ANTI-*TOXOPLASMA* ACTIVITY OF VEGETAL EXTRACTS USED IN WEST AFRICAN TRADITIONAL MEDICINE

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Summary :

Both *Toxoplasma gondii* and *Plasmodium* are Apicomplexan protozoa that share common metabolic pathways and potential drug targets. The objective of this study was to examine the anti-*Toxoplasma* activity of nine West African plants with known activity against *P. falciparum*. The extracts were obtained from parts of plant commonly used, by most traditional healers, in the form of infusion or as water decoction. The in vitro activity of plant extracts on *T. gondii* was assessed on MRC5 tissue cultures and was quantified by enzyme-linked immunoassay. Aqueous extracts from *Vernonia colorata* were found to be inhibitory for *Toxoplasma* growth at concentrations > 10 mg/L, with an IC_{50} of 16.3 mg/L. A ten-fold gain in activity was obtained when organic solvents such as dichloromethane, acetone or ethanol were used to extract *V. colorata*'s active principles. These extracts were inhibitory at concentrations as low as 1 mg/L, with IC_{50} of 1.7, 2.6 and 2.9 mg/L for dichloromethane, acetone and ethanol extracts respectively. These results indicate a promising source of new anti-*Toxoplasma* drugs from *V. colorata* and African medicinal plants.

KEY WORDS : *Toxoplasma gondii*, medicinal plant, ethnopharmacology, Ivory Coast, *in vitro*.

Résumé : ACTIVITÉ ANTI-TOXOPLASMOSE D'EXTRAITS VÉGÉTAUX UTILISÉS DANS LA MÉDECINE TRADITIONNELLE D'AFRIQUE DE L'OUEST

Toxoplasma gondii *et* Plasmodium falciparum *sont deux protozoaires Apicomplexa avec des voies métaboliques communes et des cibles potentielles de médicaments proches. L'objectif de notre étude est d'examiner l'activité anti-*Toxoplasma *de neuf plantes d'Afrique de l'Ouest qui ont une activité antipaludique connue. Les extraits sont obtenus à partir des parties des plantes communément utilisées par la plupart des guérisseurs traditionnels, sous forme d'infusion et de décoction. L'activité* in vitro *des extraits de plante est effectuée sur* T. gondii *cultivé dans des cellules MRC5 et quantifiée par ELISA. Les extraits aqueux obtenus à partir de* Vernonia colorata *sont les plus actifs sur la croissance de* T. gondii *avec une* CI_{50} *de 16,3 mg/L. Une activité jusqu'à 10 fois supérieure est obtenue avec des extractions par divers solvants organiques tels que le dichlorométhane* (CI_{50} *= 1,7 mg/L), l'acétone* (CI_{50} *= 2,6 mg/L) et l'éthanol* (CI_{50} *= 2,9 mg/L). Ces résultats indiquent une source prometteuse de nouveaux médicaments anti-*Toxoplasma *à partir de* V. colorata *et des plantes médicinales africaines.*

MOTS CLÉS : Toxoplasma gondii, *plantes médicinales, ethnopharmacologie, Côte d'Ivoire,* in vitro.

INTRODUCTION

Traditional medicine could be a promising source of new parasitic drugs. Significant successes were achieved on antimalarial products with new derivatives extracted from plants like Qinghaosu (Li & Rieckmann, 1992). In Africa, the use of indigenous plants plays an important role in the treatment of a variety of parasitic diseases as reviewed by Phillipson (1986). Plants are used by traditional healers to treat and cure malaria symptoms and we previously confirmed the in vitro anti-malarial activity of several aqueous plant extracts against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* (Benoit *et al.*, 1995, 1996).

Toxoplasma gondii and *Plasmodium falciparum* are both *Apicomplexa* protozoa and share common metabolic pathways and potential drug targets. Several drugs are already known to be effective against both parasites, such as dihydrofolate reductase inhibitors which are effective on folate synthesis pathway of both parasites, and several antibiotics (macrolides, quinolones, cyclines) which probably act on protein biosynthesis in the apicoplast, *i.e.* a structure which is present in *Plasmodium* and in *T. gondii* (Derouin, 1999; Soldati, 1999). Thus, the objective of this study was to determine if nine west African plants with known anti- *P. falciparum* activity could also be inhibitory for *T. gondii* growth *in vitro*.

MATERIAL AND METHODS

PLANT EXTRACTS

Nine plants collected in Ivory Coast were studied: *Azadirachta indica, Cinnamonnum camphora, Lippia multiflora, Vernonia colorata, Guiera*

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senegalensis, Combretum micranthum, Ximenia americana, Cochlospermum planchonii and *Sida acuta.* Each plant was identified by Professor Koné-Bamba (Pharmacy University, Abidjan, Ivory Coast) by comparison with authentic samples present in Laboratory of Pharmacognosy and voucher herbarium specimens were deposited (no. 2704).

Extracts were prepared from different parts of the plants (see Table I) that had been air-dried for 10-days at 33° C, then pulverized into powder (sift: 250). Aqueous extracts (decoction and infusion) were prepared from 50 g of dried powder as previously described (Benoit, 1995). For infusion, dried powder was added into 500 ml of distilled boiling water that was then removed from heat and allowed to stand for 10 min. For the decoction, powder was added to boiling water, kept at 100° C for 10 min and then allowed to sediment for 10 min. The suspensions were centrifuged at 4,000 r.p.m. for 20 min. The supernatants were collected and sterilized by filtration at 0.22 µm (Gelman Sciences SA, France). Five milliliters of each solution was air-dried, the resulting powder weighed and then used to prepare stock solutions at 10-15 mg/ml in distilled water.

For *Vernonia colorata*, additional extractions were performed using organic solvents as nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds (Cowan, 1999). Here, extraction were performed with ethylic alcohol, acetone, dichloromethane and hexane with the aim of concentrating and determining the chemical affinity of the active components. For each extraction, 10 g of vegetal powder were mixed with 200 mL of each solvent and then kept for maceration at 4° C under agitation during four weeks. After centrifugation at 4,000 r.p.m. for 20 min., the superna-

Plant (aqueous extract)	Family	Part used	Inhibition of *T. gondii* growth IC_{50} (mg/L)	Cell toxicity (mg/L)	IC_{50} (mg/L) on *P. falciparum*[a]
Azadirachta indica A Juss	Meliaceae	Stem, leaf			
- Infusion			> 1,000	> 1,000	6.25-7.29
- Decoction			494	> 1,000	4.17-7.29
Cinnamonnum camphora Nees	Lauraceae	Cortex			
- Infusion			789	1,000	10.5-50
- Decoction			565	1,000	9.37-37.5
Cochlospermum planchonii (Hook)	Bixaceae (Cochlospermaceae)	Tubercle			
- Infusion			> 1,000	1,000	0.4-1.31
Combretum micranthum L.	Combretaceae	Stem, leaf			
- Infusion			217	> 1,000	0.7-1.2
- Decoction			254	> 1,000	0.7-1.2
Guiera senegalensis J.F. Gmel	Combretaceae	Stem, leaf			
- Infusion			351	500	0.79-12.5
- Decoction			177	250	0.79-12.5
Lippia multiflora Mold	Verbenaceae	Leaf			
- Infusion			201	> 1,000	21.0-2.2
- Decoction			127	> 1,000	1.65-3.75
Sida acuta L.	Malvaceae	Flower, leaf			
- Infusion			> 1,000	1,000	0.46-0.9[b]
Vernonia colorata Drake	Composeae	Stem, leaf			
- Infusion			17	250	2.35-4.7
- Decoction			18	250	2.35-7.82
Ximenia americana L.	Oleaceae	Stem, leaf			
- Infusion			> 1,000	1,000	1.05-6.25
- Decoction			469	1,000	0.8-1.83
Reference drugs					
- Pyrimethamine			0.04	50	ND
- Chloroquine			ND		0.12

[a]: IC_{50} were determined by a radioactive micromethods as described by Benoit *et al.*, 1996, using the FcB1-chloroquine-resistant strain.
[b]: data not published.

Table I. – *In vitro* effect of 16 aqueous extracts from nine plants on *T. gondii* growth in MRC5 tissue culture.

tants were collected, filtered sterilized at 0.22 mm and then evaporated. Stock solutions at 10 mg/mL were prepared in DMSO.

IN VITRO STUDY ON *TOXOPLASMA GONDII*

In vitro studies were performed with the virulent RH strain of *T. gondii* which was maintained in mice by intraperitoneal passage every two days. For each experiment, tachyzoites were collected from the peritoneal cavity of infected mice then resuspended in physiological saline. Tissue cultures and drug tests were carried out using MRC5 fibroblast tissue cultures as previously described (Derouin *et al.*, 1989), with minor modifications. Briefly, confluent monolayers prepared in 96-well tissue culture plates were inoculated with 2,000 fresh tachyzoites. After four hours, plant extracts at various concentrations were added into the culture medium and culture plates were incubated for an additional 72 h. Each culture plate comprised eight negative control (without *T. gondii*) and eight positive control wells (without drug). After incubation, the plates were examined microscopically for cytopathic effects and thereafter fixed with cold methanol for five min. *Toxoplasma* growth was assessed by enzyme linked immunoassay (ELISA) performed directly on the fixed cultures using a peroxydase labeled monoclonal antibody directed against the SAG-1 surface protein of *T. gondii*. After addition of the substrate, spectrophotometric readings were performed at a wavelength of 405 nm with blanking on the negative control well. For each well, the results were expressed as optical density (OD) values.

In a first experiment, serial twofold dilutions of each stock solution were prepared in the culture medium. For each dilution, four replicates were constituted by introducing 25 µL of solution in the culture wells. Duplicate culture plates were used. The final concentrations in the wells ranged between 2 and 1,000 mg/L. In a second set of experiments, organic solvent extracts of *V. colorata* were tested at final concentrations ranging from 0.1 to 50 mg/L. Each concentration was tested on eight replicates wells in two duplicate culture plates. In a preliminary experiment, we checked whether DMSO (used for dissolution of organic solvent extracts) had any effect on *Toxoplasma* growth at the dilutions tested. Each experiment was repeated twice.

The effect of each extract at various concentrations was described by data plotting. The OD values were plotted as a function of the logarithm of the concentration and a linear regression model was used to summarize the concentration-effect relationship and to determine the 50 % inhibitory concentrations (IC_{50}) (Derouin *et al.*, 1989).

RESULTS

In the first screening experiment, the inhibitory effect of each compound on *T. gondii* was examined for serial dilutions of the stock solution ranging from 2 to 1,000 mg/L. At each concentration, the toxicity on the host cells was recorded by microscopic examination of the monolayers. For each aqueous plant extract the IC_{50} against *T. gondii* and record of the IC_{50} against *P. falciparum* are presented in Table I. For *Aazadirachta indica, Cinnamonmum camphora, Cochlospermum planchoni, Sida acuta* and *Ximenia americana*, there was almost no inhibitory effect against *Toxoplasma*; for both infusion and decoction extracts, IC_{50} were not evaluable. Extracts from *Lippia. multiflora* and *Combretum micranthum* exhibited moderate anti-*Toxoplasma* activity at concentrations that were non toxic for the monolayers; IC_{50} were between 127 and 254 mg/L for *L. multiflora* and *C. micranthum* respectively. The activity of *Guiera senegalensis* was not evaluable because of a marked toxic effect on the monolayer at concentrations close to those that were inhibitory for *T. gondii.*

With *Vernonia colorata*, a marked inhibition of *Toxoplasma* growth was observed. Infusion and decoction extracts exhibited a similar pattern of activity consisting in a marked inhibition for concentrations > 10 mg/L and total inhibition at concentrations > 30 mg/L whereas a toxic effect was observed on the monolayer for concentrations > 250 mg/L (Fig. 1). From the regression curve analysis, the IC_{50} was 16.3 mg/L (5 % confidence interval 11.8-24.8) for the infusion extract and 18.5 mg/L (5 % confidence interval 13.6-27.6) for the decoction extract. From this first set of experiments, *V. colorata* was the only selected plant for further experiments using organic solvent extracts. The anti-*T. gondii* activities of acetone, ethanol, hexane and

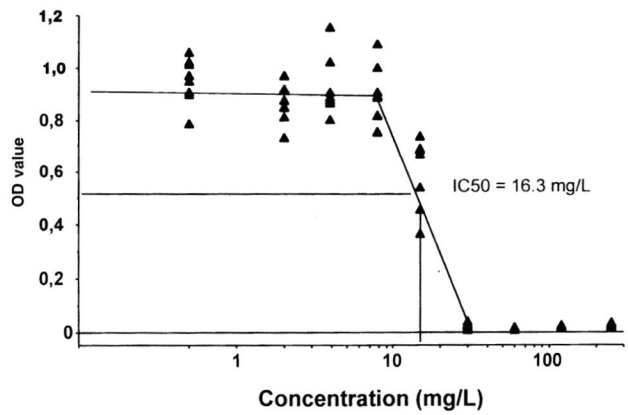

Fig. 1. – *In vitro* effect of *Vernonia colorata* aqueous extracts (decoction) on *T. gondii* growth in MRC5 tissue culture. OD value for ELISA with infected monolayers versus concentrations of *V. colorata* extracts.

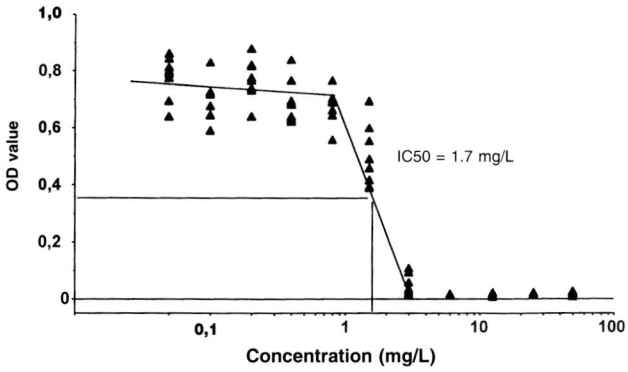

Fig. 2. – *In vitro* effect of *Vernonia colorata* dichloromethane extract on *T. gondii* growth in MRC5 tissue culture. OD value for ELISA with infected monolayers versus concentrations of *V. colorata* extract.

dichloromethane extracts of *V. colorata* were tested at concentrations comprised between 0.1 and 50 mg/L. A marked inhibition was noted for concentrations > 1 mg/L for the dichloromethane extract (Fig. 2), > 2 mg/L for the acetone and ethanol extracts and a toxic effect on the monolayers was recorded for concentrations > 25 mg/L. With the hexane extract, no toxicity was noted at the highest concentration tested (50 mg/L) but an inhibition was only noted at 25 and 50 mg/L. The IC_{50} was 2.9 mg/L (2.8-3.1) for ethanol, 2.6 mg/L (2.2-3.2) for acetone, 1.7 mg/L (1.6-1.9) for dichloromethane and 28 mg/L (14-90) for hexane extracts.

DISCUSSION

Protozoan parasites of the phylum *Apicomplexa* are responsible for a large spectrum of diseases among which malaria and toxoplasmosis are highly prevalent in humans. For both diseases, there is a crucial need for new therapeutic agents, because of the development of drug resistance by *Plasmodium* and the limited efficacy and poor tolerance of drugs that are used for treatment of toxoplasmosis (Derouin, 1999; Remington *et al.*, 1995; Slater, 1993; Pradines *et al.*, 1998). In this regards, indigenous plants could be a promising source of new anti-parasitic drugs, as already evidenced for several antimalarials such as Qinghaosu (artemisinin). Interestingly, the anti-parasitic effect of this drug and its derivatives is not restricted to *Plasmodium* since it was also found to be inhibitory for *Toxoplasma gondii in vitro* (Holfels *et al.*, 1994).

In a previous study, we examined the in vitro anti-plasmodial activity of ten plants chosen by native West African healers to treat malaria symptoms. We found that extracts from *L. multiflora, V. colorata, C. micranthum, X. americana, C. planchonii* and *S. acuta* had better inhibitory activity on two strains of *P. falciparum* than *A. indica*, which is usually considered as a "reference" for its anti-plasmodial activity (Udeinya *et al.*, 1993). Assuming that these plants extracts may be potential inhibitors for other apicomplexan parasites, we examined their anti-*T.gondii* activity *in vitro*. The present data shows no correlation between the IC_{50} values against *T. gondii* and those previously determined for *Plasmodium,* but provide evidence that several of these plant extracts are inhibitory for *Toxoplasma. L. multiflora* and *C. micranthum* aqueous extracts were found to have moderate but significant inhibitory effect on *Toxoplasma* growth. *C. micranthum* is already well-know by ethnopharmacologists for its activity against *Plasmodium*, bacteria and fungi (Gessler *et al.*, 1994; Sohni *et al.*, 1995; Valsaraj *et al.*, 1997). Similarly, *L. multiflora* presents antibacterial and antiplasmodial activities (Valentin *et al.*, 1995).

Despite these antimicrobial activities, the use of the aqueous extracts of *C. micranthum* or *L. multiflora* to cure toxoplasmosis may be limited because the IC_{50} values against *T. gondii* were high (> 100 mg/L) and probably not achievable *in vivo*.

Vernonia spp. and in particular *V. colorata* was found to be the most active plant extract against *T. gondii*. This plant is already used in traditional African medicine against fever (Fournet *et al.*, 1994), malaria (Almeida *et al.*, 1997), and other parasitic diseases (Oketch-Rabah *et al.*, 1998). In our laboratory, it was previously found inhibitory for chloroquine-sensitive and resistant *P. falciparum* strains at concentrations of 9.38 and 2.35 mg/L respectively.

Herein, we show that *V. colorata* may have potential therapeutic interest for toxoplasmosis as it strongly inhibits *Toxoplasma* growth at concentrations that were non toxic for cell cultures. Aqueous extracts obtained by decoction or infusion, *i.e.* two traditional methods widely used in Africa, were both inhibitory for *Toxoplasma* growth at concentrations > 10 mg/L. A ten-fold gain in activity was obtained when organic solvents such as ethanol, acetone or dichloromethane were used for extraction as these extracts were inhibitory for IC_{50} < 3 mg/L. The present results suggest a weak polarity of the active principle and represent a solid base for further purification processes.

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