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INVESTIGATION OF ANTIPLASMODIAL COMPOUNDS FROM TWO PLANTS, *COCHLOSPERMUM TINCTORIUM* A. RICH AND *GARDENIA SOKOTENSIS* HUTCH

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Abstract

Efforts in malaria treatment are currently directed towards the discovery and development of new antimalarial compounds. In this way two plants *Cochlospermum tinctorium* A. Rich. (Cochlospermaceae) and *Gardenia sokotensis* Hutch (Rubiaceae) traditionally used to treat symptoms of malaria in Burkina Faso were screened for antimalarial activity *in vivo* with *Plasmodium berghei.* Dichloromethane extract of *Cochlospermum tinctorium* and dichloromethane-methanol (7/3v/v) of *Gardenia sokotensis* showed a promising *in vivo* antiplasmodial activity with 50% effective dose of 17.59 mg/kg and 115 mg/kg respectively. Water extracts from the two plants showed an interesting antiplasmodial activity of *Cochlospermum tinctorium* and a weak activity for *Gardenia sokotensis*. Paradoxically chromatographic fractions issued from the active crude extract of *Cochlospermum tinctorium* appear less active. The fraction FGs_2 of Gardenia has showed a pronounced activity with 42% inhibition rate at 50 mg /kg. These results reflected in part the previous *in vitro* studies conducted on the two plants. Phytochemical screening revealed mostly the presence of triterpenes, carotenoids and flavonoids more or less in pure state.

Key words: *Cochlospermum tinctorium; Gardenia sokotensis;* Malaria; *Plasmodium berghei*

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Introduction

The treatment of malaria has become more and more difficult over the past three decades, with the appearance of resistant parasites and the increasing cost of effective antimalarial drugs. New drugs against the disease are greatly needed.

In Africa, as in many other parts of the tropical world the cost of western medicine and the weakness of health care infrastructure force the larger part of the population to use traditional plant medicine as the first choice for treatment of malaria and other diseases. This has consequently led to a traditional practice for treatment of disease with folk medicine to be preserved. Assuming that some of this might have an effect, folk medicine could be a source of new active principles and phytomedicines.

Cochlospermum tinctorium A. Rich (Cochlospermaceae) and *Gardenia sokotensis* Hutch (Rubiaceae) are two medicinal plants widely used in traditional medicine in malaria treatment in our country (Aké Assi and Guinko, 1991; Traoré, 1983). A series of bioguided fractionation conducted by us led to the identification of molecules from *Cochlospermum tinctorium* (Ballin et al., 2002). In contrast no pure fraction has been isolated from *Gardenia sokotensis* and the fractions of the plant showed a very moderate *in vitro* antiplasmodial activity. The decoctions of roots of *Cochlospermum planchonii* have been found to be effective for the treatment of uncomplicated *Plamodium falciparum* malaria without any major side effects (Benoit-Vical et al., 2003). According to the authors the decoctions of the two plants are indifferently used by traditional healers to treat fevers and malaria. Antiplasmodial efficacy of some natural products may be potentiated *in vivo* by other compounds which occur in the extracts and yet may need to be metabolized to the active form. Such compounds may be missed by assays using *in vitro* models. So the aim of this study is to identify the potential antiplasmodial compounds of these two plants *in vivo*.

Material and methods

Uses in traditional medicine

Rhizomes of *Cochlospermum tinctorium* are used against fever, hepatitis (Ekanem, 1994), abdominal pain, helminth and bilharzia infestations. The rhizomes are also used as antifungal and antibacterial (Nikiani et al., 1990); have been found to have hepatoprotective effects (Diallo et al., 1992) and possess *in vitro* antimalarial activity (Benoit et al. 1995).

Leaves of *Gardenia sokotensis* are indicated in the treatment of malaria (Dakuyo, 1992), fever, asthenia, gastro-enteritis, (Ake Assi and Guinko, 1991); few pharmacological studies have been conducted on the plant.

Previously isolated class of constituent

Rhizomes of *Cochlospermum tinctorium*: triacylbenzenes, triterpenes (arjunolic acid), apocarotenoids (cochloxanthine and dihydrocochloxanthine), flavonoids, Tannins (ellagitannins, gallic acid) (Diallo, 1991).- Leaves of *Gardenia sokotensis: s*terols and triterpenes, flavonoids, tannins, saponines (Traoré et al., 2000*)*

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Plant materials and extraction

Cochlospermum tinctorium and *Gardenia sokotensis* have been candidates for *in vivo* antiplasmodial screening from a previous study on eight plants used by our traditional healers in the treatment of malaria. The two plant materials were respectively collected in the same sites as the traditional healers. Rhizomes of *Cochlospermum tinctorium* A Rich (Cochlospermaceae) were collected in the Banfora area of Burkina Faso and the leaves of *Gardenia sokotensis* Hutch (Rubiaceae) in the East part of the country. Voucher specimens of the two plants were collected and deposited in the Herbarium of CNRST (Centre National de la Recherche Scientifique et Technologique) respectively under accession numbers 8709 and 8710.

Petroleum ether extract (PE)

300g of plant material were soaked with 500ml of petroleum ether during 24 hours. The extract was percolated with 500 ml of petroleum ether and concentrated.

Dichloromethane extract (CH_2CL_2)

The remaining plant material extracted by the first solvent was extracted a second time with 500ml of Dichloromethane, percolated again with 500 ml of the solvent. Diclorométhane-methanol extract (7/3) (CH_2CL_2/MeOH 7/3), Diclorométhane-methanol (3/7) (CH_2CL_2/MeOH 3/7), methanol (MeOH), and aqueous extracts (H_2O) were successively extracted in the same way.

Fractionation process

The extract with the most antiplasmodial activity from each plant was fractionated. Merck silicagel 60 (0.040-0.063 mm) was used for column chromatography and Merck silicagel $60F_{254}$ for Thin Layer Chromatography (TLC). Elution was started with dichloromethane followed by dichloromethane /methanol mixtures with increasing the proportion of methanol (2.5, 5, 10, 12,5, 15%). 10 ml portions of the eluate were collected and monitored by TLC. Same eluates were combined.

Antiplasmodial test performed in mice

The test was run according to the four day suppressive test of Peters on mice weighing between 20-25g maintained in same conditions of temperature (22±3ºC), diet and light. Mice divided in groups (according to doses) of five mice received daily oral extracts dissolved in sterile water containing Tween 85 and DMSO. Mice were treated from day 0 and monitored for seven days. The control group received the standard solution of extract dilution. Quinine was used as positive control at the doses 10, 15, 20, 25, 50, 100, 200 mg/kg. On the last day of the test blood smears were made and stained with Field's reagent; then the number of parasitized red blood cells was determined. The mean percentage of inhibition at each dose was calculated. The 6 first extracts of each plant were tested at the same dose (12.5 mg/kg for Cochlospermum and 500mg/kg for Gardenia) to identify the most active extract; and its

Table 1: Inhibitory effect of the two plant extracts on parasites

		PE	CH_2Cl_2	CH_2Cl_2/CH_3OH (7/3 v/v)	CH_2Cl_2/CH_3OH (3/7 v/v)	CH_3OH	H_2O
Yield (%)	Ct	5.25	0.48	0.79	2.11	1.78	3.06
	Gs	2.11	11.25	3.57	2.85	2.45	7.9
Mean reduction rate (%)	Ct (12.5mg/kg)	20±2.52[a]	60±2.52	35±4.16	45±5	44±4.35	46±9.24
	Gs (500mg/kg)	27±5.51	72±2.08	87±8.54	26±9.71	48±8.50	17±2.52
ED_{50} (mg/kg)	Ct		17.59				
	Gs			116			

[a]= Mean ±Standard Deviation , Ct: *Cochlospermum tinctorium*, Gs: *Gardenia sokotensis*, initial plant material:300g, Quinine: ED_{50}= 16mg/kg

Table 2 : Characteristics of fractions isolated from Dichloromethane extract of *Cochlospermum tinctorium*

Fractions	**Quantity (mg)**	**State**	**Spots (RF)**	**TLC coloration**			
				Visible	UV 366nm	Liebermann	Group
F_1	730 (33.72%)	Oily	0.89	yellow orange	Absorbed	blue	carotenoid
F_2	1300 (60%)	Oily	0.89	yellow orange	Absorbed	blue	carotenoid
			0.61	-	Not absorbed	violet	triterpene
			0.11	-	Not absorbed	violet	triterpene
F_3	40(0.02%)	Oily	0.11	-	Not absorbed	violet	triterpene

Eluent: cyclohexane, toluen, ethyl acetate, acetic acid (2, 1, 2.5 ,0,1 v/v)

Table 3: Characteristics of fractions isolated from Dichloromethane-methanol(7/3v/v) extract of *Gardenia sokotensis*

	Quantity (mg)	**State**	**Spots (RF)**	**TLC coloration**				
				Visible	UV 366nm	Diphenylborinate de sodium	Liebermann	Group
FGs_1	602 (30%)	oily	0.98		Not absorbed		violet	triterpene
			0.83	yellow	Absorbed	yellow		flavonoid
			0.94	yellow	Absorbed	yellow		flavonoid
FGs_2	201 (10%)	oily	0.12	colourless			violet	saponoside
			0.66	Yellow	Absorbed	Yellow		flavonoid
			0.83	Yellow	Absorbed			flavonoid

Elution solvant: petroleum ether, toluen, ethyl acetate, acetic acid (5-2-2-1.5 v/v)

Table 4: Activity of fractions (percentage reduction in parasitaemia)

Plants	***Cochlospermum tinctorium* (roots)**			***Gardenia Sokotensis* (leaves)**	
Doses (mg/kg)	12.5			50	
Fractions	FCt_1	FCt_2	FCt_3	FGs_1	Gs_2
Mean % reduction	11±7.07[a]	13±4.95	11±7.78	20±3	42±10.50

[a] = Mean ±Standard Deviation

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effective dose 50, dose required to inhibit growth of 50% of the parasites in mice was determined from following doses administrated (*Cochlospermum tinctorium*: 2.5, 7.5, 12.7, 50, 150mg/kg; *Gardenia sokotensis*: 50, 100, 200, 300, 400, 500mg/kg). The chromatographic fractions isolated from the actives extracts were also tested on the parasites.

Results and discussion

Table I presents the percentage inhibition of extracts and the effective dose 50 (ED_{50}) of the most active extracts. The applied doses of extracts showed a dose–dependent inhibition on the parasites. Fractions obtained from the two plants revealed mainly phenolic compounds (flavonoids) and terpenoids (triterpene, carotenoids and saponines).

The dichloromethane extract of *Cochlospermum tinctorium* was the extract with the most antiplasmodial activity (ED_{50} =17.59 mg/kg), followed by the aqueous extract with an inhibition rate inferior to 50%% at 12.5mg/kg. The activity of aqueous extract although less active than the dichloromethane extract seems interesting according to the proposed thresholds for *in vivo* activity of antimalarial extracts (Rasoanaivo et al., 2004.) The fractionation of dichloromethane extract of the plant produced 3 main fractions labelled FCt_1, FCt_2, FCt_3. The characteristics of the fractions are represented in Table II. The fractions FCt_1 and FCt_3 seem pure. The antiplasmodial activity of fractions from the dichloromethane extract of *Cochlospermum tinctorium* did not show a significant antiplasmodial activity compared to that of the initial extract. Three hypotheses could explain this loss of activity of the dichloromethane extract during the fractionation process: firstly the result obtained could confirm the synergistic action among the chemical compounds in an extract and that has been demonstrated several times; secondly the decline of activity of fractions may be due to the denaturation of compounds during the storage. Indeed some secondary metabolites protect other metabolites (as antioxidants for example) and the break of this association can accelerate the degradation. Thirdly the active compound may be degraded by contact with silicagel. Indeed it has been demonstrated that the cochloxanthines (carotenoids) previously identified from the plant lose their inhibition against the Epstein Barr Virus when separated on silicagel (Diallo et al.,1999). The deactivation must be due to the cis–trans isomerisation, the trans form being practically inactive. Previous studies conducted with the plant have identified molecules with antiplasmodial activity (Ballin et al., 2002). Elsewhere, the crude aqueous extract has been found to have *in vitro* antiplasmodial activity (Benoit et al., 1995). According to our study, the plant extract was slightly toxic *in vivo* (LD_{50} = 230mg/kg) (Hodge and Sterner 1943, Done,1980). The toxicity has been also indicated *in vitro* (Benoit et al., 1999, Ballin et al., 2002).

Concerning the extracts of *Gardenia sokotensis*, the dichloromethane-methanol (7/3v/v) extract presented the highest percentage rate of parasite inhibition. (ED_{50} was around 116mg /kg), and the aqueous extract showed the lowest inhibition rate. The fractionation of the dichloromethane–methanol (7/3v/v) extract gave two fractions, FGs_1, FGs_2 (Table III) and the fraction F_2 was found to be active on the parasites (42% inhibition rate at 50 mg /kg)(Table IV). Neither fraction appeared pure but we did not succeed in isolating a pure fraction (Table III). The same difficulties have been met with a series of *in vitro* bioguided fractionation. In contrast with *Cochlospermum tinctorium*, the extracts of *Gardenia sokotensis* seem not to be toxic. (LD_{50}>3000 mg/kg) (Hodge and Sterner 1943, Done,1980)

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Conclusion

This study has been a contribution to the assessment of possible antiplasmodial compounds from two plants from Burkina Faso. The total extract of *Cochlospermum tinctorium* demonstrated potent antiplasmodial activity but this activity decreased with the partitioning of the compounds. Indeed different fractions have been separated and some of them did not have antiplasmodial activity *in vivo*. Thus, crude extracts of *Cochlospermum tinctorium* were found to be more active than fractions and these results confirm those of previous studies carried out on the plant. Unlike the first plant, the screening of *Gardenia sokotensis* did not reach a pure fraction even if the antiplasmodial activity was promising. With regard to interpreting these results in relation to the traditional uses of the plants studied, great care should be taken since the aqueous extract (traditional form) of *Gardenia sokotensis* did not present antiplasmodial activity, but the traditional aqueous extract of C. tinctorium was active.

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