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

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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 µg/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

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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. 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.

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.

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*. Moreover, an investigation of antiplasmodial activity of selected Sudanese plants revealed that most plants from the family. Meliaceae showed highly potent antimalarial activity against the two tested strains (3D7-chloroquine and pyrimethamine resistant and pyrimethamine sensitive *Plasmodium falciparum* strains). *Khaya senegalensis* (Mahogany), *Azadirachta indica* (Neem) and *Trichilia emetic* (Dabkar) showed 1C50 values less than 5 µg/ml.

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

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.

**Phytochemical methods**

Phytochemical screening for the secondary plant constituents present in the plant extracts was carried out using methods adopted in similar surveys. 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 (Fecl3 test); saponins (frothing test); cyanogenic glycosides (sodium picrate paper); anthraquinone (5 g plant material in 20 ml 20% H2SO4 and 2 ml 2% Fecl3, refluxed for 30 minutes, cooled, filtered and extracted with CHcl3 + 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. 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
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 µl 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:

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

### 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 | For fevers 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 for Scorpion stings and anti-inflammatory, leaves for malaria | WP | Khs |
| *Citrus vulgaris* (Cucurbitaceae) | El-Handal | For haemoroids, arthritis, eczema, laxative and for malaria | S | GS |
| *Croton zambesicus* (Euphorbiaceae) | Um-Geleigla | Anti-hypertensive and for malaria | Fr | CS |
| *Gardenia lutea* (Rubiaceae) | Um Gawy | Fruit is eaten by human | FrP | Khs |
| *Pulicaria crispa* (Asteraceae) | El-Rmeit | As a source of essential oil | WP | Khs |
| *Nigella sativa* (Ranunculaceae) | Kamun-Aswad Habat ElBaraka | Anti-inflammatory, allergies, eczema and for malaria | S | NS |
| *Solenostema argel* (Asclepiadaceae) | El-Hargel | Carminative, Antispasmodic and for malaria | L | NS |
| *Tinospora bakis* (Menispermaceae) | Erg-El-Hagar | For fevers, diarrhoea and dysentery | WP | CS |

**Abbreviations:** 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.
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 colocynthis*, *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 antimalarial 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.

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

| Plant species           | 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 | 3.35 |
| *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**         |         | 0.2 | 0.4 | 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 \( \leq 50 \, \mu g/ml \).\(^8\) Moreover, in all of the plant extracts more than 50% inhibition of the parasites at concentration \( \leq 50 \, \mu g/ml \) 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 *Aristolochia* 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, teratogenicity, 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 \( \mu g/ml \)), *Nigella sativa* and *Aristolochia bracteolata* 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 antimalarials 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.

| No. | Botanical name       | Plant part tested | Sterols | Triterpenes | Alkaloids | Flavonoids | Cardenolides | Tannins | Saponins | Cyanogenic glycosides | Anthraquinones |
|-----|----------------------|-------------------|---------|-------------|-----------|------------|--------------|---------|----------|----------------------|---------------|
| 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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