# *IN VITRO* ANTIPLASMODIAL PROPERTIES OF *FLACOURTIA FLAVESCENS* WILLD. (FLACOURTIACEAE) AND *RYTIGYNIA CANTHIOIDES* (BENTH.) ROBYNS (RUBIACEAE)

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## Abstract

The present study was conducted to investigate the *in vitro* antimalarial activity of *Flacourtia flavescens* Willd. (Flacourtiaceae) and *Rytigynia canthioides* (Benth.) Robyns (Rubiaceae). These two plants are used in Benin folk medicine to treat malaria and fever. Antimalarial activity was assayed on fresh clinical isolates of chloroquine resistant *Plasmodium falciparum* using the *in vitro* semi-microtest. The results revealed that the $IC_{50}$ varied from 1.55 to 22.36µg/ml. *F. flavescens* hydro methanol extract was more active than *R. canthioides*. The study demonstrated scientific rationale behind the traditional usage of these plants, however further bioactivity guided phytochemical analyses are necessary to identify the active principles.

**Keywords:** *Flacourtia flavescens*, *Rytigynia canthioides*, *Plasmodium falciparum*, antiplasmodial.

## Introduction

Malaria remains a serious public health problem in sub-Saharan developing countries. According to the World Health Organization, more than 300 millions people are annually infected by *Plasmodium falciparum* the main pathogenic protozoa responsible for malaria (WHO, 2009). In majority of these malaria's regions, plants continue to be the major source of management of the disease. It has been estimated that up to 80% of the people rely on traditional medicine (TM) for their primarily health care (WHO, 2009; Hostettman and Marston 2002). Due to this strong dependence on plants, a large number of studies have been conducted on traditional usage of plants and some often showed scientific rationale or resulted in the isolation of bioactive compounds for direct use in medicine (Castellanos et al., 2009; Karou et al., 2007a,b). In malaria chemotherapy in particular, African medicinal plants were found to be very active against the parasites *in vitro* (Karou et al., 2003, Akomo et al., 2009). Some compounds such as cryptolepine from *Cryptolepis* species (Cimanga et al., 1996) and *Sida acuta* (Banzouzi et al., 2004) or, isostrychnopentamine and dihydrousambarensine from *Strychnos* species showed good activities on chloroquine sensitive and chloroquine resistant strains ((Federich et al., 1999). Even compounds with chloroquine potentiating or chloroquine resistance reversion properties such as malagashanine or strychnobrazilline were isolated from African medicinal plants (Rosanaivo et al., 1994).

Benin people have old tradition in plants usage for the treatment of several diseases, including malaria. *Flacourtia flavescens* Willd. (Flacourtiaceae) and *Rytigynia canthioides* (Benth.) Robyns (Rubiaceae) are two of such plants used in Benin traditional medicine. They have wide ethnomedicinal claim for the cure of malaria, fevers, microbial infections and anemia. Some people use them as food supplements (Djikpo-Tchibozo, 2007). However, no scientific data exist about the pharmacological properties of theses plants. The present study reports the in *vitro* antiplasmodial activities of the two plants.

## Materials and Methods

### Chemicals

RPMI 1640, bovine foetal serum, HEPES and chloroquine phosphate were obtained from Sigma Chemical Company (St. Louis). L-Glutamine and streptomycin/penicillin were obtained from Gibco BRL (Paisley, Scotland). All the solvents were of analytical grade.

### Plant material

*Flacourtia flavescens* is a plant occurring in two sub species: one sub specie bears fruits and is referred to as female while the second never carry fruits and is designated as male. The leaves and roots of *F. flavescens* (male and female); and the leaves of *R. canthioides* were collected in April 2002 in Benin (Pahou/Ouidah). The samples were authenticated at the Department of Plant Biology, University of Abomey - Calavi in Benin, where voucher specimens (numbers: Fvml 01, Fvfl 02 and Rcl 01, respectively) were deposited. The plants were air- dried ground to powders and extracted.

**Extractions**

Aqueous extraction was performed by boiling under reflux 20 g powder of each of the plant samples separately in 200 mL distilled water for 30 min. After cooling at room temperature the extracts were filtered and lyophilized. Each of the plant samples were also separately extracted using methanol and methanol-water as solvents. Percolation procedure was used by soaking 20 g powder in 200 mL of methanol or 50% methanol separately for 24 hrs. Afterwards the extracts were filtered and methanol was evaporated under reduced pressure. The extracts were then lyophilized to eliminate the residual water.

**Parasites**

Fresh isolates of *Plasmodium falciparum* were obtained from healthy children aged between four and seven years living in Pahou (a malaria endemic area) located 29 km from Cotonou (Benin). Giemsa-stained thin smears were examined for *Plasmodium* species identification. The parasite density was determined by counting the number of infected erythrocytes among 20,000 erythrocytes. From each patient, 4ml of venous blood was collected in a tube coated with EDTA (Greiner Labortechnik). Samples with monoinfection due to *Plasmodium falciparum* and a parasite density between 1 and 2% were used for the *in vitro* antimalarial tests.

***In vitro* antimalarial tests**

*Plasmodium falciparum* was grown in 96-well plates as described by Trager and Jensen (1976). Blood cells were washed three times with RPMI 1640 before use in culture. Erythrocytes were then suspended in RPMI supplemented with l-glutamine (4.2 mM), HEPES (25 mM), bovine foetal serum (10% (v/v)), streptomycin (100 g/ml) and penicillin (100 IU/ml). The haematocrit was 5%. The Giemsa stained smears of the *P. falciparum* parasites on slides were counted using the light microscopy as described by Le Bras and Deloron (1983). Lyophilized methanolic extract were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium to a final concentration of 0.5% (v/v) DMSO in the first wells. Chloroquine phosphate and aqueous extract were dissolved in distilled water. The aliquots of drug solutions were added. A control experiment was performed separately using 0.5% DMSO to check the effect of these solvents on parasite maturation. Drug concentrations in the wells ranged from 1000 to 0.24 µg/mL for the extracts; and from 100 to 0.02 µg/mL for chloroquine phosphate. The final volume in the wells was 200 µL. The plates were incubated at 37 °C in a $CO_2$ incubator under 5% $CO_2$ and humid atmosphere for a total period of 36–40 hrs.

**Evaluation of the activity**

Parasite maturation was determined by counting matured schizonts among all asexual parasites for 20,000 erythrocytes. The percentages of parasite maturation were plotted against the logarithm of drug concentrations. The concentrations causing 50% inhibition of the maturation ($IC_{50}$ values) were determined with regression equations. $IC_{50}$ values were compared with epi info version 6 software by calculating the qui square, the statistical significance set at $p<0.05$.

## Results and discussion

The study was aimed at investigating the *in vitro* antiplasmodial activity of two plants of Benin folk medicine: *R. canthioides* and two subspecies of *F. flavesecens.* Three extracts were obtained from each vegetable sample. These were the aqueous extract, the methanol extract and the methanol-water extract. Antiplasmodial assay was performed with these extracts separately. Chloroquine phosphate was used as reference antimalarial agent. The microscopy examinations showed that the presence of DMSO at a final concentration of 0.5% in the wells neither decrease parasite maturation nor alter their morphology as indicated in the control experiments. Table 1 displayed the $IC_{50}$ values recorded in these tests which ranged from 22.36 to 1.55 µg/mL for the extracts. $IC_{50}$ of 0.12 µg/ml was obtained with chloroquine. This value indicated that the tested strain was chloroquine resistant *Plasmodium falciparum*. Low $IC_{50}$ values were recorded with methanol-water extracts, showing that methanol-water was the appropriable solvent for the extraction of the antimalarial agent of the selected plants.

**Table 1.** In vitro antimalarial activity of plant extract

| Extract | Aqueous | Methanol-water | Methanol | CQP |
|---|---|---|---|---|
| *F. flavescens* (male) roots | 7.79±1.75 | 1.95±0.07 | 11.89±6.06 | 0.12±0.02 |
| *F. falavescens* (male) leaves | 13.41±0.90 | 2.54±0.46 | 22.36±3.50 | |
| *F. falvescens* (female) leaves | 4.25±0.01 | 1.55±0.05 | 10.40±1.06 | |
| *F. flavescens* (female) roots | 7.42±1.06 | 4.10±1.01 | 17.47±1.06 | |
| *R. canthioides* (leaves) | 2.13±0.01 | 7.92±3.50 | 4.47±0.02 | |

The analysis of the different of $IC_{50}$ values showed that methanolic extract of leaves of *F. flavescens* "female" was the most active ($IC_{50}$ = 1.55 µg/mL) followed by the leaves of *F. flavescens* "male" ($IC_{50}$ = 1.95 µg/mL). Referring to $IC_{50}$ values recorded in other screenings, these plants can be considered as having good antiplamodial activity (Sanon et al.,

2003a, b; Karou et al., 2003). According to our results, the two subspecies of *F. flavescens* are more active than *R. canthioides.* However, there is no significant difference between the $IC_{50}$ of the most active extract of the subspecies (p = 0.153). This finding suggests these two subspecies can be used without any distinction in the treatment of malaria. The present study is the first report on the antiplasmodial activity of *F. flavescens*. The active compounds responsible for the antiplasmodial activity are yet to be determined

This study demonstrates the scientific rationale behind plant usage in Benin traditional medicines, however further phytochemical studies are needed to isolate and identify the active principles.

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