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Authors: Ahmed, El-Hadi M., Nour, Bakri Y.M., Mohammed, Yousif G., and Khalid, Hassan s.

Source: Environmental Health Insights, 4(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/EHI.S4108

Environmental Health Insights

ORIGINAL RESEARCH

Antiplasmodial Activity of Some Medicinal Plants Used in Sudanese Folk-medicine

El-Hadi M. Ahmed[1], Bakri Y.M. Nour[2], Yousif G. Mohammed[1] and Hassan S. Khalid[3]

[1]Department of Chemistry and Pharmacognosy, Faculty of Pharmacy, University of Gezira, P.O. Box 20 Wad Medani, Sudan. [2]Blue Nile National Institute for Communicable Diseases, University of Gezira, P.O. Box 20 Wad Medani, Sudan. [3]Medicinal and Aromatic Plants Research Institute, National Council for Research, Khartoum, Sudan. Email: yahadi54@hotmail.com

Abstract: Ten plants indigenous to Sudan and of common use in Sudanese folk-medicine, were examined *in vitro* for antimalarial activity against schizonts maturation of *Plasmodium falciparum*, the major human malaria parasite. All plant samples displayed various anti-plasmodial activity. Three plant extracts caused 100% inhibition of the parasite growth at concentrations of plant material ≤ 500 ug/ml. The two most active extracts that produced 100% inhibition of the parasite growth at concentration of plant material ≤ 50 µg/ml were obtained from the seeds of *Nigella sativa* and the whole plant of *Aristolochia bracteolata*. The ten plants were phytochemically screened for their active constituents. The two most active plants showed the presence of sterols, alkaloids and tannins.

Keywords: medicinal plants, antiplasmodial activity, folk-medicine

Environmental Health Insights 2010:4 1–6

This article is available from http://www.la-press.com.

Introduction

Malaria, a mosquito-borne disease is global. It was estimated that there were over 300 million cases of malaria every year in developing countries especially in Africa Sub-sahara (90%) and other developing countries. Malaria kills over one million people a year-mainly children under five years and pregnant women.[1] 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 and the failure of malaria control is largely due to the increasing parasite resistance to chloroquine and vector resistance to insecticides used.[2]

In all malaria endemic countries, 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 may provide template molecules on which to base further novel structures by organic synthesis.[3]

In Sudan, out of 21 compounds isolated from 9 medicinal plants used in traditional medicine, only gedunin and quercetin showed IC50 of 1 µM as antiplasmodial activity when tested *in vitro* against *Plasmodium falciparum*.[4] Moreover, 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 pyrimethamine sensitive and Dd2-chloroquine resistant and pyrimethamine sensitive *Plasmodium falciparum* strains). *Khaya senegalensis* (Mahogany), *Azadirachta indica* (Neem) and *Trichilia emetic* (Dabkar) showed $1C_{50}$ values less than 5 µg/ml.[5]

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

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 1).

Extraction method

Twenty grams of dried and coarsely powdered plant materials were extracted by maceration in a conical flask for 24 hours using petroleum ether/chloroform (1:1) and continuous shaking.[6]

Each extract was first filtered, then was concentrated by evaporation under vacuum at 60 °C using a rotary evaporator to give 20 ml (1 g of plant material/ml crude extract) and kept in a refrigerator until use (for easy calculation and practical procedure).

Preparation of working solution

The required concentration corresponding to 10, 100 and 1000 µg/ml was taken from the concentrated extract and evaporated to dryness and were prepared in complete medium (RPMI 1640 Invetrogen, UK + 10% human serum), on the day of the test.[5]

Phytochemical methods

Phytochemical screening for the secondary plant constituents present in the plant extracts was carried out using methods adopted in similar surveys.[6] This quantitative and phytochemical analysis of the 10 plants was determined as follows: Sterols and terpenoids (Lieberman's—Burchard's reaction; alkaloids (Mayor's/Wagner's/Dragendroff's reagents); flavonoids (conc Hcl + magnesium ribbon); cardiac glycosoides (Keller—Kiliani test); tannin ($Fecl_3$ test); saponins (frothing test); cyanogenic glycosides (sodium picrate paper); anthraquinone (5 g plant material in 20 ml 20% H_2SO_4 and 2 ml 2% $Fecl_3$, refluxed for 30 minutes, cooled, filtered and extracted with $CHcl_3$ + 5 ml 10% ammonium hydroxide. Pink—red colour in the alkaline layer indicated the presence of anthraquinones).

Parasite cultivation and *in vitro* testing

In vitro testing of extracts were carried out according to the method recommended by WHO.[7] 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 uL blood. The sample was maintained in blood-medium mixture BMM (1:9), i.e. each 100 uL blood

Table 1. Plants screened for their antiplasmodial activity and phytoconstituents.

Botanical name and (family)	Local name	Folk use	Morphological part tested	Geographical source
Aerva javanica (Amaranthaceae)	Um-Shariaa	Forfevers and to relief intestinal gases	WP	Khs
Ambrosia maritima (Asteraceae)	Damsisa	Anti-inflammatory in kidney diseases, diabetes mellitus and malaria	WP	Khs
Aristolochia bracteolata (Aristolochiaceae)	Um Galagel	Roots used fo Scorpion stings and anti-inflammator, leaves for malaria	WP	Khs
Citrullus colocynthis (Cucurbitaceae)	El-Handal	For haemoroids, arthiritis, eczema, laxative and for malaria	S	GS
Croton zambesicus (Euphorbiaceae)	Um-Geleigla	Anti-hypertensive and for malaria	Fr	CS
Gardenia lutea (Rubiacene)	Um Gawy	Fruit is eaten by human	FrP	Khs
Puliearia crispa (Asteraceae)	El-Rmeit	As a source of essential oil	WP	Khs
Nigella sativa (Ranuneulaceae)	Kamun-Aswad Habat ElBaraka	Anti-inflamatory, allergies, eczema and for malaria	S	NS
Solenostema argel (Ascepiadaceae)	El-Hargel	Carminative, Antispasmodic and for malaria	L	NS
Tinospora bakis (Menispermaceae)	Erg-El-Hagar	For fevers, diarrhoea and dysentery	WP	CS

Abberviations: L, leaf; WP, Whole plant; Fr, Fruit; FrP, Fruit Pulp; S, Seed; Kh S, Khartoum State; GS, Gezira State; CS, Central Sudan; NS, Northern State.

sample (isolate) required 0.9 ml of RPMI 1640 liquid medium to make a total of 1 ml BMM.

The unpredosed wells (12/plate) of tissue culture plates (WHO, *in vitro* microtest plate. VCRU, USM, Malaysia), were dosed with 50 u µ I of prepared working solution of drugs of 10,100 and 1000 µg/ml separately 50 µl of the BMM were added to each well using fixed volume Eppendrof pipette and a disposable sterile tip. The resultant testing solutions were diluted thereafter by 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) 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 to go off. The candle jar was then placed in an incubator at 37 °C for 42 hours. At the end of the incubation period, the tissue culture plates were removed 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 glass slide. Smears were made and stained with 1% Giemsa in phosphate buffer, pH 7.2 for 30 minutes, then dried and examined under the microscope. Schizonts containing 3 or more merozoites per 200 trophozoites were enumerated, they were considered successful if ≥10% of the parasites in the control well developed into schizonts. 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, and the mean was calculated as follows:

a. Maturation percentage = $\frac{\text{No. of developed schizonts for test}}{\text{No. of developed schizonts for control}} \times 100$

b. Inhibition percentage = 100-maturation percentage.

Statistical method

The collected data were analyzed using one—way ANOVA.

Ethical approval

The ethical approval for this study was obtained from the Ethical Committee of the Blue Nile Research and Training Institute/University of Gezira (Wad Medani, Sudan) and from Gezira State Ministry of Health.

Results

Antiplasmodial activity

Table 2 shows the effects of extracts from 10 Sudanese medicinal plants on schizonts maturation of *Plasmodium falciparum*.

It was shown that, at plant material concentration ≤5 µg/ml, six plant extracts were found to possess more than 50% inhibition of the parasite growth; these are: *Ambrosia maritime, Aristolochia bracteolata, Citrullus colocnthis, Gardenia lutea, Nigella sativa* and *Solenostema argel*. All plant extracts tested showed >50% inhibition of the parasite at plant material concentration ≤50 µg/ml. *Aerva javanica*, whole plant, caused 100% inhibition of the parasite growth at the incubation concentration ≤500 µg/ml. The two most active extracts that produced 100% inhibition of the parasite growth at plant material concentration ≤50 µg/ml were obtained from the seeds of *Nigella sativa* and the whole plant of *Aristolochia bracteolata*.

Phytochemical screening

phytoconstituents detected in plant samples as: sterols, triterpenes, alkaloids, flavonoids, cardenolides, tannins, saponins, cyanogenic glycosides and anthraquinones are shown in Table 3. Out of the 10 plant samples tested, none had shown the presence of cyanogenic glycosides. Alkaloids and sterols were detected in 8 plant samples while tannins were found to occur in 7 plant samples. Cardenolides were detected in trace amounts in 3 plant samples; *Ambrosia maritima, Citrullus colocynthis* and *Croton zambesicus*. Flavonoids were evident in 5 plant samples while triterpenes were found to exist in only 2 samples and traces of anthraquinones were detected in 2 samples.

Discussion

The present study examined the antiplasmodial activity of 10 plants used traditionally as crude drug powders as for Nigella and Pulicaria or in a form of water extracts as for others, to treat fever and/or malaria,

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

Plant species	Maturation of the parasite (%)*					
	Control	Concentrations used in ug/ml				
		5		50		500
Aerva javanica	100	88.24		2.35		0.0
Ambrosia maritime	100	35.29		17.65		5.88
Aristolochia bracteo-lata	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		3.35
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		Concentration used in ug/ml				
		0.2	0.4	0.8	1.6	3.2
	100	76	8.24	1.18	0.0	0.0

*In relation to negative control, the difference being statistically significant ($p < 0.01$).

on the number of developed schizonts expressed in maturation percentage of the parasite (Table 2).

The *in vitro* test results (Table 2), also showed that, significantly higher amounts of plant drugs were required, as compared with positive control drugs represented by the standard antimalarial chloroquine. However, these plant drugs could be considered as active antimalarial drugs, since plant extracts are considered active if they demonstrate 50% growth inhibition of the parasite at concentration ≤50 µg/ml.[8] Moreover, in all of the plant extracts more than 50% inhibition of the parasites at concentration ≤50 µg/ml was obtained, indicating that, such antiplasmodial activity of these plants have proven the ethomedical claims to treat fever and/or malaria. *Nigella sativa* seeds are edible and used widely as condiment and/or spice.[9] Thus, it has the advantage as crude antimalarial over *Aristoloshi*a species which had been shown to be nephrotoxic, mutagenic and carcinogenic due to the cytotoxicity of the aristolochic acid constituents.[10,11]

Hence the use of *Aristolochia bracteolata* as an antimalarial plant is not recommended in its crude form. However, the antimalarial activity may reside in a nontoxic molecule which needs to be investigated. 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, teratogenicty, carcinogenicity and other biological evaluation should be pursued to end with safe and effective affordable drug.

The preliminary phytochemical screening of plants under investigation (Table 3), revealed that none of them had shown the presence of cyanogenic glycosides. The tituents was found two plants which showed high antiplasmodial activity (<50 µg/ml), *Nigella sativa* and *Aristolochia brateolata* showed the presence of sterols, alkaloids and tannins. It was noticed that in all plant samples more than one group of constituents were found in each morphological part tested. Physiological activity may be due to one or more than one group of constituents. Several investigations have been published in the field of antiplasmodials of plant origin related to different bioaditive functional groups classified as: terpenoids;[3–5,12] alkaloids;[3,13–16] unsaturated fatty acids;[17] volatile oils[18,19] and phenolic compounds including flavonoids[4,17] and quinones.[20,21]

In conclusion, we have demonstrated the antimalarial effects and preliminary phytochemical profiles of extracts from ten commonly used Sudanese medicinal plants. Effort will be undertaken to continue biological and phytochemical evaluation to isolate and identify the active constituents as well as to understand the mechanism of action.

Table 3. Plants screened for their phytoconstituents.

No.	Botanical name	Plant part tested	Sterols	Triter penes	Alkaloids	Flavon oids	Carden olides	Tan nins	Sapo nins	Cyanog enic glycosides	Anthraquin ones
1	*Aerva Javanica*	WP	–	–	±	+	–	+	–	–	–
2	*Aristolochia bracteolata*	WP	+	–	+	–	–	±	–	–	–
3	*Solenostema argel*	L	+	–	±	±	–	+	±	–	–
4	*Pulicaria crispa*	WP	±	–	–	+	–	±	+	–	–
5	*Ambrosia maritima*	WP	+	–	+	–	±	–	–	–	–
6	*Citrullus colocynthis*	S	±	±	±	±	±	–	±	–	–
7	*Croton zambesicus*	Fr	±	–	–	–	±	+	–	–	–
8	*Tinospora bakis*	WP	–	–	+	–	–	+	–	–	–
9	*Nigella sativa*	S	+	±	±	+	–	+	+	–	±
10	*Gardenia lutea*	FrP	±	+	±	–	–	–	+	–	–

Abbreviations: L, leaf; WP, whole plant; Fr, fruit; FrP, fruit pulp; S, Seed; –, Negative test (not detected); ±, Slightly positive test (traces); +, Strongly positive test (high concentration).

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

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