EVALUATION OF THE ANTIMALARIAL ACTIVITY OF SOME ETHIOPIAN MEDICINAL PLANTS AGAINST *PLASMODIUM FALCIPARUM IN VITRO*

A Thesis
presented to the School of Graduate Studies
Addis Ababa University

In partial fulfilment of the requirements
for the degree of
Master of Science in Biology

By
Mesfin Bogale

June, 1994.

ACKNOWLEDGEMENTS

I express my deepest gratitude to my research advisor Dr Beyene Petros who always has had the time to guide me through the research work. I would also like to thank Dr Ermias Dagne for his valuable advices and support of the work; the Natural Products Research Network for Eastern and Central Africa (NAPRECA) for covering the research and subsistence costs during my stay in Madagascar, and also for covering 25% of the cost of transportation, through its IPICS-supported Exchange of Researchers Scheme; the Ethiopian Science and Technology Commission for covering 75% of the cost of transportation.

I am indebted to Prof R. Ratsimamanga, Prof P. Rasoanaivo, Mr R. Milijaona and Mrs B. Rafatro (Institut Malagache de Recherches Appliquées, IMRA, Madagascar) who helped me with the work, and all the staff of IMRA who made my stay in Antananarivo very pleasant.

Dr Sebsibe Demissew and Dr Ensermu Kelbessa are thanked for identification of the plants used in this study. My thanks are also due to Dr Wendimagegn Mammo, W/t Senait Asmellash, Ato Melaku Alemu, Ato Tekabe Ayalew, Ato Mamuye Hadis and all who helped me with one or another aspect of the research work.

The National Programme for the Control of Malaria and other Vector-Borne Diseases is also thanked for allowing me access to its research facilities. I also thank all its staff for the technical support they offered me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Thirty-three crude extracts from nine Ethiopian medicinal plants that are used to treat malaria were screened for antimalarial activity against a chloroquine-resistant FCM-29 strain of *Plasmodium falciparum in vitro*. The measurement of radiolabelled hypoxanthine incorporation by *P. falciparum* was used to assess inhibition of growth of the parasite. Three extracts, namely, chloroform and methanol extracts of leaves of *Withania somnifera*, and chloroform extract of leaves of *Vernonia amygdalina*, showed substantial antimalarial activity with IC_{50} and IC_{90} values less than 20 and 100 μg/ml, respectively. The IC_{50} and IC_{90} values of the remaining extracts were out of this range. The antimalarial activity of the three crude extracts improved by several-folds upon column-fractionation. These three crude extracts were also tested for cytotoxicity against human HeLa cells *in vitro;* all of them showed high cytotoxic activity. However, the antimalarial and cytotoxic components of the extracts have not been determined.

I. INTRODUCTION

A. Malaria

Malaria is a protozoal disease transmitted to man by female mosquitoes of the genus *Anopheles*. It is characterized by a cyclic course of periods of febrile attacks. Splenomegaly, anaemia and occasional severe lesions of the nervous system, kidneys, and other organs also characterize the disease.

Malaria has been known since the beginning of civilization. In Ebers Papyrus (1500 B.C.) are mentioned '*rigors*,' fevers, splenomegaly, and the use of an oil expressed from Balanites trees as a mosquito repellant (Loban and Polozok, 1985). The aetiology remained a mystery until Laveran identified the causative agents of human malaria in 1880; it was only in 1897 that the role of mosquitoes in the transmission of malaria was discovered by Ronald Ross (Bruce-Chwatt, 1985).

Plasmodium falciparum, P. vivax, P. ovale and *P. malariae* are the causative agents of human malaria. They belong to the Phylum Protozoa, Class Sporozoa, Order Haemosporidia, Family Plasmodiidae and Genus *Plasmodium*. The genus *Plasmodium* is further divide into two Subgenera: Subgenus Plasmodium, containing *P. vivax*, *P. ovale* and *P. malariae*; Subgenus Laverania, containing *P. falciparum* (Beaver *et. al.*, 1984; Black *et. al.*, 1986).

The types of causative agents differ on the basis of their morphological characteristics, virulence, duration of the incubation period, immunological and epidemiological characteristics and their sensitivity to the effect of chemotherapeutic drugs.

Malaria occurrence is determined by the zone of prevalence of the vectors, female mosquitoes of the genus *Anopheles*, and suitable ambient temperature ensuring the

completion of sporogony in the mosquito. Out of the four types of causative agents of human malaria, the greatest dissemination has been achieved by *P. vivax* (TDR, 1988). In some countries of temperate climate, vivax malaria has been the single or predominant form. This is explained by the capacity of *P. vivax* to develop in the vector at lower temperatures and also by the longer duration of the course of vivax malaria (Lysenko *et. al.*, 1978; Marcus, 1976). *P. vivax* can survive for a long time in the body of the vertebrate host in the inactive dormant state in the form of hypnozoites (Markus, 1978. In: Bruce-Chwatt, 1980; WHO, 1991).

In the tropical zone, particularly on the African continent, the predominant malaria parasite is *P. falciparum* (Bruce-Chwatt, 1985). However, in the equatorial zone of South America, *P. falciparum* is inferior to *P. vivax* in terms of geographical distribution (WHO, 1990). The geographical prevalence of *P. malariae* and *P. ovale* is characterized by a discrete and focal pattern (WHO, 1989). Despite its comparatively restricted geographical area of incidence, *P. falciparum* is responsible for 50% of the morbidity and 98% of the mortality from malaria throughout the world (Bruce-Chwatt, 1985).

In Ethiopia, the two epidemiologically important species are *P. falciparum*, comprising 60% of all the cases of malaria, and *P. vivax*, constituting nearly 40% of all malaria cases (Assefa Nega, 1993). *P. malariae* comprises less than 1% of all cases and is most frequently reported from the Arba Minch area whereas *P. ovale* is rarely reported (Assefa Nega, 1993). About three quarters of the total area of Ethiopia is estimated to be malarious and about two thirds of the inhabitants of the country are at risk of infection (Assefa Nega, 1993).

The sexual phase of the life cycle of malaria parasites begins with entry of the male (micro) and female (macro) gametocytes into the alimentary tract of the female *Anopheles* mosquito with its blood meal. The nucleus of the male gametocyte undergoes division to

give rise to eight nuclei, which immediately migrate to the periphery of the gametocyte. The eight nuclei travel into the long and thin cytoplasmic processes that protruded outside the cell membrane. The microgametes break away from the parent body and wander about the contents of the midgut in search of female gametes.

The macrogametocytes undergo a process of maturation; the nucleus moves towards the surface where a small projection forms, and into which wriggles the microgamete during fertilisation. The process continues amid the gut contents of the insect with the formation of the ookinete or travelling vermicule. The ookinete crosses the epithelial layer and rounds up on the outer side of the stomach. There it secretes a thin cyst wall and proceeds to grow into a sphere. The diploid nucleus of the oocyst undergoes meiotic divisions followed by mitotic divisions. Upon maturity the oocyst bursts and the sporozoites are discharged into the haemocoel, and proceed to invade all parts of the body of the insect.

Through its bites as it takes its next meal, the infected mosquito introduces sporozoites along with its saliva into the vertebrate host. The sporozoites then enter the liver and pass through the next stage of the life cycle, *i.e.*, exoerythrocytic schizogony, where the parasite assumes a rounded or oval form and the nucleus undergoes repeated divisions.

All malaria parasites have basically the same life cycle. Primary exoerythrocytic schizogony ends with the invasion of red blood cells by tissue merozoites (Black *et. al.*, 1986). However, in *P. vivax* and *P. ovale*, some of the tissue stages remain in the liver and form hypnozoites (Lysenko *et. al.*, 1978; Markus, 1978. In: Bruce-Chwatt, 1980; WHO, 1991) which are responsible for secondary exoerythrocytic schizogony and relapses in patients.

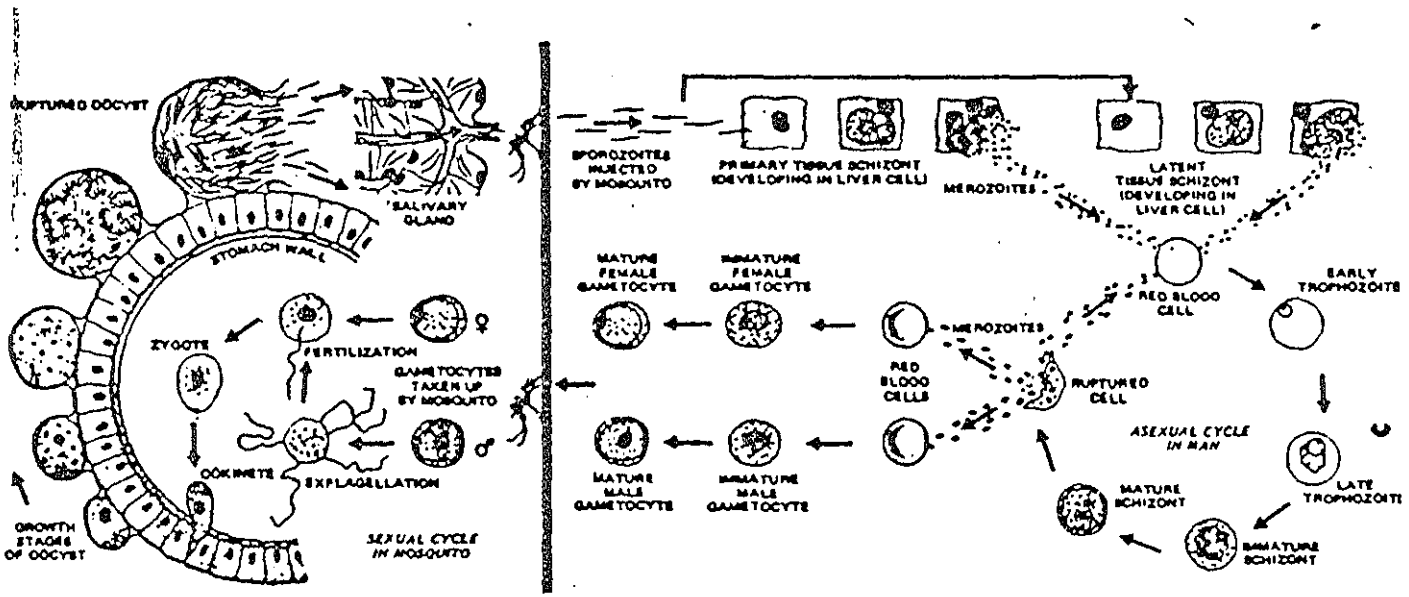

Fig. 1. Cycles of development of malaria parasites in the anopheline mosquito and in man.
From: Black *et. al.*, 1986.

Trophozoites in the red blood cells grow in size, and their nuclei continue to divide by mitosis until mature schizonts with the respective number of merozoites are produced. The red blood cell envelope bursts and merozoites escape into the blood stream, where those that are not destroyed by the immune mechanism quickly invade other red blood cells to start a new cycle.

Malaria is one of the most wide-spread transmissible diseases especially in the tropics and subtropics. A malaria patient whose peripheral blood contains mature gametocytes serves as the source of infection. In endemic areas where most adult populations possess acquired immunity, children aged 3 months-15 years serve as the major source of malaria (Cohen and Butcher, 1974). This is because they lack acquired immunity and usually have a very high parasitaemia as a result. Adults lacking acquired immunity also serve as sources of infection. However, the infectivity of parasites from immune adults appears to be less marked than the non-immune children and adults (Cohen and Butcher, 1974).

The duration of sporogony, which usually parallels with the length of the gonotrophic cycle of the mosquito, shortens at higher temperatures within the optimal range, *i.e.*, 20-30°C (Ravetch *et. al.*, 1985). Mean relative humidity higher than 60% lengthens the life of the mosquito (Bruce-Chwatt, 1980). Such high mean temperature and relative humidity are characteristics of tropical and subtropical climates and account for the high prevalence of mosquitoes (WHO, 1989) and hence the high transmission of malaria in these regions.

Routes of malaria transmission other than through the bites of infected mosquitoes are also possible. Intra-uterine infection may occur through the placenta (McGregor *et. al.*, 1983). Most malaria parasites may remain viable in blood for 10 days or more, especially

when anticoagulants contain dextrose (Bruce-Chwatt, 1974). As a result, transmission of malaria is also possible in transfusion of blood obtained from malaria parasite carriers or of preparations made from this blood.

Pathological changes in malaria are produced in various ways. Red blood cells parasitized with *P. falciparum* become more adherent to the lining of blood vessels (Miller, 1972; Hall, 1976; Aikawa, 1988). As the thin rings (trophozoites) mature into the multinucleate form (the schizont), the parasitized cells become even more adhesive, especially in the viscera (Hall, 1976). This explains why schizonts may be present in the viscera but not in the circulating blood and why the disease may be severe with a low concentration of parasites in the circulating blood (Hall, 1976).

Blockage of cerebral capillaries by *P. falciparum* infected erythrocytes appears to be the principal cause of cerebral malaria (Aikawa, 1988). Deposition of immune complexes in brain capillaries also contributes to the development of cerebral malaria (Clark, 1987).

Parasitized cells carry less oxygen which will contribute to anoxia. In malaria, the degree of anaemia is often greater than could be explained on the basis of parasitized red blood cells alone (Hall, 1976). This is mainly because anaemia in malaria results from an increased rate of red blood cell destruction and also reduction in red blood cell formation, *i.e.*, bone marrow function is depressed (Srichaikul *et. al.*, 1976).

Almost all individuals who have never had malaria are susceptible to this disease. Malaria evokes a poor protective immunity in humans. Residents of endemic areas acquire a partially protective immunity after several years of exposure to intense malaria transmission (Mendis *et. al.*, 1991). In malaria endemic areas, infants born to immune mothers receive passive immunity from their mothers and never contract malaria in the first

three months of life (Cohen and Butcher, 1974).

Most West Africans and their descendant black Americans have a congenital, genetically determined resistance to *P. vivax*. The nature of this phenomenon lies in the insufficiency in these people of isoantigens in Daffy blood group (Fy^a or Fy^b), which serve as parasite receptors, *i.e.*, the factor of parasite attachment to the red blood cell (Miller *et. al.*, 1976. In: Loban and Polozok, 1985).

Carriers of the sickle cell gene (haemoglobin S) are less susceptible to malaria than non-sicklers (Livingstone, 1976). Malaria is characterized by low parasitaemia and a considerably milder course in carriers of the sickle cell anomaly than non-sicklers when both lack the acquired immunity to malaria (Livingstone, 1976). Most of the homozygotes for the sickle cell gene die in childhood whether or not they live in malarious areas (Carlson, 1984).

About 40% of the world's population lives where malaria is a risk; 300-500 millions fall ill and 1-2 million die from the disease every year (WHO, 1993). Over 80% of the morbidity and mortality from malaria occurs in tropical Africa (WHO, 1993).

An estimated 150,000 people died and 3 million others contracted malaria during the epidemics that struck Ethiopia in 1958 (Fontaine, 1961). Also morbidity exceeding 75% of the population was encountered in many rural settlements. In spite of control efforts the prevalence and incidence of malaria in Ethiopia has greatly increased since the mid-1980s (Assefa Nega, 1993). In general, there has been a resurgence of malaria in recent years all over the world (WHO, 1993). This is mainly due to the widespread development of resistance by *P. falciparum* to chloroquine and to an increasing extent to quinine and other antimalarial drugs (Payne, 1987). In addition, the use of two useful drugs, amodiaquine and fansidar has had to be restricted because of serious adverse effects

(Phillipson and Wright, 1991). Furthermore, the increasing resistance of vector mosquitoes to insecticides (Wright and Phillipson, 1990) and the environmental unsafeness of the insecticides has hampered malaria control measures.

From among 250,000 compounds which were synthesized and tested for antimalarial activity only mefloquine was developed into antimalarial drug at a cost of 150 million US dollars (Salako, 1985. In: Nkunya, 1992). Because of the disadvantages in such blind screening programmes the attention has now been turned onto medicinal plants, most of which have been used as antimalarials for centuries.

B. Medicinal Plants Against Malaria

According to Sofowora (1982), a traditional medicine can be described as "the total combination of knowledge and practice, whether explicable or not, used in diagnosing, preventing, or eliminating a physical, mental, or social disease and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing." A medicinal plant is any plant which contains substances that can be used for therapeutic purposes or serve as precursors for the synthesis of useful drugs (Sofowora, 1982).

Until the beginning of the 19th century, all medicine was traditional (Jellife and Jellife, 1977). A large number of people in the world depend on medicinal plants to treat various types of diseases, mainly because they have no access to modern medicine (Elmi, 1990). A number of plants have been used in the traditional medicinal system for the treatment of malaria for many years (Chopra *et. al.*, 1956; Fransworth, 1985; Gelahun Abate, 1989). Some do seem to work although there may not be sufficient scientific data to confirm their efficacy; such plants also should qualify as medicinal (Sofowora, 1982).

Plants from the Family Simaroubaceae have been in use in traditional medicine as febrifuges, especially species of *Brucea, Castela, Harrisonia, Picramnia, Quassia* and *Simaba* (Steck, 1972). Fransworth (in Elmi, 1990) points out that one quarter of the total prescription drugs in industrialized countries contain one or more components derived from plants.

In view of the number of resistant strains of *P. falciparum* in Africa (Brandicourt *et. al.*, 1986; Brasseur *et. al.*, 1987; Brasseur *et. al.*, 1988; Brasseur *et. al.*, 1992a; Brasseur *et. al.*, 1992b), Asia (Baird *et. al.*, 1991), South and Central America (Nguyen-Dinh *et. al.*, 1981; Payne, 1987), the need for new antimalarial agents with novel modes of action cannot be overemphasized.

In vitro investigations for possible antimalarial agents are greatly facilitated by the availability of continuous culture lines of *P. falciparum* (Trager and Jensen, 1976; Haynes *et. al.*, 1976; Osisanya *et. al.*, 1981; Fairlamb *et. al.*, 1985). *In vivo* test systems that use *P. berghei* (Peters, 1980) are also available. Though expensive and time consuming, these *in vivo* systems have advantages in that they are easier and are done in the actual physiological conditions. They also enable to assess antimalarial efficacy and acute toxicity of the test materials simultaneously.

Quinine, from the bark of Cinchona, whose legend dates from the 17th century (Bruce-Chwatt, 1985) is an outstanding example of a plant product which has been used in the treatment of malaria. Artemesinin from the Chinese medicinal herb *Artemisia annua* (Li *et. al.*, 1983; O'Neill *et. al.*, 1985a; Basco and Le Bras, 1993) is another example of this kind.

Many plants have also been tested for antimalarial activity and promising results have been obtained. For instance, antimalarial activity was detected *in vivo* in species of

Ailanthus, Castela, Picramnia, Picrasma, Picrolemma, Quassia, Samadera, Simaba, Simarouba (Spenser *et. al.*, 1947. In: Guru *et. al.*, 1983) and *Brucea* (Thu *et. al.*, 1979. In: Guru *et. al.*, 1983; O'Neill *et. al.*, 1987), *Spathodea campanulata* (Makinde *et. al.*, 1988).

In vitro antimalarial activities were also observed in *Brucea javanica* (O'Neill *et. al.*, 1985b; O'Neill *et. al.*, 1987), *Eurycoma longifolia* (Chan *et. al.*, 1986), *Ailanthus altissima* (O'Neill *et. al.*, 1985a), *Croton macrostachys, Calpurnia aurea* and *Dodonea angustifolia* (Solomon Sorssa, 1992).

Trager and Polonsky (1981) found that quassinoids Simalikalactone D, glaucarubinone, and soularubinone have high activity against chloroquine-resistant strains of *P. falciparum in vitro*. The most highly active quassinoids are many times more potent than chloroquine against multi-drug resistant (K1 strain) *P. falciparum*; for example, bruceantin has an IC_{50} of 0.008 μg/ml compared to 0.210 μg/ml of chloroquine (O'Neill, 1986). Li *et. al.* (1983) have also reported on the effects of qinghaosu and other related compounds on the incorporation of radio labelled hypoxanthine by *P. falciparum in vitro*.

Several plants have been reported to be of use in the treatment of malaria in traditional medicinal practices in Ethiopia (Gelahun Abate, 1989; Mesfin Tadesse and Sebsibe Demissew, 1992). The main objective of this research work is to assess, in an *in vitro* system, the antimalarial properties of some of these medicinal plants. Attempt has also been made to evaluate the cytotoxicity of some of them, *in vitro*.

II. MATERIALS AND METHODS

1. Plant materials

The plant materials were collected from Biteyou Forest, Butajira, Southern Shoa, during the month of November, 1993; Ares Kebele, Jihur Woreda, Northern Shoa, during the month of February, 1993; Arat kilo area, Addis Ababa, during the month of October, 1993. The identity of each plant material was confirmed and herbarium specimens to each has been deposited at the National Herbarium, Department of Biology, Addis Ababa University.

The plants collected were: *Clutia abyssinica* Jaub & Spach (Euphorbiacea) (Amharic: *fiyel-fej*), *Cucumis ficifolius* A. Rich (Cucurbitaceae) (Amharic: *yemidir-inbuyi*), *Jasminum abyssinicum* Hochst ex DC. (Oleaceae) (Amharic: *tambelel*), *Justicia schimperiana* (Hochst. ex Nees) T. Anderson (= *Adhathoda schimperiana* Hochst ex Nees) (Acahantaceae) (Amharic: *sensal*), *Leonotis velutina var. rugosa* Bark (Lamiaceae) (Amharic: *yefares-zang*), *Securidaca longepedunculata* Fres (Polygalaceae) (Amharic: *etsemenahe*), *Tmarindus indica* L. (Fabaceae) (Amharic: *komar*), *Vernonia amygdalina* Del. (Asteraceae) (Amharic: *girawa*), *Withania somnifera* (L) Dunal. (Solanaceae) (Amharic: *gizawa*).

1.1. Description of the plants

***Clutia abyssinica* Jaub. & Spach.** "*Clutia* is a genus of very similar herbaceous shrubs with lanceolate leaves usually upto 3 in. long and obtuse, entire, and narrowing to the base: they turn orange-red when old. Flowers small and usually dioecious, the males pedicellate in axillary fasciales, the females often solitary with longer pedicels; pentamerous, stamens 5, ovary 3-celled. Fruit a small subglobose capsule. The plants usually occur in grassy scrub in the highlands" (Dale and Greenway, 1961).

"*C. abyssinica* is a dioecious woody shrub to 2 m with a variable leaf shape (ovate to obanceolate or elliptic, obtuse or acute), a variable indumentum (nearly glabrous to densely velvety-pubescent) but with very constant floral characters" (Agnew, 1974).

***Cucumis ficifolius* A. Rich.** "Perennial. Stem slender, angled; petioles and peduncles scabrid with stiff white prickles. Leaves very variable, coriaceous, very scabrid, 1-2 in. in diameter, generally longer than broad, 3-7-lobed to or below the middle, sinus dilated and rounded at the base; lobes obovate, acutely toothed, rounded or acute; petioles rather short. Tendrils short, rather stiff. Male fl.: calyx hispid. Anthers with long projecting gland-tipped connectives, obscurely ciliate. Female fl.: Stigmas sessile, oblong obcordate. Ovary densely clothed with stiff, short, conic, rigid setae, each with a transparent pungent tip. Fruit 3/2 in. long; broadly ovoid, yellow, densely clothed with soft spines 1/3 in. long. Seeds small, 1/6 in. long, elliptic-oblong, smooth, white or pale brown, without thickened margins or depressed disk" (Oliver, 1871).

***Jasminum abyssinicum* Hochst. ex DC.** "Generally a climbing or a scrambling shrub with the young branches, the inflorescences, and the calyces puberulous or tomentose to nearly or quite glabrous. Leaves trifoliolate; opposite; leaflet blades elliptic to ovate, more or less acute, very variable in size but usually about 5-6 cm long and 3-4 cm broad (up to 11 cm long and 5 cm broad), midrib and lateral veins impressed on upper and prominent on lower surface; petiolule to terminal leaflet 1-2 cm long, to lateral leaflets 0.2-0.6 cm long. Inflorescences terminal and lateral, the final cymes having three to about 50 flowers, the lower numbers on the lateral and the higher on the terminal ones. Calyx (at anthesis) 3-4.5 mm long and 2.5-4 mm in diameter at the apex which is often more or

less truncate. Corollas scented, white; tube 1.3-2.5 cm long" (Turril and Milne-Redhead, 1952).

Habitat: Forest undergrowth and margins, hill sides, bushland, often near streams; 690-2700 m (Turril and Milne-Redhead, 1952).

***Justicia schimperiana* (Hochst. ex Nees) T. Anderson.** "Shrub. Leaves 6 by 2 and 1/4 in., narrowed at either end, obtuse, glabrous; petiole 1/4 -3/4 in. long. Spikes 3-7 by 1 in., several peduncled, often forming a terminal thyrsus, strobilate; bract 2/3 by 1/2 in., minutely pubescent, with a prominent scarious margin; bracteoles 1/3-1/2 in. long, ovate. Sepals 5, nearly separate, subsimilar, 1/3 in. long., oblong-lanceolate, pubescent. Corolla 1 in. in total length, yellowish, purple-spotted, lips 2/3 in. long. One anther-cell slightly below the other, mucronate at the base; pollen ellipsoid, with two stopples, and rows of spots on the longitudinal smooth bands. Ovary and style-base shaggy. Capsule 3/4-1 in. long, pubescent, usually 4-seeded, lower 1/3 part a cylindric stalk; placentae not rising elastically; seeds glabrous, rugose" (Thiselton-Dyer, 1900).

***Leonotis velutina var. rugosa*. Baker.** "The genus *Leonotis* consists of plants whose calyx-tube is funnel shaped, arcuate, 8-10-ribbed; throat oblique; teeth 8-10, more or less unequal, the upper the largest. Corolla-tube as long as the calyx; limb bilabiate; upper lip elongated, concave, hairy outside; lower short, deflexed, with 3 subequal lobes. Stamens 4, didynamous, arcuate; lower pair longest; anthers 2-celled; cells divaricate, subconfluent. Disk equal. Style shortly bifid. Nucules ovoid-triquetrous, obtuse or truncate, glabrous. Coarse tall, annual perennial herbs. Leaves petioled, ovate, crenate. Whorls very dense, axillary. Flowers white or yellow" (Thiselton-Dyer, 1900).

L. velutina var. rugosa "is a tall, branched perennial herb, with stout finely hairy stems. Leaves distinctly petioled, ovate, crenate, very hairy beneath. Whorls very dense, 3 in. diam.; bracts small, linear. Calyx-tube very hairy, 1/3 in. long; teeth deltoid uppermost much the largest. Corolla deep bright yellow, 1 in. long; upper lip 1/3 - 1/2 in. long" (Thiselton-Dyer, 1900).

***Securidaca longepedunculata* Fres.** "Savanna tree or shrub usually 10-15 ft. high, but sometimes attaining 30 ft. Branchlets slender, erect or drooping, pubescent. Bark pale brown to grey-brown, rough with very small dark coloured scales. Slash yellow. Leaves minutely pubescent below oblong to oblong-lanceolate, 1-1.5 in. long, 1/2-3/4 in. broad, rounded at the apex, lying more or less parallel to the wings. Flowers reddish purple to pink, very fragrant (smelling of violets), borne in loose racemes; sepals 2; petals 3, the lower one appendaged and larger than the others; stamens 8. Samaras about 2 in. long, yellow-green to red, usually in bunches of 6-8. Wood pale yellow, the annual rings distinctly marked in dark brown, the dry wood parting at these rings into a series of concentric cylinders; Weight 55 lb. per cu. ft., air dry. Wet savanna; from the coast to 5, 000 ft." (Dale and Greenway, 1961)

***Tamarindus indica* L.** "Evergreen tree 3-4 m high. Leaves 5-12(-16) cm long; leaflets c 10-18 pairs, narrowly oblong, (0.8-)1.2-3.2 x 0.3-1.1 cm, usually glabrous or nearly so. Racemes 1-15(-22) cm long. Sepals 0.8-1.2 cm long, reddish outside. Large petals elliptic or obovate-elliptic, 1-1.3 cm long, golden with red veins. Pods (3-)6.5-14 x 2-3 cm, 1-10 seeded. Seeds chestnut brown, 11-17 x 10-12 mm" (Polhill and Thulin, 1989).

Habitat: grassland, woodland and *Combretum* bushland, frequently riparian; up to 1500 m. Native of tropical Africa (doubtfully also Asia), now widely cultivated in tropical and subtropical regions for ornament and for the fruits (Polhill and Thulin, 1989).

The acid pulpy part of the fruits is used for preserves, in cooking and as a cooling, mildly laxative drink (Polhill and Thulin, 1989) valuable in fevers (Dale and Greenway, 1961); the seeds are also edible. The very hard durable wood is useful for various constructional and domestic purposes. Now recognised as an important species for conservation and use in agroforestry systems (Polhill and Thulin, 1989).

***Vernonia amygdalina* Del.** "A freely branched shrub of 6-10 ft. high, or a small tree. Branches terete or somewhat angular above, striate, nearly or quite glabrous below, puberulous above. Leaves alternate, lanceolate or lanceolate-oblong, more rarely ovate-elliptical, narrowed at both ends, acute, firmly membranous, serrulate or subentire, puberulous or glabrescent, subsessile or petiolate, 4-6-8 in. long by 5/2-3 in. wide; petioles in most forms quite short, in others upto 1/2-3/4 in. wide, on pedicels 1/8-1/4 in. long, in dense much branched globose or pyramidal terminal panicles, 4-12 in. diameter. Scales of the involucre pluriseriate, ovate elliptic or oblong, obtuse or subacute, pale green with darker spot near tip, scarcely glabrous or ciliolate; inner ones 1/5 in. long by 1/12 in. wide, deciduous. Receptacle glabrous, flat, areolate. Corolla gradually narrowed, white. Anther-tips linear-lanceolate, -base produced. Achenes setulose, obscurely 10-costate, sessile glandular. Pappus barbellate, sub-uniserate; setae equal or some shorter, tawny, or in some specimens rufous" (Oliver, 1877).

A thicket-forming species in savanna and on the edge of forest. The branchlets are resistant to termites, and are useful as stakes for lining-out plantations (Dale and

Greenway, 1961). In Angola, the root and stem bark of *V. amygdalina* are used as a tonic in fevers and as a remedy for intestinal upsets (Watt and Breyer-brandwijk, 1962).

***Withania somnifera* (L.) Dunal.** "Small shrub to 8 ft. Leaves elliptic, ovate, obovate or oblong, 2-4 in. long and 1 in. wide, more or less stellate-pubescent or tomentose. Flowers greenish or yellowish in axillary bunches of 2-6. Calyx accrescent and inflated in age enclosed and orange-red berry. Widely spread" (Dale and Greenway, 1961).

A decoction of the roots and leaves of *W. somnifera* are used in the treatment of infections and eruptic diseases such as smallpox. The roots and leaves have also shown marked antibiotic effect against *Staphylococcus aureus*, the leaves being the more active (Watt and Breyer-brandwijk, 1962).

1.2. Extraction and fractionation

The plant materials were sun-dried and ground using a grinder (Straub, model 4E, Philadelphia, USA). The powdered materials (50 g each) were then successively extracted with 400 ml each of petroleum ether, chloroform and methanol by shaking overnight on an orbital shaker (GFL, Model 3020, Germany) at room temperature. The extracts were filtered through whatman filter paper (Whatman Ltd., England). The organic solvents were removed from the filtrate under reduced pressure on a rotary evaporator (Buchi, model 140, Switzerland).

Extracts that were found to have substantial antimalarial activity (IC_{50} and IC_{90} values below 20 and 100 μg/ml, respectively) were applied (4 g each) on silica gel column and fractionated by eluting with chloroform or mixtures of chloroform and methanol. Organic solvents were removed from these fractions under reduced pressure.

2. Antimalarial test

2.1. Preparation of media

The culture medium was prepared according to the method described by Trager and Jensen (1976) and Brockelman *et. al.* (1985) but with slight modifications. Briefly, sodium bicarbonate (5.25% w/v) was prepared by dissolving 5.25 g powdered salt in 100 ml of glass redistilled water. Glucose (2.5 g), 26 g RPMI (Rosewell Park Memorial Institute) 1640 powder with glutamine but without sodium bicarbonate (Sigma, St. Louis, USA), and 14.85 g HEPES (N-2-Hydroxyethylpiperazine-N'-2- ethanesulfonic acid) (Gibco, Paisicy, Scotland) were weighed and dissolved separately. These and the bicarbonate solution were mixed and the volume adjusted to 2.5 l in glass redistilled water. It was sterilized by filtering into sterile flasks through membrane filters of pore size of 0.22 μ (Nalgene, USA).

Sterility of the medium was checked by incubating 2 ml of the incomplete medium with 10 ml ready-to-use trypto casein soy broth for 24 hours at 37°C. Appearance of turbidity indicated non-sterility of the medium which meant that the medium needed re-sterilization. The sterile medium was labelled as incomplete medium. It was used to wash infected and non-infected red blood cells, and also to prepare the complete medium. It was possible to store this medium for seven days at 4°C, or for six months at -30°C.

A complete medium was composed of 10% type AB^+ human serum in incomplete medium. It also was sterilized as the incomplete medium. Complete medium was used in the preparation of infected red blood cell suspensions and also in test cultures. It was also possible to store this medium for a week at 4°C, or for six months at -30°C.

2.2. Preparation of non-infected red blood cells

Non-infected human O^+ type blood was obtained from individuals free from malaria. It was collected in sterile vacutainers containing acid citrate dextrose. These were stored at 4°C for one week in order to lyse leucocytes selectively (Trager and Jensen, 1976). The stored blood was aseptically transferred into graduated conical centrifuge tubes and centrifuged at 1800 rpm for 10 minutes. The plasma, the buffy coat and the upper layer of the red blood cells were removed, and the remaining cells were suspended in an equal volume of incomplete medium. The suspension was centrifuged again, the supernatant removed and the cells resuspended in incomplete medium. This washing with incomplete medium was done twice. After the final removal of the supernatant, the packed red blood cells were suspended in equal volume of complete medium and stored at 4°C. Washed non-infected blood was used to adjust parasitaemia of infected blood.

2.3. Preparation of infected red blood cells

Infected erythrocytes were obtained from previous cultures or from cryopreserved cultures of blood infected with FCM-29 *P. falciparum* strain. This chloroquine-resistant strain was originally obtained from Cameroon and maintained *in vitro* at Institut Malagache de Recherche Appliquees (IMRA), Madagascar.

To initiate a cryopreserved culture, a microcentrifuge tube containing the FCM-29 was removed from the liquid nitrogen and thawed at 37°C in a water bath. It was washed once with 3.5% (w/v) sterile sodium chloride solution, and twice with incomplete medium. The sediment after the final washing was suspended in equal volume of complete medium.

After adjusting the parasitaemia to 1-2% and the haematocrit to 2.5%, the infected red blood cell suspension was dispensed at 2 ml in a 35-mm, 6 ml in a 55-mm, or 18 ml in an 85-mm Petri dish. The contents of the Petri dish were homogenized by shaking on a vibro mixer (Titertek, Flow laboratories) for one minute. The dish was then placed in a candle jar and incubated at 37°C after lighting the candle in the jar.

The cultures were removed from the candle jar to provide them with fresh medium every day. This was done by tilting the dishes gently and aspirating off the supernatant with a Pasteur pipet. Fresh complete medium was added, the cells were resuspended by gentle swirling and the dishes were returned to the candle jar and the incubator.

Thin blood films were prepared from each culture after 48 and 96 hours. They were stained in Diff Quick reagents. That is, the thin films were first fixed with methanol by keeping the slides in methanol for 1 minute. Afterwards, they were stained in eosin G (in phosphate buffer, pH = 6.6) for 20 seconds and then in thiazine dye (in phosphate buffer, pH = 6.6) for further 3 minutes. The proportion of parasitized cells out of a total of 10,000 red blood cells was taken as an indication of the parasitaemia of the culture.

Antimalarial tests were carried out when a required parasitaemia in a desired volume of infected blood cell was obtained. The contents of the Petri dish were aseptically transferred to a graduated centrifuge tube and centrifuged at 1800 rpm for 10 minutes. The supernatant was removed and the cells resuspended in an equal volume of fresh complete medium. The parasitaemia was adjusted to 0.5-1% using non-infected washed red blood cells.

2.4. *In vitro* antimalarial test

The semiautomated microdilution technique (Desjardins *et. al.*, 1979) was used with some modifications. That is, test materials were dissolved to a concentration of 1 mg/ml in either distilled water, methanol or dimethyl sulfoxide. These were diluted to appropriate concentrations in complete medium and then filter-sterilized by passing through 0.22 μ membrane filters.

A 24-well flat-bottomed semimicrotiter plate (Dynatech Laboratories, Inc., USA) was assigned to each test material. The test material was applied in triplicates at seven different concentrations over a range of 128-folds. The remaining three wells were used as negative controls for culture medium (2 wells) and the solvent used in test wells. The maximum amount of solvent used in the test wells was also applied in the negative control well. For all tests, the amount of the solvent both in the test and control wells was kept below 0.05% (v/v) of the final volume of cell suspension in each well. A positive control, chloroquine diphosphate (4-(1-methyl-4-diethylaminobutylamnio)-7-chloroquinoline diphosphate), was tested at different concentrations.

After allowing the dosed plates to dry in the hood, 700 μl of a red blood cell suspension with 2.5% haematocrit and a parasitaemia of 1% was dispensed to each well. The contents of the plate were homogenized by shaking on the vibro mixer for one minute. The plates were then placed in a candle jar and incubated at 37°C for 18 hours after lighting the candle. At the end of the 18 hours, 0.5 μCi ^{3}H-hypoxanthine (Amersham Corp., USA) was added to each well. The contents of the plates were homogenized again and the plates were placed in the candle jar and incubated at 37°C for further 24 hours.

At the end of the final incubation period the plates were transferred to and left overnight in a deepfreezer at -30°C. They were then thawed and the contents of each well filtered through a microfiber glass filter paper discs (Whatman Glass Microfiber Filters) using a filter (Skatron AS, Norway). Discs were dried at 50°C in an oven and placed into scintillation vials. Toluene-based organic scintillant (2 ml) was added to each vial. The radioactivity in each vial was then measured as counts per minute using a liquid

scintillation counter (Intertechnique, Liquid scintillation counter, SL 32, France). The reading in the negative control wells was taken as an indication of a 100% incorporation of the radioactive material. The percentage inhibition in the test wells was calculated as follows:

$$\% \text{ inhibition} = 100 - \left[\frac{\text{Radio activity in test well}}{\text{Radio activity in negative control well}} \times 100 \right]$$

Doses of the test materials resulting in 50 and 90% decrease in ^{3}H-hypoxanthine incorporation, *i.e.*, IC_{50} and IC_{90} values respectively, were determined from the corresponding linear regression curves on the log dose *Vs* percentage inhibition graphs. The minimum inhibitory concentration (MIC), at which complete inhibition of growth was observed, was also determined similarly. The IC_{50}, IC_{90}, MIC and the slope of the linear regression lines were used to evaluate the potency of the test materials.

3. Cytotoxicity test

3.1. Preparation of medium

Eagle's Minimum Essential Medium, EMEM, (Flow Laboratories, North Ryde, Australia) (23.8 g) and $NaHCO_3$ (5.5 g) were dissolved in about 2 l glass redistilled water. To this was added 3.75 ml of 1N HCl, 2.5 ml penicillin (100,000 UI/ml) and 1.25 ml streptomycin (100 mg/ml). The volume was adjusted to 2.5 l and filter-sterilized by suction through membranes with 0.22 μ pore size. EMEM containing 10% fetal calf serum made up the complete medium.

3.2. Test procedure

Human HeLa cell suspension (60,000 cells/ml) in EMEM were dispensed at 2 ml into all the wells of a 24-well flat bottomed plate. They were grown at 37°C for 24 hour in an atmosphere composed of 75% N_2, 20% O_2 and 5% CO_2. After this incubation period the old medium was aspirated off; cells in control wells were fed with a new batch of complete medium whereas those in the test wells were given graded doses of test materials as well. The plate was incubated under the same condition for further 72 hour. Cell growth was measured following the procedure described by Oliver *et. al.* (1989), but with slight modifications. That is, at the end of the second incubation period, the supernatant was removed from all the wells and methylene blue (1% in distilled water) was dispensed at a volume of 2 ml to each well. After 30 minutes, the methylene blue was discarded and the cells were detached from the glass surface by trypsin-EDTA solution and their optical density was measured by using a spectrophotometer (Jouan, Optical density meter, France).

Since only actively growing cells stain in methylene blue, the optical density in the control wells was taken as indicative of a 100% per cent growth. Percentage inhibition of growth at various concentrations of a test material was determined by using the following formula:

$$\% \text{ inhibition} = 100 - \left[\frac{\text{Optica density in the test well}}{\text{Optical density in the control well}} \times 100 \right]$$

IC_{50}, IC_{90} and MIC values were determined from linear regression lines of the log dose-percentage inhibition curves.

III. RESULTS

The plant materials were successively extracted with petroleum ether, chloroform and methanol. In Table 1 are shown the amount of crude extracts obtained from the nine plants upon extraction of 50 g each of the plant materials with 400 ml of the respective solvent. In general, higher amounts of extracts were obtained from leaf materials and by extraction with methanol as compared to those obtained from other plant materials and by extraction with petroleum ether and chloroform.

Table 1. Yields of petroleum ether, chloroform and methanol extracts from the nine plants.

		Yield (g)		
Plant name	Part used	Petroleum ether	Chloroform	methanol
C.a.	rt	3.2	4.0	3.6
C.f.	rt	1.5	2.4	2.9
J.a.	rt	3.3	4.0	3.7
J.s.	rt	2.4	2.3	3.8
L.v.	fl + ft	4.3	6.5	8.2
S.l.	rt	2.1	3.1	3.0
T.i.	pd	4.2	6.4	8.6
V.a.	lf	3.4	5.5	9.2
	rt	2.1	1.8	3.0
W.s.	lf	3.1	6.4	8.4
	rt	2.0	4.5	5.5

Key: *C.a.* = *C. abyssinica; C.f.* = *C. ficifolius; J.a.* = *J. abyssinicum; J.s.* = *J. schimperiana; L.v.* = *L. velutina; S.l.* = *S. longepedunculata; T.i.* = *T. indica; V.a.* = *V. amygdalina; W.s.* = *W. somnifera*; ap = aerial part; fl = flower; ft = fruit; pd = pod; rt = root; lf = leaf

All the thirty-three crude extracts obtained from the nine medicinal plants (Table 1) were screened for antimalarial activity against the FCM-29 strain of *P. falciparum*. Chloroquine diphosphate (CQ), a standard antimalarial drug, was used as a positive control (Table 5) to assess the relative antimalarial activity of the crude extracts/column-fractions. The screening results for the crude extracts and the positive control are shown in Tables 2-5 and Figures 2-7. Due to the high cost of the test only those extracts/column-fractions which showed substantial antimalarial activity were tested in nine replicates whereas the less active ones were tested in triplicates only.

All the petroleum ether extracts did not show 50% inhibition of ^{3}H-hypoxanthine incorporation at concentrations less than 100 μg/ml (Table 2). As shown in Tables 3 and 4, chloroform and methanol extracts of leaves of *W. somnifera*, and methanol extract of leaves of *V. amygdalina* were found to have substantial antimalarial activity. They showed more than a 60% decrease in the incorporation of ^{3}H-hypoxanthine by the parasite at a concentration of 25 μg/ml and above.

Methanol extract of pods of *T. indica* showed 53.85% inhibition at 12 μg/ml, whereas the inhibition at the next higher dose (25 μg/ml) was only 42.76 μg/ml (Table 4). This might have been due to experimental errors.

Table 5. Mean ± standard deviation (n = 3) of the percentage inhibition of maturation of *P. falciparum* at various concentrations of **petroleum ether** extracts.

Plant	Concentration (μg/ml)						
	3	6	12	25	50	100	200
C. ficifolius (rt)	2.44 ± 0.47	7.77 ± 0.92	20.47 ± 1.56	33.83 ± 1.58	46.41 ± 0.39	57.46 ± 1.15	64.60 ± 0.75
C. abyssinica (ap)	0.65 ± 0.24	2.98 ± 1.48	9.81 ± 0.71	15.86 ± 0.75	22.33 ± 0.80	28.19 ± 1.44	37.48 ± 4.43
J. abyssinicum (rt)	0.36 ± 0.01	7.36 ± 0.74	12.06 ± 5.17	38.69 ± 3.03	47.58 ± 1.89	66.30 ± 4.45	85.08 ± 1.41
J. schimperiana (rt)	0.36 ± 0.25	5.18 ± 2.45	13.93 ± 0.72	21.66 ± 1.89	28.50 ± 0.43	48.37 ± 0.77	58.13 ± 0.92
L. velutina (fl+ft)	2.01 ± 2.45	9.12 ± 1.75	14.68 ± 1.15	20.34 ± 3.14	37.45 ± 4.71	49.1 ± 1.62	60.11 ± 0.93
S. longeped-unculata (rt)	1.17 ± 0.01	13.24 ± 0.95	21.60 ± 0.75	42.80 ± 2.3	34.87 ± 0.70	46.05 ± 1.20	69.03 ± 0.92
T. indica (pd)	3.25 ± 0.54	8.07 ± 1.01	16.71 ± 0.29	28.74 ± 1.54	39.87 ± 0.84	48.87 ± 1.58	58.65 ± 1.42
V. amygdalina (lf)	0.16 ± 0.01	1.89 ± 1.27	11.10 ± 3.81	14.67 ± 0.73	22.32 ± 1.83	41.35 ± 1.95	45.67 ± 1.73
V. amygdalina (rt)	6.96 ± 0.87	14.33 ± 3.37	39.20 ± 8.64	37.95 ± 4.82	44.53 ± 1.25	61.69 ± 3.63	83.90 ± 1.00
W. somnifera (lf)	0.44 ± 0.01	6.72 ± 0.51	15.22 ± 1.52	19.77 ± 3.28	43.9 ± 1.28	49.84 ± 0.73	57.75 ± 1.64
W. somnifera (rt)	1.00 ± 0.14	4.46 ± 1.03	14.73 ± 0.96	17.29 ± 0.84	30.57 ± 1.03	44.66 ± 1.61	51.68 ± 1.68

Table 6. Mean ± standard deviation (*n =3; **n = 9) of the percentage inhibition of maturation of *P. falciparum* at various concentrations of **chloroform extracts.**

Plant	Concentration (μg/ml)						
	3	6	12	25	50	100	200
C. ficifolius* (rt)	3.75 ± 0.68	16.21 ± 0.93	28.83 ± 0.79	37.61 ± 1.39	46.01 ± 0.84	54.89 ± 0.40	67.08 ± 1.76
C. abyssinica* (ap)	1.09 ± 0.28	3.04 ± 0.70	8.77 ± 1.33	13.56 ± 1.21	16.33 ± 1.21	22.32 ± 1.03	33.45 ± 2.03
J. abyssinicum* (rt)	3.50 ± 0.58	15.17 ± 0.48	28.20 ± 0.66	54.65 ± 1.09	66.16 ± 1.49	72.51 ± 1.16	85.67 ± 0.81
J. schimperiana*(rt)	1.79 ± 0.59	9.17 ± 6.92	15.17 ± 0.40	23.98 ± 4.70	32.17 ± 3.29	46.83 ± 2.83	62.30 ± 1.97
L. velutina* (fl+ft)	1.12 ± 0.38	4.52 ± 1.08	15.63 ± 0.66	29.01 ± 1.19	37.25 ± 2.14	46.77 ± 0.83	64.96 ± 2.16
S. longeped-unculata* (rt)	1.53 ± 0.42	4.30 ± 0.77	15.89 ± 0.77	22.82 ± 1.18	39.86 ± 0.75	54.12 ± 1.05	65.80 ± 1.58
T. indica* (pd)	9.17 ± 2.04	26.8 ± 2.58	39.89 ± 0.67	46.88 ± 1.05	54.73 ± 0.55	61.37 ± 1.02	70.98 ± 0.87
V. amygdalina** (lf)	9.12 ± 5.13	26.37 ± 5.64	36.18 ± 4.37	63.27 ± 4.64	84.11 ± 1.11	92.31 ± 1.21	98.65 ± 0.25
V. amygdalina* (rt)	0.69 ± 0.71	3.35 ± 2.51	15.86 ± 1.45	28.14 ± 0.63	39.18 ± 1.10	44.16 ± 1.56	55.31 ± 0.72
W. somnifera** (lf)	9.54 ± 3.17	40.59 ± 5.23	59.83 ± 4.02	83.74 ± 1.31	87.79 ± 1.13	96.35 ± 0.68	96.63 ± 0.58
W. somnifera* (rt)	7.08 ± 1.03	15.39 ± 0.49	26.85 ± 1.96	42.89 ± 1.03	56.79 ± 1.27	64.52 ± 1.01	73.16 ± 4.03

Table 7. Mean ± standard deviation (*n =3; **n = 9) of the percentage inhibition of maturation of *P. falciparum* at various concentrations of **methanol extracts.**

Plant	Concentration (μg/ml)						
	3	6	12	25	50	100	200
C. ficifolius* (rt)	1.59 ± 0.87	6.76 ± 2.27	16.07 ± 1.40	34.86 ± 1.52	46.62 ± 0.76	57.34 ± 1.26	64.94 ± 1.19
C. abyssinica* (ap)	2.21 ± 1.14	14.70 ± 0.63	27.36 ± 1.02	36.73 ± 1.43	45.30 ± 1.51	58.77 ± 1.53	65.00 ± 3.05
J. abyssinicum* (rt)	2.43 ± 0.52	12.60 ± 0.86	24.95 ± 0.56	44.69 ± 0.76	64.70 ± 1.44	78.88 ± 1.21	85.96 ± 0.95
J. schimperiana* (rt)	1.01 ± 0.75	9.08 ± 1.73	18.10 ± 0.70	31.03 ± 1.00	45.14 ± 4.21	58.77 ± 0.80	67.55 ± 2.32
L. velutina* (fl+ft)	2.21 ± 1.37	9.57 ± 1.37	16.79 ± 1.18	36.78 ± 2.91	49.46 ± 0.93	54.66 ± 1.16	65.17 ± 5.51
S. longeped-unculata* (rt)	3.19 ± 0.91	5.53 ± 0.48	12.04 ± 0.53	21.22 ± 1.47	33.47 ± 0.97	52.58 ± 4.59	62.04 ± 1.21
T. indica* (pd)	9.31 ± 1.31	22.19 ± 2.65	53.85 ± 1.50	42.76 ± 0.88	50.25 ± 1.08	59.23 ± 0.92	71.08 ± 1.23
V. amygdalina* (lf)	2.53 ± 1.04	15.27 ± 0.60	27.54 ± 1.05	34.74 ± 0.43	41.97 ± 2.67	50.00 ± 1.48	67.80 ± 1.30
V. amygdalina* (rt)	0.50 ± 0.15	11.75 ± 2.79	15.68 ± 0.69	24.64 ± 6.99	34.81 ± 3.31	43.06 ± 0.93	67.57 ± 0.82
W. somnifera** (lf)	6.35 ± 5.17	19.00 ± 4.26	47.27 ± 7.37	78.13 ± 6.17	88.98 ± 5.00	97.97 ± 0.77	98.75 ± 0.23
W. somnifera* (rt)	9.58 ± 0.73	18.19 ± 0.68	31.39 ± 0.68	38.09 ± 1.19	45.79 ± 1.23	58.53 ± 1.45	64.67 ± 0.56

Table 5. Mean ± standard deviation (n = 9) of the percentage inhibition of maturation of *P. falciparum* at various concentrations of chloroquine diphosphate.

Chloroquine (μg/ml)	Percentage inhibition
0.258×10^{-3}	12.69 ± 2.40
0.516×10^{-3}	24.98 ± 1.50
1.032×10^{-3}	35.33 ± 2.21
1.548×10^{-3}	48.03 ± 2.23
2.064×10^{-3}	68.95 ± 1.34
2.580×10^{-3}	85.35 ± 1.01
4.128×10^{-3}	91.84 ± 1.08

The linear regression curves obtained from the log dose *Vs* percentage inhibition of incorporation of ^{3}H-hypoxanthine by all the crude extracts, and chloroquine are shown in figures 2-4. Linear regression lines for crude extracts that correspond to one organic solvent are shown divided in two figures for convenience of presentation. For instance, linear regression curves for the eleven petroleum ether extracts are shown in figures 2a and 2b; five of them in Fig. 2a, and the remaining six in Fig. 2b. Figures showing regression curves were scaled to include all. As a result, percentage inhibitions less than 0% and greater than 100% are shown. Inhibitions above 100% can be taken for the maximum possible percentage inhibition, 100% whereas inhibitions less than 0% can be taken for the minimum possible percentage inhibition, 0%.

The regression lines show the general trend of the antimalarial activity of each extract. The slopes of the three extracts which showed substantial antimalarial activity (Fig. 3b and 4b) were found higher when compared to the other crude extracts.

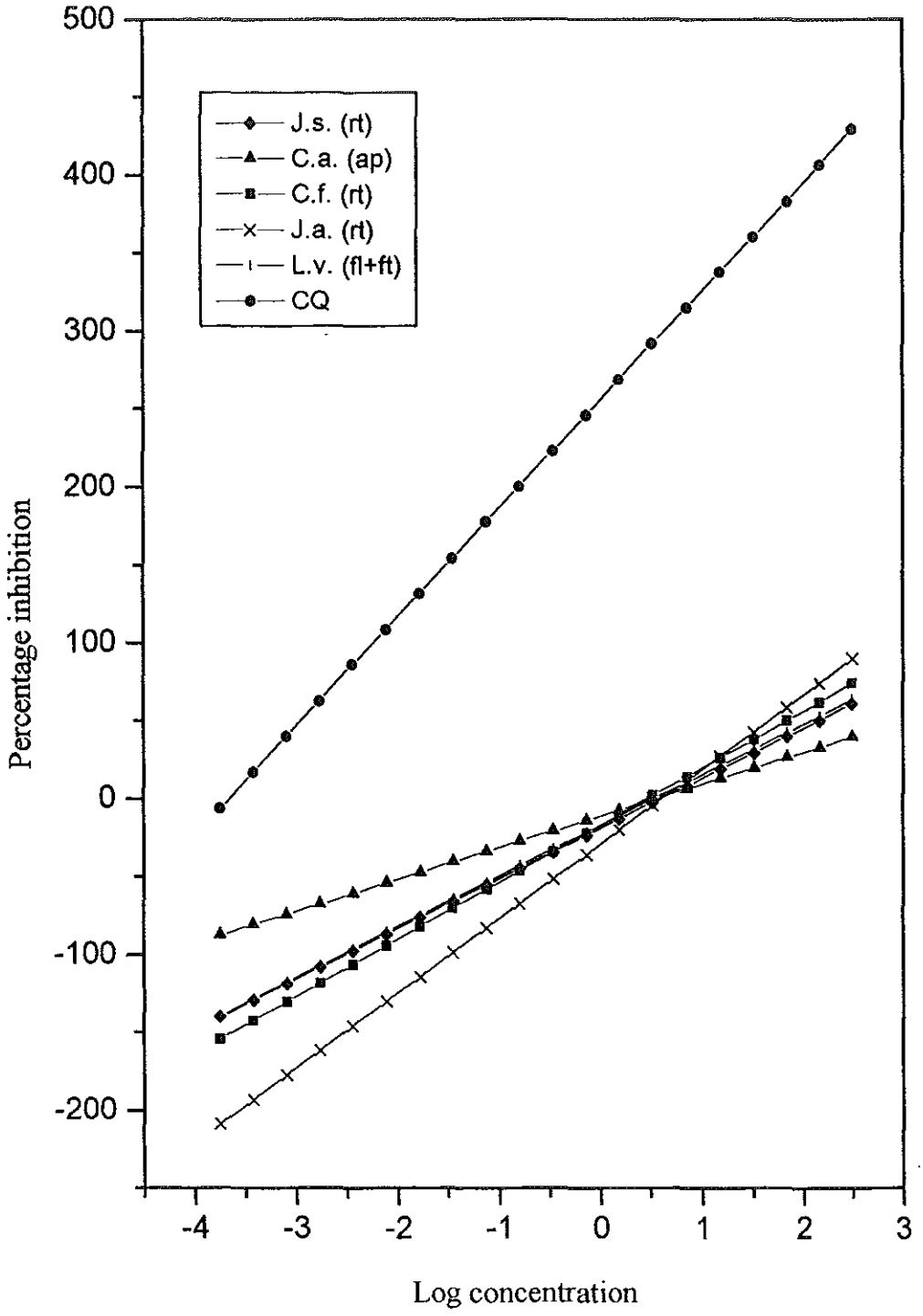

Fig. 2a. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and petroleum ether extracts from the nine plants.

---

abbreviations as in Table 1.

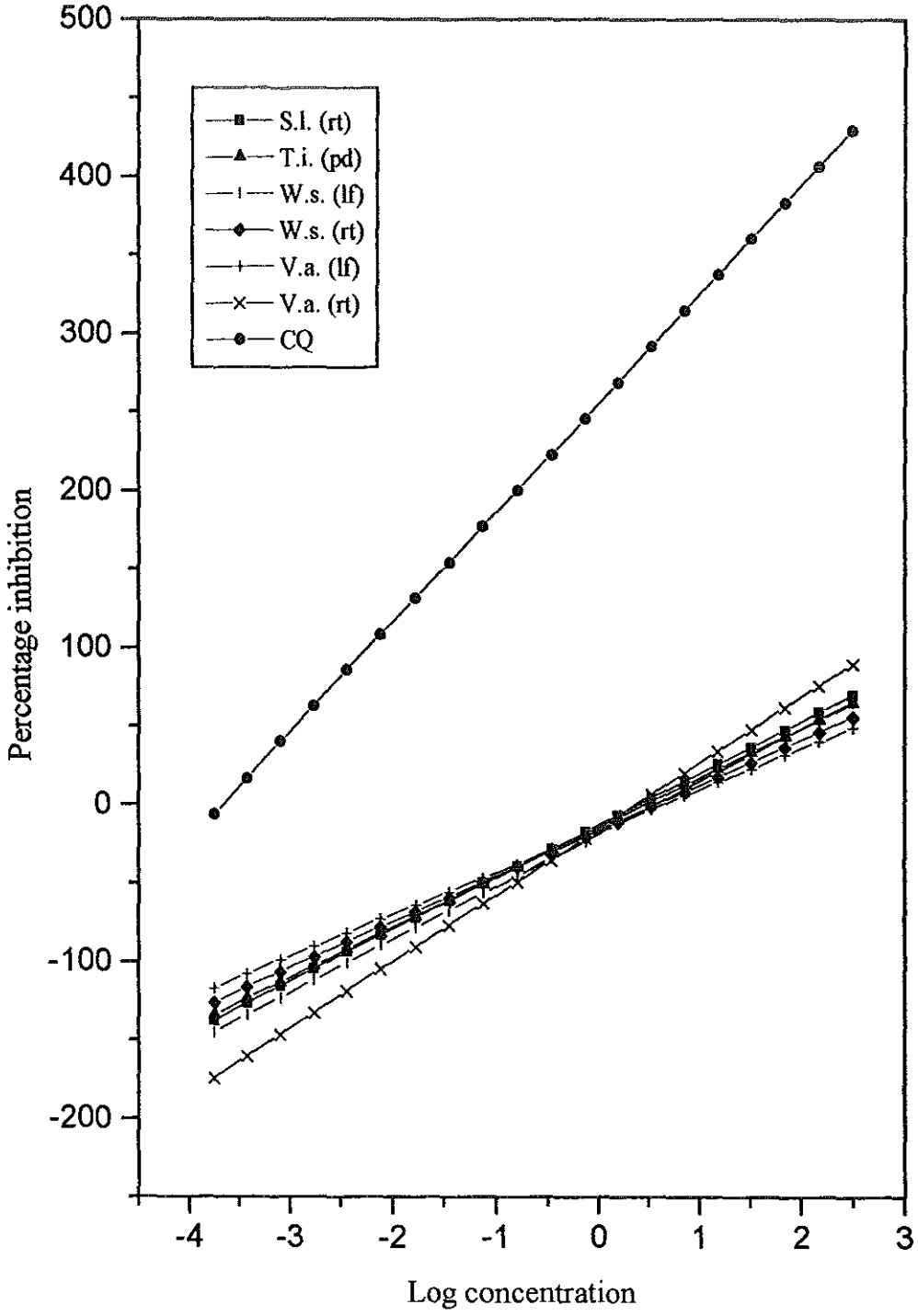

Fig. 2b. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and petroleum ether extracts from the nine plants.

---

abbreviations as in Table 1.

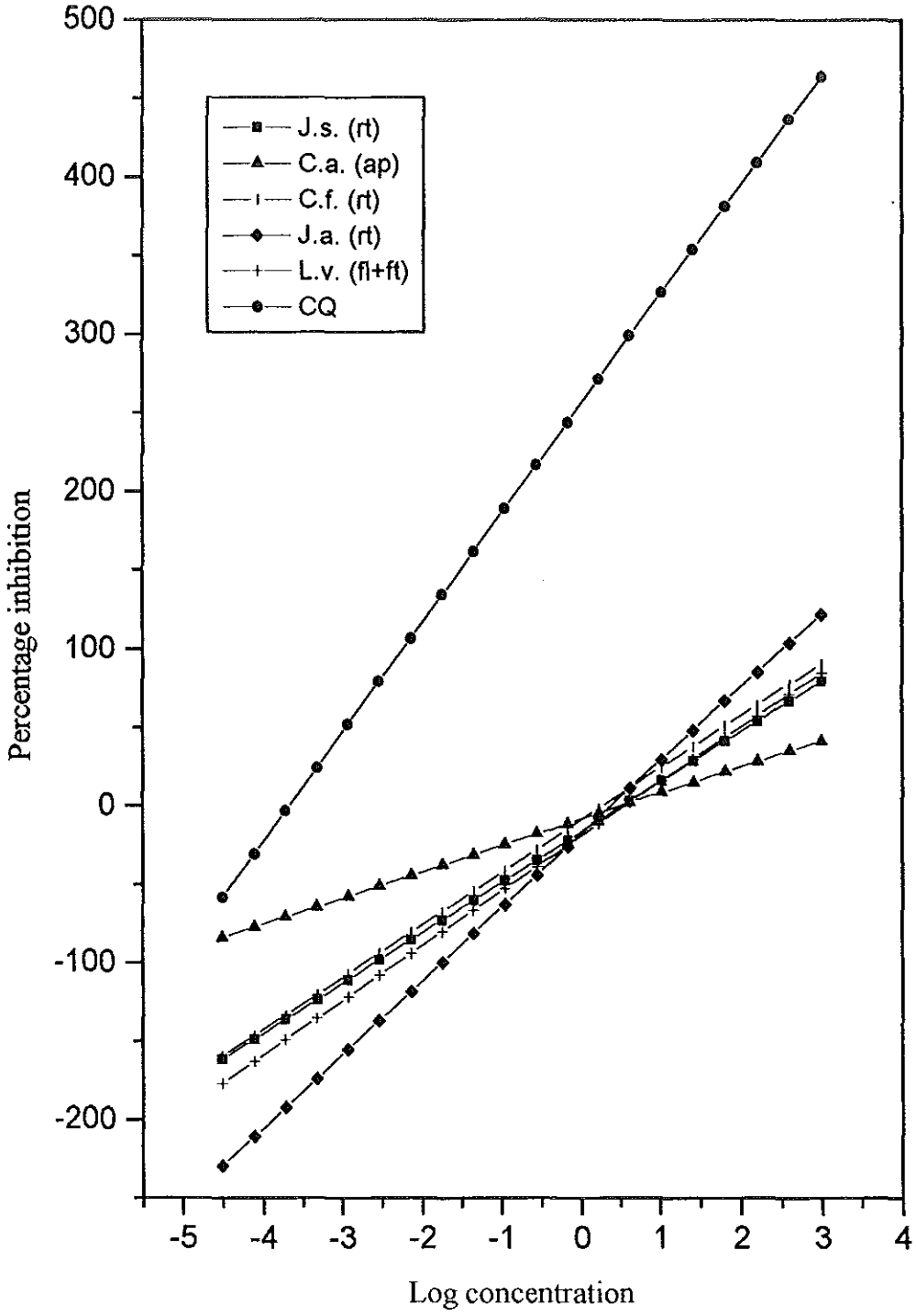

Fig. 3a. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and chlororform extracts from the nine plants.

---

abbreviations as in Table 1.

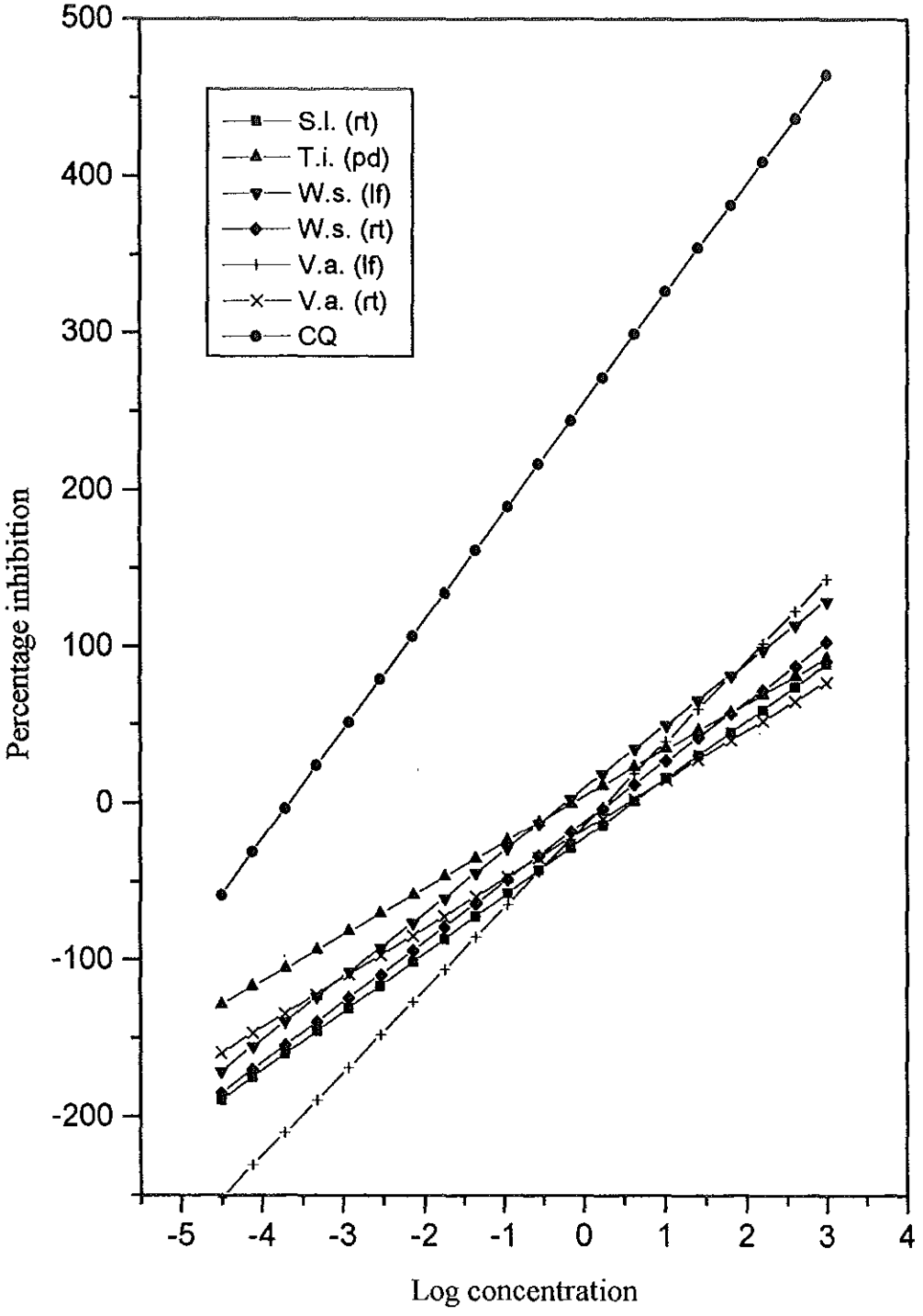

Fig. 3b. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and chlororform extracts from the nine plants.

---

abbreviations as in Table 1.

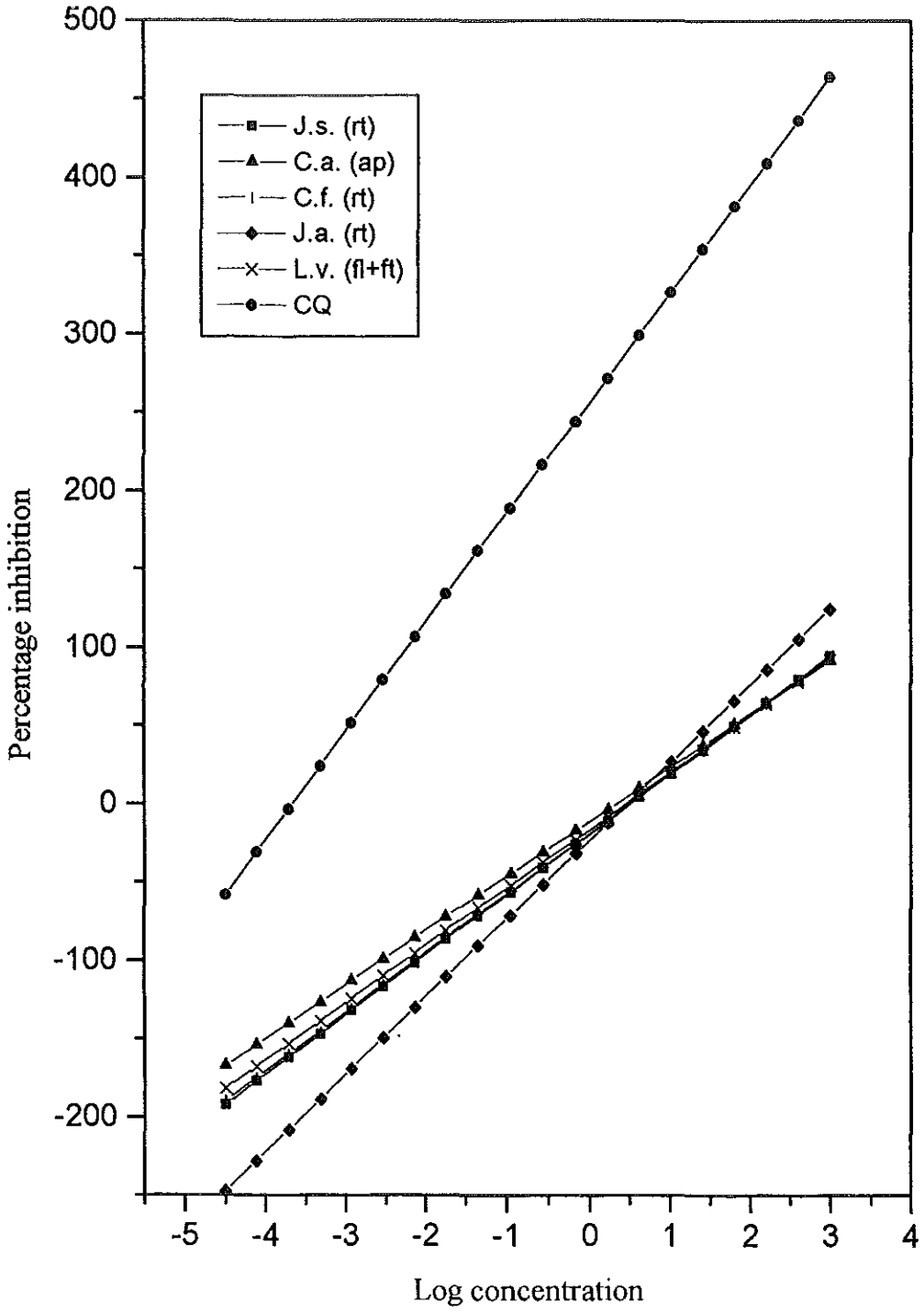

Fig. 4a. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and methanol extracts from the nine plants.

---

abbreviation as in Table 1.

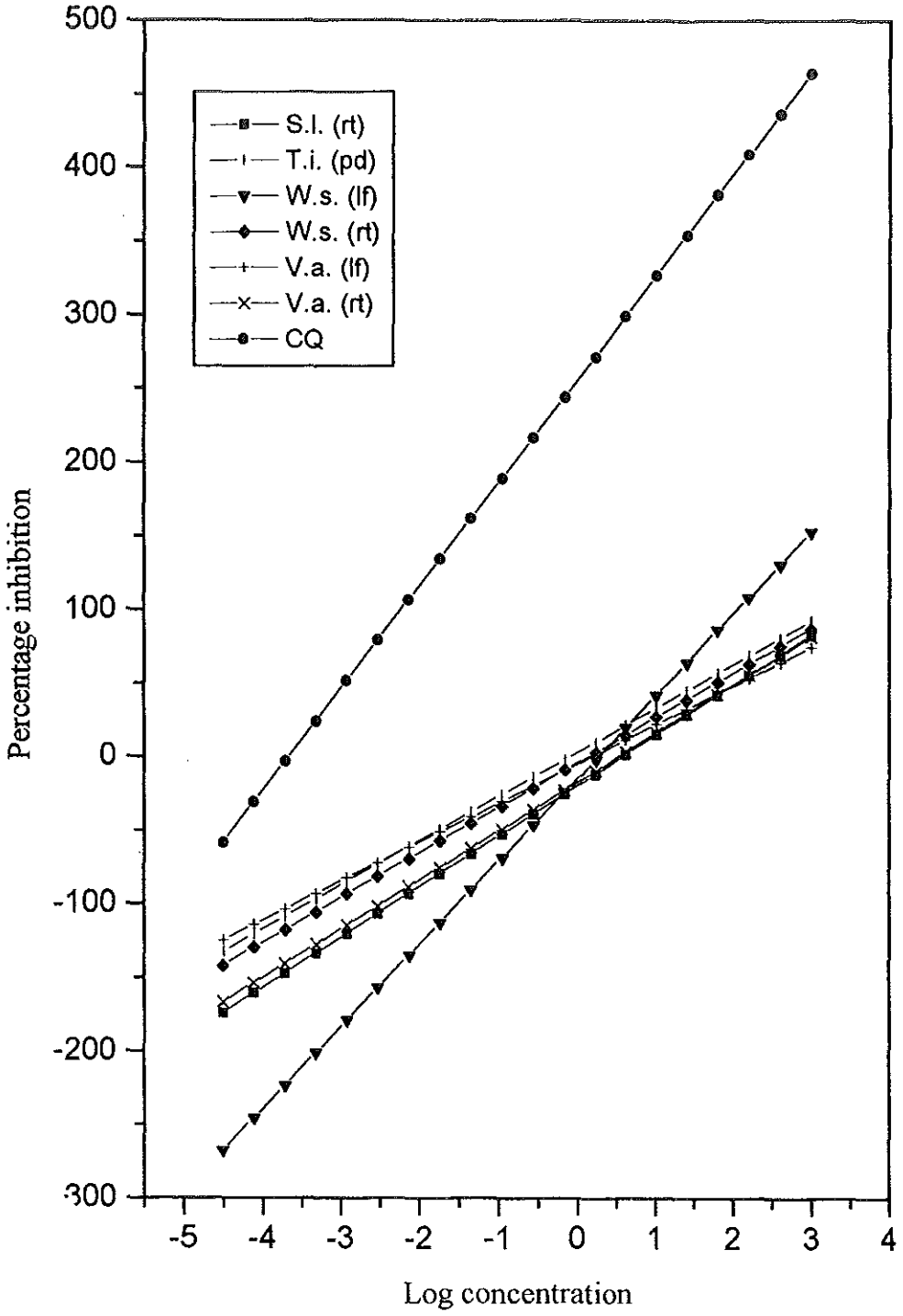

Fig 4b. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and methanol extracts from the nine plants.

---

abbreviation as in Table 1.

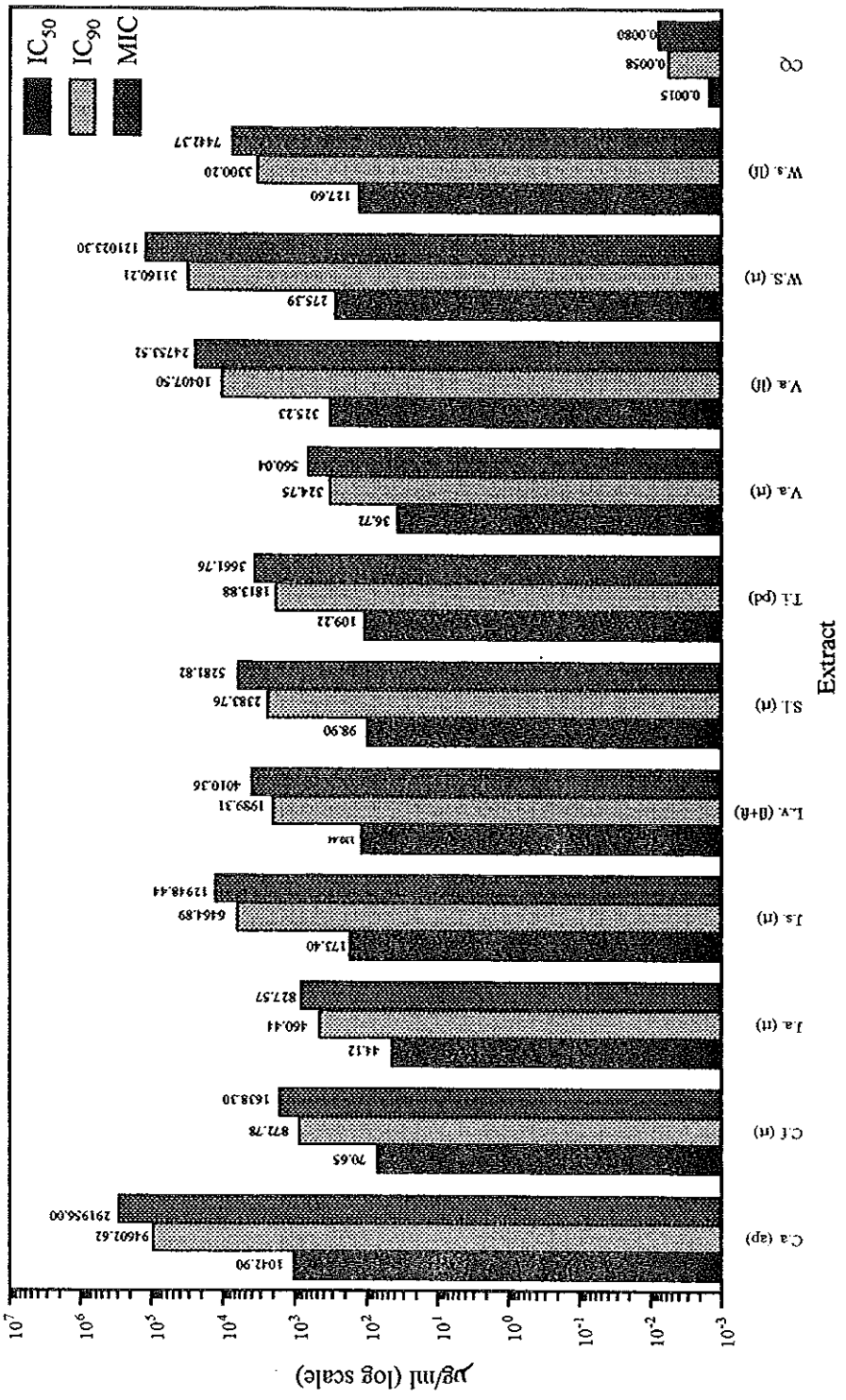

Fig. 5. IC_{50}, IC_{90}, and MIC values for chloroquine and petroleum ether extracts of the nine plants. abbreviations as in Table 1.

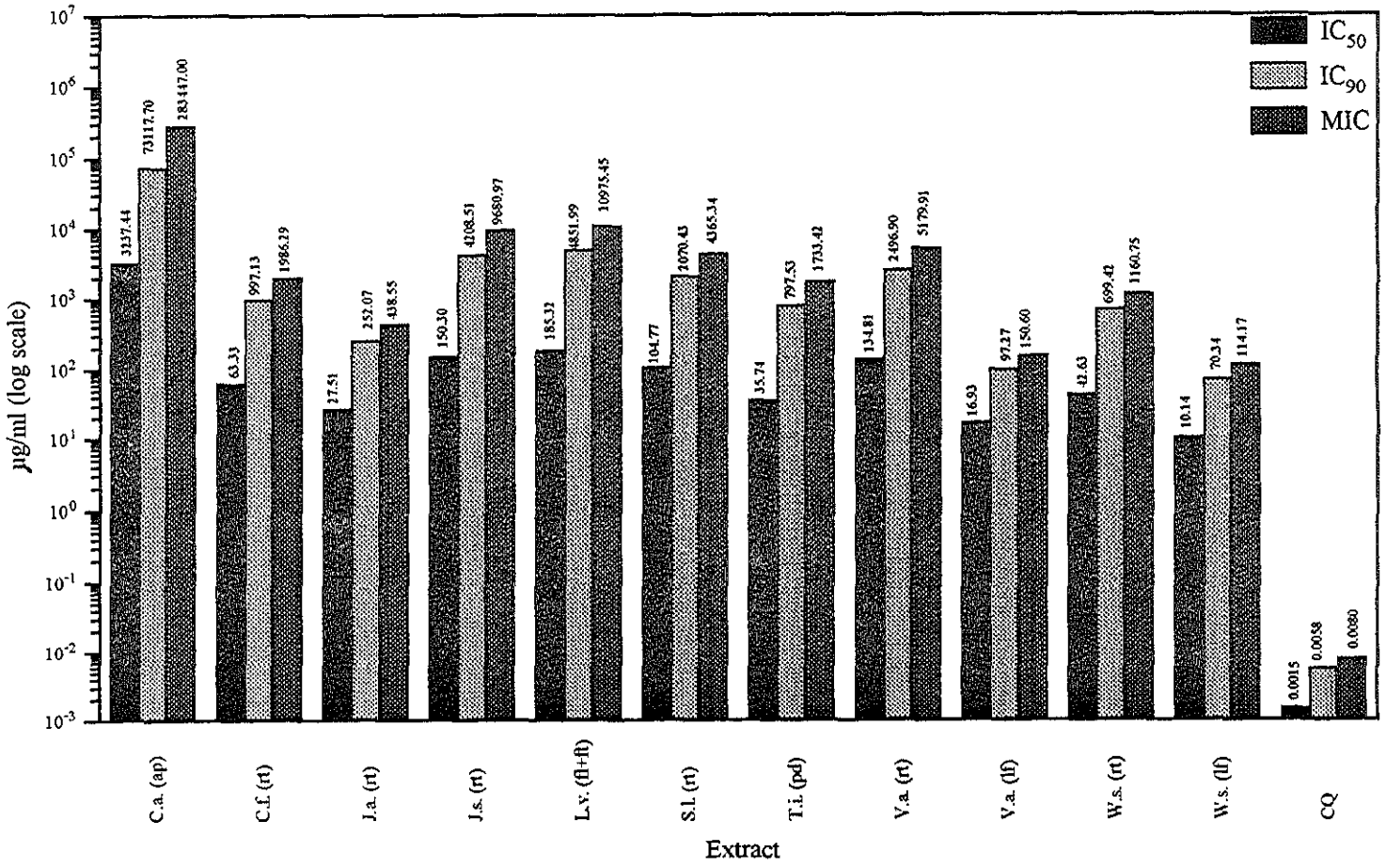

Fig. 6. IC_{50}, IC_{90}, and MIC values for chloroquine and chloroform extracts of the nine plants.

---

abbreviations as in Table 1.

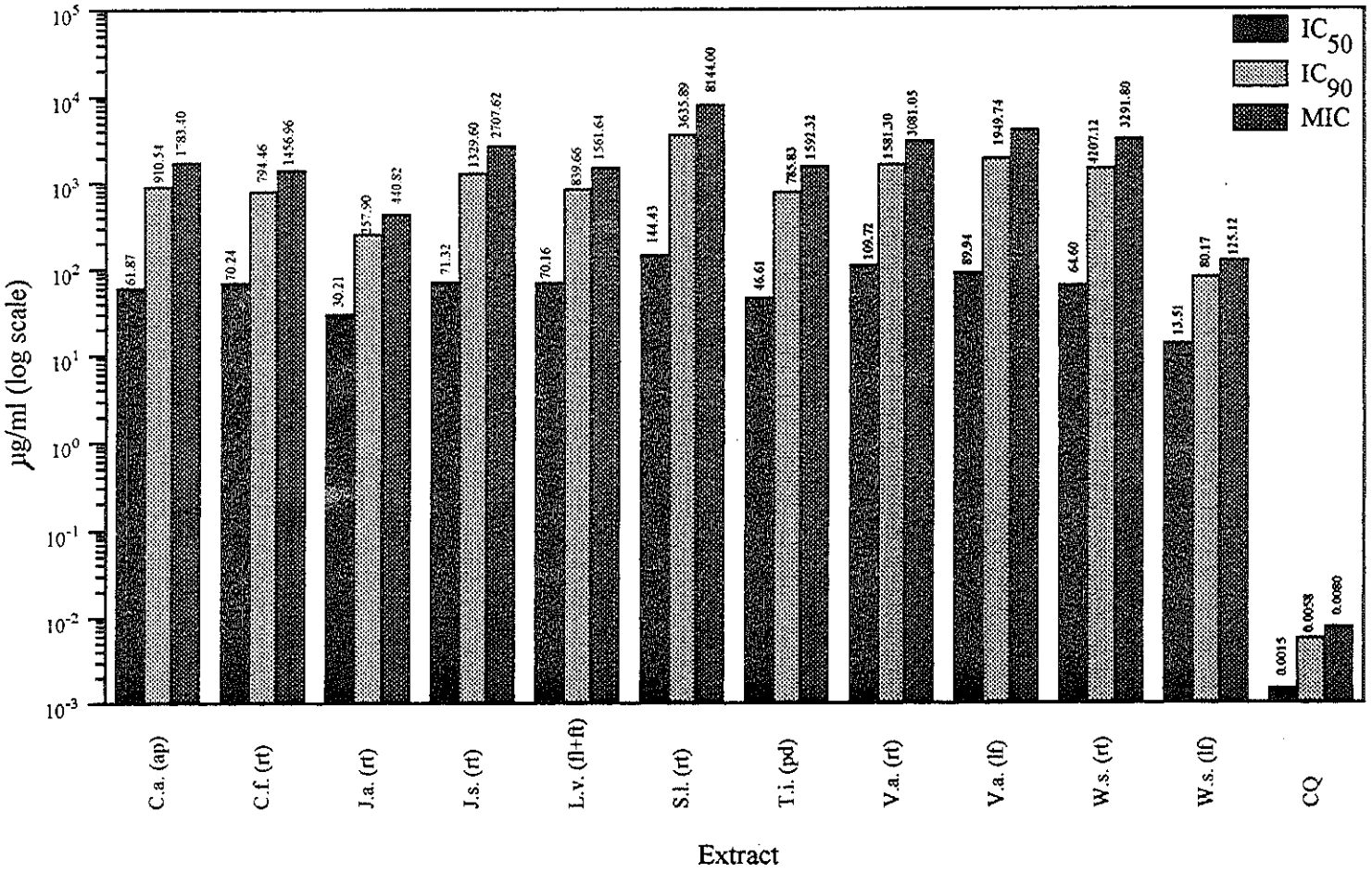

Fig. 7. IC_{50} IC_{90} and MIC values for chloroquine and methanol extracts of the nine plants.

---

abbreviations as in Table 1.

As mentioned above, the IC_{50}, IC_{90}, and MIC values of the test materials were determined from the corresponding linear regression lines of the log dose *Vs* percentage inhibition curves. These values for the crude extracts and chloroquine are shown in figures 5-7. The IC_{50} and IC_{90} values of all the petroleum ether extracts (Fig. 5) were found to be above 20 and 100 μg/ml, respectively. Only the three crude extracts, *i.e.*, chloroform and methanol extracts of leaves of *W. somnifera* (Fig. 6 and 7), and chloroform extract of leaves of *V. amygdalina* (Fig. 7), were found to have substantial antimalarial activity (IC_{50} and IC_{90} less than 20 and 100 μg/ml).

These three crude extracts were applied (4 g each) on silica gel column and fractionated by eluting with chloroform, methanol or a mixture of the two. Tables 6-8 show the kind and amount of organic solvent used, and the amount of column-fractions obtained after removing the solvents.

Table 6. Kind and amount of organic solvents used and column-fractions obtained from chloroform extract of leaves of *W. somnifera*

	Eluent (ml)		
Column-fraction	Chloroform	Methanol	Amount (mg)
1	40.0	0.0	13
2	38.0	2.0	22
3	32.0	8.0	25
4	0.0	40.0	31

Table 7. Kind and amount of organic solvents used and column-fractions obtained from methanol extract of leaves of *W. somnifera.*

	Eluent (ml)		
Column-fraction	Chloroform	Methanol	Amount (mg)
1	15.0	0.0	10
2	15.0	0.0	13
3	13.5	0.5	20
4	10.5	4.5	8
5	9.0	6.0	9

Table 8. Kind and amount of organic solvents used and column-fractions obtained from chloroform extract of leaves of *V. amygdalina.*

	Eluent (ml)		
Column-fraction	Chloroform	Methanol	Amount (mg)
1	40.0	0.0	5
2	60.0	0.0	8
3	80.0	0.0	10
4	80.0	0.0	14
5	59.4	0.6	18
6	18.0	2.0	12

All the column-fractions were then screened against the FCM-29 for antimalarial activity. The screening results are shown in tables 9-11 and figures 8-13.

Table 9. Mean ± standard deviation (* n = 3; ** n = 9) of the percentage inhibition of maturation of *P. falciparum* at various concentrations of column-fractions obtained from chloroform extract of leaves of *W. somnifera*.

μg/ml	Column-fraction (Fr)			
	Fr-1*	Fr-2*	Fr-3**	Fr-4*
1	0.81 ± 0.00	0.28 ± 0.26	18.18 ± 6.43	11.06 ± 2.65
2	2.17 ± 0.64	6.02 ± 1.20	47.65 ± 4.43	27.96 ± 1.18
4	8.00 ± 1.75	14.25 ± 0.98	78.96 ± 1.47	38.89 ± 1.93
8	18.97 ± 2.57	23.07 ± 0.41	86.23 ± 0.76	47.04 ± 0.52
16	27.58 ± 0.93	40.10 ± 1.20	93.50 ± 0.86	55.26 ± 1.47
32	37.03 ± 1.06	47.55 ± 0.75	97.83 ± 0.64	65.37 ± 1.24
64	44.41 ± 1.09	57.15 ± 3.68	98.70 ± 0.21	72.95 ± 1.75

Among the four column-fractions obtained from the chloroform extract of leaves of *W. somnifera*, the highest inhibition in the incorporation of ^{3}H-hypoxanthine by *P. falciparum* was observed in the presence of the more polar fractions, fraction 3 and 4 (Table 9). Fraction 3 showed more than 90% inhibition of ^{3}H-hypoxanthine incorporation at 16 μg/ml and above. The next more active fraction (fraction 3) showed more than 50% inhibition at 16 μg/ml.

Fraction 5 obtained from the methanol extract of leaves of *W. somnifera*, decreased the incorporation of ^{3}H-hypoxanthine by the FCM-29 by more than 90% at concentrations higher than 16 μg/ml (Table 10). The highest inhibition observed in the presence of the less polar fractions, fractions 1-4, was less than 62%.

Table 10. Mean ± standard deviation (* n = 3; ** n = 9) of the percentage inhibition of maturation of *P. falciparum* at various concentrations of column-fractions of the methanol extract of leaves of *W. somnifera*.

μg/ml	Column-fraction (Fr)				
	Fr-1*	Fr-2*	Fr-3*	Fr-4*	Fr-5**
1	1.58 ± 1.12	2.21 ± 1.53	2.81 ± 0.6	2.56 ± 0.85	12.86 ± 2.76
2	4.44 ± 1.20	6.58 ± 0.85	7.55 ± 0.96	8.64 ± 1.24	27.90 ± 1.07
4	14.77 ± 0.6	15.16 ± 1.19	17.59 ± 0.78	15.67 ± 1.17	53.59 ± 2.46
8	27.41 ± 0.85	23.46 ± 1.63	26.77 ± 5.48	24.47 ± 3.31	81.47 ± 2.63
16	38.00 ± 0.72	30.75 ± 1.11	42.04 ± 2.01	30.43 ± 0.13	88.02 ± 1.59
32	46.74 ± 0.45	39.08 ± 0.73	49.97 ± 0.76	48.16 ± 0.14	97.64 ± 0.36
64	57.35 ± 1.22	44.16 ± 1.53	60.12 ± 0.68	61.91 ± 3.53	98.79 ± 0.21

Table 11. Mean ± standard deviation (*n = 3; **n = 9) of the percentage inhibition of maturation of *P. falciparum* at various concentrations of the column-fractions from the chloroform extract of leaves of *V. amygdalina*.

μg/ml	Column-fraction (Fr)					
	Fr-1*	Fr-2*	Fr-3*	Fr-4*	Fr-5*	Fr-6**
1	0.97 ± 0.53	0.71 ± 0.49	9.25 ± 0.52	1.15 ± 0.00	4.28 ± 3.94	18.16 ± 3.11
2	8.04 ± 0.91	2.05 ± 1.20	16.52 ± 1.36	4.18 ± 1.16	12.43 ± 6.84	41.40 ± 1.27
4	14.73 ± 1.42	9.26 ± 1.44	28.38 ± 1.14	12.49 ± 3.11	22.46 ± 4.31	54.63 ± 1.51
8	20.52 ± 2.34	17.45 ± 0.60	35.01 ± 4.49	19.92 ± 0.72	34.90 ± 1.49	72.84 ± 1.60
16	29.80 ± 0.26	29.05 ± 1.65	45.16 ± 0.43	29.30 ± 0.30	54.49 ± 2.28	85.35 ± 1.08
32	39.89 ± 0.80	36.93 ± 1.86	58.32 ± 0.80	35.10 ± 1.55	86.03 ± 3.86	92.20 ± 1.08
64	44.18 ± 0.82	48.07 ± 0.91	63.76 ± 2.26	47.74 ± 1.46	92.71 ± 1.33	98.68 ± 0.15

As shown in Table 11, fractions 5 and 6 showed very high antimalarial activity compared to fractions 1-4 obtained from the same crude extract. Fraction 5, which was found to be less active than fraction 6, decreased the incorporation of ^{3}H-hypoxanthine by more than 80% at 32 and 64 μg/ml. Fractions 5 and 6 are relatively more polar than the remaining four fractions.

The linear regression curves for the column-fractions obtained from the chloroform and methanol extracts of leaves of *W. somnifera*, and chloroform extract of leaves of *V. amygdalina* are shown in figures 8-10. The slope of the regression line for fraction 3 (Fig. 8) was found to be the highest compared to that of fractions 1, 2 and 4, obtained from the same plant extract. As shown in figures 9 and 10, fraction 5 obtained from methanol extract of leaves of *W. somnifera*, and fractions 5 and 6 obtained from chloroform extract of leaves of *V. amygdalina*, were found to be more potent than the other fractions in their respective groups.

Among the fractions obtained from chloroform extract of leaves of *W. somnifera*, the IC_{50}, IC_{90} and MIC values of fraction 3 were found to be very low (Fig. 11). Fraction 4 was found to have the second lowest IC_{50} value compared with the other fractions obtained from the same crude extract. But its IC_{90} and MIC values were very high. These values for the other column-fractions were also relatively very high.

As shown in Fig. 12, the IC_{50}, IC_{90} and MIC values of column-fractions 1-4 obtained from the methanol extract of *W. somnifera* were found to be very high. Fig. 13 shows the most polar fraction (fraction 6) obtained from the chloroform extract of leaves of V. amygdalina showed the lowest IC_{50}, IC_{90}, and MIC values.

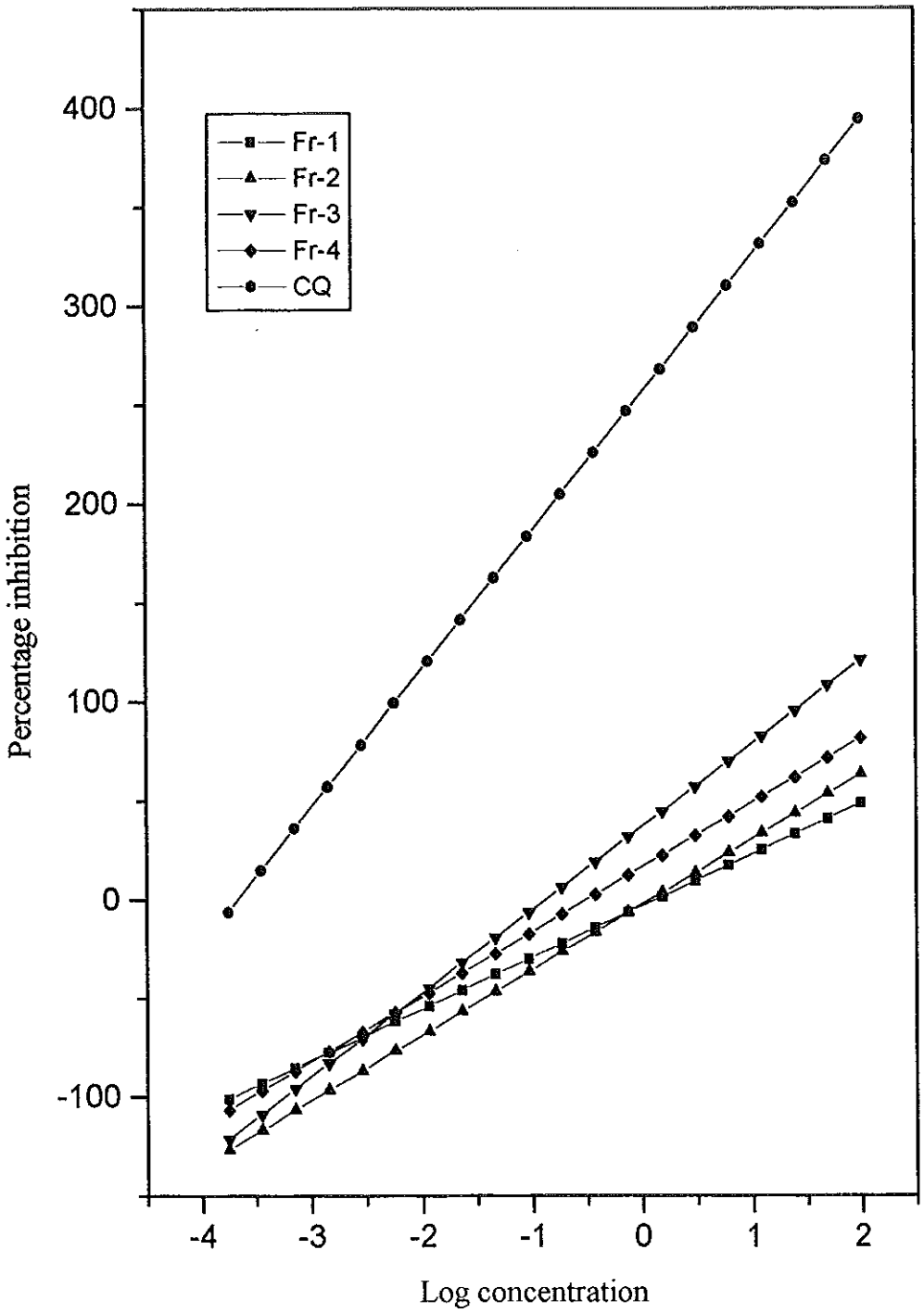

Fig. 8. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and column fractions from chloroform extract of leaves of *W. somnifera*.

---

abbreviations as in Table 9.

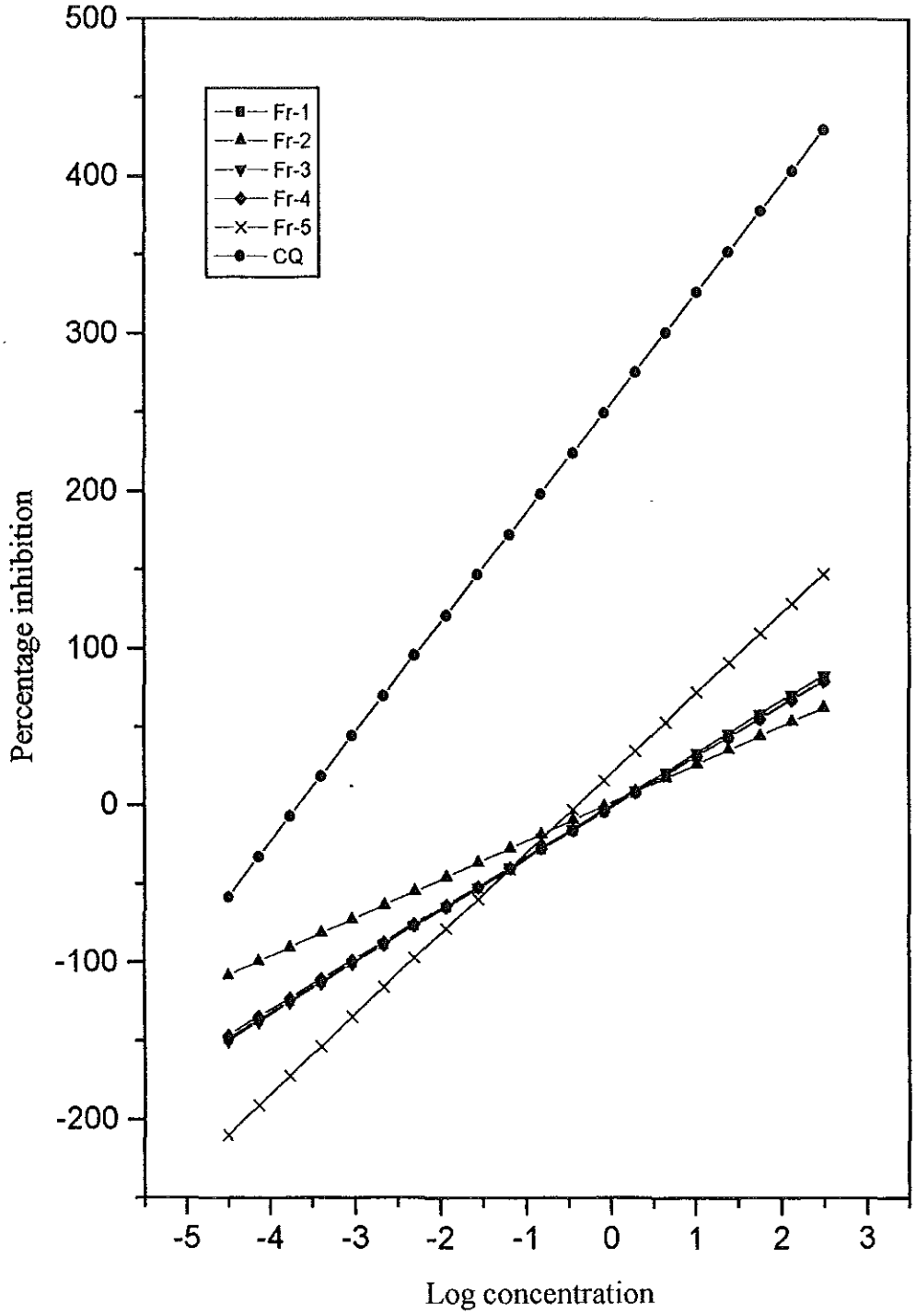

Fig. 9. Percentage inhibition of growth of *P. falciparum* at various concentrations of chloroquine and column fractions from methanol extract of leaves of *W. somnifera*.

---

abbreviations as in Table 10.

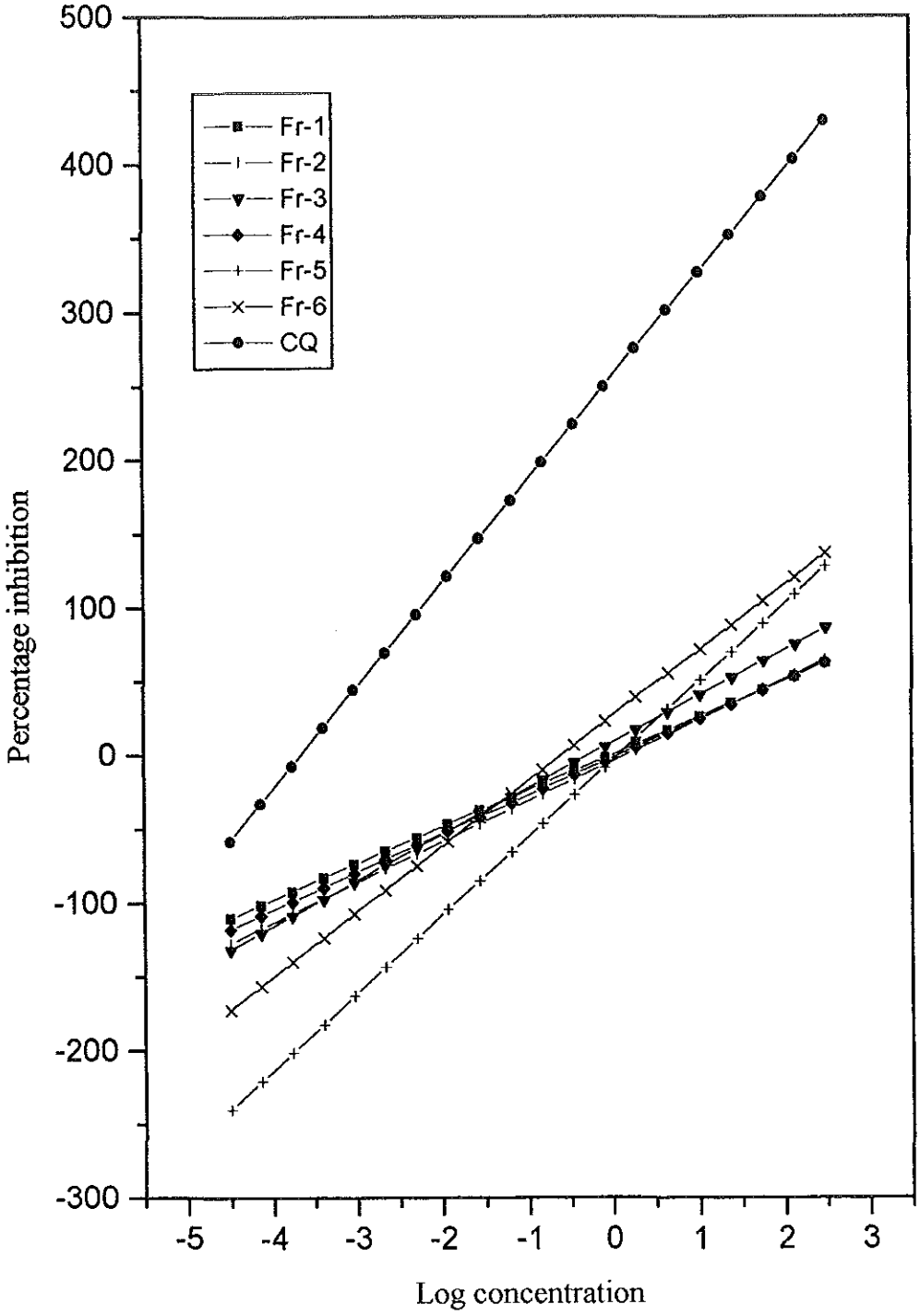

Fig. 10. Percentage inhibtion of growth of *P. falciparum* at various concentrations of chloroquine and column fractions from chloroform extract of leaves of *V. amygdalina*.

---

abbreviations as in Table 11.

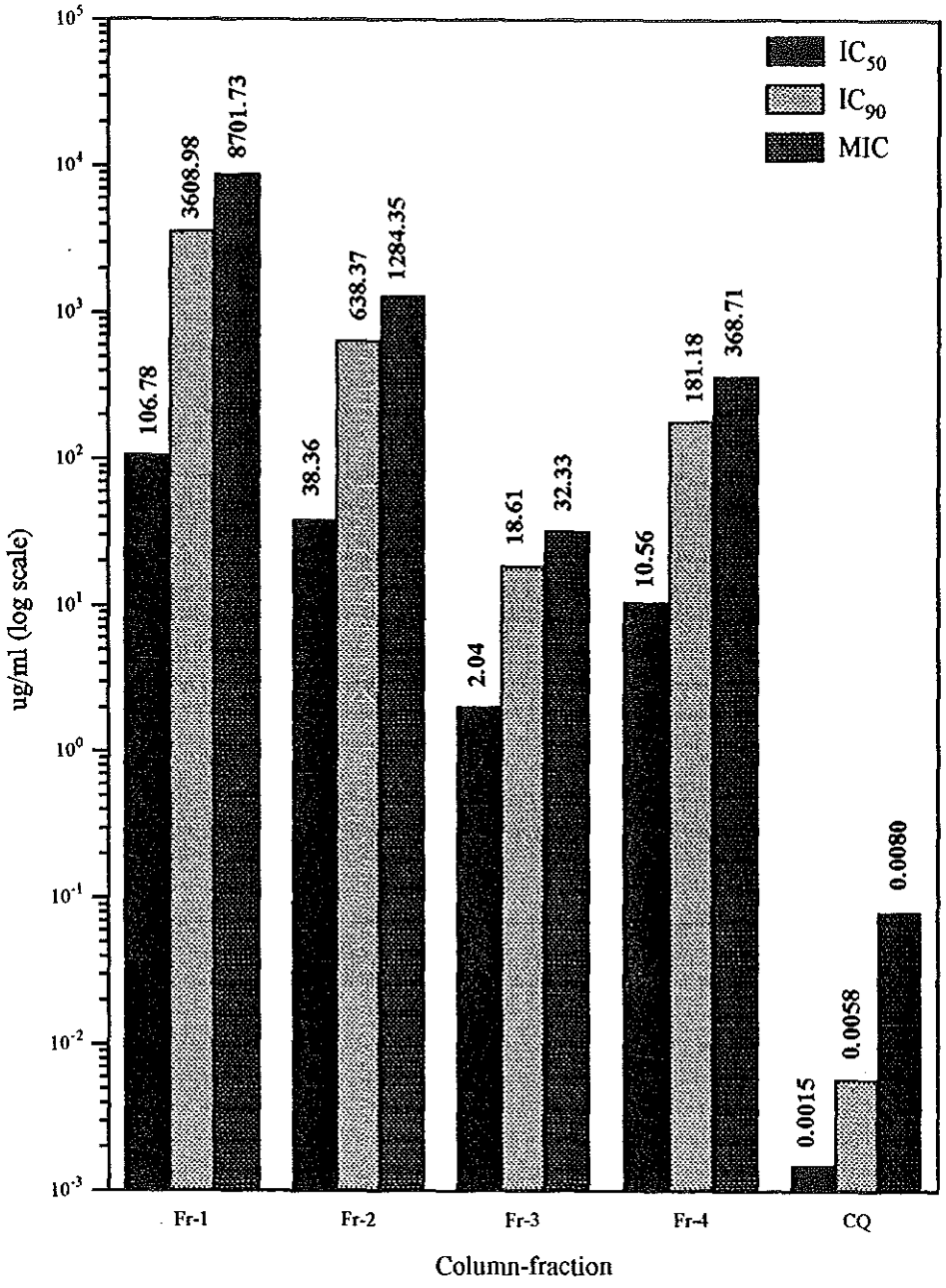

Fig. 11. IC_{50}, IC_{90}, MIC values for chloroquine and column-fractions obtained from chloroform extract of leaves of *W. somnifera.*

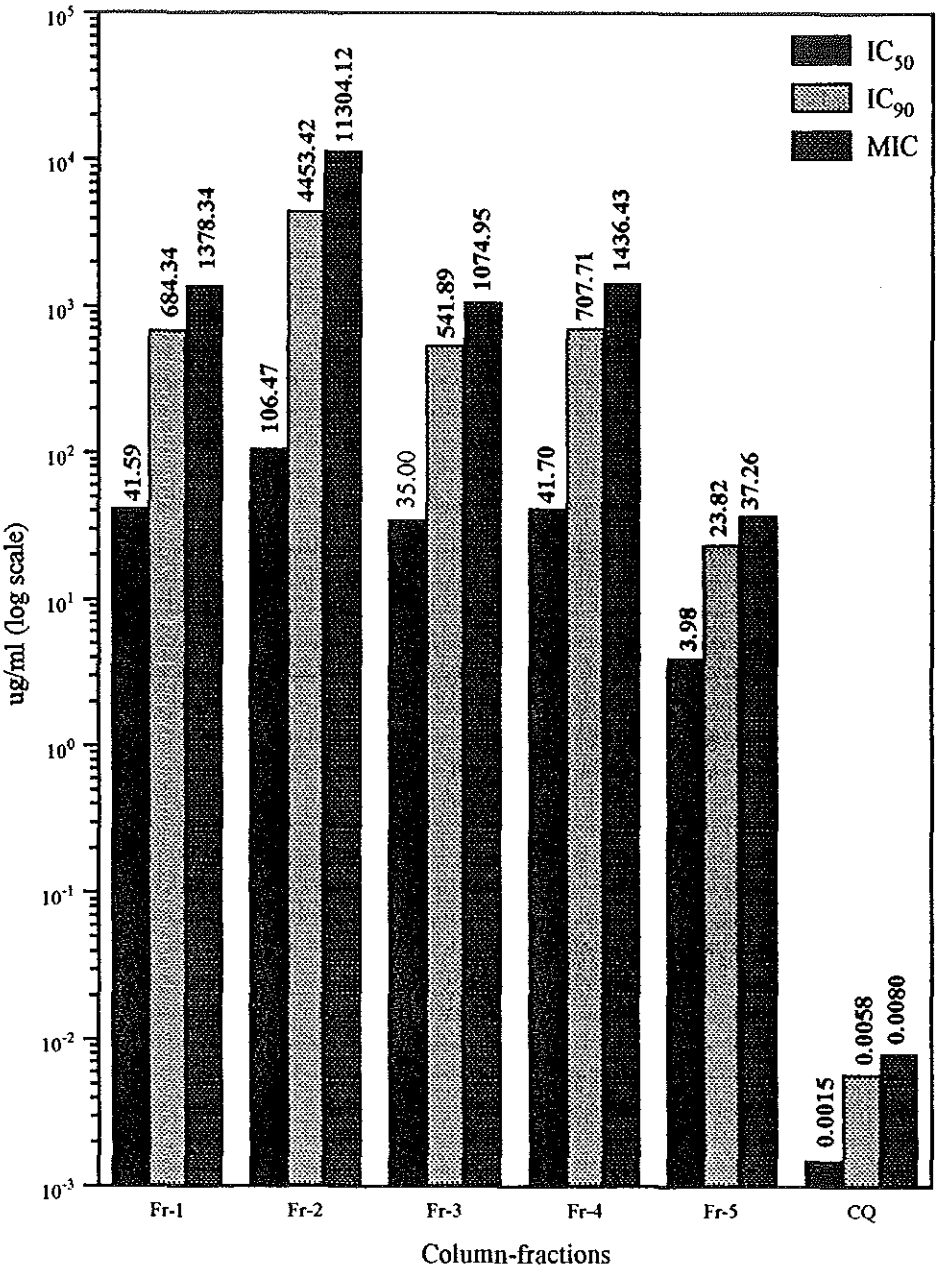

Fig. 12. IC_{50}, IC_{90}, and MIC values for chloroquine and column-fractions obtained from methanol extract of leaves of *W. somnifera*.

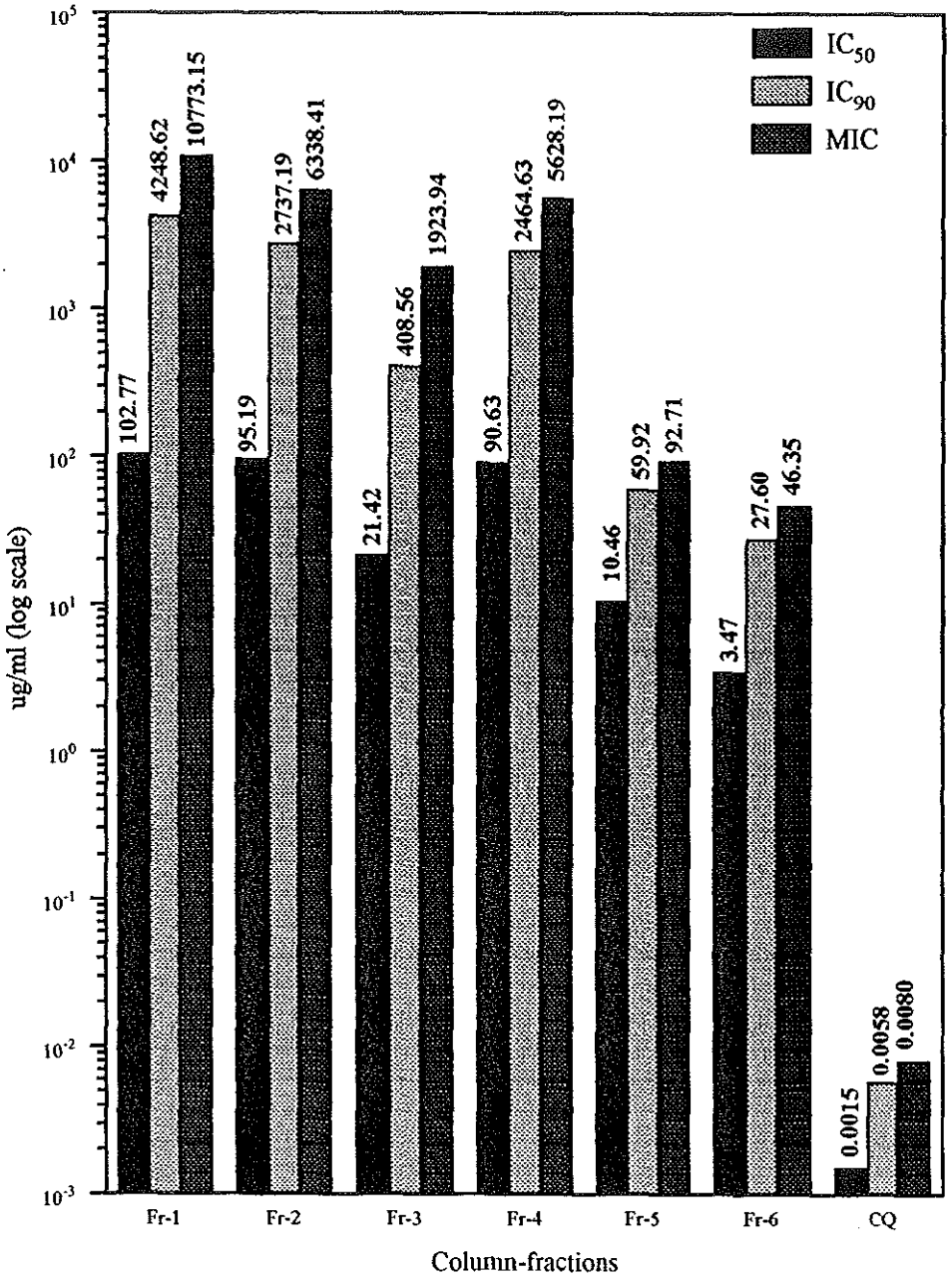

Fig. 13. IC_{50}, IC_{90}, and MIC values for chloroquine and column-fractions obtained from chloroform extract of leaves of *V. amygdalina*.

The three more active extracts, *i.e.*, chloroform and methanol extracts of leaves of *W. somnifera*, and chloroform extract of leaves of *V. amygdalina* were also tested for cytotoxicity against human HeLa cells *in vitro*. In Table 12, and figures 14 and 15 are shown the results of the cytotoxicity test.

Table 12. Mean ± standard deviation (n = 6) of the percentage inhibition of growth of HeLa cells at various concentrations of chloroform and methanol extracts of leaves of *W. somnifera*, and chloroform extract of leaves of *V. amygdalina*.

Concentration (μg/ml)	Extract (Ext)		
	Ext-1	Ext-2	Ext-3
1	25.54 ± 1.24	3.02 ± 0.05	1.02 ± 0.05
5	38.15 ± 0.55	15.67 ± 2.45	11.11 ± 0.12
10	60.82 ± 2.03	66.98 ± 2.86	56.85 ± 1.03
20	85.88 ± 1.25	93.62 ± 1.38	86.79 ± 4.41
50	98.54 ± 0.62	98.89 ± 0.47	99.03 ± 0.61

Where, Ext-1 = Chloroform extract of leaves of *W. somnifera*
Ext-2 = Methanol extract of leaves of *W. somnifera*
Ext-3 = Chloroform extract of leaves of *V. amygdalina*

All the three crude extracts inhibited the growth of HeLa cells by more than 50% at a concentration of 10 μg/ml and above (Table 12). As shown in Fig. 15, the IC_{50} and IC_{90} values of all the three extracts were between 5-10 and 10-50 μg/ml, respectively.

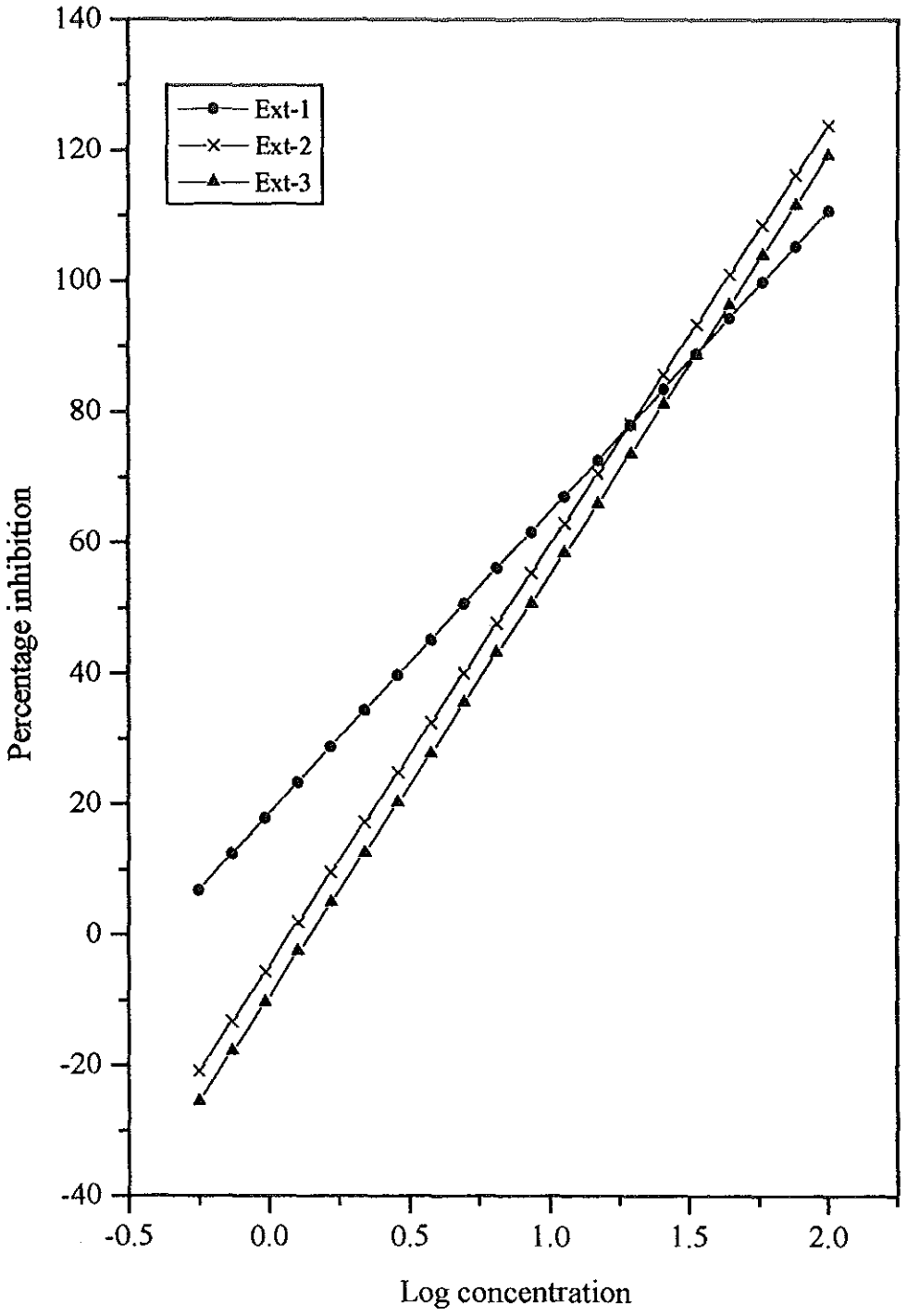

Fig. 14. Percentage inhibition of growth of HeLa cells by the chloroform and methanol extracts of *W. somnifera*, and chloroform extract of *V. amygdalina*.

---

abbreviations as in Table 12.

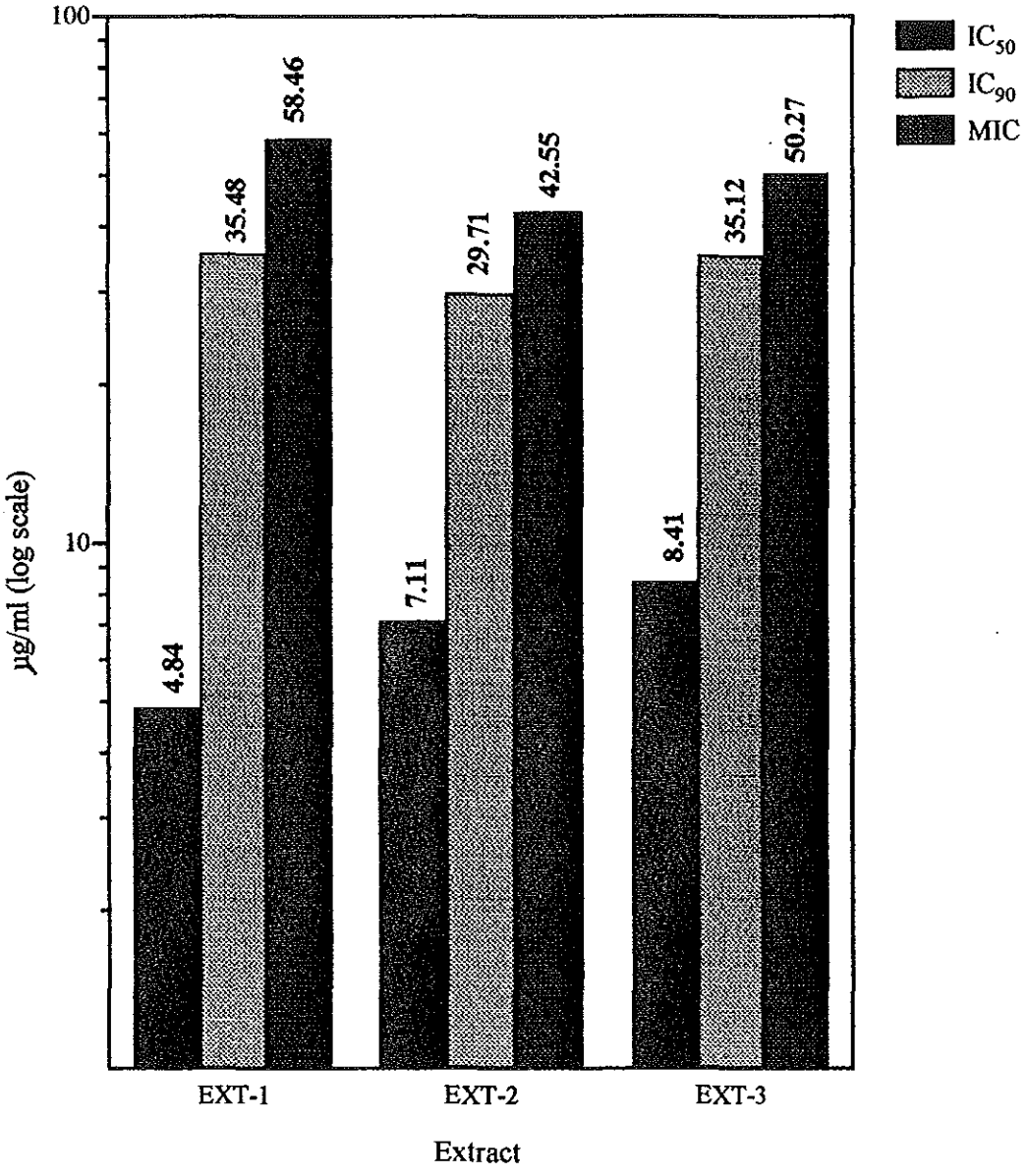

Fig. 15. IC_{50}, IC_{90}, and MIC values of chloroform and methanol extracts of leaves of *W. somnifera*, and chloroform extract of *V. amygdalina* against HeLa cells.

IV. DISCUSSION

Plant materials were first extracted with petroleum ether, the least polar of the three organic solvents used in this study. Further extraction of the marc was done with more polar solvents, chloroform and methanol, successively. This extraction method and the organic solvents used were presumed to enable extraction of most of the secondary metabolites from the plant materials.

Stock solutions of test materials were dissolved either in methanol or dimethyl sulfoxide. These were further diluted in complete medium so that the final concentration of solvents in the test wells would be less than 0.05% (v/v). Dosed-plates were allowed to dry in a sterile hood. These treatments enabled to rule out possible inhibitory effects of the solvents on the maturation of the malarial parasite.

The dilution of stock solutions in culture medium might have helped extracts/column-fractions to stay in solution. Protein substances presumably provide binding sites for compounds which might otherwise precipitate in aqueous solutions (Desjardins *et. al.*, 1979).

^{3}H-hypoxanthine incorporated into parasite nucleic acids was used as an indirect measurement of the growth of *P. falciparum* (Desjardins *et. al.*, 1979). "Hypoxanthine is the major purine base used by *P. falciparum* for the synthesis of adenosine and guanosine nucleotides and nucleic acids. Since the harvesting method traps only macromolecules, the radioactivity measured represents primarily ^{3}H-hypoxanthine incorporated into parasite nucleic acids. Background ^{3}H-hypoxanthine incorporation by uninfected erythrocytes is low since these cells synthesize neither RNA nor DNA (Chulay *et. al.*, 1983)."

Measurement of ^{3}H-hypoxanthine incorporation into the nucleic acids of *P. falciparum* is a more rapid method of growth of the parasite. It also has less variability among replicates (coefficient of variation < 5%) than does the measurement of percent parasitaemia by the traditional microscopic method (coefficient of variation generally 10 - 20%) (Chulay *et. al.*, 1983).

Test materials were compared based on their IC_{50}, IC_{90}, and MIC values. The lower these values for a test material, the higher the antimalarial activity of the material. In this study, IC_{50}, IC_{90} and MIC values were obtained from linear regression curves on the log dose *Vs* percentage maturation graphs. Therefore, these values are only approximations and not actual values as such. The slope of these linear regression lines were also taken as indications of the potency of antimalarial activity; *i. e.*, the higher the slope, the greater the potency.

The *P. falciparum* FCM-29 strain used in this study was resistant to chloroquine (IC_{50} = 0.0015, IC_{90} = 0.0058, and MIC = 0.008 μg/ml). But, it was found to be about 1,000 times more sensitive to chloroquine than the multi-drug resistant K1 strain (IC_{50} = 0.210 μg/ml) (O'Neill *et al.*, 1986) following the measurement of radiolabelled hypoxanthine incorporation method.

As shown in figures 5-7, three extracts showed substantial antimalarial activity (IC_{50} and IC_{90} lower than 20 and 100 μg/ml, respectively) against the FCM-29 strain. These were chloroform and methanol extracts of leaves of *W. somnifera*, and chloroform extract of leaves of *V. amygdalina*.

Chloroform extract of leaves of *W. somnifera* completely inhibited maturation of the parasites at 114.17 μg/ml (Fig. 6). The IC_{50} value of this extract was about 7,000 times higher than that of chloroquine. The MIC value of the methanol extract of leaves of this

same plant was found to be 125.12 μg/ml, which was more than 15,000 times higher than that of chloroquine (Fig. 7).

The IC_{50} and IC_{90} of the chloroform extract of the leaves of *V. amygdalina* (Fig. 6) were 16.93 and 87.27 μg/ml respectively. Incorporation of ^{3}H-hypoxanthine was completely inhibited by this extract at 150.60 μg/ml. The IC_{50} and IC_{90} values of this extract were found to be 9,000 and 14,000 times, respectively, higher than the corresponding values for chloroquine.

Methanol extract of a *Cinchona* species grown in Usambara, Tanzania, was found to have IC_{50} value of 0.5 μg/ml (Weenen *et. al.*, 1990a) against the multi drug resistant Thailand K1 strain of *P. falciparum* following the method described by Desjardins *et. al.* (1979). Compared with this, the three crude extracts that showed substantial antimalarial activity in this study, were found to be more than 20 times less active.

In comparison to the work of Weenen *et. al.* (1990a, 1990b) the findings of this study have revealed improvement of antimalarial activity upon column-fractionation. That is, both the dichloromethane extract of tubers of *Cyperus rotundus* (Cyperaceae) and the most active antimalarial compound isolated from this extract (α-cyperone) were found to have IC_{50} value between 5-9 μg/ml against the K1 strain of *P. falciparum in vitro* (Weenen *et. al.*, 1990b). On the other hand, the antimalarial activity of the three most active crude extracts in this study improved upon column-fractionation (Tables 9-11 and Fig. 8-13). IC_{50} and IC_{90} (Fig. 11) values of chloroform extract of the leaves of *W. somnifera* decreased by five- and four-folds respectively. The corresponding values for methanol extract of leaves of this same plant (Fig. 12) decreased by four-folds. IC_{50} and IC_{90} values of chloroform extract of leaves of *V. amygdalina* (Fig. 13) also improved by four-folds.

Compared with α-cyperone, fraction 2 and fraction 5 obtained from chloroform and methanol extracts, respectively, of leaves of *W. somnifera*, and fraction 6 obtained from chloroform extract of leaves of *V. amygdalina* were found to be more active. The higher antimalarial activity of the column-fractions as compared to the crude extracts suggests that the observed antimalarial activity was not due to synergistic effect of compounds present in these extracts. It also indicates that further purification of the column-fractions would improve the antimalarial activity. In all cases the highest antimalarial activity was observed in the most polar fractions of the crude extracts.

Based on IC_{50} values, chloroform extract of leaves of *W. somnifera* was found to have the highest inhibitory activity to the growth of HeLa cells *in vitro* (Table 12 and Figs 14 & 15). It was also found to be two-times more cytotoxic to HeLa cells than that of chloroform extract of leaves of *V. amygdalina*.

The ninety percent inhibitory concentration of the chloroform extracts of the leaves of *W. somnifera* and *V. amygdalina* were about the same. Based on IC_{90} values methanol extract of leaves of *W. somnifera*, was found to be more inhibitory to the growth of HeLa cells *in vitro*.

The methanol extract of leaves of *W. somnifera* completely inhibited the growth of HeLa cells at 42.55 μg/ml. This value for the chloroform extract of leaves of *V. amygdalina* and *W. somnifera* were found to be 50.27 and 58.46 μg/ml, respectively. Considering the IC_{50}, IC_{90}, MIC values, and the slope of the linear regression curves from which these values were obtained, the methanol extract of leaves of *W. somnifera* were found to have the highest inhibitory effect on the growth of HeLa cells. Chloroform extract of leaves of *V. amygdalina* was found to be the next higher cytotoxic extract whereas chloroform extract of leaves of *W. somnifera* were found to be the least cytotoxic of the three.

The inhibitory activity of the three crude extracts was higher against HeLa cells than *P. falciparum*. The IC_{50} and IC_{90} of these extracts against HeLa cells were more than two times less than the corresponding values against *P. falciparum*. This shows the cytotoxicity of these extracts on HeLa cells to be higher than their antimalarial activity against *P. falciparum*.

In man the adverse effects of chloroquine are generally related to dosage and modes of administration. For instance, intravenous injections of chloroquine (at the usual dose of 200-300 mg base) are generally well tolerated by adults. However, even proportionally adjusted doses of chloroquine given parenterally to children may occasionally produce serious effects on the central nervous and cardiovascular systems (Black *et. al.*, 1986). Acute oral poisoning may occur when a dose of 1.5-2 g is swallowed at once; in children half of this dose may be fatal (Black *et. al.*, 1986).

In traditional practices these plant materials (except for *T. indica* and *C. abyssinica*, for which no information was available) are used in combination with several others: *Cucumis prophetarum* (Cucurbitaceae), *Phytolacca dodecandra* (Phytolaccaceae), *Colutea abyssinica* (Fabaceae) (Gelahun Abate, 1989). According to Gelahun Abate (1989), the plant materials are used by grinding all the plant materials together and mixing two glasses of this with four glasses of honey to make a stock. For use, two large spoonful of this stock is diluted in two glasses of water and boiled. One cup of this mixture in the morning and one in the evening, for three days, makes the traditional prescription. This shows that the vehicle used in the preparation and administration of these materials in traditional practices is different from the organic solvents used for extraction in this study. Moreover, traditional healers claim that the mixing of several herbs together for use against a certain disease improves the efficacy and safety of the traditional medicine. For this reason, the

antimalarial and cytotoxic activities of the extracts/column-fractions in this study give only an indication and are not conclusive of the actual effects of the materials in traditional practices.

V. CONCLUSION AND RECOMMENDATIONS

Many plants produce economically important products such as resins, oils, pesticides, flavours, and pharmaceuticals. Moreover, each pharmacological class of drugs includes a natural product prototype (Vlietinck, 1987). Nevertheless, most species of higher plants have not been described, much less surveyed for biologically active constituents (Vlietinck, 1987), and new sources of commercially valuable materials remain to be discovered.

More than 80% of the world's population especially those in developing countries rely on traditional medicine to treat various types of ailments (Phillipson and Wright, 1991). Most of the materials in traditional medicinal practices have been used over centuries. This study has demonstrated that some of the materials used to treat malaria in the traditional medicinal practices of Ethiopia are indeed effective, at least, *in vitro*. A similar work on the *in vitro* evaluation of some Ethiopian traditional medicinal plants against *P. falciparum* (Solomon Sorssa, 1992) has also demonstrated the effectiveness of some of these medicinal plants *in vitro*.

One of the major problems in traditional medicinal practice is that there are no scientific studies made regarding the safety and efficacy of the materials used. In this study it has been determined that at least two of the medicinal plants used to treat malaria in the traditional medicinal practices of Ethiopia do also have cytotoxic effects. If the toxicity of these materials is not specific to HeLa cells, a malaria patient who received a traditional treatment with these plants may encounter serious toxicological problems. The fact that there are no scientifically established doses in traditional medicinal practices can also aggravate these problems. So, it is very important that the safety and efficacy of materials used in traditional medicinal practices be determined and appropriate dosage formulations

be prepared and made available for use in primary health care systems.

Further studies on these plants should be targeted to isolate the antimalarial constituents, evaluate their *in vitro* and *in vivo* efficacy and toxicity.

REFERENCES

Agnew, A.D.Q. 1974. Upland Kenya wild flowers. A flora of the ferns and herbaceous flowering plants of upland Kenya. Oxford University Press, London.

Aikawa, M. 1988. Human cerebral malaria. *Am. J. Trop. Med. Hyg.* 39(1): 3-10.

Assefa Nega. 1993. Malaria. In: H. Kloos, A.Z. Zein (eds). *The Ecology of Health and Disease in Ethiopia.* Westview Press, Oxford pp. 341-352.

Baird, J.K., Basri, H., Jones, T.R., Purnomo, Bangs, M.J. and Ritonga, A. 1991. Resistance to antimalarials by *Plasmodium falciparum* in Arso Pir, Irian Jaya, Indonesia. *Am. J. Trop. Med. Hyg.* 44(6): 640-644.

Basco, L.K. and Le Bras, J. 1993. *In vitro* activity of Artemesinin derivatives against African isolates and clones of *Plasmodium falciparum. Am. J. Trop. Med. Hyg.* 49(3): 301-307.

Beaver, P.C., Jund, R.C. and Cupp, E.W. 1984. *Clinical Parasitology*. 9th edn Lea and Fabiger, Philadelphia.

Black, R.H., Canfield, C.J., Clyed, D.F., Peters, W. and Wernsdorfer, W.H. 1986. *Chemotherapy of Malaria.* Revised 2nd edn. WHO Monograph Series No. 27.

Brandicourt, O., Druilhe, P., Diouf, F., Brasseur, P., Turk. P. and Danis, M. 1986. Decreased sensitivity to chloroquine and quinine of some *Plasmodium falciparum* strains from Senegal in September 1984. *Am. J. Trop. Med. Hyg.* 35(4): 717-721.

Brasseur, P., Kouamouo, J., Moyou-somo, R. and Druilhe, P. 1992a. Multi-drug resistant falciparum malaria in Cameroon in 1987-1988 I. Stable figures of prevalence of chloroquine- and quinine-resistant isolates in the original foci. *Am. J. Trop. Med. Hyg.* 46(1): 1-7.

Brasseur, P., Kouamouo, J, Moyou-somo, R. and Druilhe, P. 1992b. Multi-drug resistant falciparum malaria in Cameroon in 1987-1988 II. Mefloquine resistance confirmed *in vivo* and *in vitro* and its correlation with quinine resistance. *Am. J. Trop. Med.Hyg*. 46(1): 8-14

Brasseur, P., Kouamouo, J., Brandicourt, O. Moyou-somo, R. and Druilhe, P. 1988. Patterns of *in vitro* resistance to chloroquine, quinine, and mefloquine of *Plasmodium falciparum* in Cameroon, 1985-1986. *Am. J. Trop. Med. Hyg*. 39(2): 166-172.

Brasseur, P., Pathe-Diallo, M. and Druilhe, P. 1987. Low sensitivity to chloroquine and quinine of *Plasmodium falciparum* isolates from Guinea in March 1986. *Am. J. Trop. Med. Hyg*. 37(3): 452-454.

Brockelman, C.R., Tan-Ariya, A. and Manabandhu, C. 1985. Cultivation of *P. falciparum*. In: S. Panyim, P. Walairat and Y. Yuthavong (eds). 1985. *Application of genetic engineering to research on tropical disease pathogens with special reference to plasmodia*. UNDP/World Bank/WHO special programme for Research Training in Tropical Diseases (TDR), Mahidol University, Bangkok, Thailand. pp 365-378.

Bruce-Chwatt, L.J. 1974. Transfusion malaria. *Bull. Wld. Hlth. Org*. 50: 337-346.

Bruce-Chwatt, L.J. 1980. *Essential Malariology*. William Heinemann Medical Books Ltd., London

Bruce-Chwatt, L.J. 1985. *Essential Malariology*. 2nd edn William Heinemann Medical Book Ltd., London.

Carlson, E.A. 1984. *Human Genetics*. D.C. Health and Company. Lexingthon, Massachusetts, Toronto.

Chan, K.L., O'Neill, M.J., Phillipson, J.D. and Warhurst, D.C. 1986. Plants as sources of antimalarial drugs. Part 3: *Eurycoma longifolia*. *Planta Med.* 52: 105-107.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. *Glossary of Indian medicinal plants.* Council of Scientific and Industrial Research, New Delhi.

Chulay, J.D., Haynes, J.D. and Diggs, L.C. 1983. *Plasmodium falciparum*: Assessment of *in vitro* growth by ^{3}H-hypoxanthine incorporation. *Experi. Parasitol.* 55: 138-146.

Clark, I.A. 1987. Cell-mediated immunity in protection and pathology of malaria. *Parasitol. Today.* 3: 300-305.

Cohen, S. and Butcher, G.A. 1974. Mechanisms of immunity to malaria. *Bull. Wld. Hlth. Org.* 50(3): 251.

Dale, I.R. and Greenway, P.J. 1961. *Kenya trees and shrubs.* Buchanan's Estate Ltd., Nairobi.

Desjardins, R.E., Canfield, C.J., Haynes, J.D. and Chulay, J.D. 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* 16: 710-718.

Elmi, A.S. 1990. Research into medicinal plants. The Somali experience. In: Mshigeni, K.E.; Nkunya, M.H.H.; Fupi, V.; Mahunnah, R.L.A. and Mshiu, E.N. (eds). Proceedings of International Conference on Traditional Medicinal Plants. Arusha, Tanzania. Dar es Salaam University Press. pp 27-33.

Fairlamb, By. A., Warhurst, D.C. and Peters, W. 1985. An improved technique for the cultivation of *Plasmodium falciparum* without daily medium change. *Ann. Trop. Med. Parasitol.* 79(4): 379-384.

Fontaine, R.E., Najar, A.E. and Prince, J.S. 1961. The 1958 malaria epidemic in Ethiopia. *Am. J. Trop. Med. Hyg.* 10: 795-803.

Fransworth, N.R. 1985. Plants and modern medicine: where science and folklore meet. World Health Forum, WHO, Geneva. pp. 76-80.

Gelahun Abate. 1989. *Etse Debdabe* (in amharic). Research and Publications Office, Addis Ababa University.

Guru, P.Y., Warhurst, D.C., Harris, A. and Phillipson, J.D. 1983. Antimalarial activity of bruceantin *in vitro*. *Ann. Trop. Med. Hyg*. 77(4): 433-435.

Hall, A.P. 1976. The treatment of severe falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg*. 71: 367-378.

Haynes, J.D., Diggs, C.L., Hines, F.A. and Desjardins, R.E. 1976. Culture of human malaria parasites, *Plasmodium falciparum*. *Nature*. 263: 767-769

Jellife, D.B. and Jellife, E.F.P. 1977. The cultural Cul-de-sac of Western Medicine. *Trans. R. Soc. Trop. Med. Hyg*. 71(4): 331-334.

Li, Z.L., Gu, H.M., Warhurst, D.C. and Peters, W. 1983. Effects of qinghaosu and related compounds on incorporation of G-^{3}H-hypoxanthine by *Plasmodium falciparum in vitro*. *Trans. R. Soc. Trop. Med. Hyg*. 77(4): 522-523.

Livingstone, F.B. 1976. Haemoglobin history in West Africa. *Hum. Biol.* 48: 487-500.

Loban, K.M. and Polozok, E.S. 1985. *Malaria*. Mir Publishers, Moscow.

Lysenko, A. Ya., Belyaev, A.E. and Rybalko, V.M. 1978. Population studies of *Plasmodium vivax* I. The theory of sporozoite polymorphism and the epidemiological phenomenon of vivax malaria. *Bull. Wld. Hlth. Org*. 55(5): 549-564.

Makinde, J.M., Amusan, O.O.G. and Adesogan, E.K. 1988. The antimalarial activity of *Spathoda campanulata* stem bark extract on *Plasmodium berghei* in mice. *Planta Med.* 54: 122-125

Marcus, M. 1976. Possible support for the sporozoite hypothesis of relapse and latency in malaria. *Trans. R. Soc. Trop. Med. Hyg.*, 70(5-6): 535.

McGregor. I.A., Wilson, M.E. and Billewicz, W.Z. 1983. Malaria infection of the placenta in the Gambia, West africa; its incidence and relationship to stillbirth, birthweight and placental weight. *Trans. R. Soc. Trop. Med. Hyg.* 77(2): 232-244.

Mendis, K.N., David, P.H. and Carter, R. 1991. Antigenic polymorphism in malaria: is it an important mechanism for immune evasion? Elsevier Science Publishers Ltd, UK.

Mesfin Tadesse and Sebsibe Demissew. 1992. Medicinal Ethiopian plants: inventory, identification, and classification. In: Sue Edwards and Zemede Asfaw (eds). **1992.** *Plants used in African traditional medicine as practised in Ethiopia and Uganda.* Botany 2000: East and Central Africa. NAPRECA Monograph Series No. 5. NAPRECA, Addis Ababa University , Addis Ababa.

Miller, L.H. 1972. The ultrastructure of red cells infected by *Plasmodium falciparum* in man. *Trans. R. Soc. Trop. Med. Hyg.*, 66: 459-462.

Nguyen-Dinh, P., Hobbs, J.H. and Campbell, C.C. 1981. Assessment of chloroquine sensitivity of *Plasmodium falciparum* in Cholutea, Honduras. *Bull. Wld. Hlth. Org.* 59(4): 641-646

Nkunya, H.H. 1992. Progress in the search for antimalarials. NAPRECA Monograph Series No. 4. NAPRECA, Addis Ababa University, Addis Ababa.

O'Neill, M.J. 1986. Plants as source of antimalarial drugs, part 2: *In vitro* antimalarial activity of some quassinoides. *Antimicrob. Agents Chemother.* 30: 101-103.

O'Neill, M.J., Bray, D.H., Boardman, P., Phillipson, J.D. and Warhurst, D.C. 1985a. Plants as sources of antimalarial drugs. Part I. *In vitro* test method for the evaluation of crude extracts from plants. *Planta Med.* 61: 394-398.

O'Neill, M.J., Boardman, P., Chan, K.L., Bray, D.H., Phillipson, J.D. and Warhurst, D.C. 1985b. Antimalarial activity of *Brucea javanica* fruit. *J. Pharm. Pharmac.* 37(suppl.): 49.

O'Neill, M.J., Bray, D.H., Boardman, P., Chan, K.L. and Phillipson, J.D. 1987. Plants as sources of antimalarial drugs. Part 4: activity of *Brucea javanica* fruit against chloroquine resistant *P. falciparum in vitro* and against *P. berghei in vivo. J. Nat. Prod.* 50: 41-48.

Oliver, D. 1871. Flora of tropical Africa. Vol. II. Leguminoceae to Ficoideae. L. Reeve & Co., London.

Oliver, D. 1877. Flora of tropical Africa. Vol. III. Umbelliferae to Ebenaceae. L. Reeve & Co., London.

Oliver, M.H., Harrison, N.K., Bishop, J.E. Cole, P.J. and Laurent, G.J. 1989. A rapid and convenient assay for counting cells cultured in microwell plates: application for assessment of growth factors. *J. Cell Sci.* 92, 513-518.

Osisanya, J.O.S., Gould, S. and Warhurst, D.C. 1981. A simplified culture technique for *Plasmodium falciparum. Ann. Trop. Med. Parasitol.* 75(1): 107-109.

Payne, D. 1987. Spread of chloroquine resistance in *Plasmodium falciparum. Parasitol. Today.* 3: 241-246

Peters, W. 1980. Chemotherapy of malaria. In: *Malaria.* Academic Press, New York.

Phillipson, J.D. and Wright, C.W. 1991. Can Ethnopharmacology contribute to the development of antimalarial agents? *J. Ethnopharm.* 32: 155-165.

Turril, W.B. and Milne-Redhead, E. (eds). 1952. Flora of Tropical East Africa: Oleaceae. The Crown Agents for the Colonies, London.

Vlietinck, H.K. 1987. Biologically active substances from traditional drugs. In: K. Hostettmann and P.J. Lea (eds), **1987**. *Biologically active natural products*. pp 33-47.

Watt, J.M. and Breyer-brandwijk, M.G. 1962. *The medicinal and poisonous plants of southern and eastern Africa.* 2nd edn E & S Livingstone Ltd. Edinburgh and London.

Weenen, H., Nkunya, M.H.H., Bray, D.H., Mwasumbi, L.B., Kinabo. L.S. and Kilimali, V.A.E.B. 1990a. Antimalarial activity of Tanzanian medicinal plants. *Planta. Med.* 56(4): 368-370.

Weenen, H, Nkunya, M.H.H., Bray, D.H., Mwasumbi, L.B., Kinabo, L.S., Kilimali, V.A.E.B. and Wijnberg, J.B.P.A. 1990b. Antimalarial compounds containing α, ß-unsaturated carbonyl moeity from Tanzanian medicinal plants. *Planta Med.* 56(4): 371-373.

World Health Organization. 1989. Geographical distribution of arthropod-borne diseases and their principal vectors.

World Health Organization. 1990. WHO news and activities. World malaria situation 1988. *Bull. Wld. Hlth. Org.* 68(5): 667-673.

World Health Organization. 1991. Basic malaria microscopy. Part I. Lerner's Guide. WHO, Geneva. p. 72

World Health Organization. 1993. WHO's plan of work for malaria control 1993-1999. Part 1: Background and overview. Meeting of interested parties on malaria control. Geneva.

Wright, C.W. and Phillipson, J.D. 1990. Natural products and the development of selective antiprotozoal drugs. *Phytotherapy Research.* 40(4): 127-139.