Antimalarial Activity of Certain Sudanese Medicinal Plants Used in Folk –Medicine

EL-Hadi M. Ahmed[1] , Hassan S. Khalid[2] and Yousif G. Mohammed[I]

1 Department of Pharmacognosy and Chemistry, Faculty of Pharmacy، University of Gezira, Wad Medani, Sudan.

2 Medicinal and Aromatic Plants Research Institute, National Council for Research, Khartoum, Sudan.

ABSTRACT

Ten indigenous plants of common use in Sudanese folk-medicine، vitro for antimalarial activity against *Plasmoclium* were examined in *falciparum*, the major human malaria parasite. All plant samples displayed various antimalarial activity. Three plant extracts caused 100% inhibition of the parasite growth at a concentration ≤ 500μg/ml. The most active extracts that produced 100% inhibition of the parasite growth at a concentration ≤ 50 μg/ml were obtained from the seeds of *Nigella* sativa and the whole plant of *Aristolochia bracteolata.* These two plants were phytochemically screened for their active constituents and both showed the presence of sterols, alkaloids and tannins.

INTRODUCTION

Malaria, a mosquito-borne disease is global. It was estimated that there were over 300 million cases of malaria every year-mainly in sub- Saharan Africa (90%) and other developing countries. Malaria kills over one million people a year-mainly children under five years and pregnant women. Malaria is a major health problem in Sudan. It constitutes 30% of all attendance to health facilities. It is the main cause of hospital death. The failure of malaria control is largely due to the increasing parasite resistance to chloroquine and vector resistance to insecticides used (Christopher, 2002)

In all countries where malaria is endemic, plants are used in traditional medicine for treatment of the disease. Examples are numerous with the urgent need to develop new, safe and effective drugs against malaria.

Plants may provide such drugs directly as with quinine from *Cinchona* bark or artemisinine from the Chinese herb *Artemisia annua* and/or they nay provide template molecules on which to base further novel structures by organic synthesis (Phillipson and O'Neill, 1986).

Several investigations have been published in the field of antiplasmodials of plant origin (Bray *et al*., 1985; Phillipson and O'Neill، 1986 Weenen *et al*., 1990; Jurg *et al*, 1991Gakunju *et al*، 1995, Mackinnon *et al*., 1997; Najib *et al*., 1999; Willcox, 1999; Antoun *et al*، 2001 Nundkumar and Ojewole 2002).

In Sudan, out of 21 compounds isolated from 9 medicinal plants used in traditional medicine, only gedunin and quercetin showed an IC_{50} of about lμ.M as antiplasmodial when tested *in vitro* against *Plasmodium falciparum* (Khalid *et al*., 1986). Recently, an investigation of antiplasmodial activity of selected Sudanese plants revealed that most plants from the family Meliaceae showed highly potent antiplasmodial activity against the two tested strains(3D7-chloroquine and pyrimetham- ine sensitive and Dd2-chloroquine resistant and pyrimethamine sensitive *Plasmodium falciparum* strains). Khaya *senegalensis* (Mahogany) Azadirachta indica (Neem) and *Trichilia emetica* (Dabkar) showed IC_{50} values less than 5μg/ml (El-Tahir *et al*., 1999).

The present study was carried out to screen the antiplasmodial activity of 10 plant samples, representing 10 species and 9 families.

MATERIALS AND METHODS

Plant material

Plants used for this study were kindly supplied by the Medicinal and Aromatic Herbs Research Institute of the National Council for Research، Khartoum (Table l).

Extraction method

Twenty grams of the dried and coarsely powdered plant material was extracted by maceration in a conical flask for 24 hours using petroleum ether/chloroform (l: l) and continuous shaking. Each extract was evaporated under vacuum at 60^0C using a rotary evaporator to give 20 ml (l g/ml).

Preparation of working solution

The dried extracts of each plant material were dissolved separately in RPMI 1640 liquid medium (Invetrogen, UK). Three concentration 10, 100and 1000 μg/ml were prepared in complete medium (RPMI 1641 + 10 % human serum), on the day of the test (EL-Tat1ir *et al*, 1999).

Phytochemical screening

Phytochemical screening for the secondary plant constituents present in the plant extracts were carried out using methods adopted in similar surveys (Ahmed, 1983).

Parasite cultivation and in vitro testing

Screening tests for parasite cultivation and *in vitro* testing of different extracts were carried out according to the method recommended by WHO (W.H.O, 2001). In this method, sterile heparinized capillary tube was used to take blood samples (isolate) from patients with symptomatic malaria and who had not recently received antimalarial drug and who had mono-infection with *Plasmodium falciparum* and asexual *parasitaemias* in excess of 1000 parasites but less than 80.000 parasite per μL blood. The sample was maintained in blood - medium- mixture BMM (1 :9), i.e each 100 μL blood sample (isolate) required 0.9 ml of RPMI 1640 liquid medium to make a total of Iml BMM.

The unpredosed wells (12/plate) of tissue culture plates (WHO, invitro microtest plate, VCRU, USM, Malaysia), were dosed with 50 μ I of prepared working solution of drugs of 10, 100 and 1000 μg/ml separately 50 μI of the BMM were added to each well using fixed volume Eppendrof pipette and a disposable sterile tip. The resultant testing solutions were diluted the addition of equal volume of BMM to give: 5, 50 and 500 μg/ml. Dosing of wells with BMM was always done starting with control wells. Chloroquine (standard) was tested concomitantly on each occasion. Blood/drug concentration was mixed well by shaking plates gently. Plates were then placed in candle Jar with candle on. The candle Jar was closed when the candle is about (go off The candle jar was then placed in an incubator at 37 ^{o}C

for 42 hours. At the end of the incubation period, the tissue culture plates were moved and placed on a clean bench. The supernatant was removed using glass Pasteur pipette and the red blood cells deposited at the bottom were mixed and transferred to a clean glass side. Smears were made and stained with 2% Giemsa in phosphate buffer, pH 7.2, for 30 minutes to dry and then examined under the microscope. The parasites were counted and the ratio. of schizonts to red blood cells was determined. All slides were gently washed by dipping into a beaker or tap water, allowed parasites at the schizont stage with 3 or more nuclei were counted out of a total of 200 asexual parasites. The number of schizonts in the control smears should be 20 or more per 200 asexual forms for the test to be valid. The highest drug concentration at which no schizonts grow was considered to be the end point value for the test. Each extract was evaluated in triplicate. The mean was calculated as follows:

a. Maturation percentage = $\frac{\text{No. of developed schizonts for test}}{\text{No. of developed schizonts for control}}$ XI00

b. Inhibition percentage = 100 - maturation percentage

Table 1. Plants screened for their antimalarial activity.

Botanical name and family	Local name	Folk use	Morphological part tested	Geographical Source
Aerva javanica Amaranthaceae	Um- Shariaa	For fevers and to relief intestinal gases	WP	KhS
Ambrosia maritima Asteraceae	Damsisa	Anti-inflammatory in kidney diseases and *diabetus mellitus*, malaria.	W P	Kh S
Aristolochia bracteolata Aristolochiaceae	Um Galagel	Roots used for scorpion stings and anti-inflammator, leaves for malaria	W P	G S
Citrullus *colocynthis* Cucurbitaceae	EL-Handal	For haemoroids, arthiritis, eczema, laxative and for malaria.	S	G S
Croton zambesicus Euphorbiaceae	Um- Geleigla	Anti-hypertensive and for malaria	Fr	C S
Gardenia lutea Rubiaceae	UmGawy	Fruit is eaten by human	Fr P	Kh S
Pulicaria crispa Asteraceae	EL-Rmeit	As a source of essential oil	W P	Kh S
Nigella sativa Ranunculaceae	Kamun-Aswad - Habat EL-Baraka	Anti-inflam-matory, allergies, eczema and for malaria.	S	N S
Solenostema argel Asclepiadaceae	EL-Hargel	Carminative, antispasmodic and for malaria.	L	N S
Tinospora bakis Menispermaceae	Erg-EL-Hagar.	For fevers, diarrhoea and dysentery	W P	C S

L: Leaf; W P : Whole plant; Fr : Fruit; Fr P : Fruit Pulp and S: Seed; Kh S :Khartoum State; G S: Gezira State; C S : Central Sudan; N S : Northern State.

RESULTS AND DISCUSSION

Table 2 shows the effects of 10 plants, used traditionally to treat fever and/or malaria, on the number of developed schizonts expressed in maturation percentage of the parasite.

Table 2. *In vitro* antimalarial activities of extracts from certain Sudanese medicinal plants on *Plasmodium falciparum*.

Plant species	Maturation of the parasite(%)				
	Control	Concentrations used in μg/ml			
		5	50	500	
Aerva javanica	100	88.24	2.35	0.0	
Ambrosia maritime	100	35.29	17.65	5.88	
Aristolochia bracteolata	100	2.35	0.00	0.00	
Citrullus colocynthis	100	17.65	3.53	2.35	
Croton zambesicus	100	52.94	42.35	17.65	
Gardenia lutea	100	4.71	3.53	2.35	
Pulicaria crispa	100	58.82	14.12	3.53	
Nigella sativa	100	2.35	0.00	0.00	
Solenostema argel	100	6.06	4.71	1.18	
Tinospora bakis	100	72.94	29.41	7.06	
Chloroquine	Concentrations used in µg/ml				
		0.2	0.4	0.8	1.6	3.2
	100	76	8.24	1.18	0.0	0.0

It was shown that, at a concentration ≤5µg/ml, six plant extracts were found to possess more than 50% inhibition of the parasite growth; these are: Ambrosia maritime, *Aristolochia bracteolata*, *Citndlus colocynthis*، *Gardenia lutea*, *Nigella sativa* and *Solenostema argel*. All plant extracts tested showed > 50% inhibition of the parasite at a concentration ≤ 50µg/ml. The results obtained from this preliminaw study indicate that all plant samples displayed various antimalarial activity against *Plasmodium falciparum* in *in vitro* tests (Table 2). Such antiplasmodial activity of these plants have proven the ethomedical claims to treat fever and/or malaria and hence we propose that these plants should be further investigated.

Aerva javanica, whole plant, caused a 100% inhibition of the parasite growth at the incubation concentration ≤500µg/ml. The two most active extracts that produced 100% inhibition of the parasite growth at a concentration ≤ 50µg/ml were obtained from the seeds of *Nigella sativa* and the whole plant of *Aristolochia bracteolata*. *Nigella sativa* seeds are edible and used widely as condiment and / or spice

(Cevdet and Semih، (1993). Thus, it has the advantage as crude antimalarial over *Aristoloshia* species which was shown to be nephrotoxic, carcinogenic and mutagenic due to the cytotoxicity of the aristolochic acid constituents (Evans, 1989؛ Pena *et al*، (1996). Hence the use of *Aristolochia bracteolata* as an antimalarial plant is not recommended in its crude form. The antimalarial activity may reside in a nontoxic molecule which needs to be investigated.

The plants which showed high antiplasmodial activity (≤ 50μg/ml) were phytochemically screened (Table 3). Both *Nigella sativa* and *Aristoloshia bracteolata* showed the presence of sterols, alkaloids and tannins. None of them was proved to contain cardenolides, cyanogenic glycosides and anthraquinones while flavonoids and saponins were detected in *Nigella sativa*.

The antiplasmodial activity was not confined to any particular family and not restricted to any morphological part of the plant. Nonetheless، we believe that studies on these plants concerning their toxicity، teratogenicity, carcinogenicity and other biological evaluation should be pursued to end with safe and effective affordable drug.

Table 3. Phytochemical screening of the most active antimalarial plant.

Botanical name	Plant part tested	Sterols	Trite-rpenes	Alka-loids	Flavo-noids	Carde-nolides	Tann-ins	Sapo-nins	Cyano-genic glycosides	Anthra-quinones
Nigella sativa	S	+	±	±	+	-	+	+	-	-
Aristolochia bracteolata	WP	+	-	+	-	-	±	-	-	-

WP: Whole plant, S : Seed. - : Negative test (not detected); ± : Slightly positive test (traces); + : Strongly positives test (high concentration).

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