

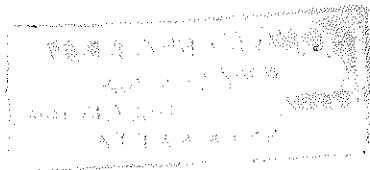
**IN VITRO EVALUATION OF THE ACTIVITY OF SOME
ETHIOPIAN TRADITIONAL MEDICINAL PLANT CRUDE
EXTRACTS AGAINST PLASMODIUM FALCIPARUM**

**BY
SOLOMON SORSSA**

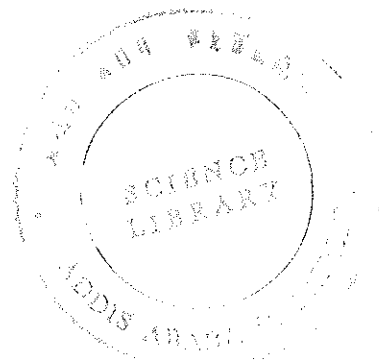


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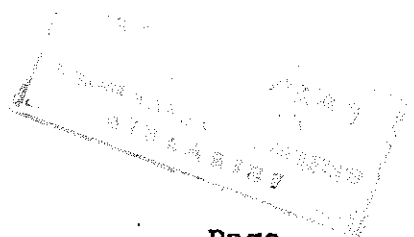
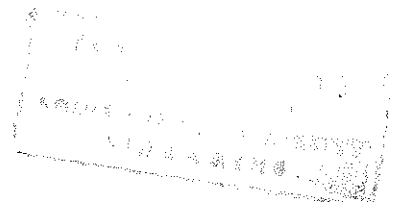


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ABSTRACT

The crude extracts of six Ethiopian medicinal plants used traditionally for the treatment of malaria were screened for their in vitro anti-malarial activity. Methanol, chloroform and ethanol extracts of the six species of plants, Croton macrostachyus Del. (Euphorbaceae); Calpurnia aurea (Alt.) Benth. (Fabaceae); Buddleia polystachya Fresen. (Loganiaceae); Terminalia schimperiana Hochst. (Comberetaceae); Dodonaea angustifolia L.f (Sapindaceae) and Jasminum grandiflorum L. (Oleaceae), were tested for possible schizonticidal effects. Of the extracts screened, methanol extract of C. macrostachyus fruits and D. angustifolia seeds; and chloroform extracts of C. aurea leaves and C. macrostachyus fruits had IC_{50} values of less than 10 $\mu\text{g/ml}$ showing a high anti-malarial activity. The ethanolic extracts of C. macrostachyus fruits, T. schimperiana leaves; methanol extracts of T. schimperiana leaves and C. aurea leaves, J. grandiflorum root and bark; chloroform extracts of B. polystachya leaves, D. angustifolia seeds, J. grandiflorum root and bark; and T. schimperiana leaves possessed low activity with IC_{50} values between 11-50 $\mu\text{g/ml}$. All other solvent extracts of the plants had no activity.



1. INTRODUCTION

The microorganisms causing human malaria belong to the Order Haemosporidia, Family Plasmodiidae and genus Plasmodium of the Protozoa. This genus can be further divided into two sub-genera, Plasmodium and Laverania. The sub-genus Plasmodium comprises Plasmodium vivax, P. ovale and P. malariae, the respective agents of benign tertian, ovale tertian and quartan malaria. The sub-genus Laverania contains P. falciparum, the causative agent of malignant tertian malaria (Beaver, et. al., 1984; Black, et. al., 1986).

Of the four species of human malaria parasites, P. vivax is the most prevalent having the widest geographical range and occurring throughout most of the temperate zone and in large parts of the tropics and the subtropics (Bruce-Chawatt, 1985; TDR, 1988). P. falciparum is generally confined to the tropical or subtropical regions (Bruce-Chawatt, 1985). Although P. falciparum is inferior to P. vivax in terms of geographical distribution (Loban and Polozok, 1983), it is the chief source of infection in endemic malaria areas and also responsible for malaria epidemics (Bruce Chawatt, 1985). P. malariae is less common than P. vivax and P. falciparum (TDR, 1988) and its geographical distribution extends over both tropical and subtropical areas, although its presence in various regions tends to be patchy. P. ovale is the least prevalent species and although much less common, present over the same range as P. falciparum (Bruce-Chawatt, 1985).

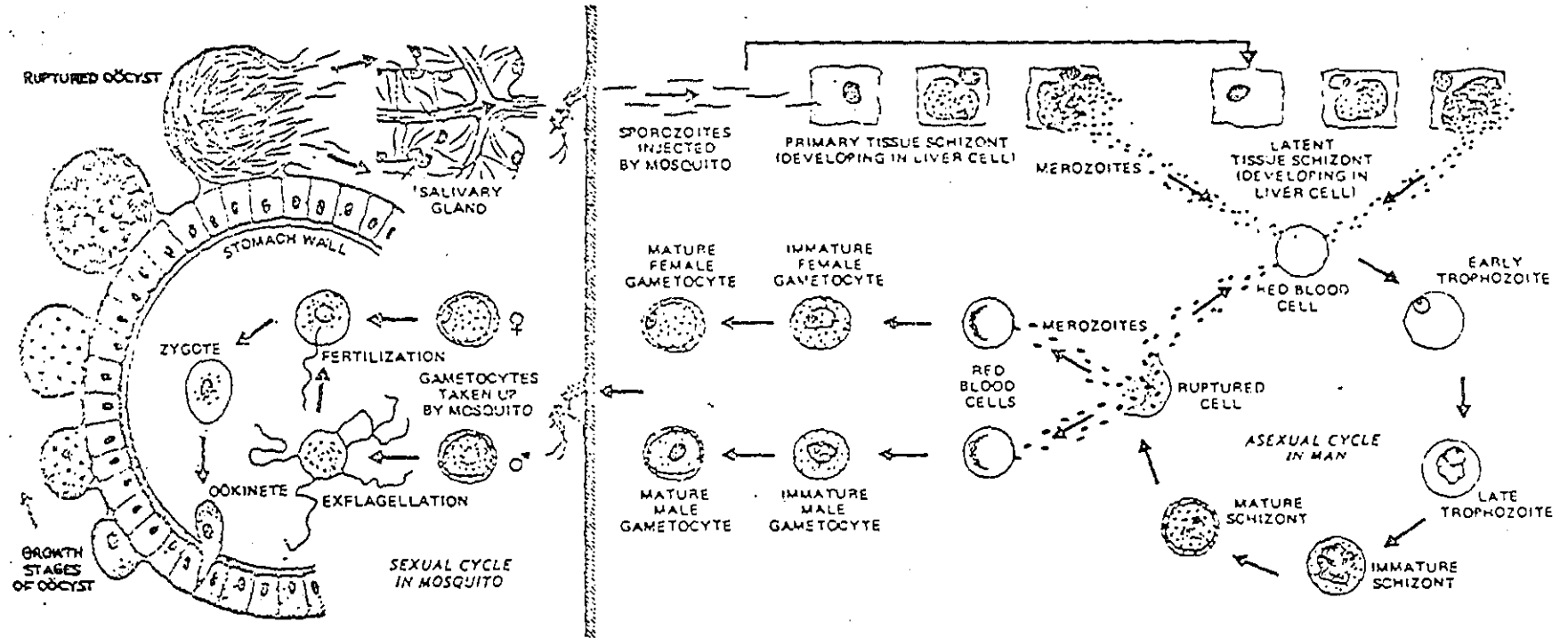
In Ethiopia, all four human malaria parasite species are present. P.falciparum is the dominant species followed by P.vivax, P.malariae and P.ovale (Gebremariam, 1984). During the epidemics of 1958 the proportions reported were, 75% P.falciparum, 22% P. vivax, and 3% P.malariae (Fontain, et.al.,1961). According to Gebremariam (1988), P.falciparum was the dominant species in most localities in the years 1974, 1979, and 1984.

Human malaria is transmitted by female anopheline mosquitos. In Ethiopia 42 species of Anopheles have so far been recorded (Gebremariam, 1988). These include Anopheles arabiensis, a member of A. gambiae complex, A.funestus, A.pharoensis and A.nili (Giaquinto-Mira, 1950;).

The life cycle of all species of human malaria parasites is essentially the same (Fig. 1). That is, development of each of the four human Plasmodium species starts with the phase in which the direct progeny of sporozoites injected into the circulation by the bite of an infected Anopheles mosquito enter the liver, where they undergo growth and multiplication in the parenchymatous cells. This pre-erythrocytic tissue schizogony is completed towards the end of incubation period of the infection when large numbers of tissue merozoites from ruptured tissue schizonts are released into the blood stream (Black et.al.,1986). However, in P.vivax and P.ovale infections, some of the parasites remain in the liver and do not reproduce immediately forming dormant parasites (tissue hypnozoites) which are responsible for the relapse in patients (WHO, 1991b). Tissue merozoites released into circulation invade the erythrocytes, grow and multiply

Fig. 1. Cycles of development of malaria parasites in the anopheline mosquito and in man.

(adapted from Black, et al., 1986)



cyclically from trophozoites to matured blood schizonts and produce the clinical symptoms of the disease. Some erythrocytic forms develop into two types of sexual parasites (male and female gametocytes) which will unite when taken by suitable female Anopheles mosquitoes as they ingest the blood of the infected individual. After further development inside the mosquito, large number of sporozoites which are infective are produced and stored in the salivary glands of the mosquito. (Black, et.al., 1986).

From time immemorial malaria has been one of the most prevalent human diseases affecting particularly the population of tropical regions. It is also one of the oldest infections mentioned in early writings in Egypt, India and China. Its clinical symptoms were fully described by Hippocrates 400 years before the Christian era (Black, et.al., 1985). Over the centuries malaria has had a profound impact in curbing socio-economic development by reducing the productive potential of mankind (Wernsdorfer, 1980; TDR, 1988).

The four human malarias can be sufficiently similar in their early clinical symptoms. That is, high fever, headache, severe chills, general pains and in some cases vomiting and diarrhea (WHO, 1991b). In all types of infections clinical symptoms are associated with the time of rupture of parasitized erythrocytes and subsequent release of merozoites into the blood circulation (Strickland, 1982; Bruce-Chawatt, 1985). If malaria is not cured at this stage, it is usually succeeded by a period in which the course of the infection is influenced and complicated by the hosts immune response (Black, et.al.;

1986). Serious complications of malaria seem to have an immunological basis for example are, kidney lesions (acute and chronic), tropical splenomegaly syndrome (big spleen disease), cerebral malaria, anemia, immunosuppression and autoimmunity (Cohen and Lambert, 1982; Houba, 1982).

In general, falciparum malaria is the most serious disease and prompt treatment is essential even in mild cases since irreversible complications may appear suddenly; case fatality among untreated children and non-immune adults considerably exceeds 10%. Vivax malaria, quartan and ovale malaria, generally are not life threatening except in the very old or in patients with concurrent disease (Benenson, 1985).

In addition to common features for all human malaria parasites, P.falciparum has an essential characteristic that accounts for severity of infection. It is capable of invading erythrocytes of all age and thus produces overwhelming parasitemia (Neva, 1977). The duration of the pre-erythrocytic stage, which influences the length of incubation period, is usually short (5.5 - 7 days), and the number of tissue and erythrocyte merozoites released is very large (Black, et.al., 1986). The erythrocytes that are infected tend to stick to the endothelium of the blood vessels and to each other resulting in mechanical obstruction of small blood vessels with anoxia of affected organs (Pinder, 1973). These unique features are responsible for severe and complicated conditions within the blood cells and outside (in circulation and organs) leading to pathophysiological cascade of the infection (Hall, 1977).

On the basis of available figures, the global incidence of clinical malaria is estimated at about 110 million cases annually, out of which 90 million cases are in tropical Africa, and the prevalence of the infection is in the order of 270 million parasite carriers (WHO, 1990a; 1990c; 1991a). Global deaths are estimated at approximately 1 and 2 million a year (WHO, 1990c; 1991a). In Africa more than 75% of malaria deaths are believed to occur in children under age 5; the disease is believed to account for at least 10% of annual deaths in infants and children under 14 (Schlesinger, et.al., 1988; WHO, 1991a).

In Ethiopia, more than 64% of the population lives in malaria endemic areas while about 75% of the land mass is regarded as endemic for the disease (Gebremariam, 1988). According to Zein (1988), malaria takes the tenth place among the causes of out-patient morbidity accounting for 34% of the visits to health institutions. In a WHO (1985) report, the prevalence of malaria in Ethiopia was 5.1% and 31% of the blood smears taken in febrile patients were positive for malaria.

Malaria control programmes are based on controlling the vector and treating the patients. Vector control strategies include environmental management to prevent breeding of the mosquitos, applying various methods to destroy the immature stage of mosquitos, spraying residual insecticides to kill

the adult mosquitos, Biological methods can also be used for the control of vectors. Treatment of patients is based on administration of curative and prophylactic drugs.

Attempts at the treatment of malaria by preparation from various plant parts had variable outcome. However, the powdered roots of Ch'ang Shan (Dichoria febrifuge) were used in China for at least 200 years with a confirmed medicinal effect. This is due to the presence of an alkaloid, febrifugine, isolated and analyzed only recently. Qing hao (Artemisia annua) also used for a similar period in China, has been shown to be a schizonticide of very low toxicity (Black, et.al., 1986). However, the first and the last time a natural product gained wide acceptance as a treatment for malaria was in the sixteenth century. It was then that the cinchona tree which was disclosed by the natives of Peru to Jesuit missionaries, which inturn, brought the word of its utility to Europe (Klayman, 1985). For 200 years the crude bark of cinchona tree was used for the preparation of powder and infusion to treat malaria. Following many attempts to isolate the active principle of the drug, at the beginning of the nineteenth century, Antonio Gomez in Portugal and Th.I. Gize in Rassia obtained a crystalline substance from an alcoholic extract of the bark. But the final isolation of the two basic alkaloids of cinchona, namely quinine and cinchonine, was not accomplished until 1820 when the French chemist Pierre Pelletier and Joseph Caventou succeeded in doing so. Following the isolation of quinine, two other alkaloids (quinidine and

Table 1. Action of commonly used drugs on the cycle of development of malaria parasites (adapted from Black, *et. al.*, 1986)

Drug	Sporozoites	Tissue phase during the incubation period	Erythrocytic phase		Latent tissue phase (responsible for relapses)	Development of gametocytes in the mosquito (sporontocidal action)	Chemical class of the relevant antimalarial compound
			Asexual parasites	Sexual forms (gametocytes)			
Quinine	No action	No action	Fast action	Active against <i>P. vivax</i> and <i>P. malariae</i> . No direct action on <i>P. falciparum</i>	No action	No action	Cinchona alkaloids
Mepacrine	No action	No action	Fast action	As quinine	No action	No action	9-aminoacridines
Chloroquine Amodiaquine	No action	No action	Fast action	As quinine	No action	No action	4-aminoquinolines
Primaquine	No action	Active but not used for prophylaxis	Active but only in toxic doses	Direct and fast action on all species but particularly <i>P. falciparum</i>	Highly active	Highly active	8-aminoquinolines
Proguanil	No action	Active, particularly on <i>P. falciparum</i>	Active, but relatively slow	No direct action	No action	Highly active	Biguanides
Pyrimethamine	No action	Probably as proguanil	As proguanil	No evidence of direct action	Some action on <i>P. vivax</i>	Little evidence	Diaminopyrimidines
Sulfones and Sulfonamides	No action	Possible action	Moderate action when given alone	As pyrimethamine	Little evidence		Sulfones and sulfonamides comprise a large number of short-acting and long-acting compounds
Mefloquine	Probably no action	Probably no action	Marked action	As quinine	Probably no action	Probably no action	Quinolnemethanols

achieve better results in treatment. For example, the combination of sulfadoxine with pyrimethamine or Fansidar and sulfalene with pyrimethamine has been used to treat chloroquine resistant P.falciparum infections (Donno, 1974 ; WHO, 1990c).

The factors limiting success in the use of antimalarial drugs, whether for prophylactic or for curative purposes, is the varying response of the species or strains of the parasite. Each drug in use has been selected because it has one or more specific actions against the malaria parasite when administered to the patient in an appropriate dose. When this recognized action does not occur, it may be that not enough of the drug or its active metabolites has not reached the parasite (in which event we are dealing with drug failure), or the drug has reached the parasite but the parasite has become adapted to the introduced chemical environment and, by surviving, has entered the state of drug resistance (Black, et.al., 1986).

According to WHO(1965 ;1973) and Black, et.al. (1986), drug resistance in malaria has been defined as the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in dose equal to or higher than those usually recommended but within the limits of tolerance of the subject to the effect that the form of drug active against the parasite must gain access to the parasite or infected red blood cells for the duration of time necessary for its normal action.

Wernsdorfer and Kouznetsov (1980) state that, resistance of human plasmodia to modern synthetic antimalarials was observed soon after these drugs have become widely used. Drug resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding maximum dose tolerated by the host) (WHO, 1984).

At present the only species of human malaria parasite that has developed significant resistance to chloroquine is P.falciparum (Black, et.al.,1986). Chloroquine resistant P.falciparum was first suspected in South America in the late 1950's (Peter, 1970) and confirmed in 1959 in Thailand (Harinasuta, et.al.; 1982). Since then it has been spreading further and there are a few countries where this problem has not been reported. However, this phenomenon has mostly a focal distribution (WHO,1990a ; 1990b) and upto 1988 it has been confirmed in more than 50 countries from different parts of the world (WHO, 1988). It has also occurred throughout East, Central and West Africa (Ekanem, et.al.,1990) and since 1978 when it was documented in America tourist returning from the United Republic of Tanzania (Campbell, et.al.,1979) it has been reported from more than 15 African countries (Lobel and Campbell, 1986). In Ethiopia, it has recently been reported in the southern and western lowland bordering Somalia, Kenya and Sudan (Gebremariam and Teklehaimanot, 1986; Teklehaimant, 1986) and a more recent in vitro study on isolates of P.falciparum from different parts of the country confirmed

that most Ethiopian isolates are resistant to chloroquine (Mengesha, et.al ;1991). According to Black,et.al., (1986), P.falciparum strains are resistant to amodiaquine and its geographical distribution resembles that of chloroquine - resistant falciparum malaria.

Strains of P.falciparum are resistant also to other antimalarials. Quinine - resistance has been reported from in vitro studies in west and central Africa (Brandicoult, et.al., 1986 ; Brasseur,et.al., 1987 ; Brasseur,et.al., 1988). According to Black,et. al., (1986), resistance to one or both of the tetrahydrofolate - dehydrogenase inhibitors is present in certain localities of all the endemic regions, including Africa. Also P.falciparum has become resistant to some alternative combinations and new drugs. For example, resistance of Fansidar has now become well established in several South-east Asian countries (Reacher,et.al., 1981 ; Pinichopngse,et.al., 1982 ; WHO, 1991a). Even the new drug, mefloquine, has encountered resistance (Boudreau, et.al., 1982).

In general, multidrug resistant P.falciparum occurs in most of the malarious areas of the world and represents a formidable problem to the management and control of malaria (Webster,et.al; 1985). According to Harinasuta,et.al., (1982) and Webster,et.al., (1982), in South-east Asia, and in particular Thailand, conventional drugs such as chloroquine and Fansidar are no longer useful in the treatment or prophylaxis of falciparum malaria.

Most of the known antimalarial drugs have a relatively short and incomplete action when given in a single dose and must be administered regularly over a number of days to have the desired effect. Also no single antimalarial drug is effective against all stages of malaria parasites, and this is difficult in developing countries where health services are inadequate. In addition, some drugs have side-effects at prolonged administration, high dosage and even at normal dosage. Phillips-Howard and Bjorkman (1990) states that, the three drugs considered to have caused serious reactions in recent years are Fansidar, pyrimethamine-dapose combination (maloprim) and amodiaquine. According to WHO (1990c), chemoprophylaxis has become progressively more difficult in recent years, owing in part to increasing recognition of the adverse side-effect of several prophylactic drugs. Therefore, these problems now triggered off mass screening of new antimalarial compounds, either synthetic or from plants which are commonly used in traditional medicine for the treatment of malaria.

Traditional medicine is a vague term loosely used to distinguish ancient and culture-bound health care practice which existed before the application of science to health in official modern scientific medicine (Bannerman, et.al., 1983). According to Akerele (1984), traditional medicine is the sum total of knowledge and practices, whether they are explained or not, used in prevention, diagnosis, and elimination of physical, mental or social imbalance, and relying exclusively on practical

experiences and observations handed down from generation to generation, wether orally or in writing. It has been practiced since the dawn of mankind in all cultures (Bannerman,et.al.,1983).

The use of traditional medicine for treatment of disease has its own advantages and disadvantages. Of the advantages, the major one is that it is a potential source of new drugs. Its major disadvantages are that, most preparations expected to have medicinal value are not evaluated scientifically for their efficacy and safety, and its potions are not standardized, nor dispensed to patients in specific doses or strictly regulated quantities (Sofowora , 1982).

The historical background suggests that medicinal plants have been used for the treatment of diseases in all countries whether developed or developing. In the former, such plants constitute the raw material for industrial processing and preparations of pure chemical derivatives. In many developing countries medicinal plants are utilized as extract or infusions and constitute the basis of almost all forms of therapy. Thus, both modern scientific medicine and traditional medicine find a use for plants (WHO, 1979).

In many tropical countries medicinal plants are used for the treatment of malaria. According to Weenen, et.al.(1990 a), plants continue to be used in the treatment

of malaria, either for their antiparasitic activity or because they possess other activities with therapeutic value for a patient with malaria. Medicinal plants used for the treatment of malaria are tropical in their distribution and are found in Africa (Watt and Breyer-Brandiwijk, 1962 ; Ayensu, 1978), in the Americas (Morton, 1981 ; Von Reis and Lipp, 1982) and in Asia (Chopra, et. al., 1956). According to Farnsworth (1985), the natural product data base, NAPRALERT, lists some 152 genera which are used for the treatment of malaria.

According to Attiso (1983), appropriate utilization of local resources to cover drug need is dependant on preliminary scientific study to determine the efficacy and safety of the preparations based on plants that are used on an empirical basis in traditional medicine. This is why intensive research efforts are required in this field. The extraction of bioactive agents from medicinal plants as well as its evaluation by using in vitro and in vivo test methods is the most interesting area of natural product research today.

Farnsworth (1985) states that, if traditional medicine in general and medicinal plants in particular are validated:

1. they will provide locally accessible alternative to imported drugs that will be accepted by the population

both because of their cheapness and because they have been used for centuries;

2. they may be used together with western pharmaceutical products, i.e integrated within the framework of official health service (following the Chinese model), in order to take advantage of the positive feature of both traditional and modern medicine;
3. they will in the long run enable pharmaceutical industry based on local resources to evolve what would benefit the national economy and provide a basis for the discovery of new substances that might be useful against intractable ailments.

In the course of their long history, the people of Ethiopia have learned much about the therapeutic qualities of the country's flora and other traditional systems of health care, to which about 80% of the population resorts. The Ministry of health established a Department of traditional medicine by considering the existence of several traditional medical practices, the availability of great man power potential in the system, and the natural resource of medicinally used plants, animals and minerals. The research and development activities carried out show that there are an estimated 22,000 traditional medical practitioners in the country, of which 7,000 are registered and are categorized according to their specialities. In addition, about 670 traditionally known medicinal plant

species are collected, identified and stored with necessary empirical information on their use and methods of formulation and administration (Abebe, 1986; Department of traditional medicine, 1991).

Although the efficacy and safety of Ethiopian medicinal plants, that are used for the treatment of malaria, is not yet scientifically known, lists of some species of medicinal plants identified by some investigators in the Department of Biology, Addis Ababa University, are available. For example, Tadesse (1986), lists some 16 species of medicinal plants of central Shoa and south western Ethiopia. Also, Abate (1989), mentions 7 species of plants for the treatment of malaria.

Basic research aimed at a better understanding of tropical pathogens is often hindered by the lack of materials. Although tremendously significant observations on malaria parasites were made between 1950 and 1975 using rodent, avian and primate malaria as models, there were constraints with studies on human malaria. Thus, the successful continuous cultivation of erythrocytic stages of P. falciparum developed by Trager and Jensen (1976) not only provided a large quantity of parasites but also made detailed investigation into all aspects of malaria possible (Brockelman, et. al., 1985). This has created new opportunities for studying the Biology, Biochemistry, Chemotherapy and Immunology of these parasites (Trager, 1982 ; Chalay, et. al., 1983).

Trager (1976), first reported prolonged cultivation method of malaria parasites (P.coatneyli and P.falciparum). Other improved methods such as, a more simple dilution system in petri dishes (Trager and Jensen,1976), the use of outdated erythrocytes and description of the candle jar method (Jensen and Trager,1977), the improved continuous flow method (Trager, 1979), a simplified culture technique for P.falciparum (Ososanya,et.al.,1981), continuous in vitro culture of the human malaria parasites, P.falciparum (Trager,1982), an improved technique of the cultivation of P.falciparum without daily medium change (Fairlamb, et.al.,1985), etc, were introduced later. The basic principle of all the methods is to have a shallow stationary layer of erythrocytes covered by a shallow layer of medium. The technique of in vitro cultivation of P.falciparum has led to the subsequent development of assay systems for drug susceptibility tests and evaluation of crude extracts and isolated active compounds (Riekmann,1968 ; Riekmann,et.al.,1978 ; O'Neill, et. al., 1985a; Webster,et.al.,1985).

Because of an obvious need for new non-cross-resistant and less toxic antimalarial drugs, it is essential that the efficacy of plants used for the treatment of malaria be validated. Both in vivo and in vitro test methods have been used to evaluate plant extracts against malaria parasites. The largest screening programme for testing plants for the presence of antimalarial compounds was published in 1947 (Spencer,et.al.,1947). In this programme, from 126 Families

of plants, about 600 species were evaluated for their ability to suppress in vivo avian malaria utilizing P.gallinaceum in chicks, and P.cathemerium and P.lophurar in duckings. About 30 of the genera tested yielded active extracts and two plant families, Simarobuaceae and Ameryllidaceae proved to be significantly active. The most significant advances in testing was reported in 1979 when a method was published for a test by a semiautomated microdilution technique (Desjardins, et.al., 1979).

A number of in vivo test systems are now available, including the four day suppression of parasitemia test which utilizes P.berghei infected mice (Peter, 1980) and this test method has been used for the evaluation of plant extracts (Fandeur, et.al., 1985).

Since in vivo methods are expensive and time consuming, in vitro methods have been developed for P.falciparum. Short-term in vitro cultivation of erythrocytic stage of malaria parasites has been used since the 1920's to study the activity of various antimalarial drugs (Richards and Williams, 1975 ; Trigg, 1976). In recent years in vitro methods have been used for various quantitative measurements of anti-malarial compounds. Some of these are based on [³H]-hypoxanthine incorporation of P.falciparum in test culture (Desjardins, et.al., 1979), a morphological endpoint based on schizont maturation by examination of thick films prepared after an incubation period of 24 - 30 hrs depending on initial parasite (Rieckmann, 1968 ;

Rieckmann,et.al.,1978 ; Wernsdorfer,1980), and the end-point for assessing antimalarial activity based on parasite re-invasion of erythrocytes as determine by examination of thin films prepared after an incubation period of 48 hrs (Nguyen-dinh, et.al.,1981 ; Fandeur,et.al.,1985 ; Pavanand,et.al., 1986).

Extracts from various plant species such as the Chinese medicinal herb, Artemisia annua (Li, et.al.,1983 ; O'Neill, et.al. 1985a), the British mugwort, Artemisia vulgaris, (Klayman,et.al., 1984), Brucea javanica from Thailand (O'Neill,et.al.,1985a ; Pavanand,et.al., 1986), Ailanthus altissima from U.K., Simba cedron from Panama (O'Neill,et.al., 1985a), Coutarea lotifoloria, Exostema caribaeum (Noster and Kraus,1990), Spatoda campanulata (Makinde,et.al.,1988) else where, etc have been evaluated for antimalarial activity and most of them found to be highly active. Some African medicinal plants are also screened for antimalarial activity. For example, recently, extracts from eight medicinal plants of Togo, West Africa, were tested against P.falciparum in vitro and three of them, Jatropha gossypifolia, Cassia siamea, and Pavetta crassipes, found to show 100% inhibition (Gbeassor, et.al., 1989); and out of 49 Tanzanian medicinal plants investigated, extract from three species, Cyperus rotundus, Hosiudia opposita and Lantana cambara, were found to have high activity (Weenen, et.al., 1990a).

In recent years various research activities were carried out on the active constituents of medicinal plants with reported antimalarial activity. The major research efforts have concentrated on qinghausu, the active principle of A.annua, and several derivatives such as artemether(methyl dihydroartemisinin) and sodium arteunate(sodium dehydroartemisinin hemisuccinate) that have been used clinically (Li, et. al., 1983). Yingzhausu A, the active principle of another Chinese herb used in the treatment of malaria was obtained from Artabotry hexapetalus (Annonaceae). This contains the endoperoxide moiety which is a feature of the artemisinin structure (Xiao, 1983).

Researches into the active principles of several species of Simaroubaceae have shown that their activity can be attributed to a series of bitter terpenes known as quassinoids (Trager and Polonsky, 1981 ; Guru, et. al., 1983 ; Chan, et. al., 1986). Bruceantin, simalikalactone D, glucarbinone and sergeolide are remarkably active in vitro tests against P.falciparum and sergeolide is highly active against P.berghie in mice (Fandeur, et. al., 1985). A series of 14 quassinoids have in vitro antiplasmodial activity against a multi-drug resistant strains of P.falciparum (O'Neill, et. al., 1986). Fruits of B.javanica have been investigated in some detail and a series of quassinoids were isolated and identified. These have been evaluated in vitro against a chloroquine resistant strains of P.falciparum (O'Neill, et. al., 1985b; 1986 ; 1987) and nine

of them found to be active (O'Neill,et.al.,1987). Also in vivo test against P.berghie infection in mice confirmed four quassinoids to be active, although they showed some toxicity (O'Neill,et.al.,1986 ;1987). Eurycoma lactifolia is used in Malaysia for the treatment of malaria (Gimlette and Thomson,1977; Perry, 1980) and a series of quassinoids and alkaloids were isolated from these plants, but their activity was encountered only in those fractions which contained quassinoids (Chan,et.al.,1986). Several limonoids from the family Meliaceae have been found to be responsible for the antimalarial activity of the plant and the most active compounds in this series is gedunin, which was obtained from Azadirachta indica (Badam,et.al., 1987). Recently, pure compounds were isolated from Tanzanian medicinal plants, Cyperus rotundus, Zanthoxylum gilletti and Margaritaria discoidea, and the most active compound that are obtained as alpha-beta-unsaturated carbonyl moiety included alpha-cyperone, N-isobutyldeca-2,4- dienamide and securinine. A mixture of autoxidation products of beta-selinene was found to be the most active substance obtained from C.rotundus (Weene, et.al., 1990b).

In general, due to fast spreading multi-drug resistant strains of P.falciparum against existing antimalarial drugs and the need for new antimalarials with novel actions and with less toxic effect, intensive research activities are required in evaluating extracts and active constituents of medicinal plants for antimalarial activity. Especially in most developing countries, like Ethiopia, where highest

proportion of the population live in rural and often inaccessible areas; very low health facilities are available; the price of imported drugs or raw materials of locally manufacturable drugs is exorbitant and ever increasing and where more than 80% of the population are forced to rely on traditional medicine, it is very important to validate medicinal plants for their efficacy and safety to integrate gradually with modern health care system especially at the primary health care level to take advantage of both.

In this study:

- crude extracts from six species of Ethiopian medicinal plants will be evaluated in vitro against local isolates of P.falciparum, and
- of the solvents used for the extraction of plant materials, the one that contained the most potent extract will be identified.

ovate, 4-15(-20)cm long, 2.5-10(-13)cm wide, obtusely or subacutely acuminate, rounded or cordate, with 2-4(-8) subsessile or stipitate discoid basal glands visible or not from above. Recemes terminal, (7-)15-32cm long, upto 3cm wide, either all male or all female, or mostly male with a few female flowers at the base, or sometimes half male and half female or mostly female with a few male flowers at the base. Pedicels of male flowers are 4-10 (-14)mm long, densely stellate-pubescent; sepals 5, ovate, 3mm long, 2.5mm broad, pale green; petals are 5, oblanceolate-oblong, 3.5mm long, 1.5-2mm wide, pale yellow; stamens 15-20, filaments 4mm long, anthers 1mm long; receptacle densely villous. Pedicels of female flower are 2-5(-8)mm long; sepals 5, lanceolate or ovate-lanceolate, 3mm long, 1.5mm broad; petals zero, or, if present linear and smaller than the male flower; stamenods 0-1(2), filament-like; ovary trigonous, 2mm diameter; styles 3(-4), spreading, 2.5mm long. Fruit subtrilobed, rarely 4-lobed, often more particularly so at the apex, 8-9mm long, 8-10(-15)mm wide, loculicidal. Seeds ellipsoid, 7mm long, 4mm wide, longitudinally rugulose, grey, some what shiny, with a large waxy caruncle 4.5mm long and 4mm wide (Smith, 1987).

C. macrostachyus is known to have molluscicidal action (Olver-Bever, 1986) and in East Africa the bark is reported to be used as cathartic (Githens, 1949). In Ethiopia, the fruits (either fresh or dried) are pounded with water and the supernatant is drunk for the treatment of malaria (personal communication with traditional medical practitioners).

C.aurea is a shrub or small tree 1-10m tall. Young branches and inflorescences densely pubescent. Leaves (4-6)-24cm long, leaflets 10-30, ovate or oblong to ovate. Recemes (5-7)-24cm long, 8-30 -flowered, pendent. Calyx 3-10mm long. Corolla 9-20mm long; limb of standard strongly reflexed. Pod 50-120 X 8-16m, straw-coloured or brownish, with a 1-25m broad wing along the upper edge, 4-8 - seeded. Seeds 5-6mm long, brownish. This plant grows in forest margins, bushland or grassland, favored by overgrazing. The seeds may be used as fish - poison or as cure for dysentery (Thulin , 1989).According to Tadesse (1986), the dried leaves of C.aurea are pounded,the powder is mixed with water and the mixture is drunk for the treatment of malaria in Ethiopia.

B.polystachya is a robust shrub, upto 5m tall, with pale brown bark; young parts densely flocculent, glabrescent. Leaves shortly petiolate; petiole upto 5mm long; lamina upto 16cm long, 4cm wide, lanceolate, gradually narrowing above to an acute apex, convergent below, serrate at least in part.Inflorescence a compound receme of short subglomerulate clusters; lateral recemes upto 15cm long and less than 2cm in diameter; pedicels obscure. Corolla reddish-orange; corolla-lobes almost square, with rounded apex. Anthers sessile, inserted just within the throat. Ovary narrowly ovoid shortly hirsute (Bruce and Lewis, 1960).In Ethiopia, dried leaves of this plant are pounded, mixed with water and the infusion is drunk for treatment of malaria. This

preparation is often given together with that of C. aurea (Tadesse, 1986).

T. schimperiana is a tree upto 10m long; bark dark to blackish grey, fissured; branchelets glabrous to rufous (rarely whitish) tomentellous, remainign fibrous in second year. Leaves dull green or olive green, rufous (rarely whitish) tomentose when young, becoming pubescent to densely so (rarely glabrous even when young); petiole 1-5.5cm; lamina elliptic to obovate, (6-)10-28 X (1.5-)4-10(-11.5)cm; acuminate to acute (rarely rounded); base attenuate to rounded; midrib 1(-2)mm wide near base; tertiary veins raised or not above, reticulation impressed, beneath with conspicuous but not raised veins and reticulation. Spikes 3-13cm long, tomentose; bisexual flowers in basal 1-2cm. Flowers white or cream, heavily scented, pubescent to tomentose (calyx often may contain or not globlous on lobes); pedicels of male 1-2(-3)mm; calyx 2-3mm long; filament (2-)4-5mm. Fruit (2.5-)4-7 X 1.5-3.5cm, oblong; yellowish to yellowish brown, densely puberulos (Vollesen, Flora of Ethiopia, in preparation). Leaves (either fresh or dried) of this plant are pounded with little water and the supernatent is drunk for the treatment of malaria in Ethiopia (Tadesse, 1986).

D. angustifolia is a shrub upto 3m long; all parts are glabrous and resinous when young. Leaves simple; petiole 1-5mm; lamina narrowly elliptic to narrow obovate, 2-12.5 X 0.7-3cm, entire; apex acute (rarely rounded to tuncate);

base attenuate. Inflorescence terminal and axillary, paniculate 1-4cm long, often merging into a compound terminal inflorescence; pedicels 2-7 (-15)mm in female flower. Sepals 4, ovate, 2-3.5 X 1.5-2mm, yellowish green, shortly connate. Stamens (5-)7-9(10), subsessile; anthers 3mm long. Ovary 2-3 - locular, 3mm long; style 4-7mm, 2-3 fid; stigma 2-3mm. Fruit circular in outline, 0.7-2 X 1-2.1cm, yellowish, with 3-6mm wide reddish tinged wings. Seeds lenticular, 3 X 2mm. This plant grows at the edge of upland forest, upland and grassland, secondary forest and scrub, invading areas recently cleared off forest and invading overgrazed Acacia commiphora bushland, also in cultivated areas (Vollesen, 1989). According to Tadesse (1986), the dried fruits (seeds) of D. angustifolia are pounded, the powder mixed with water and the mixture is drunk to treat malaria in Ethiopia.

According to Green (1986), J. grandiflorum L. contains two sub-species (grandiflorum and floribundum). However, the variations within these sub-species are not taxonomically significant. This plant is a branched, glabrous, low shrub or scrambler, with slender twig-like branches. Leaves imparipinnate, typically with 5 leaflets (sometimes upto 9 or reduced to 3), opposite; leaflet blades ovate to elongate-ovate, the lateral sometimes asymmetrical especially at the base, the terminal is large, ranging considerably in size even in the same leaf, apex of terminal leaflet and sometimes of lateral leaflet is long acuminate, of lateral often acute to obtuse, 0.5-4cm long,

0.2-1.7cm broad, midrib impressed on upper and prominent on lower surface, lateral veins not conspicuous; petiolus to terminal leaflet 3-6mm long, to lateral leaflet 1-3mm long. Inflorescences terminal or axillary, the final cymes generally 3-7 flowered, appearing all the year round, but especially about November. Corollas scented, white, streaked on outside with red or crimson; tube 1.5-2cm long (Turill, 1952). J.grandiflorum is widely grown in warm temperature and tropical regions throughout the world and often used in perfume industry as a source of jasmine (Green, 1986). According to Abate (1989), root and bark of this plant is dried and pounded, mixed with bee honey, dissolved in water, the solution is boiled and drunk for the treatment of malaria in Ethiopia.

2.2 Plant extraction method

Parts of the plant were dried in an open air in the laboratory and ground to powder using grinding mill machine (Straub, model 4E, Philadelphia. U.S.A.).

The crude extract was prepared by cold maceration technique as outlined by O'Neill, et.al. (1985 a) with minor modifications. In brief, the dried, powdered leaves of C.aurea (42.5g), B.polystachya (63.5g), and T.schimperiana (63.5g); fruits of C.macrostachyus (100g); seeds of D.angustifolia (20.7g); and the root and bark of J.grandiflorum (50.8g) were transferred into 100ml Erlenmeyer flasks, dissolved in petroleum ether (boiling

point 40-60°C), placed on orbital shaker (at 120 revolution per minute) and defatted for 48hr. The marc (the remaining plant material) was allowed to dry and extracted with methanol, chloroform and ethanol as outlined in Fig.2.

The dried marc was dissolved in methanol, extracted for 48hr and the resulting extract was filtered by using Whatman filter paper number 1. The filtered extracts from each plant species were transferred into two quickfit flasks and concentrated to dryness by removing the solvent under reduced pressure in the rotary evaporator (Buchi, model 140, Switzerland). The first quickfit flask yielded methanol extract (fraction 1) of approximately 1.2g (C.macrostachyus), 3.8g (C.aurea), 4g (B.polystachya), 1.1g (T.schimperiana), 0.6g (D.angustifolia) and 0.95g (J.grandiflorum). The second flask yielded approximately 3.8g (C.macrostachyus), 2.6g (C.aurea), 4.1g (B.polystachya), 2.5g (J.grandiflorum), 4.2g (T.schimperiana) and 0.6g (D.angustifolia). The second methanol extract was partitioned between chloroform and water (dissolved in the ratio 2:1). Then the chloroform layer was transferred into a quickfit flask, concentrated to dryness and yielded chloroform extract (fraction 2) of approximately 1.8g (C.macrostachyus), 0.4g (C.aurea), 0.4g (B.polystachya), 0.6g (T.schimperiana), 0.1g (D.angustifolia) and 0.1g (J.grandiflorum).

Ethanol extract was obtained from the defatted marc by dissolving it in methanol, extracted for 48hr and the

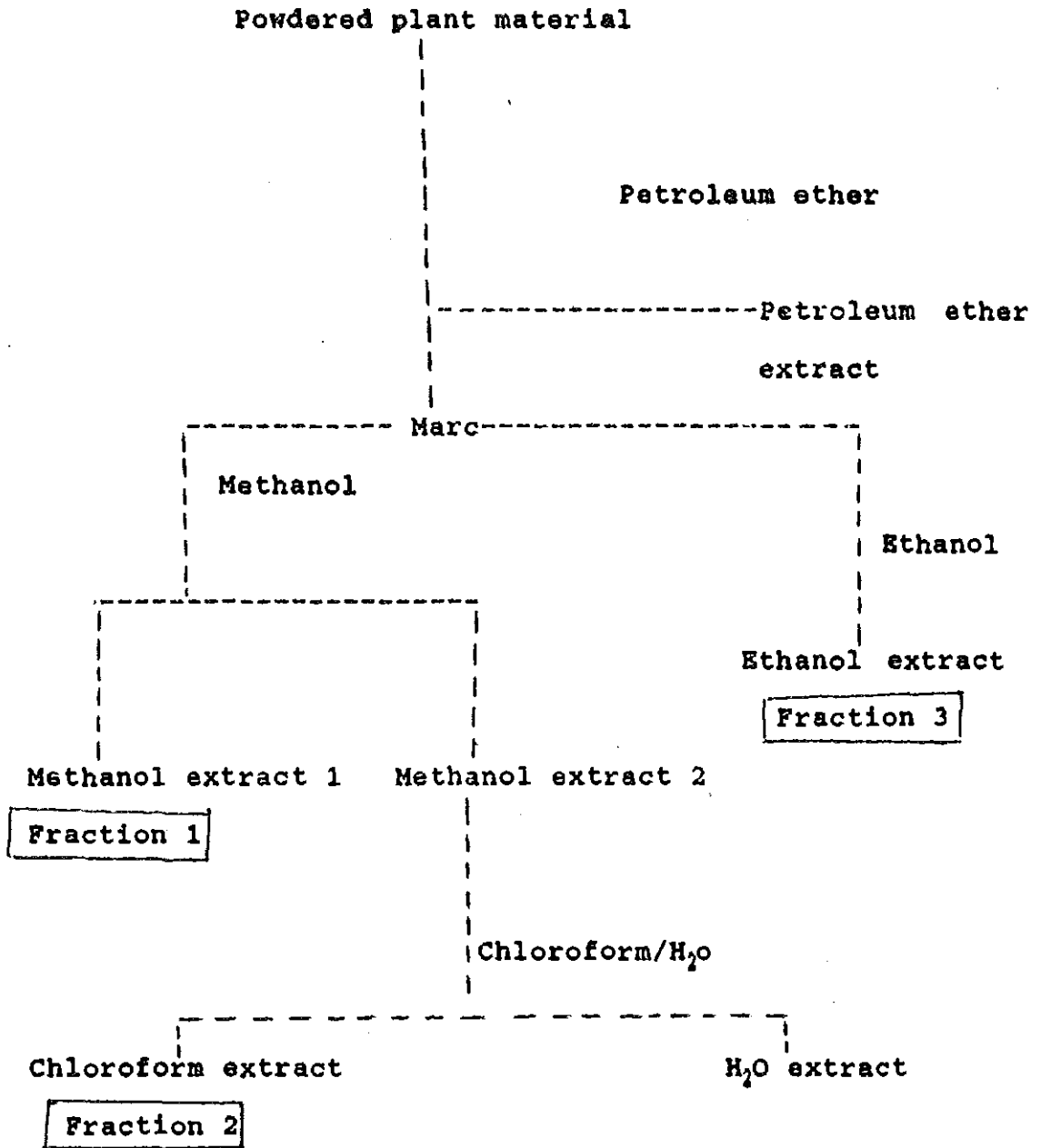


Fig.2 Fractionation of plant crude extracts for in vitro antimalarial testing (modified from O'Neill, et.al.,1985a)

resulting extract was filtered and concentrated to dryness. This yielded ethanol extract (fraction 3) of approximately 1.8g (C.macrostachyus), 0.8g (C.aurea), 2.8g (B.polystachya), 2.3g (T.schimperi), 3g (D.angustifolia) and 0.6g (J.grandiflorum).

For each species of plant extract, stock solutions were prepared in different solvents. That is, crude methanol extract was dissolved in 70% ethanol; chloroform extract was dissolved in water containing 3% Tween-80 and ethanol extract in 70% ethanol as described by Pavanand, et.al. (1986) with minor change. Then the stock extracts were sterilized through a 0.45 micron syringe filter (Nalgen, cat. no. 190 2020, New York, U.S.A.), the concentration of all extracts was adjusted and stored in refrigerator at -20 °c.

2.3 Culture medium preparation

For an in vitro P.falciparum culture, many laboratories in the world have adopted the culture technique reported by Trager and Jensen (1976). For the successful long-term cultivation of P.falciparum in vitro, a synthetic medium, RPMI 1640, is used (Trager and Jensen, 1976; Haynes, et.al., 1976). According to Brockelman, et.al. (1985), this synthetic medium supports parasite growth and multiplication very well and is thus preferred for routine cultivation.

For this study, the culture medium was prepared according to the methodology described by Jensen and Trager (1977) and Brockelman, et.al.(1985) and performed in aseptic condition. In brief, 10.4g of powdered RPMI 1640 medium (Gibco, cat.no. 074-01800A, SCOTLAND, U.K.) with glutamine but without sodium bicarbonate was dissolved in 900ml of glass redistilled water continuously stirring with magnetic stirrer. To this solution 5.9g HEPES(N-2-Hydroxyethyl piperazine-N-2--ethanesulfonic acid) (Gibco, cat.no.066-01344M, Paisicy, Scotland) was added, diluted to 960ml with glass redistilled water and sterilized by filtering through 0.22 micron millipore(Nelgen, cat.no. 190 2020, New York, U.S.A). This medium was dispensed aseptically into 100ml screw capped flasks and stored at 4⁰C refrigerator. Sodium bicarbonate (5%) was prepared by dissolving 5g of sodium bicarbonate in 100ml Of glass redistilled water, filter sterilized, aliquoted in 5ml volume into screw capped tubes and stored at 4⁰C .

For use, 4.2ml of sodium bicarbonate was added to 100ml of the medium to make up a medium designated as medium without serum and the p^H adjusted to 7.4. This medium was used to wash both non-infected and infected red blood cells and to prepare the complete medium (with serum). The complete medium was prepared just prior to use and to prepare it 11.5ml of human type AB⁺ serum was added to 104.2ml of medium with out serum. This medium

was used to make suspensions of non-infected and infected blood cells after washing, and also used in test cultures.

2.4 Preparation of non-infected blood cells

The non-infected human O⁺ type whole blood stored at 4^oc in acid citrate dextrose (ACD) was obtained from the Blood Bank of the Ethiopian Red Cross. From the storage bag the blood was transferred aseptically into sterile 15ml conical centrifuge tubes and centrifuged at 1000 rpm for 10 minutes. The serum, the buffy coat and the upper layer of the red blood cells were removed and the remaining cells were suspended with equal amount of culture medium without serum. Then, the cell medium suspension was centrifuged, the supernatant removed, the cells resuspended and centrifugation repeated twice. After final removal of supernatant, the packed blood cells were resuspended in equal volume of complete medium (50% suspension) and stored at 4^oC. This blood was used to adjust parasitemia of infected blood taken from patients.

2.5 Infected blood cell preparation

Infected blood was obtained from patients who attended the Malaria and Other Vector Borne Diseases Control Center and Teklehaimanot malaria control center in Addis Ababa. First, blood samples from patients were examined for the presence of asexual forms of P.falciparum.

This was done on thin and thick blood smear taken from the patients. Stock Giemsa stain was prepared according to the standard methodology. The films were stained for 30 minutes by placing them in a coplin jar containing the stain. The stained blood films were washed with 0.067 M phosphate buffer for a few minutes and air dried. By using a microscope with an oil immersion lens (100X magnification) the blood film was examined and for films with asexual forms, parasitemia was determined. Patients who have taken antimalarial drugs recently, have very low parasitemia count, with mixed infections or have large number of gametocytes of P.falciparum were not used to take blood for testing.

Infected venous blood was taken from patients by using a sterile heparinized vacutainer. From the vacutainer the blood was transferred into graduated conical centrifuge tube, centrifuged at 1000 rpm for 10 minutes and the supernatant was removed. Then the infected blood was washed twice by using warmed (in water bath at 37 °C) culture medium without serum and 50% suspension of the non-infected blood was made by using the complete medium. The parasitemia was adjusted to 1% by dispensing washed human type O⁺ red blood cells as outlined by Webster, et.al. (1985). The blood suspension was adjusted to 10% in warmed complete medium and this infected blood was used immediately for in vitro testing of plant extracts against P.falciparum.

2.6 In vitro test method

In this study the 24-hour micromethod described by Rieckmann, et.al, (1978) was used with minor modifications to screen the crude extracts against P.falciparum. The test was performed aseptically on 96 well flat bottom microtiter plates (cat.no. 001-012-9050, Dynatech Laboratories, INC., Chantilly, Virginia U.S.A). In the test, 25µl of the initial crude extract (approximately 0.02g/ml) was dispensed into appropriate wells on the microtiter plate by using multichannel micropipettor and the extract solvent was allowed to evaporate for few minutes to reduce the the possible solvent effect on the parasite isolates. Following this, 50 µl of complete culture medium was dispensed and serial dilutions made to yield the following test concentrations ; 3333.3, 555.5, 92.5, 15.4, 2.5 and 0.4µg/ml. Duplicates of each dilution were used in the test. Three series of controls were used in each test. These included two negative controls and one positive control. The negative controls were the solvents at the same volume as the test preparation and culture medium. Chloroquine phosphate solution at a concentration equivalent to that of the extract was used as a positive control. To each well that contained the test preparations, 50ul of infected red blood suspension was dispensed and mixed thoroughly. Then the plates were placed in the candle jar. The candle was lit and the cover placed over the jar with stopcock open. Just before the candle flame

went out, the stopcock was closed and the jar was placed inside an incubator at 37⁰c for 26hrs.

After incubation, the culture supernatant was removed from each well and thick films prepared from the sediment and stained in 3% Giemsa-stain for 30 minutes. The thick films were examined by oil-immersion light microscopy. Schizonts with more than two nuclei were counted per 200 asexual parasites, and parasites that exhibited greater than 10% schizogony (more than 20 schizonts per 200 asexual parasites) in the negative control wells were considered to be successful. For successful cultures (from 3 experiments), the in vitro schizonticidal activity of crude extracts was determined by calculating the percentage inhibition of schizogony at various extract concentrations, assuming that the growth (schizont maturation) in the control well was 100% schizogony. That is, percentage maturation = average count in test preparation/average count in control X 100. For each plant extract with 3 kinds of solvents, minimum inhibitory concentration (MIC) was determined. According to Ekanem, et.al. (1990), MIC was defined as the lowest extract concentration at which there was complete inhibition of maturation to schizonts. The 50% inhibitory concentration (IC₅₀) and 99% inhibitory concentration (IC₉₉) for each extract was determined from a probit linear regression analysis plot of the percentage inhibition of schizont maturation (probit scale) against the concentration of extracts (logarithmic scale).

The test method used in this study measures the extent to which the maturation of ring forms to normal schizonts is inhibited after the incubation of parasitized blood at various drug concentrations for a period of 24-30hrs. According to WHO (1984), in this short-term culture system, a marked difference in the maturation of parasite isolates can be observed. This difference was used to measure the anti-malarial activity of extracts from different species of plants and different solvents.

Table 2 . Average schizont count at different concentrations of methanol extract (triplicate experiment)

Extract Concent. (MG/ml)	1	2	3	4	5	6	Controls		
							Negative	Solvent	Chlorquine
0	139 ± 96.1	139 ± 96.1	139 ± 96.1	139 ± 96.1	139 ± 96.1	139 ± 96.1	139 ± 96.1	139 ± 96.1	139 ± 96.1
0.4	112 ± 96.6	131 ± 90.2	137 ± 94.2	132 ± 92.2	135 ± 93	131 ± 90.2		139 ± 96.1	0
2.5	23 ± 39.3	123 ± 91.2	133 ± 95.5	124 ± 94	134 ± 93.4	127 ± 88.5		139 ± 96.1	0
15.4	0	120 ± 89.9	129 ± 93.6	107 ± 95	9 ± 15.6	120 ± 95.3		139 ± 96.1	0
92.5	0	55 ± 90.4	120 ± 98.7	0	8 ± 14.4	46 ± 74.8		139 ± 96.1	0
555.5	0	0	0	0	0	0		139 ± 96.1	0
3333.3	0	0	0	0	0	0		139 ± 96.1	0

Key: 1= C. macrostachyus

2= C. aurea

3= B. polystachya

4= T. schimperiana

5= D. angustifolia

6= J. grandiflorum

Table 3. Average schizont count at various chloroform extract concentrations (triplicate experiment)

Extract Concent. (MG /ml)	1	2	3	4	5	6	Controls		
							Negative	Solvent	Chlorquine
0	135 ± 96.3	135 ± 96.3	135 ± 96.3	135 ± 96.3	135 ± 96.3	135 ± 96.3	135 ± 96.3	135 ± 96.3	135 ± 96.3
0.4	123 ± 96	130 ± 93.5	133 ± 94.5	135 ± 96	135 ± 96.3	129 ± 92.1		136 ± 96.3	0
2.5	84 ± 97.2	112 ± 92.2	126 ± 98	130 ± 94.4	123 ± 94.3	123 ± 88		135 ± 96.3	0
15.4	9 ± 11	20 ± 9.5	83 ± 94.7	113 ± 88.2	118 ± 90.6	110 ± 80.5		135 ± 96.3	0
92.5	0	0	3 ± 4.6	49 ± 39.1	49 ± 55.4	7 ± 12.7		135 ± 96.3	0
555.5	0	0	0	4 ± 7.5	0	1		135 ± 96.3	0
3333.3	0	0	0	0	0	1		134 ± 96.3	0

Key: 1= C. macrostachyus

2= C. aurea

3= B. polystachya

4= T. schimperiana

5= D. angustifolia

6= J. grandiflorum

Table 4. Average schizont count at different ethanol extract concentrations (triplicate experiment)

Extract Concent. (µg/ml)	1	2	3	4	5	6	Controls		
							Negative	Solvent	Chlorquine
0	136 ± 97.3	136 ± 97.3	136 ± 97.3	136 ± 97.3	136 ± 97.3	136 ± 97.3	136 ± 97.3	136 ± 97.3	136 ± 97.3
0.4	130 ± 99.1	135 ± 96.4	136 ± 97.7	136 ± 97.3	135 ± 96.7	135 ± 96.2		136 ± 97.3	0
2.5	127 ± 99	134 ± 95.2	136 ± 97	135 ± 96.4	133 ± 94.7	130 ± 93		136 ± 97.3	0
15.4	66 ± 107.5	131 ± 93.3	135 ± 96.1	78 ± 97.3	132 ± 94.7	129 ± 93.5		136 ± 97.3	0
92.5	46 ± 78.2	67 ± 83.6	134 ± 95.6	72 ± 100.7	124 ± 88.3	56 ± 82.9		136 ± 97.3	0
555.5	8 ± 13.9	46 ± 68.7	62 ± 92	7 ± 12.1	79 ± 86.7	1 ± 15.8		136 ± 97.3	0
3333.3	5 ± 9.8	33 ± 47.4	1 ± 2.2	1 ± 2.2	5 ± 8.1	0		136 ± 97.3	0

Key: 1= C. macrostachyus
2= C. aurea

3= B. polystachya
4= T. schimperiana

5= D. angustifolia
6= J. grandiflorum

seen at a test concentration of 15.4 μ g/ml and above for C. macrostachyus while the inhibitory concentrations for the rest were much higher. That is, for T. schimperiana 92.5 μ g/ml and for B. polystachya, D. angustifolia C. aurea and J. grandiflorum extracts it was 555.5 μ g/ml. At the lowest test concentration (0.4 μ g/ml) 80.5% of ring forms matured into schizonts for C. macrostachyus while in the case of other plant extracts, 94-97% schizont maturation was observed.

Chloroform extract test results (Table 6) showed that, schizont maturation was totally inhibited by C. macrostachyus and C. aurea at concentration greater or equal to 92.5 μ g/ml. The same effect was obtained for B. polystachya and D. angustifolia extracts at above 555.5 μ g/ml while for T. schimperiana it was at 3333.3 μ g/ml. It was only with extracts of T. schimperiana and D. angustifolia that 100% schizont maturation was seen at 0.4 μ g/ml, the lowest concentration tested.

With ethanol extract, although complete inhibition of schizont maturation was not observed, percentage maturation decreased as the concentration of the extracts increased. At lowest extract concentration tested (0.4 μ g/ml), there was almost total lack of schizont maturation inhibition by all plant extracts except for C. macrostachyus (Table 7).

Table 5. Percentage schizont maturation at various methanol extract concentrations

Extract Concent. ($\mu\text{g/ml}$)	1	2	3	4	5	6	Controls		
							Negative	Solvent	Chlorquine
0	100	100	100	100	100	100	100	100	100
0.4	80.5 \pm 69.6	94.2 \pm 64.9	98.6 \pm 67.8	95 \pm 66.4	97 \pm 66.9	94.2 \pm 65		100	0
2.5	16.5 \pm 28.2	88.5 \pm 65.6	95.7 \pm 68.7	89.4 \pm 67.6	96.4 \pm 66.4	91.4 \pm 63.6		100	0
15.4	0	86.3 \pm 64.6	92.8 \pm 67.3	77 \pm 68.4	6.5 \pm 11.2	86.3 \pm 68.5		100	0
92.5	0	39.6 \pm 65	86.3 \pm 70	0	5.8 \pm 10.3	33 \pm 53.8		100	0
555.5	0	0	0	0	0	0		100	0
3333.3	0	0	0	0	0	0		100	0

Key: 1= C. macrostachyus

2= C. aurea

3= B. polystachya

4= T. schimperiana

5= D. angustifolia

6= J. grandiflorum

Table 6. Test for percentage schizont maturation at various chloroform extract concentrations

Extract Concent. (Mg/ml)	1	2	3	4	5	6	Controls		
							Negative	Solvent	Chlorquine
0	100	100	100	100	100	100	100	100	100
0.4	91.1 ± 71	96.3 ± 69.2	98.5 ± 70	100 ± 71.1	100 ± 71.7	95.6 ± 68.2		100	0
2.5	62.2 ± 71.9	83 ± 62.3	93.3 ± 72.5	96.3 ± 69.9	91.1 ± 69	91.1 ± 65.1		100	0
15.4	6.7 ± 9.2	14.8 ± 9.8	61.5 ± 70.1	83.7 ± 65.2	87.4 ± 67	81.5 ± 59.6		100	0
92.5	0	0	2.2 ± 4.8	36.3 ± 28.9	36.3 ± 41	5.2 ± 9.4		100	0
555.5	0	0	0	3 ± 5.5	0	0.7 ± 1.2		100	0
3333.3	0	0	0	0	0	0.7 ± 0.02		99.3	0

Key: 1= C. macrostachyus

2= C. aurea

3= B. polystachya

4= T. schimperiana

5= D. angustifolia

6= J. grandiflorum

Table 7 Percentage schizont maturation at different ethanol extract concentrations.

Extract Concent. (μ g/ml)	1	2	3	4	5	6	Controls		
							Negative	Solvent	Chlorquine
0	100	100	100	100	100	100	100	100	100
0.4	95.6 \pm 72.7	99.3 \pm 70.7	100 \pm 71.3	100 \pm 71.3	99.3 \pm 70.8	99.3 \pm 70.4	100	100	0
2.5	93.4 \pm 72.5	98.5 \pm 69.8	100 \pm 71.1	99.3 \pm 70.7	97.8 \pm 69.4	95.5 \pm 68.1	100	100	0
15.4	48.5 \pm 78.7	96.3 \pm 70	99.3 \pm 70.4	57.4 \pm 71.3	97 \pm 63.1	94.8 \pm 68.5	100	100	0
92.5	33.8 \pm 57.5	63.9 \pm 61.3	98.5 \pm 70	52.1 \pm 73.9	91 \pm 64.7	41.1 \pm 60.8	100	100	0
555.5	5.9 \pm 10	33.8 \pm 50.3	45.6 \pm 67.4	5.1 \pm 8.8	58 \pm 63.5	0.7 \pm 1.2	100	100	0
3333.3	3.7 \pm 7.2	24 \pm 34.7	0.7 \pm 1.6	0.7 \pm 1.6	3.7 \pm 5.9	0	100	100	0

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Key: 1= C. macrostachyus

2= C. aurea

3= B. polystachya

4= T. schimperiana

5= D. angustifolia

6= J. grandiflorum

3.3 Assessment of the inhibitory effect of the extracts

An important parameter for determination of the effect of extracts against isolates of P.falciparum is percentage inhibition of schizont maturation. This was calculated from percentage schizont maturation by subtracting the values of percentage schizont maturation from 100. That is, percentage schizont inhibition = 100 - percentage maturation. The values calculated in this manner for different solvent extracts can be obtained from Tables 5, 6 and 7.

In all tests, examination of thick smears from solvent controls used for extraction of plant parts has no inhibitory effect on the maturation of P.falciparum. On the contrary, chloroquine introduced as a positive control completely inhibited schizont maturation.

At a concentration of 0.4 µg/ml, the highest inhibition of schizont maturation (19.5%) was seen for methanol extract (Table 5) of C.macrostachyus fruits. Methanol extract from other species of plants has low percentage inhibition. Complete inhibition (100%) was observed for C.macrostachyus at a concentration of greater or equal to 15.4 µg/ml and for the other four plant species the same was achieved at 555.5 µg/ml and at 92.5 µg/ml for T.schimperiana.

Chloroform extract of C. macrostachyus has relatively high percentage inhibition (8.9%) at 0.4 µg/ml when compared with other plant extracts at the same concentration (Table 6). In tests that contained greater or equal to 92.5µg/ml chloroform extracts of C. macrostachyus and C. aurea, schizont maturation was completely inhibited. For the rest of the test species chloroform extracts almost completely inhibited schizont maturation at a concentration of 555.5µg/ml (Table 6).

When compared with methanol and chloroform extract, ethanol extracts were not significantly inhibitory at lower concentrations (Table 7) of all species of plants tested. However, extract of C. macrostachyus showed some inhibition (4.6%). Furthermore, except for J. grandiflorum, complete inhibition of schizont maturation was not observed for other species in ethanolic extract tests.

The values at which complete inhibition of schizont maturation was observed (MIC) are shown in Table 8. Of all the extracts, lowest effective MIC value of 15.4µg/ml was seen in test with methanol extract of C. macrostachyus. T. schimperiana which possessed MIC of 92.5µg/ml was the second most potent plant species tested. The other species of medicinal plants had MIC of at 555.5µg/ml. Chloroform extract of C. macrostachyus and C. aurea had an MIC value of 92.5µg/ml while B. polystachya and D. angustifolia had 555.5µg/ml; T. schimperiana had 3333.3µg/ml and J. grandiflorum greater than 3333.3µg/ml. Ethanol extracts

Table 8. In Vitro antimalarial activity (approximate MIC values in $\mu\text{g/ml}$)
of crude extracts.

PLANT SPECIES	EXTRACTS		
	Methanol	Chloroform	Ethanol
1. <u>C. macrostachyus</u>	15.4	92.5	> 3333.3
2. <u>C. aurea</u>	555.5	92.5	> 3333.3
3. <u>B. polystachya</u>	555.5	555.5	> 3333.3
4. <u>T. schimperiana</u>	92.5	3333.3	> 3333.3
5. <u>D. anyustifolia</u>	555.5	555.5	> 3333.3
6. <u>J. grandiflorum</u>	555.5	> 3333.3	3333.3

with the MIC value of 3333.3ug/ml for all plants had the lowest anti-malarial potency.

Computer analysis (SPSS-X₁₁) of probit linear regression displayed probit plots (for three kinds of extracts from six species of plants) (Fig. 3, 4 and 5) of the percentage inhibition of schizont maturation (probit scale) against the concentration of extracts (logarithmic scale). As is evident from the plots, the slope of the probit linear regression lines for percentage inhibition of schizogony against methanol extract concentrations were steep for C. macrostachyus and D. angustifolia; intermediate for T. schimperiana and more gradual for J. grandiflorum; C. aurea and B. polystachya (Fig.3). In the case of chloroform extracts (Fig.4), the slope lines were steep for C. macrostachyus, C. aurea and B. polystachya, and gradual for the rest species of plants. When compared with methanol and chloroform extracts, steepness of the slope lines of ethanol extracts has decreased (Fig.5). From these plots, the 50% inhibitory concentration (IC₅₀) and 99% inhibitory concentration (IC₉₉) were determined (the actual values ranging from 1-99% were displayed by the computer) and the IC₅₀ and IC₉₉ values are given in Table 9.

The methanol extract of C. macrostachyus fruits possessed the lowest IC₅₀ and IC₉₉ values (0.94 and 10.28ug/ml, respectively) when compared with the extracts of the other five species of plants. But when this extract was partitioned with chloroform/water, the IC₅₀ value of

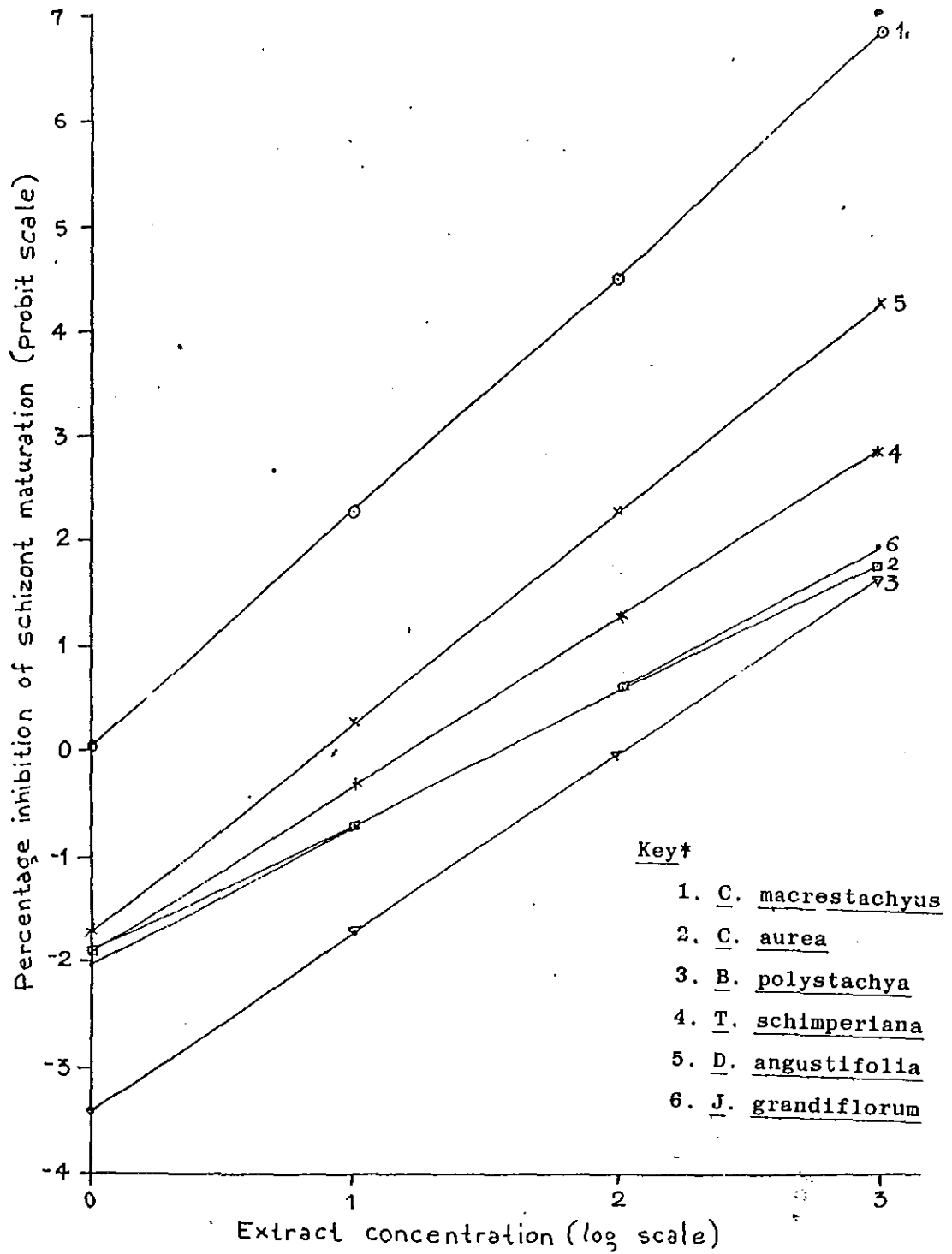


FIG. 3, Probit linear regression plots showing the in vitro inhibition of schizont maturation of methanol extract from 6 species of plants.

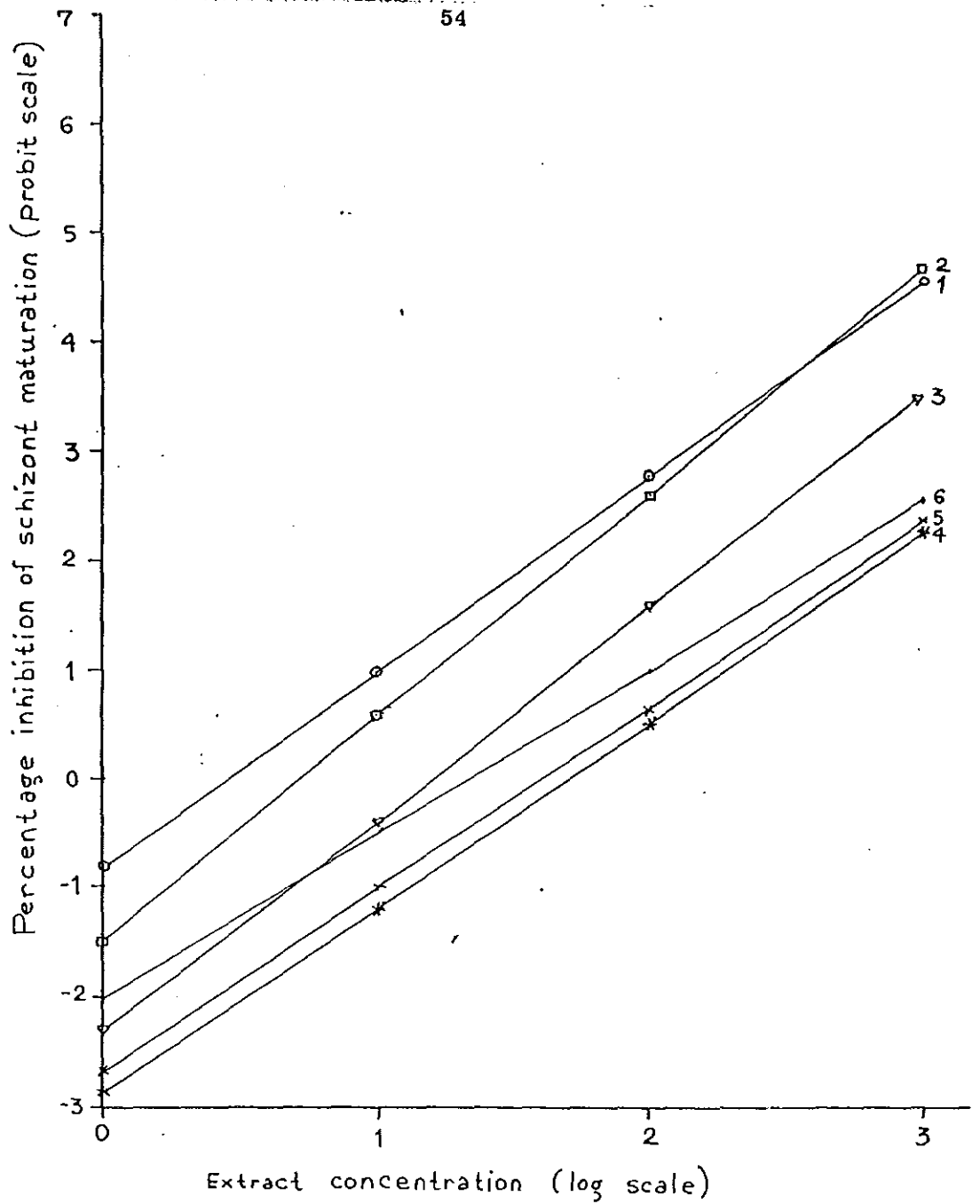


FIG. 4. • Probit linear regression plots showing the in vitro inhibition of schizont maturation of chloroform extract from 6 species of plants.

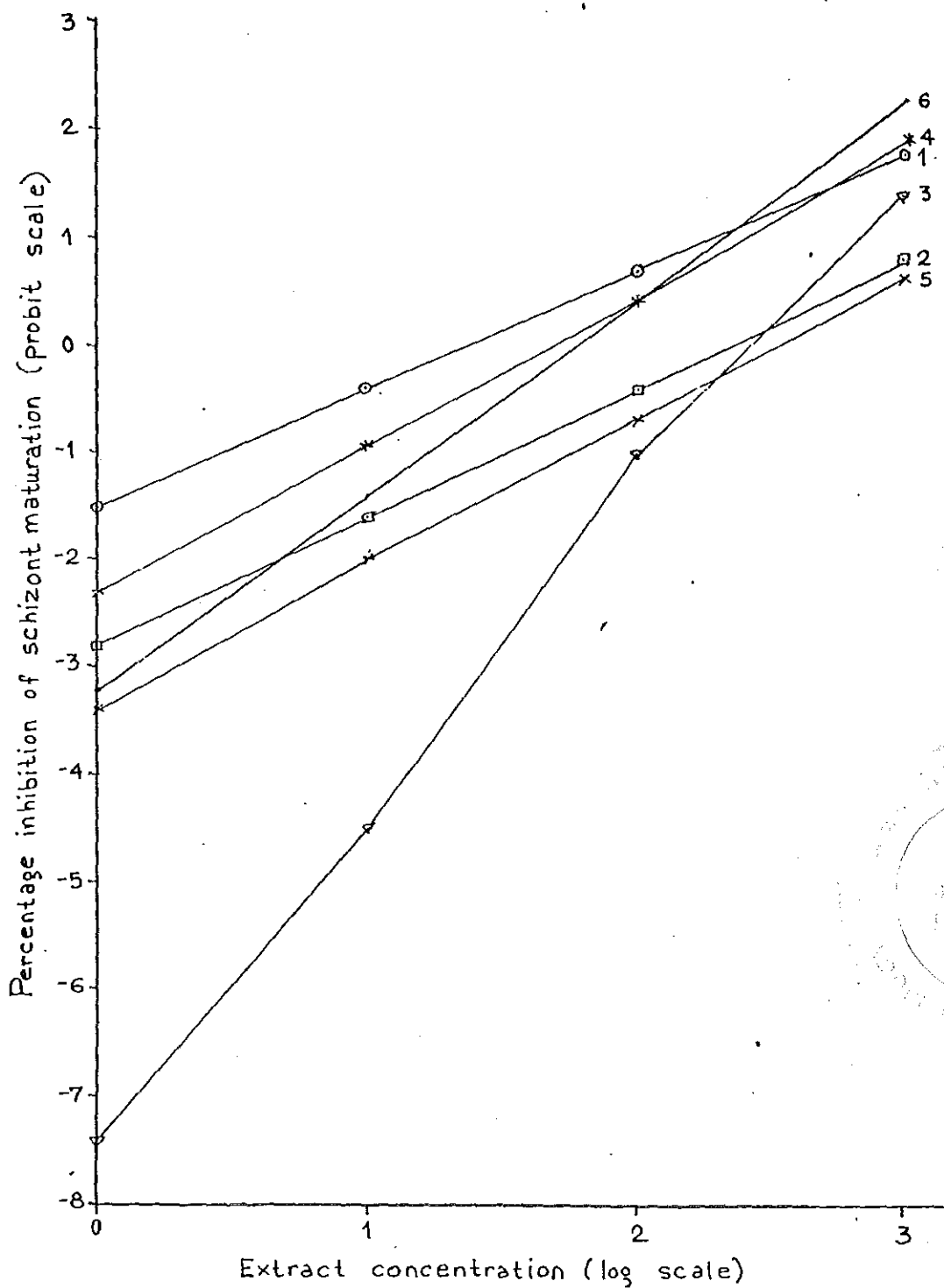


FIG. 5. Probit linear regression plots showing the *in vitro* inhibition of schizont maturation of ethanol extract from 6 species of plants.

chloroform extract increased 3 times (2.88 μ g/ml) and the corresponding IC₅₀ increased 5 times (54.16 μ g/ml) than that of methanolic extracts. The ethanol extract of the same plant possessed the highest IC₅₀ and IC₉₉ values than the methanol and chloroform extracts. When methanol extract was partitioned, a similar increase in IC₅₀ and IC₉₉ values were noted with the extract of D. angustifolia. The IC₅₀ of the methanol extract of this plant was determined to be 7.06 μ g/ml while that of chloroform extract was 42.47 μ g/ml and the ethanol extract was 315.97 μ g/ml. In the case of C. aurea, B. polystachya and J. grandiflorum, chloroform extracts possessed low IC₅₀ values than their corresponding methanol and ethanol extracts, the latter possessing the highest value. This indicates that, in partitioning the methanol extract, the IC₅₀ of chloroform extracts of these plants has decreased. Leave extract of T. schimperiana possessed low IC₅₀ values for ethanol (44.57 μ g/ml) and chloroform (48.43 μ g/ml).

antimalarial activity and those with between 10-50 $\mu\text{g/ml}$ are considered to be less active. Among the six species of plants tested for antimalarial activity in this study, the methanol extract of the fruit of C. macrostachyus possessed the lowest IC_{50} values (0.94 $\mu\text{g/ml}$). Furthermore, complete inhibition of schizont maturation was also attained at a concentration of 15.4 $\mu\text{g/ml}$. The above two inhibitory concentrations are good evidences of the plant's antimalarial activity. The IC_{50} values obtained for the methanol extract of C. macrostachyus was almost twice higher than that reported for the methanol extract of Cinchona (0.5 $\mu\text{g/ml}$) grown in Tanzania. However, the fact that it compares well with a renowned antimalarial Cinchona (quinine) indicates that methanolic extract of C. macrostachyus has a good potential for use as an antimalarial agent.

On the otherhand, the high antimalarial activity of the methanolic extract decreased almost three times upon partitioning it between chloroform/water. This is an indication of the compound nature of the active principle of the plant. The higher IC_{50} value of 23.9 $\mu\text{g/ml}$ determined for the ethanol extract of C. macrostachyus shows that the most potent antimalarial principle contained in the plant does not come out (dissolve) in ethanol or otherwise its activity is reduced when dissolved in ethanol.

According to El-Kheir and Salih (1979), chromatographic analysis of the seeds of C. macrostachyus showed that it

contains alkaloids, amino acids and sterols as well as triterpens or their saponins. These chemical characteristics are shared by those that have proven antimalarial activity. For example, in the extracts of A. annua, the novel sesquiterpene lactone, a series of related terpenes, other terpenes, a flavonoid and scopoletin have been isolated (Tu You-you, et.al., 1982). Also significant level of activity was found in the extracts of species from Simaroubaceae. In these, the active ingredients were found to be degraded terpenes known collectively as quassinoids (bitter terpenes). The biological activity of these compounds include antimalarial and amoebicidal (Steck, 1972; Gillin, et.al., 1982), antiviral (Pierre, et.al., 1980), antifeedant (Polynsky, 1983), anticancer (Suffness and Douros, 1979) and anti-inflammatory (Hall, et.al., 1983). In addition, the alkaloid, quinine, isolated from Cinchona species has been used for treatment of malaria. Although isolation of refined constituents of C. macrostachyus requires further chemical and biochemical studies, it can be suggested that the antimalarial activity of extracts from this plant species observed in this study, could be due to either one or more of the reported bioactive compounds (most probably triterpene or alkaloids).

The methanol extract of the leaves of C. aurea possessed relatively low activity (IC_{50} value 34.56 $\mu\text{g/ml}$) but when partitioned, the resulting chloroform fraction of the leaves had an increased activity (IC_{50} 5.36 $\mu\text{g/ml}$). Complete

inhibition of schizont maturation was attained at MIC value of 92.5 µg/ml. The ethanolic extract of this plant appeared to be essentially inactive. These findings suggest that the antimalarial active principle in C. aurea are different from that found in C. macrostachyus.

The IC₅₀ value (7.06 µg/ml) of the methanol extract of D. angustifolia seeds is within the range of the highest antimalarial activity. The resulting chloroform fraction and ethanol extract of this species of plant were found to be inactive. It can therefore, be assumed that there is similarity in the active principles of D. angustifolia and C. macrostachyus.

The chloroform extracts of B. polystachya leaves and that of J. grandiflorum root and bark, as well as the methanol extract of T. schimperiana leaves possessed low activity with IC₅₀ values outside the range of high antimalarial activity. Also methanol and ethanol extracts of these plant species were inactive even at much higher concentrations.

The solvent that contained the most potent extract in this study varied from one species of plant to another. Hence, there was no single solvent which contained potent extract for all six species of plants tested. However, for a particular species of plant, one kind of solvent was found to be more potent than others. For example, for C. macrostachyus and D. angustifolia, methanol extract

5. CONCLUSION AND RECOMMENDATIONS

Ethiopia is rich in medical lore and various species of plants have been used locally for the treatment of diseases, including malaria. However, the therapeutic value of these plants has not been substantiated by scientific proof. Thus, the findings of the present study, are good indications that some Ethiopian medicinal plants possess substances with antimalarial effects. As a result, it is an additional example of the correctness of traditional wisdom of Ethiopian traditional medical practitioners. The present findings are also good indications that extensive screening efforts could lead to the identification of very effective drugs with novel action. Such information would enable us to gradually integrate traditional health care with modern health care system especially at the primary health care level.

The present study was an initial step in screening Ethiopian medicinal plants for their antimalarial activity. Based on the present findings a few consideration, before recommending possible use of the traditional medicinal plants investigated would be necessary:

1. Confermation of the antimalarial activity of the plant extracts based on column fractionated samples;

2. Since it is possible that cytotoxic natural products could give false positive results in any screening test based on an in vitro system, further work on determining the in vitro cytotoxicity of extracts to special cells such as guinea pig keratinocytes (GPK cells) is recommended before in vivo work is undertaken on humans (O'Neill, et.al., 1985a).
3. Isolates of P.falciparum used in this study may contain strains with different susceptibility to drugs. Therefore, it is essential to confirm the effectiveness of the active compounds in extracts against known resistant strains
4. Most traditional medical practitioners prescribe plant parts from two or more species of plants for the treatment of malaria. Therefore, further studies would be essential to investigate the possible synergistic effects of the extracts.

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