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Evaluation in vitro of antiplasmodial activity of ethanolic extracts of *Funtumia elastica*, *Rauvolfia vomitoria* and *Zanthoxylum gilletii* on *Plasmodium falciparum* isolates from Côte-d'Ivoire

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Antiplamodial activity, *Plasmodium falciparum*; In Vitro Test, Côte-d'Ivoire

1 SUMMARY

The ethanolic extracts of *Zanthoxylum gilletii* (GZA), *Funtumia elastica* (EFU) and *Rauvolfia vomitoria* (VRA) were tested for effect on the in vitro growth of *Plasmodium falciparum* isolates (IS1, IS2 and IS3) according to the isotopic variant of microtest. The isolates were obtained from patients who came for consultation at the National Institute of Public health, in Abidjan (Côte-d'Ivoire). The isolates were maintained in culture using the method of Trager and Jensen in the presence of variable concentrations of chloroquine and extracts of plants (GZA, EFU and VRA) from the Ivorian traditional medicines. The values of IC_{50} (concentration inhibiting 50 % of the growth of parasites) showed that extracts of these plants have very strong antiplasmodial action.

2 INTRODUCTION

In several countries of the West African sub-region and particularly in Côte-d'Ivoire, there is high occurrence of resistance of *Plasmodium* to the mostly used antipaludics (Chloroquine, Mefloquine) (Djaman, 1997; Ankrah *et al.*, 2003). As part of the search for new antipaludics, a collaboration was initiated between the "Unity of Formation and Research" (U.F.R.) of Biosciences, University of Cocody-Abidjan (Côte-d'Ivoire) and the National Museum for Natural History of Paris, France. Thirty three (33) medicinal plants used in the treatment of paludism in Côte- d'Ivoire were evaluated by using the FcB1 Colombian strain of *Plasmodium falciparum*, which is resistant to chloroquine (Frappier *et al.*, 1996). The results of this first study showed that plants, such as *Funtumia elastica* (EFU), *Rauvolfia vomitoria* (VRA) and *Zanthoxylum gilletii* (GZA), have strong antiplasmodial action (IC_{50} < 5 µg/ml), and also have very low cytotoxicity (Zirihi *et al.*, 2005a).

In order to evaluate the antiplasmodial activity of their products on isolates responsible for paludism in Côte-d'Ivoire, the blood parasitized by *Plasmodium falciparum* was cultured

with variable concentrations of EFU, VRA and GZA extracts. This study presents the results of the study on the antiplasmodial activity of these 3 plants on 3 isolates (IS1, IS2 and IS3) of *Plasmodium falciparum* obtained from patients suffering from uncomplicated paludism who came for consultation at the National Institute of Public Health (INSP), in Abidjan (Côte-d'Ivoire).

3 MATERIAL AND METHODS

3.1 Ethnomedicinal survey: The investigation on the traditional use of plants having antiplasmodial effect was conducted among 10 native villages in the Department of Issia (Mid-West of Côte- d'Ivoire). Traditional healers were contacted and interviewed. Each of them was interviewed twice at different times, but answering the same questions. This helped to compare and check the information for reliability and accuracy. During this ethnopharmacological investigation, information was collected relating to the plants used to treat paludism, the different parts used as drugs, their methods of collection and the modes of preparation and administration. From the collected samples and specimens of the herbarium of the National Floristic Center (C.N.F.), plants were identified by their scientific name, botanical characteristics determined and their sketches made.

3.2 Sampling materials for chemical and biological analyses: Stem barks of *Funtumia elastica*, *Rauvolfia vomitoria* and *Zanthoxylum gilletii* were collected and dried 2 weeks under shade, in a well aired atmosphere in order to avoid contamination by moulds. The dried barks were crushed separately into fine powder. The RPMI (Roswell Park Memorial Institute) 1640 containing the HEPES [N-(2-hydroxyethyl) acid piperazine-N'-(2-ethanesulfonic)] 25 mM final, sodium bicarbonate ($NaHCO_3$) (Merck, Mannheim, Germany) 25 mM final was used to prepare the initial culture medium (RPMI wash). Preparation of the complete medium was done by adding 10 % human serum to RPMI of washing. Parasitized red blood cells (GRP, "Globules Rouges Parasités") called isolates (IS) and healthy red blood cells (GRS, "Globules Rouges Saines") were also used as biological material.

Different concentrations (ranging from 25 to 100 nM) of Chloroquine (Aventis, Antony, France) were used as reference antipaludics. To carry out the phytochemical screening, we used solvents (ether of oil, methanol and distilled water) and various classic reagents (Bornsträgen, Buchard, Cyanidine, Dragendorff, Ferric chloride, Foam Test, Liebermann and Stiasny). Classical methods described in the works of Ronchetti and Russo (1971), Hegnauer (1973), Wagner (1983), Békro *et al.* (2007) and N'Guessan (2008) were used to characterize the chemical constituents.

3.3 Extraction of active principles: One hundred grams of the powdered stem barks of each plant were extracted at room temperature (Zirihi *et al.*, 2005a) with ethanol (96%) using a centrifugal machine (Blinder). After 3 cycles of extraction, the solution was filtered and then the extraction solvent was removed using a rotary evaporator. The paste that was obtained was freeze-dried. This way EFU, VRA and GZA were obtained from *Funtumia elastica*, *Rauvolfia vomitoria* and *Zanthoxylum gilletii*, respectively. These products were later used for conducting the evaluation tests on the inhibitory activity of maturation of the isolates. The herbal medicines (EFU, VRA and GZA) were diluted in DMSO (Dimethylsulfoxide) and a range of concentrations 0.9 - 125 µg/ml were prepared and used to carry out the in vitro tests.

3.4 Sampling isolates of *Plasmodium falciparum:* Five (5) ml of venous blood was drawn from the arm of each patient (carrier of *Plasmodium falciparum*) using a hypodermic syringe and needle. The blood was put in sterile bottles with an anti-coagulant (Ethylene Diamine Tetracetate). Samples containing 4000 parasitized red blood cell per microliter or more were accepted and sent to the microbiology laboratory of the National Institute of Public Health (INSP).

3.5 In vitro test of *Plasmodium falciparum* chemosensitivity: The parasitized blood was centrifuged for 5 min at 2 000 revolutions per minute to remove the leuco-platelet layer. The corpuscular base was poured into a centrifuge tube to which was added RPMI of washing (the centrifugation process was done three times) (Zirihi, 2006). A thin blood smear was performed (Djaman, 2003) to verify the parasite density that should have been between 0.1 and 0.2 % (4.000 and 8.000 GRP/µl). Where the parasite rate exceeded 0.2 %, it was reduced to the proportions indicated above by dilution with healthy red blood cells (GRS). The

isolates of *Plasmodium falciparum* were grown by using the method of Trager and Jensen (1976) in the complete medium, using the isotopic variant of microtest (WHO, 1987). After 42 hours incubation in a carbon dioxide (CO_2) incubator under appropriate atmosphere (Zirihi *et al.*, 2005b), the nucleoproteins and plasmodial erythrocyte membranes were collected on a filter paper using a cell collector (Skatron Cell Harvester Titertex, Lier, Norway), then the quantity of tritiated hypoxanthine incorporated by the parasites was recorded per minute (cpm) using a liquid scintillation meter.

3.6. Data handling: The results obtained were used to plot linear regression lines, and then to first determine the phenotype for each studied isolate, compared with chloroquine according to the IC_{50} in nanomolar (nM). The phenotype of the isolate was noted as R for resistant, S for sensitive depending on whether the IC_{50} is above or below 100 nM. The dose-effect curves of maturation or inhibition rate according to the concentrations of antiplasmodial substances extracts were used to determine the sensitivity of the isolate to EFU, VRA and GZA.

4 RESULTS

4.1 Botanical characteristics of the studied plants

Funtumia elastica is a medium sized tree of 15-30 m height. The coriaceous leaves (fig. 1) are simple, opposite and entire. The flowers form inflorescences in axillary cymes. The fruits, dry dehiscent, consist of two spreading follicles with many seeds. *Rauvolfia vomitoria* is a small tree of 8-10 m height. The leaves in whorls of 3-5, mostly 4, are simple and entire. The flowers are gathered in ombelliforme cymes. The red fruits (fig. 2) are globose drupes. *Zanthoxyllum gilletii* is a thorny, large tree of 35 m height. The pinnate leaves (fig. 3) comprise entire leaflets, asymmetric at base. The small flowers are clustered into panicles. The fruits are spherical capsules. These species of plants belong to 01 sub-phylum (Angiosperms) and 01 phylum (Spermaphytes). Tables 1 and 2 give the botanical characteristics of the studied plants.

Table 1: Botanical grouping of the plants studied for antiplasmodial activity.

Plant	Family	Order	S/C	Classes
Funtumia elastica	Apocynaceae	Gentianales	Asteridae	Dicots
Rauvolfia vomitoria	Apocynaceae	Gentianales	Asteridae	Dicots
Zanthoxylum gilletii	Rutaceae	Sapindales	Rosidae	Dicots

Table 2: Morpho-Biological types and chorological affinities of plants studied for antiplasmodial activity.

Plant	Morphological types	Biological types	Chorological affinities	Status
Funtumia elastica	Tree	Mesophanerophyte	GC	Wild
Rauvolfia vomitoria	Tree	Microphanerophyte	GC-SZ	Wild
Zanthoxylum gilletii	Tree	Mesophanerophyte	GC	Wild

Key: GC: Guineo-Congolais; SZ: Soudano-Zambesienne

4.2 Ethnopharmacological characteristics

During this ethnopharmacological study 30 traditional healers were identified in 10 villages who agreed to collaborate by providing information on antipaludic plants. They comprised of 18 men and 12 women. The oldest person among the healers was a man of about 75 years and the youngest was 25 years old. The ethnopharmacological characteristics of the plants (different parts used as drugs, methods of preparation and administration) are shown in table 3.

4.3 Sensitivity of isolates to chloroquine

The IC_{50} of chloroquine towards isolates is about 25 nM (<100 nM reference value for assessing the chemosensitivity of the strains). The isolates of *Plasmodium falciparum* used in this study were all sensitive to chloroquine (figure 4).

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Figure 1 (left): *Funtumia elastica* (P. Preuss) Stapf (Apocynaceae). A part of stem with coriaceous, simple, opposite and entire leaves. **Figure 2:** *Rauvolfia vomitoria* Afzel. (Apocynaceae): A part of stem with leaves in whorls of 3-5, simple, entire and red globose fruits

Figure 3: *Zanthoxylum gilletii* (De Wild.) Wattermann (Rutaceae) with stem showing prickles and pinnate leaves with entire leaflets.

Table 3: Traditional uses of the three plants studied for antiplasmodial effect.

Plant	Part used	Modes of preparation	Formulation	Modes of administration
Funtumia elastica	Stem bark	Maceration	Macerated	Drink
Rauvolfia vomitoria	Stem bark	Maceration	Macerated	Drink
Zanthoxylum gilletii	Stem bark	Infusion	Infused	Drink

4.4 Sensitivity of plasmodium isolates to plants (herbal) extracts

The products: EFU, VRA and GZA, obtained respectively from *Funtumia elastica*, *Rauvolfia vomitoria* and *Zanthoxylum gilletii*, inhibited the maturation of the isolates in our study to different degrees. The extract EFU gave IC_{50} values of 0.7, 2.1 and 3.9 µg/ml, respectively, for isolates IS1, IS2 and IS3 (figure 5). For the extract VRA, the IC_{50} values were 1, 2.1 and 4.5, respectively, for isolates IS1, IS2 and IS3 (figure 6). For GZA, the IC_{50} values for isolates IS1, IS2 and IS3 were 0.9, 1.5 and 3.4 µg/ml, respectively (figure 7).

4.5 Phytochemical characteristics

A primary validation of the traditional medical practices was performed by studying the chemical constituents that explain the antiplasmodial effect of the three plants. *Funtumia elastica* was chemically screened and yielded alkaloids, sterols and triterpens. *Rauvolfia vomitoria* contains alkaloids, saponosides, sterols and triterpens while *Zanthoxylum gilletii* has alkaloids, flavonoids, saponosides, sterols, tannins and triterpens. Among these compounds, the alkaloids most likely contribute the antipaludic activity of the plants.

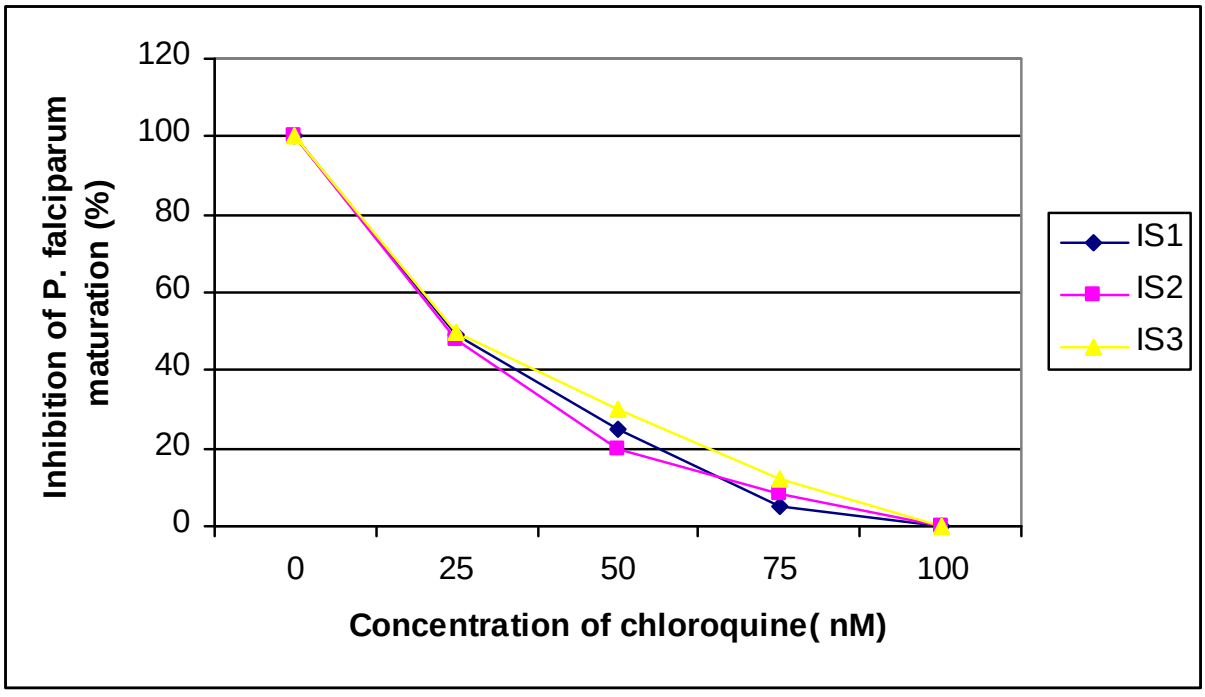

Figure 4: Inhibition curve of the maturation of *P. falciparum* (IS1, IS2, IS3) by chloroquine.

5 DISCUSSION

The results of this study show that the isolates used are all chloroquine-sensitive. Thus, despite the inexorable rise of chloroquine resistance in Côte-d'Ivoire, there are still isolates that are sensitive to this molecule. This sensitivity varies from one area to another in the country. It should be noted that although the first choice medicine currently is

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amodiaquine, chloroquine is still present in some parts of Côte-d'Ivoire.

The antiplasmodial activity of EFU, VRA and GZA obtained respectively from *Funtumia elastica*, *Rauvolfia vomitoria* and *Zanthoxylum gilleti* showed that they inhibit to different degrees the maturation of the isolates. For GZA, the isolate IS3 with an IC_{50} of 3.4 µg/ml was least sensitive compared to IS2 and IS1. With EFU isolate IS3 was again the least sensitive, isolate IS1 was most sensitive while IS2 has intermediate sensitivity.

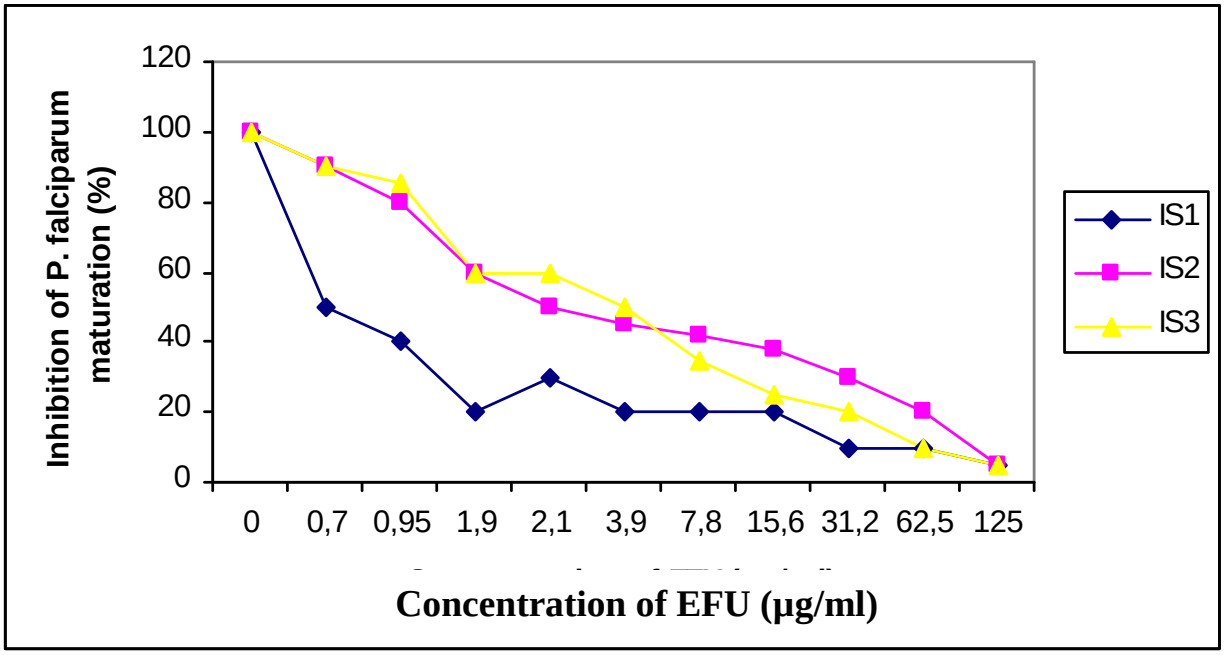

Figure 5: Inhibition curve of the maturation of *Plasmodium falciparum* isolates IS1, IS2 and IS3 by extracts from *Funtumia elastica* (EFU).

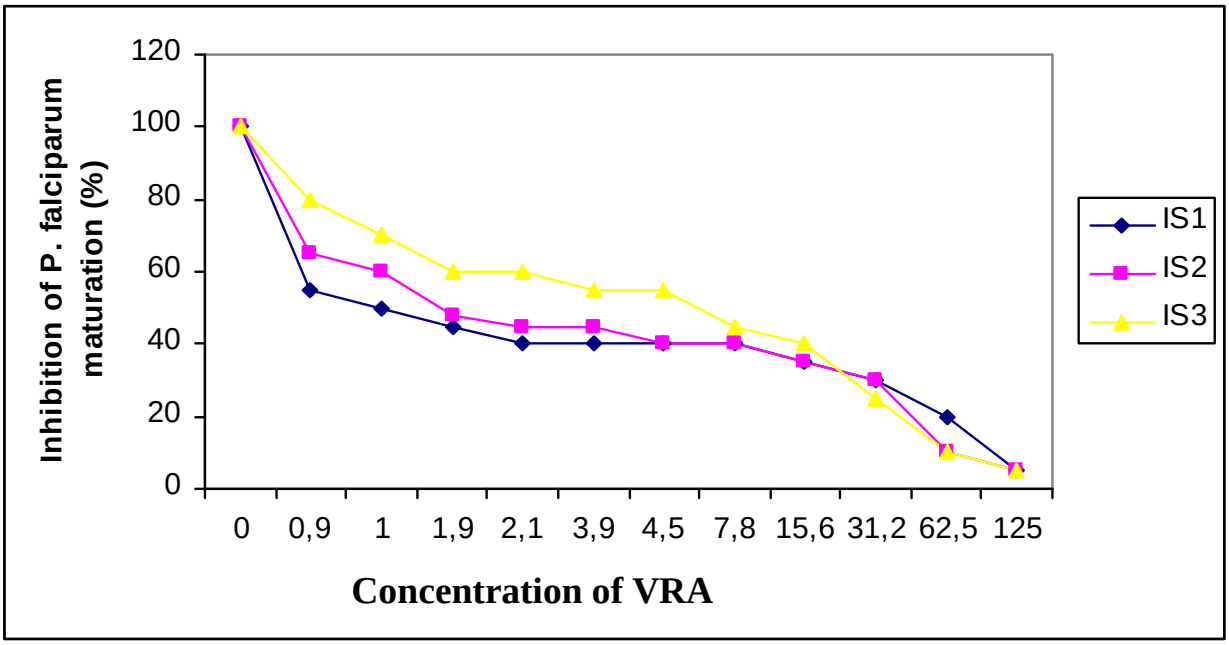

Figure 6: Inhibition curve of the maturation of *Plasmodium falciparum* isolates IS1, IS2 and IS3 by extracts from *Rauvolfia vomitoria* (VRA).

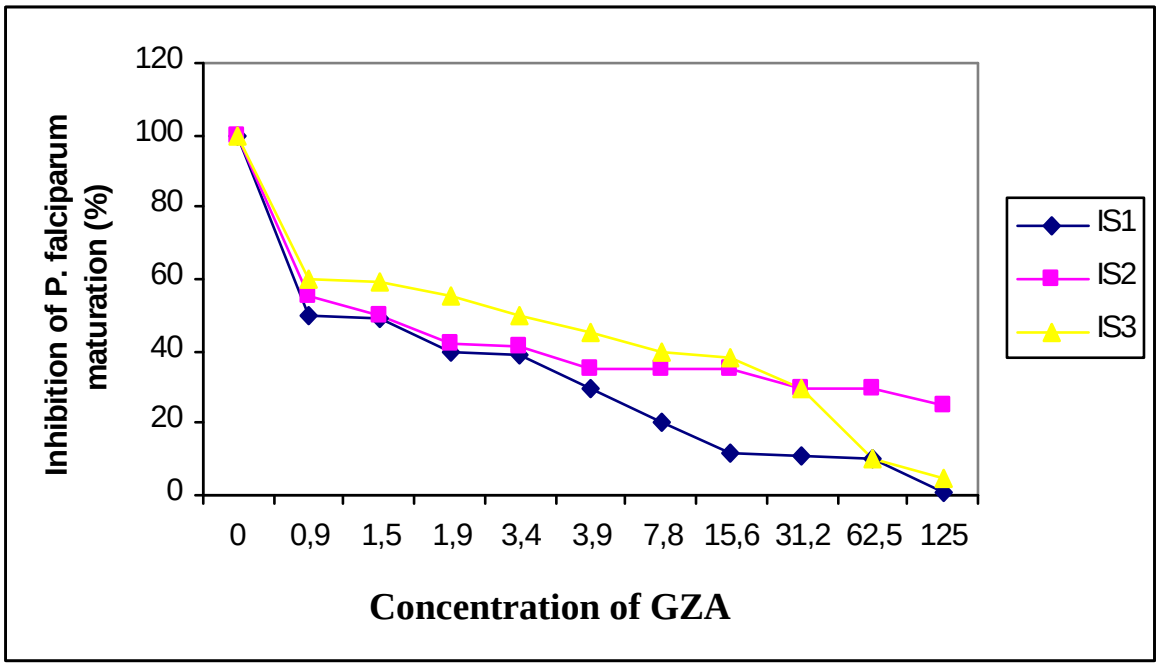

Figure 7: Inhibition curve of the maturation of *Plasmodium falciparum* isolates IS1, IS2 and IS3 by extracts from *Zanthoxylum gilleti* (GZA).

These results indicate that the ethanolic crude extract of *F. elastica*, *R. vomitoria* and *Z. gilletii* have an obvious antiplasmodial activity on the isolates of *Plasmodium falciparum*. Although further investigation is still needed, the results justify the use of these plants in the traditional treatment of paludism. What's more, *Zanthoxylum gilleti* and *Funtumia elastica* extracts showed good antiplasmodial activity on a multiresistant strain (FcB1 strain) responsible for paludism in Colombia (Zirihi *et al.*, 2005). The products have a broad spectrum activity and can be an alternative to the conventional medicines (Phillipson *et al.*, 1991). Combining the herbal extracts with common antipaludics could be another alternative in the fight against the resistance of *P. falciparum* to commonly used antipaludic medicines (Desjardins *et al.*, 1979; Djaman, 2003).

Looking at the results, bioguided fractionations of these extracts are needed to isolate the molecules responsible for the antiplasmodial action of these plants. Chemical, pharmacological and galenic studies should be considered so as to avail drugs (made from these plants) that are affordable and meet the criteria of safety, security and efficiency.

The extract of the stem barks of *F. elastica* made it possible to isolate 4 steroidic alkaloids: holarrhetine, connessine, holarrhesine and isoconessimine. The holarrhesine showed an antiplasmodial activity, similar to that of chloroquine (Zirihi, 2006). An indole alkaloidal (ajmaline) was found in the stem bark of *R. vomitoria* (Neuwinger, 1996). The ajmaline was described as chemical constituent similar to quinidine, a molecule recognized to be powerful against *Plasmodium* (Kamanzi, 2002). According to Zirihi (2006), *Z. gilletii* was chemically screened and yielded different types of alkaloids: tembetarine, oblongine, magnoflorine, nitidine. Nitidine, the main alkaloid, gives the plant its antipaludic properties, by blocking the synthesis of the DNA of *P. falciparum*.

6 CONCLUSION

F. elastica, *R. vomitoria* and *Z. gilletii* are wild plants used in traditional medicine to treat paludism. On the pharmacological level, the ethanolic extract obtained from stem barks of the plants, constituting the herbal medicines (EFU, VRA and GZA), were observed to exert antipaludic activity, thus justifying

their traditional uses. The antiplasmodial effect could result from chemical elements such as alkaloids (holarrhetine, connessine, holarrhesine, isoconessimine, ajmaline, tembetarine, oblongine, magnoflorine or nitidine). The pharmacological and phytochemical information confirm the basis for their traditional use as antipaludics.

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