried out and revealed that plant extracts may have efficacies which are comparable to fansidar. The *in vitro* studies also revealed that addition of plant extracts enhance the efficacy of fansidar [12]. It would be desirable to study the effect of mixing different plant extracts and compare the efficacies of mixtures with those of individual plant extracts. The next step would be the study of the *in vivo* effects of the plant extracts and ultimately, to carry the projects to its logical conclusion of the development of malaria drugs.

All decisions about the use of these plant extracts must be based on experimental results. The sentiments of herbal medicines practitioners that natural medicines are free of side effects and that natural medicines enhance the efficacies of modern medicines have to be viewed with caution until experimental evidence in support of the claims has been collected. We have witnessed earlier on how addition of some extracts lowered the efficacy of fansidar. However, traditional medicine practitioners who are spirit mediums argue that they consult spirits before they prescribe the drugs to use and the spirits will not give the mixtures which would give problems such as antagonism. Well. They might have a point, but the problem remains. Some herbal medicines will be antagonistic between themselves and towards conventional medicines.

It is reasonable to expect drugs in plants to behave differently to the same drugs which have been purified. There are examples of plants that consist of about 50% asprin in their extracts. Herbal medicines consumers easily consume up to 30g of powders of such plants in porridge without knowing that they were taking the equivalent of 15g asprin and they do not experience any undesirable effects. Obviously, taking 15g of asprin would lead to problems.

Herbal practitioners claim that their drugs are more effective than modern factory produced products because the constituents of the raw preparations reinforce each other. There is need to experiment on this claim, not necessarily to prove the claims true or false, but in an attempt to try and rationalize the practitioners' findings. Some plants may reinforce each other, but there are cases where extracts of plants have been antagonistic.

The recent strides in the understanding of malaria, including details of the physiology of the parasite and the vagaries it goes through as it travels from the human carrier to the mosquito and back to a human victim, the antigens acting on the parasite in the human body and in the mosquito gut will eventually translate to discovery of malaria vaccine(s). For example it is now known that only a few of the parasites taken from the human carrier will survive the attack by human white blood cell with which the parasite from the human body and the mosquito's own digestive system before the parasite travels to their safety in the mosquito body. Developments such as studies of the *Plasmodium falciparum* transmission-blocking antibodies [35], development of mosquito midgut antibodies that reduce mosquito fecundity and survivorship [36], understanding how malaria risk is linked with climate [37] and how malaria transmission-blocking immunity can be induced *in vivo* and *in vitro* will strongly aid and hasten development of a vaccine.

These developments, coupled with the awakening to the importance of herbal medicines, will likely translate to the realization of a conquest of the mosquito. A detailed knowledge of the various aspects of malaria will provide the basis for new antimalarial chemotherapeutic strategies as well as an understanding of mechanisms of antimalarial drug resistance and contribute towards conquering malaria. Chances to eradicate the mosquito are slim, but strategies to tame the killer insect must be

pursued vigorously. Eradication of the mosquito might not be a practical proposition because of the expanse of the territory the insect occupies. Nations would need to spray large expanses of uninhabited ocean coasts and jungles to get rid of the mosquito and the expense would be prohibitive.

RECOMMENDATIONS

The work on malaria cure reveals that it is possible to increase the efficacy of fansidar by including herbal extracts in formulations. However, further studies should be pursued to establish affordable alternatives to use in combination with conventional malaria drugs. The same studies should be repeated with other drugs such as quinine to establish if other malaria drugs give results which are similar to those observed for fansidar.

The active constituents from herbal malaria cures should be isolated, purified using chromatographic techniques, characterized using spectroscopic techniques and results used to enhance the herbal products through industrial synthesis and development of analogues with better efficacy. The poisonous aspects of the herbal constituents should be studied. Pharmacokinetic studies aimed at determining the half-lives, bioavailability and effective concentrations in the blood and plasma, as well as their rates of clearance, excretion routes and metabolism may lead to the identity and rates of formation of metabolites which can provide insights into the possible mechanisms whereby the drugs exert their therapeutic and toxicological effects [38]. Detailed toxicological evaluation to determine the type and degree of toxicities of the herbal drugs and to establish safe starting doses in humans can be achieved through use of animal models. Further studies should be designed to determine the relationship of toxicity to dose and schedule of administration, and to study possible reversibility of observed toxic effects.

The socio-economic, socio-political and demographic constraints of the third world demand that the results of research of this nature be converted to more practical utilization . Side effects of the herbal remedies have to be ascertained and clinical trials have to be conducted to determine the optimum therapeutic dosage of the herbal remedy; to array the general resentment some people have towards natural medicines and to open the general horizons for wide explorations in the field of natural medicines. It will be cheaper and more affordable to the general populace to use the herbal remedies without having to buy the expensive imported drugs.

FUTURE RESEARCH

Although traditional medicine is widely used to treat malaria, and is often more available and affordable than Western medicine, it is not without limitations. Firstly, there are few clinical data on safety and efficacy. Secondly, there is no consensus, even among traditional healers, on which plants, preparations, and dosages are the most effective. Thirdly, the concentration of active ingredients in a plant species varies considerably, depending on several factors.

None the less, these limitations are all remediable, through research. The Research Initiative on Traditional Antimalarial Methods has written systematic reviews and guidelines aiming to standardise and improve the quality of future research. The reviews and guidelines are not complete but they can be improved as research progresses.

The concept of adding plant extracts to established drugs might be usefully tried with other drugs besides antimalarials. Plant extracts used in this study are reputed for immune boosting. Adding them to drugs might also contribute towards immune boosting outcomes [39]. The purpose of combination therapy is to delay the development of malaria resistance to either drug when used alone [8]. The ideals of combination therapy would then be expected to be realized when raw herbal medicines are used since the raw material would contain a number of active compounds.

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