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Original Article

Assessment of antimalarial activity against *Plasmodium falciparum* and phytochemical screening of some Yemeni medicinal plants

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Developing countries, where malaria is one of the most prevalent diseases, still rely on traditional medicine as a source for the treatment of this disease. In the present study, six selected plants (*Acalypha fruticosa*, *Azadirachta indica*, *Cissus rotundifolia*, *Echium rauwalfii*, *Dendrosicyos socotrana* and *Boswellia elongata*) commonly used in Yemen by traditional healers for the treatment of malaria as well as other diseases, were collected from different localities of Yemen, dried and extracted with methanol and water successfully. The antiplasmodial activity of the extracts was evaluated against fresh clinical isolates of *Plasmodium falciparum*. The selectivity parameters to evaluate the efficacy of these medicinal plants were measured by *in vitro* micro test (Mark III) according to World Health Organization (WHO) 1996 & WHO 2001 protocols of antimalarial drug tests. Among the investigated 12 extracts, three were found to have significant antiplasmodial activity with IC_{50} values less than 4 μg/ml, namely the water extracts of *A. fruticosa*, *A. indica* and *D. socotrana*. Six extracts showed moderate activity with IC_{50} values ranging from 10 to 30 μg/ml and three appeared to be inactive with IC_{50} values more than 30 μg/ml. In addition, preliminary phytochemical screening of the methanolic and aqueous extracts indicated the presence of saponins, tannins, flavonoids, terpenoids, polysaccharides and peptides.

Keywords: Malaria – Medicinal plants – *Plasmodium falciparum* – Yemen

Introduction

Malaria is an infectious disease that continues to be associated with considerable morbidity and mortality and significant social and economic impact on developing societies. According to the World Health Organization (WHO), malaria is endemic in 91 countries, predominantly in Africa, Asia and Latin America, with about 40% of the world's population at risk and it is distributed widely, mainly due to the multi-drug resistance developed by *Plasmodium falciparum*. It remains the leading cause of death due to parasitic diseases with approximately 300 million clinical cases annually resulting in an estimated number of 23 00 000 deaths, primarily in children (1,2). Malaria is one of the most serious health problems in Yemen. Approximately 60% of the populations live in areas with malaria transmission and *P. falciparum* accounts for more than 90% of malaria cases (3).

Resistance to all known antimalarial drugs, with the exception of the artemisinin derivatives, has developed to various degrees in several countries (4). The urgency generated by drug-resistant strains of malaria has accelerated

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antimalarial drug research over the last two decades. While synthetic pharmaceutical agents continue to dominate research, attention increasingly has been directed to natural products (5). The success of artemisinin, isolated from *Artemisia annua*, and its derivatives for the treatment of resistant malaria has focused attention on the plants as a source of antimalarial drugs (6). The world's poorest are the worst affected, and many treat themselves with traditional herbal medicines. These are often more available and affordable, and sometimes are perceived as more effective than conventional antimalarial drugs (7).

Ethnobotanical information about antimalarial plants, used in traditional herbal medicine, is essential for further evaluation of the efficacy of plant antimalarial remedies and efforts are now being directed towards discovery and development of new chemically diverse antimalarial agents (8). Some of the Yemeni people living in rural areas depend on traditional herbal medicine for treatment of many infectious diseases such as malaria (9,10). The reputed efficacies of these plants have been experienced and passed on from one generation to the other. Apparently, lack of scientific proof of efficacies claimed by traditional medical practitioners in Yemen called for this study. The present research was carried out to evaluate the antiplasmodial activity (*in vitro*) of six Yemeni medicinal plants.

Methods

Plant Materials

The selected plants were collected in June 2005 from different localities of Yemen except the plants from the island Soqotra, which were collected in December 2004. The plants were identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

Extraction of Plant Materials

The air-dried and powdered plant materials (10 g of each) were extracted with 400 ml methanol (CH_3OH) by using a Soxhlet apparatus for 8 h. The residue was dried over night and then extracted with 250 ml water (H_2O) by using a shaking water bath at 70°C for 2 h. The extraction with water was repeated three times. The water filtrates were mixed together. The methanolic and water extracts were filtered and evaporated by using a rotary evaporator and freeze dryer to give the crude-dried extract. The dried extracts were stored at −20°C until used.

Phytochemical Screening of Plant Extracts

A preliminary phytochemical analysis of the plant extracts was carried out using thin-layer chromatography (TLC). Standard screening tests using conventional protocol (11) were utilized for detecting the major components.

Antimalaria Assay

Patients selection

The inclusion criteria were patients having mono infection with *P. falciparum*, parasitemia not less than 1000 and not more than 80 000 asexual parasites/1 μl of blood (12,13) and negative urine test for 4-aminoquinolines and sulfonamides following the method of Dill & Glazko's test and lignin test (13). Patients with severe malaria, pregnant women and patients who took antimalarial drugs during the previous 14 days were excluded. Three fresh blood specimens were collected from three patients suffering from fever and other malaria symptoms with confirmed infection by *P. falciparum*.

In vitro test

The assay was performed in duplicate in a 96-well microtiter plate, according to WHO method [*in vitro* micro test (Mark III)] that is based on assessing the inhibition of schizont maturation (13). RPMI 1640 (Sigma Company, USA) was the culture medium used for cultivation of *P. falciparum* (14). Dilution was prepared from the crude plant extract and the final concentrations prepared by dilution were (125, 62.5, 31.25, 15.6, 7.8, 3.9 and 1.95 μg/ml). Negative controls treated by solvent and positive controls (Chloroquine phosphate) were added to each set of experiments. Fifty microliters from blood mixture media was added to each well in plate and incubated in CO_2 condition at 37.5°C for 24–30 h. After incubation, contents of the wells were harvested and stained for 30 min in a 2% Giemsa solution pH 7.2, after that the developed schizonts were counted against the total asexual parasite count of 200. The count process was done in duplicate, and the data were analyzed by using a computerized mathematical log-concentration-response-probit analyzing model for result interpretation (15).

Results and Discussion

An experimental study was performed from November 2005 to June 2006. The main aim was to verify scientifically the local traditional uses of six Yemeni medicinal plants as antimalarial drugs, and to evaluate their therapeutic efficacy as antimalarial drugs for *P. falciparum*, which may be regarded as future promising phytotherapeutics in the treatment of malaria. Table 1 shows the botanical names, plant parts used, site of collection and the traditional uses of the six investigated plants.

Table 1. List of the selected medicinal plants used in the investigation

Botanical name	Voucher specimen no.	Family	Part used	Site of collection	Traditional uses
Acalypha fruticosa L.	YH-05	Euphorbiaceae	Leaves	Hajjah	Antiinflammatory, antimalarial, antibacterial.
Azadirachta indica A. Juss (*Melia azadirachta*)	MO-H08	Meliaceae	Leaves	Hodeida	Antimalarial, fever, digestive disturbances, skin problems, general fatigue, intestinal parasites, diabetes, fungal infections, inflammatory diseases.
Boswellia elongata Balf. f.	SP-M102	Burseraceae	Bark	Soqotra	Antiinflammatory, anti cough, arthritis and joint pain, Gum is expectorant, diuretic, for diarrhea and dysentery, coetaneous troubles (16,17).
Cissus rotundifolia (Forssk.) Vahl	MO-T12	Vitaceae	Leaves	Taiz	Loss of appetite, antimalarial, gastrointestinal troubles, skin diseases, burns; young shoots are edible, acid in taste.
Dendrosicyos socotrana Balf. f.	SP-A015	Cucurbitaceae	Leaves	Soqotra	Urinary retention, cystitis, symptoms of diabetes, problems with the liver and burns, constipation (16,17).
Echium vulgare L.	MO-I20	Boraginaceae	Leaves	Ibb	Fever, headaches, nervous complaints, inflammatory pains, kidney stones.

Table 2. In*Vitro* antimalarial activity of the investigated extracts and phytochemical screening

Plant name	Extracts	Yield of extract (%)	IC_{50} (μg/ml)	100% SMI (μg/ml)	Active constituents
Acalypha fruticosa	MeOH	13.4	10.7	125	Tannins, terpenoids, flavonoids
	H_2O	8.0	1.6	7.8	Proteins, polysaccharides
Azadirachta indica	MeOH	10.9	16.9	–	Flavonoids, terpenoids
	H_2O	4.9	2.0	7.8	Polysaccharides, proteins, tannins
Boswellia elongata	MeOH	8.9	26.7	–	Terpenoids
	H_2O	5.7	27.1	–	Tannins, polysaccharides
Cissus rotundifolia	MeOH	10.7	58.0	–	Steroids, flavonoids
	H_2O	6.9	34.7	–	Proteins
Dendrosicyos socotrana	MeOH	4.2	20.0	–	Terpenoids
	H_2O	10.9	2.3	7.8	Proteins, polysaccharides
Echium rauwalfii	MeOH	8.2	48.3	–	Terpenoids, flavonoids, alkaloids
	H_2O	8.7	18.8	–	Polysaccharides
Chloroquine			0.2	12.5	

There is no complete schizont maturation inhibition until the highest concentration that was used in this investigation (125 μg/ml)for cells with dash. MeOH, methanol extract; H_2O, aqueous extract; IC_{50}, inhibition concentration 50; SMI, Schizonts maturation inhibition.

Antimalaria Activity

The results of *in vitro* antimalarial activity of the extracts are shown in Table 2. The most interesting antiplasmodial activity was obtained with the aqueous extracts of *Acalypha fruticosa* ($IC_{50} = 1.6\,\mu$g/ml), of *Azadirachta indica* ($IC_{50} = 2.0\,\mu$g/ml) and of *Dendrosicyos socotrana* ($IC_{50} = 2.3\,\mu$g/ml). The microscopic examination of Giemsa-stained slides for these three aqueous extracts showed a virtual absence of developed schizonts in ring-stage synchronized cultures treated with crude extracts at concentrations of (7.8 μg/ml) during 30 h of incubation. These observations suggest that the active constituents in the extract may be cytotoxic for *P. falciparum* trophozoites, thereby inhibiting their development to the schizont stage.

The antimalarial activity of *A. indica* was previously reported by (18). They found that the aqueous extract of *A. indica* leaves showed IC_{50} values less than 5 μg/ml which is in agreement with our results ($IC_{50} = 2.0\,\mu$g/ml); in our investigations, the methanolic extract showed moderate activity with $IC_{50} = 16.9\,\mu$g/ml. Our results showed complete inhibition of the schizonts maturation exhibited at 7.8 μg/ml of *A. indica* aqueous extract. These findings are in agreement with the results by (19) in which extracts of *A. indica* have been suggested to contain active constituents which might target specific

metabolically active processes at the parasitic schizont stage. In a comparative study of acetone/water and aqueous extracts of *A. indica* leaves, they manifested inhibitory effect on a chloroquine sensitive *P. falciparum* at a concentration of 20 μg/ml (20). Earlier findings showed that Azadirachtin of *A. indica* was able to block the development of motile malaria gametes *in vitro* and raised the possibility of developing Azadirachtin-based compounds as antimalarial agents with transmission blocking potential (21).

This study represents the first conducted for antimalarial activity of crude extracts of medicinal plants in Yemen (aqueous extracts from *A. fruticosa* and *D. socotrana*). The results confirm that those plants which are used in traditional medicine against malaria possess *in vitro* a significant antimalaria potential and justify their use in traditional medicine. However, *in vivo* studies on these medicinal plants are necessary and should seek to determine toxicity of the active constituents, their side effects, serum-attainable levels, pharmacokinetic properties and diffusion in different body sites. Additional pharmacokinetic investigations are therefore advisable to identify host-related factors, such as poor absorption, accelerated gastrointestinal passage of the test drug, or metabolic peculiarities of some patients, which might lead to a faster-than-normal inactivation or elimination of the test drug (22). Other investigated plants which are mainly used for other purposes than malaria showed a moderate activity against malaria or were inactive.

Phytochemical Screening

The phytochemical screening of medicinal plants showed the presence of tannins, polysaccharides and proteins in the aqueous extracts (Table 2), which may be responsible for the antiplasmodial activity, whereas the methanol extracts of these medicinal plants showed the presence of saponins, tannins, flavonoids and terpenoids. The presence of triterpenoids, limonoids, in *A. indica* may take part in the antimalarial activity of this traditional medicinal herb. It is known that the limonoid gedunin, isolated from *A. indica*, exerts antimalarial effect *in vitro* (23).

If plants are to be used as sources of novel antimalarial compounds, we need to increase our knowledge about their empirical use to improve plant selection. In the hope of preserving useful resources, we should now gather and record ethnobotanical data, and should try to bridge the gaps between empirics and realism (24). Further studies to be undertaken in relation with these results are also highlighted.

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