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ABSTRACT

Ethnobotanical study of antimalarial medicinal plants in Awash Fentale district was carried out between November and December 2007. Semi-structured interview was used to gather medicinal plant knowledge of the Afar people residing in Awash Fentale District. Sampled informants were interviewed about their knowledge of use of medicinal plants, plant parts used, practices and management systems against malaria. The local names of medicinal plants and their habits were also recorded. During the survey, a total of 19 antimalarial medicinal plant species were reported. These plants were collected from four kebeles in the study area. With regard to preparations, remedies are mainly done by crushing plant parts to make infusion or decoction with cold or hot water. Oral application is widely used in almost all practitioners. The leaf is the most common part of the plants used (44 %) followed by stem (22 %). Shrubs and trees were plants with higher frequencies to treat malaria accounting for 63 % and 21 % of the plants, respectively. Crude water and ethanol extracts obtained from the leaves of *Aloe* sp. and *Cadaba rotundifolia* were tested *in vivo* for antimalarial activity on a total of 160 Swiss albino mice. Each mouse in the study was infected intraperitonially with blood samples taken from mice previously infected with *Plasmodium berghei* (chloroquine sensitive) after dilution so that 0.2ml contained 10^6-10^7 infected erythrocytes. The extracts were given orally to the infected mice starting from three hours following infection. Antimalarial activity was evaluated by taking blood smears on the fifth day of infection. This study showed that ethanol extracts obtained from the leaves of *C. rotundifolia* and *Aloe* sp. suppressed parasitemia significantly (53.73% and 49.07%, respectively) at 900mg/kg. However, aqueous crude extracts obtained from the leaves of both *C. rotundifolia* and *Aloe* sp. (40.8% and 31.7%, respectively) did not show significant suppressive effect on parasitemia as compared to their ethanol extracts. The non-toxic properties of these plants up to a dose of 1500mg/kg was also revealed.

Key words: *Aloe* sp., Antimalarial activity, *Cadaba rotundifolia*, Ethnobotany, *In vivo*, Medicinal plants, *Plasmodium berghei.*

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SCHOOL OF GRADUATE STUDIES

Ethnobotanical survey of plants used to treat malaria in the Awash –Fentale District of the Afar Region of Ethiopia and *in vivo* screening of some selected plants for their antimalarial activities.

BY

NEGA ALELIGN

Thesis Submitted to the Research and Graduate Studies of the Addis Ababa University in Partial Fulfillment of Requirements of the Degree of Master of Science in Biology (Biomedical sciences)

JULY, 2008

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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Declaration

I, the undersigned, declare that this thesis is my own work. It has not been presented in other universities, colleges or institutions, seeking for similar degree or other purposes. All sources of materials used for the thesis have been duly acknowledged.

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LIST OF ABBREVIATIONS

ALIPB = Aklilu Lemma Institute of Pathobiology
ANOVA = Analysis of Variance
CDC = Center for Disease Control
CQ = Chloroquine
Dist. H_2O = Distled water
D_O = The first day when infection was done
D_4 = The fifth day when infection was done
EtOH = Ethanol
EMA = Ethiopian Metriological Agency
IBD = Institute of Biodiversity
LD = Lethal dose
IC = Inhibition concentration
MED = Minimum effective dose
Para.= Parasitemia
PCV = Packed cell volume
RBC = Red blood cells
SEM = Standared error of the meanSMI = Schizont Maturation Inhibition
SPSS = Statistical package for social sciences
SSA = Sub Saharan Africa
Supp = Suppression
UN-EMU = United Nations Emmergencies Unit for Ethiopia
% Para = Percentage parasitemia
% Supp = Percentage suppression

INTRODUCTION

Malaria is one of the most severe diseases in the world that puts some 3.2 billion people at risk of infection in 107 countries (Bora *et al.*, 2007). It causes considerable morbidity and mortality globally, resulting in an estimated 300-500 million clinical cases and about 1.4-2.6 million deaths annually. More than 90% of this morbidity and occurs in Sub-Saharan Africa (SSA) (Okenu, 1999; Fokialakis *et al.*, 2007). According to the World Health Organization (WHO, 2001), malaria is ranked second after HIV/AIDS in SSA. In Africa, there are up to 800,000 infant mortalities and a substantial number of miscarriages and low birth weight babies (Sachas and Malaney, 2002) per year due to the disease. Despite the great intake of antimalarial drugs in the continent, the death rate is increasing (Njoroge and Bussmann, 2006).

Four species of *Plasmodium* parasites (*Plasmaodium vivax*, *P. falciparum*, and *P. ovale* and *P. malariae)* cause human malaria. In African, the predominant parasite is *P. falciparum* (Bruce-Chwatt, 1985). Although all the four species of malaria parasites infect humans and cause illness, *P. falciparrum* is known to be potentially life threatening.

In Ethiopia, the disease occurs in different parts of the country in epidemic forms and about 75% of the total area is estimated to be malarious with 65% of the total population being at risk of infection (Hodes, 1996).

In Ethiopia the two epidemiologically important species are *P. falcipanum* and *P. vivax* that contribute to 60% and 40% of all malaria cases, respectively (Bogale, 1994). *P. malariae* comprises less than 1% of all the cases and is most frequently reported in the Arba Minch area where as *P. ovale* is rarely reported.

The development of resistance to antimalarial drugs posed the greatest threat in malaria control and has been linked to increased malaria morbidity and mortality (Onori, 1982; Kihamia and Gills, 1982). Chloroquine- a prominent antimalarial drug- has been the first line drug for the treatement of malaria, over the last fourty years in Ethiopia. The first report on chloroquine failure to clear asexual *P. falciparum* parasites appeared in 1986. Since then the resistance has been increasing in Ethiopia as well as its neighbouring

countries (Teklehaimanot, 1986; Elchalal *et al.,* 1993; Fletcher *et al.*, 1993; Mengesha and Seboxa, 1998; Mengesha and Mekonen, 1999; Nuwaha, 2001).

P. falciparum has developed resistance to nearly all of the currently available antimalarial drugs, such as chloroquine, sulfadoxine/ pyrimethamine (SP), mefloquine, halofantrine, and quinine. Although resistance to these drugs tends to be much less widespread geographically, in some areas of the world, the impact of multi-drug resistant malaria can occur (CDC, 2004). Sulphadoxine-pyrimethamine has been and is currently used for treatment of uncomplicated *P. falciparum* malaria in many African countries. Nevertheless, the response of parasites to SP treatment has shown significant variation between individuals (Erasto *et al.*, 2006).

The increased problem due to malaria and emergence of drug-resistant *P. falciparum* increased the need for new antimalarial drugs with novel mechanisms of pharmacological action (Mohammed *et al*., 2007). Herbal medicines, which formed the basis of health care throughout the world since the earlier days of humankind, are still widespread and have considerable importance in international trade. Over 60% of the world's populations and about 80% of people in developing countries depend directly on plants for their primary health care needs (Bye, 1986; WHO, 2002; Shrestha and Dihillion., 2003; Huai and Pei, 2004; Bourdy *et al.,* 2005). Recognition of their clinical, pharmaceutical and economic value is still growing (Zhang, 1998).

African plants have long been the source of important products with their nutritional and therapeutic values (Hostettmann *et al.,* 2000). In many areas, especially in the tropics, several species of medicinal plants offer access to safe and effective products for use in the prevention and treatment of various illnesses including malaria (Mohammed *et al*., 2007). One of the area for the search of new antimalarias is the traditionally claimed antimalarial plants from the African flora (Whitefield, 1995).

According to WHO (2002), there is an increasing interest on traditional medicine in developing countries in general and herbal medicine in particular. Such an interest and broad use of medicinal plants is because of its accessibility and affordability to the poor.

The high cost of Western pharmaceuticals put modern health care services out of the reach of a large percentage of Africa's population, especially those living in rural areas. Instead, they rely on traditional medicine and medical plants to meet their health care needs. For example, malaria treatment in Ghana with herbal medicines is considerably cheaper than other forms of health care (WHO, 2002).

Among many diseases traditionally treated with medicinal plants, malaria ranks the most important one (Muthaura *et al*., 2007). Historically, majority of antimalarial drugs have been derived from medicinal plants or from structures modeled on plant lead compounds (Klayman, 1985 cited in Muthaura *et al*., 2007).

Quinine and Artemisinine, the drugs of choice for the treatment of malaria, were obtained directly from *Cinchona* tree and the shrub *Artemisia annua*, respectively. Chemical structures of these compounds have been used as templates (Payne, 1987; Sharma, 2006 cited in Muthaura *et al.,* 2007), for the subsequent development of synthetic derivatives such as chloroquine, amodiaquine, primaquine and mefloquine. Relatively simple chemical modifications of artemicinine have led to a series of potent antimalarial drugs that play important role in the treatement of malaria (Meshnick, 2001). This initiated the search for alternative drugs from medicinal plants.

It has been estimated that more than 150 genera of higher plants are used through out the tropical world for the treatment of malaria in the indigenous system of traditional medicine (Phillipson, 1989). According to Fokialakis *et al.* (2007), different parts of 65 plant species from the Greek Island of Crete have been extracted and have been investigated for *in vitro* antiprotozoal activity of which 22 extracts had activity against *P. falciparum.* According to Vigneron *et al.* (2005), a total of 34 different species have been registered as antimalarials in French Guiana; twenty seven are used for curative purpose. *Quassia amara* was the species most frequently used as antimalarial for curative and preventive purposes.

The study from Bora *et al.* (2007) showed that the people of the north eastern region of India use at least 65 plants belonging to 38 families to treat malaria. Different plant parts

such as the leaf, root bark, stem bark , fruit and in some cases the whole plant were used for making herbal preparations.

According to Muthaura *et al.* (2007) twenty five species in 21 general and 16 families were documented for their traditional antimalarial use in Kwale community of the Kenyan coast. Ethnobotanical study by Njoroge and Bussmann (2006) showed that both indigenous and introduced species of plants are in use in management of malaria in central Kenya. In total 58 species in 54 genera and 33 families were identified .The family Rubiaceae was found to have the highest number of reported species.

In Ethiopia, traditional medicine in general and herbal medicine in particular continue to be widely used by the majority of the rural population (Gedif and Hahn, 2002) to treat malaria and other infections since a long time (IBD, 2007). Due to poor access to health services, especially in the rural areas, the majority of the Ethiopian people relay mainly on traditional medicine for their primary health care needs (Derib *et al*., 2006). Neverthless, the system has been neglected and its therapeutic potential as well as advers effects has not been studied scientifically.

The health and drug policy of the Ethiopian Ministry of Health recognizes the role traditional health system play in health care. Unfortunately, little has been done in recent decades to enhance and develop the beneficial aspects of traditional medicine including relevant research to explore possibilities for its gradual integration in to modern medicine (Derib *et al.*, 2006).

Some ethnobotanical studies conducted in Ethiopia indicated that there is wide indigenious knowledge on antimalarial plant remedies in different parts of the country. From these studies (Bogale, 1994; Teklehaymanot *et al.*, 2006; Giday *et al*., 2006) it has been indicated that females have less knowledge than males and medicinal plants reported by the females are either cultivated or weeds that are found in their backyards and they mainly treat their own children. This is because the traditional knowledge in the family or community is passed from male parent to his first –born son. Healers may deliberately use poly-herbal treatment to disguise the plant that is used as a remedy for a given aliment.

Plant parts used for the treatment of malaria mainly depend on the practice of the local healer, and accessibility of the parts. Parts of plants such as the leaf, root, stem, flower, fruits, seeds, barks of stems and roots are used. Roots and leaves were found to be the most frequently sought parts in the preparation of remedies (Giday *et al.*, 2006). However, in the study conducted by Teklehaymanot *et al.* (2006) the leaf is more favored in preparation of herbal medicines. Some plants are green but the root and stem are more favored in herbal medicines after dried, powdered and stored.

Residents of Gual Mereb, in Tigray Region of Ethiopia, use stem bark of *combretum molle* as a cure for malaria (Asres and Balcha, 1998). *Vernonoia amygdalina* and *Withania somnifera* are used by grinding all the parts together and mixing a glass of the powder with two glasses of honey to make a stock (Bogale and Petros, 1996). A large spoonful of the stock is diluted in glass of water and boiled for use. A cup of this mixture is taken in the morning and in the evening, for three days, making the traditional prescription.

Some plants have been documented (Sorssa, 1992; Bogale and Petros, 1996; Asres and Balcha, 1998; Animut, 2002; Dikasso *et al.*, 2006; Giday *et al.*, 2006; Birhanu *et al.*, 2006; Teklehaymanot *et al.*, 2006) to have been part of life for centuries; and a number of plant products have been in extensive use in ethnomedicine, particularly, for their use to treat malaria and repel and kill insects.

Experimental evaluation of some of these medicinal plants showed significant antimalarial activities. Dichloromethane and methanol extracts of *Berberis cretica* and *hypocistis* sub sp. is responsible to cause significant activity against both chloroquine sensitive and resistant strains of *P. falciparum* (Fokialaakis *et al.*, 2007). Water and methanol extracts of *Boscia salicifolia* and *Artemisia afra* showed activity against both chloroquine sensitive and resistant *P. falciparum in vitro*. *A. afra* and *Rhus natalensis* exhibited significantly high *P. berghei* clearance and chemosuppression (>70%) *in vivo* (Gathiraw *et al.*, 2007).

Extracts of *Pleiocarpa mutica, Cleistophlis patens* and *Uvaria chamae* were found to have significant antiplasmodial activity (Kyereme *et al*., 2001). Methanol and water extracts of *Malytenus undata* which is used for the treatment of malaria in traditional health care system of kwale people in Kenya showed strong activity against *P. falciparum* (Muthaura *et al*., 2007).

Limitted number of *in vivo* and *in vitro* studies has been done on the antimalarial activities of Ethiopian medicinal. It has been reported that crude extracts of *Moringa stenopetala, Wthania somnifera* and *Vernonia amygdalina* (Animut, 2002; Teklemariam, 2005) have shown valid antiplasmodial activities. A similar study (Dikasso *et al*., 2006) showed that extracts of the roots and aerial parts of *Asparagus africanus* (Asparagaceae) inhibited *P. berghei* parasitemia in Swiss albino mice.

The plant *Combertum molle* (Combertaceae) is widely used in Ethiopian traditional medicine for the treatment of liver disease and malaria. Its acetone and methanol fractions possessed remarkable schizont inhibition effect (Asres and Balcha, 1998). Chloroform and methanol extracts of leaves of *Withania somnifera* and chloroform extracts of leaves of *Vernonia amygdalina* showed substantial antimalarial activity against *P. falciparum in vitro* (Bogale and Petros, 1996). Their antimalarial activity was improved by several folds upon column fractionation.These extracts were also shown to reduce *P. berghei* parasitemia in mice (Teklemariam, 2005). Hydroalcoholic extract of *Asparagus africanus* displayed a very good activity against *P. berghei in vivo* at the dose of 200mg/kg (Dikasso *et al*., 2006).

Acetone extract of the roots of *Erythrina abyssinica* showed potent antiplasmodial activities against *P. falciparum* with IC_{50} values of 0.64 ± 0.06 and 0.49 ± 0.07 µg/ml, respectively (Derese *et al.,* 2006). Sorssa (1992) reported that extracts of *Croton macrostachyus, Calpurnia aurea, Buddleia polystachya* and *Dodonia angustifolia* extracts have strong activity against *P. falciparum in vitro*. Evaluation of these and other plants might contribute a lot in the effort to produce new antimalarial drugs.

Malaria is diagnosed clinically by traditional healers.However, the signs and symptoms of malaria are non specific and overlap with that of the other febrile illness. Hence, the medicinal plants that are used by traditional healers for the treatement of malaria should be studied for their antimalarial, antibacterial, antiviral and/or antifever effects. Therefore, in this study, the medicinal plants that are most frequiently used to treat malaria by the residents of the Awash Fentale District were evaluated for their antimalarial activities.

2. OBJECTIVES OF THE STUDY

2.1. General Objective

- To document and evaluate antimalarial medicinal plants used by the people of the Awash Fentale District in the Afar National Regional State of Ethiopia.

2.2. Specific objectives

- To document a list of medicinal plants used against malaria by people of the Awash Fentale District in the Afar National Regional State of Ethiopia against malaria.
- To evaluate the toxicity and antiplasmodial activity of *Aloe* sp. and *Cadaba rotundifolia.*
- To recommend antimalarial medicinal plants that require further pharmacological evaluation.

3. MATERIALS AND METHODS

3.1. Ethnobotanical study

3.1.1. The study area

The study was conducted in Awash Fentale District, Zone 3 of the Afar National Regional State of Ethiopia. The zone is bordered on the south by the Oromia Region, on the west by the Amhara Region, on the north by Dulcha and on the east by Amibara woredas of the same region. Towns and cities in Awash Fentale include Awash Sebate Kilo and Sabure. The study area is situated at about 200kms east of Addis Ababa.

According to the Ethiopian Metriological Agency (EMA), unpublished climatological data gathered for the last ten years (1997-2006) at the Awash Sebat killo town, the average annual rain fall is 580 mm and the major rain falls from July to September. The mean annual temperature ranges from 21 ^{o}C to 37 ^{o}C. The highest temperature is registered in June and the lowest is in December and January. Between the ends of May and mid-June, water and grazing stress was reported in the district (Piguet, 2002).

Field study sites were selected based on the prevalence of malaria and availability of practitioners. The study sites were Awash Sebat Kilo town, Sabure, Birgeet and Kebena kebeles. Sabure and Kebena are located north of Awash Sebat Killo at 42 and 47 kms, respectively, where as Birget is located east of Awash Sebat Kilo at 5 kms.

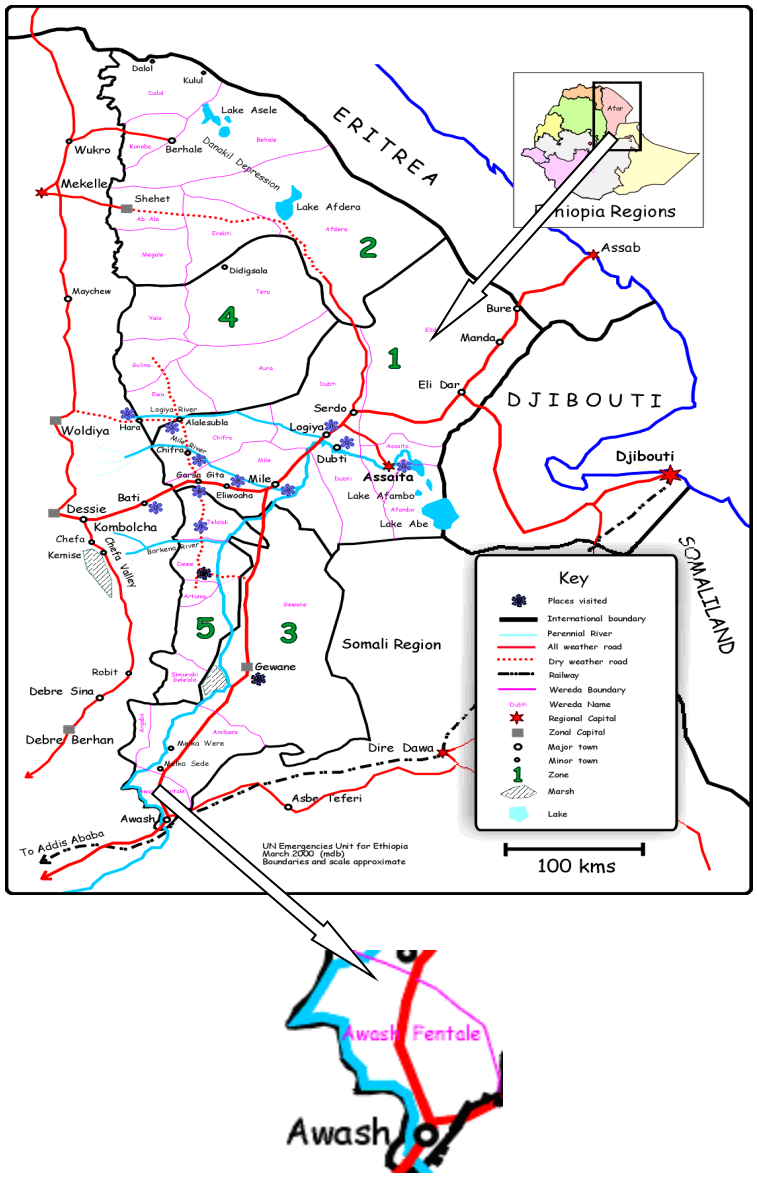

Figure1. Map of the Afar National Regional state of Ethiopia showing the study area, the Awash Fentale District (Adapted and modified from UN Emergencies Unit for Ethiopia, 2000).

3.1.2. Data collection Method

The study was conducted from November 2007 to December 2007. Study site and informant selection for ethnobtanical data collection was made based on the method given by Maritn (1995) and Hoft *et al.* (1999). Thirty informants (10 claimed traditional healers and 20 randomely picked informants) were selected. The knowledgable informants were selected based on comments and recommendations from religious leaders, elders and the local officials. The other informants were selected randomly from the community questioning every person in the house or in working fields. Ethnobotanical data were collected using semi structured interviewing (Annex I). Oral consent was obtained from every respondent.

A research assistant was identified who had grown up in the area and knew the people and the language well. The assistant accompanied the researcher during the interviews and was helpful in wining trust of the respondents as well as in translating and explaining any hidden meanings.

Various ethnobotanical skills were applied in the field work such as personal discussion on management of malaria through herbal preparations, local means of identifying anti malarial plants, direct observations of what the local people were using or selling in the markets as well as taking part in the local people's activities such as religious practices and medicinal plant harvests. Group interviews were carried out between very close family members, especially when there was need to identify most preferred antimalarial herbal remedies in the study areas (Hoft *et al.*, 1999).

For all the plants collected data on plant species and families; parts used; plant habit; the vernacular name and districts of the study area were entered in to excel work sheets and frequency of each species worked out. Voucher specimens were collected and preserved at the national herbarium, Addis Ababa University, after identification by botanists.

3.2. Toxicity and Antimalarial studies

3.2.1. Basis for the selection of plants for *in vivo* antimalarial study

Aloe sp. and *Cadaba rotundifolia*, were selected for the toxicity and *in vivo* antimalarial study among the plant species studied ethnobotanically. The selection was based on the frequency of each plant species cited by the local informants of the study area and their accessibility which made them easy to collect the plant parts for extraction.

3.2.2. Description of the plants used for the study

Aloe sp. (Afar –Urae, Amharic-Eret), member of the *Aloaceae*, is an erect unbranched shrub with yellowish flowers, it grows up to 1m long. The plant has a short underground stem and a green thick, juicy leaves grow in a bach and erect as a stem, with marginal teeth. The medicinal values of the different *Aloe* species have been documented. The species *A. arborescens* and *A. ballyi* are used to treat skin problems in Kenya (Newton, 1998). According to Demissew and Gilbert (1997) *A.camperi is* used in the treatment of skin problems in Ethiopia and Eritrea.

Cadaba rotundifolia (Afar-Adangele), belongs to the family *Capparidaceae*. It is a small shrub growing up to 2-4m, is evergreen and all parts are without scaly hairs but generally glandular- puberulous. Young twings are pale green. Older branches are grey brown, glabrous; widely branched down to the ground with small, circular green and have well spaced leaves with long petioles (Edwards *et al.*, 2000). It has been reported that *Cadaba rotundifolia* has a medicinal value in the management of malaria in other parts of the world. For example, in Yemen the leaf decoction of this plant species is used traditionally to treat malaria (Ali *et al.*, 2004). On the other hand, *C. rotundifolia* has medicinal value as a remedy for internal parasites; boiled leaf extract is taken orally (Kebebew *et al*., 2001).

3.2.3. Plant Extraction

Leaves of *Aloe* sp. and *C. rotundifolia* were ground into pieces using a kitchen blender grinder. Powders were extracted using water and ethanol. Powders of the plant parts in 1:10 (W/V) in distilled water/ethanol were mixed in separate Erlenmeyer flasks and placed on orbital shaker (GFL, Model 3020, Germany) at room temperature for 24 hrs.The extracts were then filtered through cotton and subsequently with Whatman filter paper (15.0 cm size). Ethanol was removed from the filtrate by rotary evaporator (Buchi RE 121, Swizerland) and the water extracts were freeze dried by using centrifugal freeze drier. The dried extracts were placed in small screw cuped bottles and kept in refrigerator at a tempreture of -70 ^{0}C until used (Ahmed, 2002)

3.2.4. Toxicity test

Acute toxicity test was done for both extracts of *Aloe* sp. and *Cadaba rotundifolia* leaves. Three groups of mice each group having 4 mice were treated orally at doses of 500 mg/kg (group 1), 1000mg/ kg (group 2) and 1500 mg/kg (group 3) for four consecutive days. Signs of acute toxicity such as death, changes in physical appearance and behavioral changes were observed for ten days (Wanyoki *et al*., 2004).

3.2.5. *In vivo* antimalarial tests

A 4 day suppressive standard test (Peter, 1996) was used againest *Plasmodium berghei in* Swiss albino mice 5-7 weeks of age obtained from Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University. The mice were fed with standard mice pellet ad libitum and given water. The test animals were put randomly into five groups each containing four mice.

The *P. berghei* used in the test was subsequently maintained in the laboratory by serial blood passage from mouse to mouse. Blood sample taken from donor mice with growing

parasitemia of 20% was diluted by 3% citrate, so that 0.2 ml contained $1x10^6$ - 10^7 infected erythrocytes. Mice used in the study were infected intraperitonially on day 0.

The treatment group was subdivided in to sub groups 1 (treated with 300 mg/kg of the extracts), 2 (600 mg/kg), 3 (900 mg/kg), 4 (control given the vehicle, 0.4 ml distilled water) and 5 (positive control that was given chloroquine, 10m g/kg). The above doses are for water and ethanol extracts of both *Aloe* sp. and *C. rotundifolia* leaves. Treatment continued daily for four consecutive days starting 3 hours after infection, that is, from day 0 (D_0) to day 4 (D_4).

Thin smears of blood films were obtained from the peripheral blood on the tail from each mouse and percentage parasitemia was recorded on day 4, following the procedure used by Stayvivad *et al.* (1998). The packed cell volume (PCV) was measured to predict effectiveness of the extracts.

3.2.6. Data analysis

The ethnobotanical data were entered to an excel sheet to determine the frequencies of the plant species in the respective study sites.

Comparison on the number of medicinal plants cited by the knowledgable and randomly picked informants was made.

Results from the *in vivo* study were presented as a mean plus or minus standard error of the mean (M±SEM). Statistical significance was determined by one way analysis of variance (ANOVA) using computer soft ware. Students paired t-test was used to compare parameters with in groups. For all the data values with $p< 0.05$ were considered statistically significant.

Percentage parasitemia and percentage suppression for each extracts were calculated as:

$$\% \text{ Parasitemia} = \frac{\text{number of infected RBC}}{\text{number of infected RBC+ number of uninfected RBC}} \times 100$$

$$\% \text{ Suppression} = \frac{\text{Parasitemia in negative control - Parasitemia in extracts}}{\text{Parasitemia in negative control}} \times 100$$

For all the groups weights in grams and survival time in days were recorded and the mean for each group is calculated.

4. RESULTS

4.1. Ethnobotanical Results

A total of 19 plant species were identified and documented as antimalarials in the study area (Table 1). The people in Awash Fentale District use different plant parts for making herbal preparations namely leaf, root, bark, fruit and in some cases the whole plant to treat malaria. The most frequently cited plant species among the plant remedies were *Aloe* sp., *Cadaba rotundifolia, Monadenium reflexum* and *Carica papaya.* The first species (*Aloe* sp.) is used in all the four kebeles while the others were cited in two of the study sites.

In this study, thirty informants were interviewed from the four kebeles. Among these, 24 (80%) have the experience of using at least one medicinal plant species as antimalarial remedy. Only one informant was woman while all the others were men. The average age of the informants was 52 years. The majority of the informants were from Sabure, 41.7% (N=10) and the least from Awash 7 kilo, 16.7% (N=4) (Appendix II). The majority of medicinal plants were collected from Sabure (50%) followed by Kebena (25 %) (Table 2).There was no a significant difference on the average number of medicinal plants claimed by the knowledgable (2.9) and randomly picked (2.2) informants in the study area.

Table 1. Antimalarial plant species collected from the Awash Fentale District of the Afar Region of Ethiopia.

Botanical name	Family name	Vernacular name	Habit	Part used	Administr-ation	Treatment preparation
Acacia mellifera Benth.	Fabaceae	Merkeato	shrub	whole	oral	Decoction, hot water extract
Acalypha friticosa Forssk.	Euphorbiaceae	Migameli	shrub	leaf	oral	Cold water infusion
Aloe sp.	Aloeaceae	Urae	shrub	leaf	oral	Cold water infusion
Azadirachta indica A.Juss	Meliaceae	Neem	tree	leaf	oral	Decoction in water
Barleria sp.	Acanthaceae	Yamerktu	shrub	whole	oral	Cold water infusion
Boswellia papyrifera (Del.) Hochst.	Bursseraceae	Melmele	tree	bark	oral	Hot water decoction
Cadaba rotundifolia Forssk.	Capparidaceae	Adangele	shrub	leaf	oral	Cold water infusion
Carica papaya L.	Caricaceae	Papaya	tree	leaf	oral	Cold wter infusion with honey/sugar
Casuarina equissetifolia (Fosberg) Sachet.	Casuarinaceae	Sagento	shrub	stem	oral	Decoction in water
Celosia polystachia (Forssk.) C.C. Townsend	Amaranthaceae	Kontoma	shrub	stem	oral	Cold water infusion in soup
Cucumis sp.	Cucurbitaceae	Hashrel-ajer*	shrub	root	oral	Root boiled, decoction
Grewia schweinfurthii Burret.	Tiliaceae	Hidaytu	herb	Leaf / stem	oral	Cold water infusion
Indigofera coerulea Roxb.	Fabaceae	Kimbiro-hada	shrub	root	Nasal / oral / fumigation	Decoction, root boiled in 'Nech shinkurt'
Indigofera coerulea Roxb.	Fabaceae	Hawda* (lesser)	herb	Leaf / root	oral	Hot water decoction
Monadenium reflexum Chiov.	Euphorbiaceae	Labnema	shrub	stem	oral	Cold water infusion
Salvadora persica Brenan	Salvadoraceae	Adaytu	shrub	Leaf/ stem	oral	Decoction in water
Senna italica Mill.	Fabaceae	Selmeky	shrub	leaf	oral	Cold water infusion
Senna sp.	Fabaceae	Hawda* (greater)	herb	Leaf / root	oral	Hot water decoction
Terminalia brownie Fresen.	Combertaceae	Woybu	tree	Leaf / bark	oral	Cold water infusion

* = vernacular names in Sudanese (Arabic)

Table 2. List of plant species used for malaria treatment by frequency in Awash Fentale District.

Botanical name	Family name	Vernacular name	Frequency	Place cit ed
Acacia mellifera Benth.	Fabaceae	Merkeato	1	Sabure
Acalypha fruticosa Forssk.	Euphorbiaceae	Migameli	1	Sabure
Aloe sp.	Aloeaceae	Urae	12	Awash Sebat Kilo,Birget, Kebena, Sabure
Azadirachta indica A.Juss.	Meliaceae	Neem	4	Awash 7 killo, sabure
Barleria sp.	Acanthaceae	Yamerktu	1	Kebena
Boswellia papyrifera (Del.) Hochst.	Bursseraceae	Melmele	3	Birget
Cadaba rotundifolia Forssk.	Capparidaceae	Adangele	7	Birget,Kebena
Carica papaya L.	Caricaceae	Papaya	5	Awash 7 Kilo,Sabure
Casuarina equisetifolia (Fosberg) Sachet.	Casuarinaceae	Sagento	1	Sabure
Celosia polystachia (Forssk.) C.C. Townsend	Amaranthaceae	Kontoma	1	Sabure
Cucumis sp.	Cucurbitaceae	Hashrel-ajer	2	Sabure, Kebena
Grewia schweinfurthii Burret.	Tiliaceae	Hidaytu	2	Sabure
Indigofera coerulea Roxb.	Fabaceae	Kimbiro-hada	2	Sabure, Kebena
Indigofera coerulea Roxb.	Fabaceae	Hawda (lesser)	2	Sabure
Monadenium reflexum Chiov.	Euphorbiaceae	Labnema	8	Sabure, Kebena
Salvadora persica Brenan.	Salvadoraceae	Adaytu	1	Sabure
Senna italica Mill.	Fabaceae	Selmeky	3	Kebena
Senna sp.	Fabaceae	Hawda (greater)	2	Sabure
Terminalia brownie Fresen.	Combertaceae	Woybu	3	Birget

Informants of the study areas made all the crude preparations using water as a medium. The preparations were administered orally either as crude extracts; hot water decoction or cold water infusions. In some cases the ingredients of the herbal preparation also included honey or sugar to make the preparations palatable and the remedy more powerful. Based on frequency with which the respondents mentioned the antimalarial species; it was possible to establish the most important species in each kebele (Table 3).

Table 3. Plant species commonly used in the traditional treatmentof malaria in four selected kebeles of the Awash Fentale District

Sabure		**Birget**		**Awash7 kilo**		**Kebena**	
Plant species	Frequency	Plant species	Frequency	Plant species	Frequency	Plant species	Frequency
Monadenium reflexum *Aloe* sp.	5 4	*Aloe* sp. *Cadaba rotundifolia* *Boswellia papyrifera* *Terminalia brownii*	5 4 3 3	*C. papaya*	3	*C.rotundifolia* *s. italica* *M.reflexum*	3 3 3

Various parts of the plants were cited as main ingredients useful in the preparation of antimalarial herbal remedies. Leaves were mentioned as antimalarial from most of the plants mentioned (Fig. 2). The majority of the species (44%) were obtained from the leaves. The use of stem, root, the whole plant and the bark of a given species were also mentioned to be used for the preparation of herbal remedies found to be the parts used in preparing the antimalarial herbal remedies.

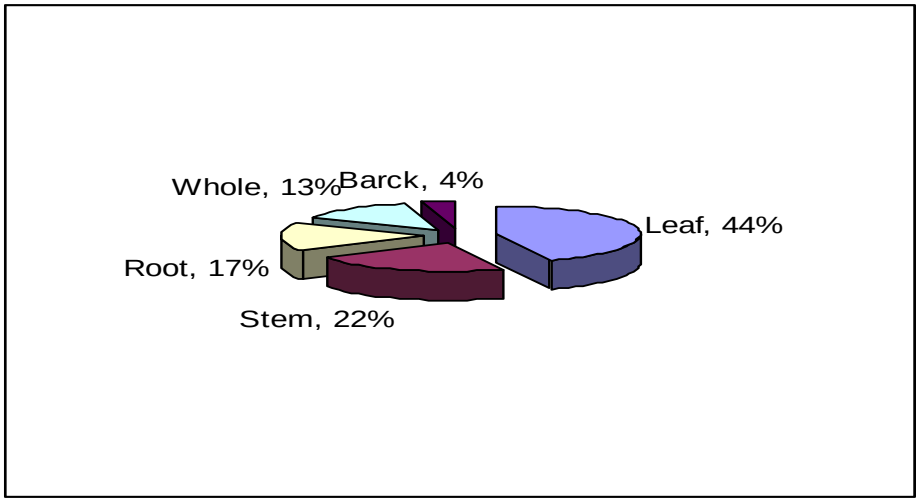

Figure 2. Plant parts utilized in management of malaria in Awash Fentale District

Most of the plants cited to have antimalarial activities by the informants (63%) were shrubs (Fig. 3). The next groups of plants mentioned (21%) were trees. Herbs gained the lowest recognition to be used in the traditional treatement of malaria by the informants.

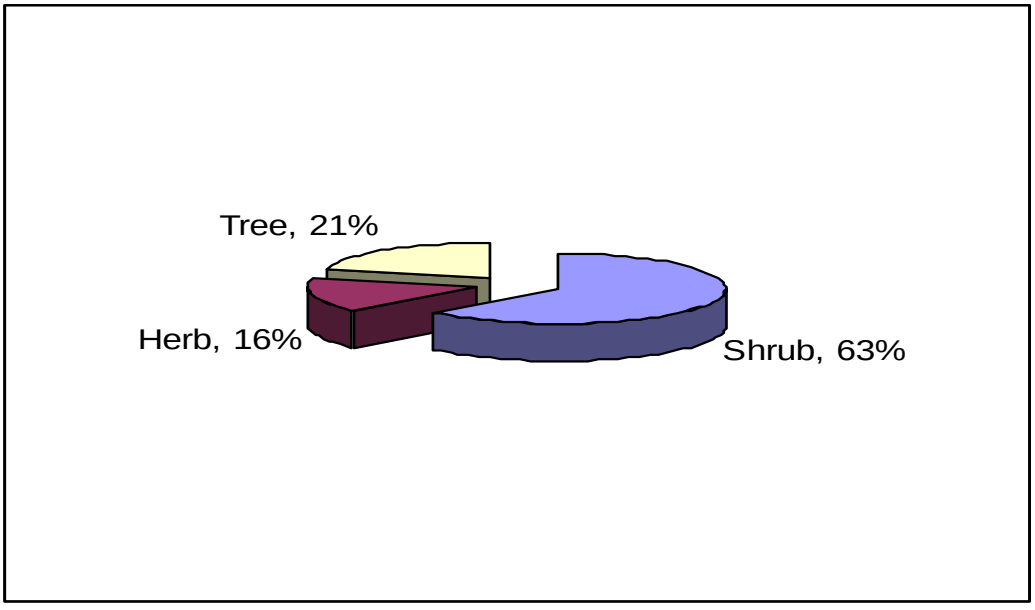

Figure 3. Habit of the plant species used in management of malaria in Awash Fentale District

4.2. Toxicity and Antimalarial activity of the plant extracts

4.2.1. Acute toxicity test

Water extracts of *C. rotundifolia* and *Aloe* sp. leaves showed no lethal effect up to 1500 mg/kg of doses, when administered orally. The mice were attended for over a week but no sign of toxicity was observed (Table 4). Gross behavioral and physical observations revealed no urination, no muscle weakness and no body weight loss. They were physically active.

Table 4. Body weight of mice after the administration of *Aloe* sp. and *C.rotundifolia* aqueous extracts.

plant species	**Dose mg/kg**	**Body wt. D_0**	**Body wt. D_4**	**%body wt.change**
Aloe sp. leaves	500	25.63 ± 0.7	26.30 ± 0.34	2.54
	1000	24.97±0.22	25.57±0.34	2.35
	1500	25.72±0.61	26.20±0.63	1.8
Dist. H_2O	1ml	30.60±0.55	28.33±0.38	-8.01
C. rotundifolia leaves	500	28.20±0.29	28.60±0.4	1.4
	1000	27.34±0.62	27.64±0.71	1.08
	1500	29.23±0.83	29.97±0.81	2.47
Dist. H_2O	1ml	26.33±0.96	26.1±0.88	-0.88

D_0= 1stday administration was started $p<0.05$

D_4= 5th day of administration The result is mean of mice ± SEM

n = 4

The plant extracts did not cause weight loss at all dose levels compared to their respective controls. The increase in body weight is not consistent with increasing dose of the extracts.

Similarly, ethanol extracts of the two plants leaves showed no lethal effect up to the dose of 1500 mg/kg. Mice treated with ethanol extracts of *Aloe* sp. leaves did not show sign of

toxicity (Table 5). Gross behavioral and physical observations revealed no urination, no muscle weakness and no body weight loss.

Table 5 .Body weight of mice after the administration of *Aloe* sp. and *C. rotundifolia* ethanol extracts

Test plant species	**Dose mg/kg**	**Body wt. D_0**	**Body wt.D_4**	**%body wt.change**
Aloe sp. leaves	500	24.34±0.53	24.83±0.71	1.9
	1000	25.25±0.38	25.98±0.88	2.8
	1500	27.42±0.62	27.83±0.72	1.4
Dist. H_2O	1ml	24.57±0.85	24.35±0.75	-0.9
C. rotundifolia leaves	500	28.21±0. 64	27.39±0.71	-2.9
	1000	26.24±0.34	26.63±0.83	1.4
	1500	28.43±0.54	28.92±0.63	1.7
Dist. H_2O	1ml	27.64±0.91	26.86±0.87	-2.9

Oral administration of ethanol extracts of *C. rotundifolia* leaves revealed no toxicity signs. The mice were physically active, no body convolution, and active to feed. However, some body weight loss was observed on the fifth day in mice given a dose of 500mg/kg. Mice delivered 1000 and 1500mg/kg of the ethanol extract of *C. rotundifolia* showed no body weight loss and were physically active.

4.2.2. *In vivo* antimalarial tests

Examination of Giemsa stained thin smears of blood from mice on day 4 showed lower parasitemia in all the mice treated with extracts of *Aloe* sp. and *C. rotindifolia* as compared to their respective negative controls. However, parasitemia was not cleared in the experimental groups unlike in the positive control groups treated with chloroquine. The highest *P. berghei* suppressive value recorded was 53.73% and the lowest was 3.85%.

Mice treated with 600mg/kg and 900mg/kg of ethanol extracts of both plants showed significantly lower parasitemia ($p< 0.05$). But treatement at the dose of 300mg/kg did not show significant effect against the parasitemia as compared to the negative control (Table 6 and 7).

As compared to the ethanol extract, water extract of *Aloe* sp. showed no significant suppressive effect on the parasite ($P> 0.05$) (Table 6).

Table 6. Activity of water and ethanol extracts of A*loe* sp.leaf against *Plasmodium berghei* in mice.

Extract	day	parameter	Dist. H_2O, n = 4 (-ve control)	300mg/kg n = 4	600mg/kg n = 4	900mg/kg n = 4	10mg/kg, n = 4 CQ (+ control)
Dist.H_2O	Day 0	Wt.	23.6±0.75	25.86±1.26	24.89±1.59	25.48±2.81	26.68±0.96
	Day 4	Wt.	21.71±0.93	25.59±0.70	25.13±2.20	27.86±0.53	27.83±0.57
		PCV	53.76±0.74	47.69±0.23	54.61±0.87	51.88±1.3	54.76+0.84
		% para.	13.62±0.57	11.67±1.88	10.97±0.21	9.76±0.89* [a]	0
		% supp.	0	18.0	23.0	31.5	100
		Mean survival time (days)	7.5 ±0.39	10.67±1.48	8.6±0.93	9.4±0.77	13.65±0.78
EtOH	Day 0	Wt.	25.63±0.87	29.4±1.14	30.16±2.6	30.84±1.84	24.35±0.77
	Day 4	Wt.	24.2±058	30.30±2.07	31.33±4.16	32.50±1.01	24.62±0.83
		PCV	48.02±0.64	51±0.01	47.17±0.41	51.01±1.41	52.34±0.47
		% para	13.92±0.91	10.01±1.71	8.88±1.21** [a]	7.09±1.28* [a]	0
		% supp.	0	28.09	36.27	49.07	100
		Mean surv.time (days)	5.5±0.65	10±1.82	10.5±1.19	13±1.06*[a]	12.97±0.75

* = $p<0.01$, ** = $p<0.05$ a=comparison against –ve control n = number of mice used in each group

Extracts of *Aloe* sp. leaves did not cause reduction of body weight in the infected mice even with increasing parasitemia. Analysis of packed cell volume (PCV) on day four indicated that water and ethanol extracts of leaves of both *Aloe* sp. and *C. rotundifolia* showed insignificant effect on PCV values of the animals. Ethanol extract of *Aloe* sp.caused 36.27 and 49.07% parasite suppression when treated with the doses of 600 and 900mg/kg, respectively.

The mean survival time of the mice treated with 600mg/kg and 900mg/kg ethanol extract of *Aloe* sp.leaves was 10.5 $\pm$1.19 and 13 $\pm$1.06, respectively. Where as the mice in the negative control lived 5.5 $\pm$ 0.65 days. On the otherhand, mean survival time of those treated with water extract of the leaves of *Aloe* sp. at a dose of 600mg/kg and 900mg/kg were 8.6 $\pm$ 0.93 and 9.4 $\pm$ 0.77 respectively.

As compared to the ethanol extract, water extract of *C. rotundifolia* showed no significant suppressive effect ($p>0.05$) on the parasite at all doses administered to the experimental group compared to the negative control (Table 7).

The percentage parasite suppression on day four in mice treated with 300mg/kg, 600 mg/kg and 900mg/kg of ethanol extract of the leaves of *C. rotundifolia* was found to be 30.46, 34.84 and 53.73, respectively.

Table 7. Activity of water and ethanol extracts of *Cadaba rotundifolia* leaf against *Plasmodium berghei* in mice

solvent	day	parameter	Dist. H_2O (-ve control)	300mg/kg	600mg/kg	900mg/kg	10mg/kg CQ (+control)
Dist. H_2O	Day 0	Wt.	25.34±0.76	28.64±1.34	24.66±2.12	22.15±0.99	24.38±0.69
	Day 4	Wt.	24.25±0.92	24.54±1.39	27.53±1.30*b	19.18±1.16	26.35±0.78
		PCV	52.14±0.87	59.04±1.87	58.48±0.82	57.32+1.00***a	58.45±0.56
		% para.	14.24±0.76	13.74±1.94	12.01±1.16	8.46±0.2	0
		% supp.	0	3.85	15.96	40.80	100
		Mean surv.time (days)	6.24±0.57	8.5±0.5	9.2±0.58	11.5±1.5	14.36±0.71
EtOH	Day 0	Wt.	34.30±1.12	22.05±0.50	27.90±1.07	25.92±0.39	25.58±0.88
	Day 4	Wt.	27.23±1.94	24.22±1.33	29.21±1.09	24.70±0.61	25.86±0.71
		PCV	53.34±0.71	53.93±1.16	52.32±0.49	56.38±1.22	57.38±0.44
		% para	13.92	9.48±2.21	9.07±1.67**a	6.44±0.34**a	0
		% supp.	0	30.46	34.84	53.73	100
		Mean surv.time (days)	7.15±0.82	11±0.91	11.75±1.10	13.5±0.96**a	13.70±0.83

*** = $p< 0.001$ b = difference between D_0 and D_4

The mean survival time of the mice treated with 600mg/kg and 900mg/kg ethanol extract of leaves of *C. rotundifolia* was 11.75 ± 1.10 and 13.5 ± 0.96, respectively. Where as that of the negative control group was 7.5 ± 0.15. On the other hand, mean survival time of water extract of the leaves of *C. rotundifolia* at a dose of 600mg/kg and 900mg/kg were 9.2 ± 0.58 and 11.5 ± 1.5 respectively which is better eventhough not significant as compared to the negative control group of 6.24 ± 0.57.

5. DISCUSSION

The absence of potent antimalarial vaccines coupled with the emergence of drug resistant *P. falciparum* necessitated the need for novel, well tolerated and more efficient antimalarial drugs affordable to the poor, living in malaria endemic tropical countries. In view of this, many species of plants have been reported to be used in different parts of the world and in Ethiopia (Sorssa, 1992; Bogale and Petros, 1996; Hunde, 2001; Animut, 2002; Teklemariam, 2005; Dikasso *et al.*, 2006; Gebre, 2006) to be used to treat the disease. In spite of this fact, the list of species of plants used by the indeginous communities for the treatement of malaria in Ethiopia is yet to be exhaustively recorded.

This study was not able to determine the knowledge difference between men and women in the study area since women interviewed were not wiling to give information on usage of medicinal plants despite the attempts made to convience them about the importance of the study for the coming generation and the country in the preparation of better antiplasmodial remedies.

The average number of medicinal plants reported by the knowledgable informants is almost the same to that of the randomly interviewed ones. This is an indicative that there is no knowledge difference on the use of medicinal plants between the informants in the study area.

Plants cited by the respondents such as *C. papaya*, *A. indica*, *Aloe* sp., *Senna sp.*, *M. reflexum* and *A. friticosa* are also reported to be used in other parts of the country and Africa (Bourdy *etal*., 2005; Birhanu *et al*., 2006; Giday *et al.*, 2006; Teklehaymanot *et al*., 2006; Njoroge and Bussman, 2006; Bora *et al*., 2007; Muthaura *et al*., 2007). The frequency results showed that the plant species such as *Aloe* sp., *C. rotundifalia, C. Papaya* and *A. indica* were found to have relatively higher occurence. This shows that these plants are widely used in the study area.

The information on the frequently utilized antimalarial plant species is also an important lead to the species that can be targeted for the production of alternative antiplasmodial

drugs. Development of new antimalarial drugs from plant sources may address the global drug resistant problem of malaria (Gessler, 1995).

The leaf was the most commonly used part of the plants in the study area and was found to be advantageous for conservation and harvesting strategies to facilitate sustainable utilization of these plant species. This may be due to the fact that some plants can replace their leaves during certain periods of the year. There is also a significant usage of the root bark of the plant in the study area which could be destructive when the whole plant has to be uprooted. Trees and shrubs are the most widely used plants used to obtain antimalarial remedies in the study area. This is may be due to the accessibility of these plants through out the year tolerating the harsh environment of the study area.

The preparation of the remedies from a single plant in the study area may indicate the confidential use of the local healers and those who use the plants for self treatement. Most of the remedies in the study area were found to be prepared from a single plant, only two plant species, *C. papaya* and *I. coerulea*, have been mentioned to be used in combination with 'Nech shinkurt' (*Allium sativum*). Honey was cited to be an ingredient added to the *C. papaya* by the local healers assuming that it will make the remedy palatable and give more strength to relieve from pain.

The methods of preparation of the remedies as a long lasting decoction and infusion using hot and cold water as a medium and dosage determinations needs a further scientific support so as to validate a standard protocol. In the study area, all the plant materials are put in a large pot, macerated in water for abut four to five days. A dose is taken from the pot, without filtering the plant material.

The dose preparations containing *Aloe* sp., designated by all informants as extremely bitter, is a small coffee cup (50ml) three times a day until the patient gets a sign of relief. Hot water decoction is applied for plant parts usually other than leaf (root, bark and whole part of the plant). The reason behind administering warm remedies is that they cause profuse sweating and through which they releave from fever and malaria.

The observed antimalarial activity of *Aloe* sp. and *C. rotundifolia* is consistent with the traditional use of the plants as herbal medication against the disease in the Awash Fentale District. The observed protection of the infected animals from losing body weight by the leaf extract of the tested plant species in the present study is in conformity with the traditional use of the plant in the management of malaria in the study area and as well as in North Ethiopia as reported by Giday *et al*.(2006).

The medicinal plants are collected from an area which is facing great pressure due to over utilization of indigenous trees and some may disappear before their uses are documented. According to the report of the *1997 IUCN Red List of Threatened Plants* records, 11 species out of the 20 endemic species of *Aloes* are rare or threatened in Ethiopia. Habitat destruction is the main threat to critically endangered species. Although *Aloe* sp. can survive in inhospitable conditions, today even marginal lands are being cleared in some areas (Demissew, 2003). According to this report Aloe species such as *A. tewoldei, A.scholleri, A.mcloughlinii, A. kefaensis* and *A.harlana* are critically endangered.

The majority of the population in Awash Fentale District especially the rural sites residents are pastoralists with low income status and very often medicinal plant use is the only affordable treatment option both for themselves and their livestock. The use of medicinal plants, therefore, may continue to be part of the health care system to the community in the future. This calls for further and urgent ethnobotanical exploration which is linked to the need for sustainable conservation strategies for medicinal plants.

According to the informants report and field observation antimalarial medicinal plants such as *M. reflexum (*Euphorbiaceae) and *Aloe* sp. (Aloeaceae) are becoming rare in the nearby localities and they traveled longer distances to collect them. Hence immediate conservational measures are nedded so as to minimize the danger of loosing these plant species in the study area.

Most of the knowledge of medicinal plants is transferred orally in many communities and there is, therefore, the danger of losing this precious cultural heritage (Muthoura *et al*, 2007). Hence ethnobotanical studies are useful in documenting, analyzing and

disseminating the knowledge on the interaction between biodiversity and human society and how it is valued in different societies and how it is influenced by human activities (Gebre, 2005). Moreover, ethnobotanical information is used for further evaluation of efficacies of plant remedies and for isolating and identifying of new antimalarial drugs.

Aloe sp. and *C. rotundifolia* were selected based on the frequency of informants towards the plants. The water and ethanol extracts of both plants did not show acute toxic effect in mice. However, the ethanol extract of *C. rotundifolia* leaf showed some body weight loss at 500mg/kg. The observed weight loss of the mice treated with *C. rotundifolia* may possibly be due to the appetite suppressive effects of the extracts, which would reasonably increase with increasing dose. This in turn can affect the feeding capability of the mice and could cause a relative reduction in body weight, which could be indicative of toxicity. However, in the present study, the reduction in body weight was not consistent with increasing dose levels. Hence, the loss of body weight at the indicated dose may be due to the mice were not using the standard pellet provided.

In other studies, *C. rotundifolia* was found to be toxic for goats showing signs of depression, diarrhea, frothing at the mouth, dyspnea, ataxia, loss of condition and recumbency (El Dirdiri *et al*., 1987). However, this in contradiction to the non toxicity of the tested plant species. *Aloe* sp. caused no death and acute toxic effect indicating that the test extracts were safe. This could also explain the safe use of the plant by the local people, who have been using it in traditional treatment of malaria, in the Afar Regional State of Ethiopia.

The genus *Aloe* consists nontoxic species such as *Aloe vera*. (Kathleen, 2001). The non toxic property of this plant makes it to be a common element in cosmetic products. While raw leaf juice was traditionally used as laxatives, its mucilaginous gel is casually used to treat burns and cuts. Although the indicated non toxicity of the *A. vera* is for external use, further in vivo studies on this plant may find out a supportive evidence for the tested aloe sp. in this study.

The 4-day antimalaria suppressive test is a standard test commonly used for antimalarial screening, and the determination of percent inhibition of parasitemia (Peters *et al*., 1975). The rodent models are found to provide valid prediction of efficacy in humans in the identification of several conventional antimalarials, such as chloroquine, halofantrine, mefloquine and more recently artemisinine derivatives. *P. berghei* are used in the prediction of treatment outcomes.

The *in vivo* antimalarial suppressive experiment showed that water extracts of leaves of *C. rotundifolia* did not induce significant *in vivo* suppression effect on *P. berghei* compared to their ethanol extracts. This is in contradiction to the traditional use by the local people. This may be due to the use of combination of plant remedies in the traditional practice which gives possible synergism between the complex and heterogeneous mixtures of different compounds (Wanyoike, 2004).

The low antiplasmodial activity of some traditionally used plants could be due to circumstances that many plants are used in the treatment of malaria not for antiparasitic effect (curing the disease) but because of other activities (like reducing fever; easing convulsions and headache; and as immunostimulatory agents) with therapeutic value for a patient with the disease. Another possible reason for low antimalarial activity is that traditional healers give a mixture of many plant homogenates for the treatment of a disease, making it difficult to get the right plant (Wanyoike, 2004; Teklemariam, 2005).

On the other hand, the results obtained from *in vivo* antimalarial activity of ethanol extracts of the leaves of *C. rotundifolia* are in agreement to the reported traditional usage in the study area. Previous ethnobotanical study in other parts of the region (Kebebew *et al*., 2001) also indicated that decoction of the leaves of *C. rotundifolia* is an effective remedy to manage malaria. The ethanol extracts of the leaves of *C. rotundifolia* reduced the parasite load significantly at doses of 600 mg/kg and 900mg/kg at the fifth day of the treatment.

The suppression induced by the extracts might be associated with the presence of chemical ingredients that have antimalarial properties. Different *Cadaba* species were reported to contain alkaloids and sesquiterpene lactones. Cadabicine and cadabicine acetate spermidine

alkaloids) were isolated from the stem bark of *Cadaba farinosa* (Viqar et *al.*, 1975, 1985, 1987). A new flavonol triglycoside, rhamnocitrin-3-O-neohesperoside-4_-O-glucoside was isolated from the ethanol extract of *Cadaba glandulosa* together with two known diglycosides rhamnocitrin- 3-O-neohesperoside and rhamnetin-3-neohesperoside (Ahmed, 2002).

On the other hand, the observed antimalarial activity of *C. rotundifolia* could also be associated with the existence of alkaloids in its leaves Water extracts of *Aloe* sp. leaves exhibited no significant antimalarial activity against *P. berghie* compared to their ethanol extracts, which is not in agreement with the traditional use of the plant to treat malaria in the region and the reports in other parts of Ethiopia (Giday *et al*., 2006). The fact that the water extracts are less acive than the ethanol extract extracts are much less active could be due to the difference in the efficiency of the solvents in extracting the antimalarial bioactive compounds in the leaves. synergism effect of the different mixtures of the combinations used by the traditional healers.

The result of the *in vivo* study of the ethanol extracts of the leaves of *Aloe* sp. showed significant suppression of parasitemia at dose level 900mg/kg. This is in agreement with the ethnobotanical information obtained in the study area, Awash Fentale district of the Afar National State.

In untreated mice, the parasite count increased and the hematocrit packed cell volume (PCV) decreased markedly from day to day until the death of the animal, which was also observed in previous studies (Ayodele, 1979). Although lesser percentage reduction in PCV was observed with in groups of the different doses of the extracts in the present study, the overall effects were not consistent and conclusive in the case of PCV.

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but this study showed that ethanol extracts of these plants were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvents (Boer *et al.,* 2005).

CONCLUSION

Indigenous plants play important roles in the treatment of many diseases including malaria. The world's 80% population is estimated to use herbal remedies. Due to the fact that the resistance of malaria parasites to available drugs continues to be a serious concern as it increasingly limits the control of the disease, the interest of the use of plant products to treat the disease has been growing in many parts of the world.

Many communities in Africa, including Ethiopia, have much elaborated plant knowledge. Most knowledge on medicinal plants is transferred orally in many communities and here is therefore, the danger of losing these precious natural heritages.

Drug resistant strains of *P. falciparum* have been found in many endemic areas of the world and majority of the available antimalarial drugs have been associated with treatement failure. Despite the problem of drug resistance, chemotherapy remains one of the key control measures against the high burden of malaria. However, drug development program is constrained by factors such as efficacy, safety and additional properties for the specific disease indication, little investment by pharmaceutical companies and lack of specialized experts.

The ethnobotanical data collected in this study has shown the presence of 19 plant species that are used traditionally for the management of malaria in the Awash Fentale District of the Afar National Regional State of Ethiopia. Among these species of plants the *in vivo* antimalarial activity studies of crude ethanol extracts of the leaves of *Aloe* sp. and *C. rotundifolia* demonstrated the presence of suppressive effects. However, the antimalarial activity of the water extracts of both plant species was lower as compared to their ethanol extracts. The *in vivo* antimalarial activities of *Aloe* sp. and *C. rotunidfolia* crude extracts of leaves may partly explain and support the traditional use of the plants for malaria treatment.

RECOMMENDATION

Despite the presence of a wide knowledge and distribution of antimalrial medicinal plants in the study area, depletion of most plant species in general and antimalarial medicinal plants in particular is on progress due to both human and natural factors. Thus, appropriate conservation measures with thorough investigation of the present status especially of rare and unique medicinal species should be promoted.

In view of the rapid loss of natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, an increasing number of ethnobotanical inventories need to be conducted. Moreover, the traditional protected area system with modern conservation principles, raising awareness of the people of the area and improving the socio-economic conditions of the local people are recommended to reduce or stop mis- use of plant resources, particularly antimalarial ones of the area.

In screening plant derived compounds for antimalarial activities, discrepancy between *in vivo* and *in vitro* results must be pointed out. Further detailed antimalarial evaluations, phytochemical analysis and toxicological studies on these plants may result in appropriate dosages as potential sources of antimalarial drugs development with better mechanisms of pharmacological action.

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Annex I. Sem-structured interviewed items used during the Ethnobotanical study

1. Facts about Respondents.

1.1. Addresses

1.1.1. Region……..

1.1.2. Zone……

1.1.3. Woreda……....

1.1.4. Kebele……..

1.1.5. Locality……..

1.2.Identification

1.2.1. Name……

1.2.2. Sex……

1.2.3. Age……..

1.2.4. Ethnic group……

1.2.5. Religion……

1.2.6. Education…….

2. History of Traditional Healer

2.1.Years of service……

2.2. From whome did you acquire the knowledge?

2.3.Acceptance of traditional medicine by the community you live in –acceptance, no acceptance,I don't know.

2.4.If it is accepted, why? – Effectively of traditional medicine, cheapness of traditional medicine, lack of access to modern medicine, all of the above,I don't know.

2.5.To whom do you want to share your knowledge? Your first son or daughter, your beloved son or daughter, to any child of yours, to all of your children, to your wife, to all members of your family, others.

2.6.Do you collaborate with other traditional healers?

2.7.Do you document your traditional medicinal practice?

3. History of plant

3.1.Common name or vernacular name……..

3.2.Location of the plant…….

3.3.Where do you obtain the medicaments? Purchase, home garden, farm land forest, grass land, open land, others.

3.4.How do people catch malaria?

3.5.What other health problems for which the plant is used as remedy…….....

.....

3.6.Plant part used-leaf, root, root bark, stem bark, flower, fruit, and seed.

3.7.Methods of preparation-decoction, poulitices,, infusion, juice, bath syrup.

3.8.Are there any plant and /or ingredient added? If yes, what type of plant or ingredient is added?

.....

.....

3.9.Mode of addminstration -ointement, swallow, drink, chew, smoking, fumigation, others.

3.10. How do you quantify the dosage?

3.11. What factors determine the dosage? - age, sex, illness, pregnancy, any others.

3.12. Number of days and number of treatments in a day

3.13. Any side effect of the administrated medicament- diarrhea, diuretic, vomiting, insomnia, others.

3.14. Any other use of the medicinal plant- edible, spice, building, charcoal, fire wood, fodder.

3.15. Are the medicinal plants you are using still available, disappeared, available in far places, I don't know.

3.16. If disappeared, name of disappeared medicinal plants

.....

.....

3.17. Are you making any attempt to conserve or maintain the medicinal plants you are using?.....

.....

4. **Other informations that will be recorded**.

4.1.Collection number of the plant…….....

4.2.Brief description of the plant (including habit, habitat, location where collection will be made)…….....

4.3.Any special information indicated by the donor of the information such as drought resistance and others.....

24	Yesuf Kedir	M	61	Kebena	*I coerulea* (Kimbiro-hada), *S. italica(*Selmeky), *Barleria sp*.(Yamerktu)

Annex II. List of informants participated in this ethnobotanical study on traditionally used antimalarial plants in the Awash Fentale district

No.	Name of informants	Sex	Age	Kebele	Cited plant species (Vernacular name)
1.	Aba Abdu Sebil*	M	78	Sabure	*Aloe debrana (*Urae),*M.reflexum. (* Labnema*)*
2	Aba Bule Hussen*	M	45	Sabure	*Aloe debrana (*Urae), *S. persic.*(Adaytu),. *M.reflexum (* Labnema*)*
3	Aba Raya Telah	M	62	Sabure	*A. friticosa (*Migamely),*Carica papaya (*Papaya*)*
4	Abrar Seid	M	42	Kebena	*Cadaba rotundifolia (* Adangele*),* *M.reflexum (* Labnema*)*
5	Ayder Sh.Telah*	M	70	Awash 7 killo	*Carica papaya (*Papaya*),* *Azadirachta indica (*Neem*)*
6	Aysha Hussen	F	40	Awash 7 killo	*Aloe debrana (*Urae)
7	Hawas Ali	M	38	Birgate	*Cadaba rotundifolia (* Adangele*), Aloe debrana (*Urae)
8	Hawas Kedir	M	42	Sabure	*Hibiscus sp. (*Hidaytu*)*
9	Jemal Kedir	M	42	Kebena	*Cadaba rotundifolia (* Adangele*),S. italica(*Selmeky*), M.reflexum (* Labnema*)*
10	Kahsu Taya	M	51	Kebena	*Cucumis sp.*(Hashrel-ajer), *S. italica (*Selmeky*), M.reflexum (* Labnema*)*
11	Mensur Beha	M	54	Kebena	*Aloe debrana (*Urae), *Cadaba rotundifolia (* Adangele*)*
12	Mohammed Abdu Endekurkura**	M	30	Birgate	*Cadaba rotundifolia (* Adangele*) Aloe debrana(*Urae) *B. papyrifera* (Melmele),*T. brownii*(Woybu)
13	Mohammed Abdu Seid*	M	47	Birgate	*Aloe debrana (*Urae), *Cadaba rotundifolia (* Adangele*) , T. brownii*. (Woybu)
14	Mohammed Sakule*	M	31	Sabure	*Aloe debrana (*Urae) *, M.reflexum (*Labnema*),I. corulea(*Kimbirohada),*A. Mellifera* (Merkeato)
15	Sakule Ako*	M	73	Sabure	*M.reflexum.(* Labnema*),* *Aloe debran (*Urae), *Azadirachta indica (*Neem*)*
16	Salah Nuri	M	35	Sabure	*Senna sp.*(Hawda-greater),*I. coerulea*(Hawda-lesser)
17	Seifedin Jemal	M	46	Birgate	*Aloe debrana (*Urae), *Cadaba rotundifolia (* Adangele*), B. papyrifera*. (Melmele)
18	Sh. Abdela Seid	M	64	Sabure	*M.reflexum (*Labnema*),Cauarina equisetifolia(* Sagento*)*
19	Sh. Bilal Mahmud*	M	57	Sabure	*Senna sp.*((Hawda-greater), *I. coerulea* (Hawda-lesser),*Cucumis sp.*(Hasherel ajer)
20	Sh. Ebrahim Aliyu*	M	45	Awash 7 killo	*Carica papaya (*Papaya*),* *Azadirachta indica (*Neem*)*
21	Sh.Hussen Mohammed Seko**	M	55	Awash 7 killo	*Aloe debrana (*Urae), *Carica papaya (*Papaya*), Azadirachta indica (*Neem*)*
22	Temam Edris	M	48	Birgate	*Aloe debrana (*Urae), *B. papyrifera* (Melmele), *T. brownii* (Woybu)
23	Temam Sh. Kedir	M	53	Sabure	*Carica papaya (*Papaya*),C. polystachia (*Kontoma*)*

* Knowledgable informant ** Informant + translator