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ANTIPLASMODIAL AND CYTOTOXIC ACTIVITIES OF RWANDAN MEDICINAL PLANTS USED IN THE TREATMENT OF MALARIA

Raymond Muganga
National University of Rwanda, Faculty of Medicine, Department of Pharmacy, PO Box 117, Butare, Rwanda
Université de Liège, CIRM, Laboratoire de Pharmacognosie, CHU B36, B4000-Liège, Belgium

L. Angenot
Université de Liège, CIRM, Laboratoire de Pharmacognosie, CHU B36, B4000-Liège, Belgium

M. Tits
Université de Liège, CIRM, Laboratoire de Pharmacognosie, CHU B36, B4000-Liège, Belgium

M. Frédérich
Université de Liège, CIRM, Laboratoire de Pharmacognosie, CHU B36, B4000-Liège, Belgium

Keywords: Antiplasmodial activity; Malaria; Cytotoxicity; Selectivity index; Rwandan medicinal plants

Abstract

Aim of the study: In our study, methanol, dichloromethane and aqueous extracts of 13 Rwandan medicinal plants used in the treatment of malaria were tested for *in vitro* antiplasmodial activity.
Materials and methods: The growth inhibition of chloroquine-sensitive *Plasmodium falciparum* strain (3D7) was evaluated using the measurement of lactate dehydrogenase activity. The active extracts were also tested against the chloroquine-resistant *Plasmodium falciparum* strain (W2) and for cytotoxicity assay using human normal foetal lung fibroblasts (WI-38).
Results: The majority of the plants tested showed an antiplasmodial activity and the best results were observed with dichloromethane leaf and flower extracts of *Tithonia diversifolia*, leaf extract of *Microglossa pyrifolia* and root extract of *Rumex abyssinicus*, methanol leaf extract of *Fuerstia africana*, root bark extracts of *Zanthoxylum chalybeum* and methanol bark extract of *Terminalia mollis*. Those extracts were active (IC_{50} < 15 µg/ml) on both chloroquine-sensitive and resistant strains of *Plasmodium falciparum*. *Zanthoxylum chalybeum*, *Solanecio mannii* and *Terminalia mollis* presented the best selectivity index.
Conclusions: The traditional use of most of the plant evaluated was confirmed by the antiplasmodial test. This study revealed for the first time the antiplasmodial activity of two plants: *Terminalia mollis* and *Rumex abyssinicus*.

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1. Introduction

Malaria is still a major public health problem, especially in tropical and sub-tropical regions. It is estimated that, in 2006, 3.3 billion people were at risk of contracting malaria and that it causes nearly one million deaths each year, mostly of African children aged below 5 years, who are susceptible to this disease. In Sub-Saharan regions, 45 countries were endemic for malaria in 2008 (WHO, 2008). In Rwanda, malaria is one of the leading causes of outpatient attendance and one of the principal causes of morbidity in each province (PNILP, 2005). This is despite the fact that bed nets, artemisinin-based combination therapies (ACT) and indoor spraying have reduced the prevalence of the disease (Fanello et al., 2007). The ACT recommended in Rwanda is artemether-lumefantrine (Coartem®), but the use of this drug is very limited in some rural areas where the population prefer traditional, less expensive preparations (WHO, 2008, Rwanda News Agency, 2009). Artemisinin, isolated from the well-known Chinese medicinal plant Artemisia annua, is one of the best compounds used to treat multi-drug resistant strains of Plasmodium falciparum. However, artemisinin-resistant malaria parasites were recently detected in Cambodia (Maude et al., 2009). There is therefore an evident need for new anti-malarial drugs, and medicinal plants constitute a reliable source of these. Many Rwandan medicinal plants are claimed to be active against malaria but, in most cases, there is insufficient explanation or, in fact, any scientific proof of the efficacy of these medicines.

In the present study, based on ethnobotanical data obtained from Rwandan traditional healers and a literature review, 13 Rwandan medicinal plants used to treat malaria were selected and submitted to *in vitro* evaluation of their antiplasmodial and cytotoxic activities. These plants are listed in Table 1. Samples collected were dried, then extracted with methanol and dichloromethane and the crude extracts obtained were evaluated for *in vitro* antiplasmodial activity. The aqueous crude extracts of the most active plants were also evaluated. Cytotoxic evaluation was only carried out for crude extracts, which showed antiplasmodial activity (IC_{50}) < 50 µg/ml.

Table 1. *Selected species, their scientific names, parts used, voucher number and place of collection (altitude).*

Scientific name of the plant (family), date	Plant part[a]	Voucher number Rwanda	Voucher number Meise Botanical Garden, Belgium	Place of collection (altitude)
Aristolochia elagans Mast. (Aristolochiaceae), 2007	Seed	2007V39	BR0000005093953	Huye (1684 m)
Conyza aegyptiaca (L.) Aiton (Asteraceae), 2007	L	2007V38	BR0000005093878	Huye (1684 m)
Markhamia lutea K. Schum. (Bignoniaceae), 2008	L	2008R17	BR0000005093979	Nyaruguru (1753 m)
Microglossa pyrifolia (Lam.) Kuntze (Asteraceae), 2007	L	2007R1	BR0000005093977	Bugesera (1413 m)
Mitragyna rubrostipulata (K. Schum.) Havil. (Rubiaceae), 2008	L and SB	2008R19	BR0000005093595	Nyaruguru (1648 m)
Fuerstia africana T.C.E. Fr. (Lamiaceae), 2008	L and S	–	BR0000005088850	Huye (1719 m)
Rumex abyssinicus Jacq. (Polygonaceae), 2008	R	2008R15	BR0000005093670	Huye (1670 m)
Rumex bequaertii De Wild. (Polygonaceae), 2008	R	2007R3	BR0000005094059	Huye (1600 m)
Solanecio mannii (Hook.f.) C. Jeffrey (Asteraceae), 2008	L	2007R2	BR0000005093472	Musanze (2033 m)
Terminalia mollis M.A. Lawson (Combretaceae), 2007 and 2008	L, SB and RB	–	BR0000005087167	Ndego (1592 m)
Tithonia diversifolia (Hemsl.) A. Gray (Asteraceae), 2008	F and L	2007R12	BR0000005093793	Huye (1680 m)
Trimeria grandifolia subsp. *tropica* Sleumer (Flacourtiaceae), 2008	L	2008R16	BR0000005093496	Nyaruguru (1722 m)
Zanthoxylum chalybeum Engl. (Rutaceae), 2007 and 2008	SB and RB	–	BR0000005087266	Ndego (1377 m)

[a] F, flower; L, leaves; R, root; RB, root bark; S, stem; SB, stem bark.

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2. Materials and methods

2.1. PLANT MATERIAL

Plant samples were collected from the Rwandan regions where malaria is most endemic, East, South and South-West, in November 2007 and in August to October 2008 (see Table 1). Each species was identified and one voucher specimen deposited in the Rwandan National Herbarium at Butare and another at the National Botanic Garden of Belgium at Meise. All samples collected were air-dried at room temperature with no direct sunlight for 3 days, except the leaf of *Solanecio mannii* that were dried during 5 days. Dried plant samples were then pulverized using an electrical grinder under strict hygienic conditions.

2.2. PREPARATION OF EXTRACTS

Crude methanolic and dichloromethane extracts were obtained by maceration of 5 g of each powdered plant sample three times in 25 ml of solvent, for 30 min under constant shaking at room temperature. For each solvent, a new plant sample was used separately. The extracts were filtered and evaporated to dryness under reduced pressure with a rotatory evaporator. For the aqueous extracts, 100 ml of distilled water was used to extract 2 g of powdered plant material and the mixture obtained was boiled for 1 h. The solutions obtained were filtered and the filtrate freeze-dried to obtain the dried crude aqueous extracts. All extracts obtained were weighed and their yield calculated.

2.3. *IN VITRO* ANTIPLASMODIAL ASSAYS

The culture of *Plasmodium falciparum* strains was carried out as previously described (Frédérich et al., 2001). All crude extracts were evaluated *in vitro* for their activity against a chloroquine-sensitive strain of *Plasmodium falciparum* (3D7) and the most active extracts were also evaluated against a chloroquine-resistant strain (W2). For each crude extract, a series of eight threefold dilutions (from 200 to 0.09 µg/ml) was prepared, placed in two rows of a 96-well microplate and tested in triplicate. Artemisinin (98%, Sigma–Aldrich) and Chloroquine diphosphate salt (Sigma–Aldrich) were used as standards, and infected and uninfected erythrocytes were added as positive and negative controls, respectively. After 48 h of incubation at 37 °C, the level of parasitaemia was estimated by measuring lactate dehydrogenase activity, as previously described (Kenmogne et al., 2006). The results were expressed as the mean IC_{50} (the concentration of a drug that reduced the level of parasitaemia to 50%).

2.4. *IN VITRO* CYTOTOXIC ASSAY

Cells from the human normal foetal lung fibroblast cell line, WI-38, was cultivated *in vitro* in DMEM: Dubecco's Modified Eagle's Medium (Lonzo, Belgium), which contains 5% of L-glutamate (Lonzo, Belgium), 5% of penicillin–streptomycin (Lonzo, Belgium) and 10% of heat inactivated foetal bovine serum (Lonzo, Belgium). Then, the cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. For each sample, 6 threefold dilutions (from 200 to 0.82 µg/ml) were prepared, placed in 3 rows of a 96-well microplate and tested at least twice. Camptothecin (Sigma) was used as a positive control.

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After 48 hours' incubation, cell viability was determined by measuring the fibroblast mitochondrial enzyme activity, as previously described (Stevigny et al., 2002). The results were expressed by the mean of IC_{50}s of at least two independent assays and the selectivity index (the ratio between the cytotoxic (WI-38 cells) and antiparasitic (3D7 strain) activity) was calculated.

3. Results and discussion

Thirteen plants were selected, 19 samples collected and from these 46 extracts tested for antiplasmodial activity on the 3D7 chloroquine-sensitive strain of *Plasmodium falciparum*. According to WHO guidelines and previous results from our team (Pink et al., 2005, Jonville et al., 2008), antiplasmodial activity was classified as follows: highly active at IC_{50} < 5 µg/ml, promising at 5–15 µg/ml, moderate at 15–50 µg/ml and inactive at >50 µg/ml.

Most of the plants tested (77%) showed an antiplasmodial activity with IC_{50} < 50 µg/ml. Twelve extracts showed a promising level of activity (IC_{50} < 15 µg/ml) and seven of them had a very high level of activity (IC_{50} < 5 µg/ml) against 3D7 and W2 (*Plasmodium falciparum* chloroquine-resistant strain). We found that the dichloromethane extracts were generally more active against *Plasmodium falciparum*, but for some plant samples the methanolic extracts were the more active ones. Nine extracts with a promising level of activity (IC_{50} < 15 µg/ml) were assessed for their cytotoxic activity in order to determine the selectivity index (see Table 2).

Aristolochia elegans, *Rumex bequaertii* and *Trimeria grandifolia* did not show any antiplasmodial activity *in vitro*, although they are traditionally used to treat malaria (Hakizamungu and Weri, 1988, Rwangabo, 1993). This does not mean that those plants are not active against *Plasmodium falciparum*. Indeed, the *in vitro* antiplasmodial activity was assessed on the asexual erythrocytic stage of *Plasmodium falciparum* and those plants may act on other stages of the parasite. Those plants may also be active in vivo where the pharmacokinetics and metabolic process are involved and could therefore make the plant samples active. This is the case with the methanolic leaf extract of *Markhamia lutea*, which had already been reported to be active in vivo (Hakizamungu and Weri, 1988) with an important growth inhibition on *Plasmodium berghei* (62.1%) but was found to be inactive *in vitro* (IC_{50} > 50 µg/ml). Most of the time, traditional healers use a mixture of different plants so as to increase the activity but also to hide the real active plant. This means that some plant samples, which did not show any activity in our study, might still be active against *Plasmodium falciparum*. It must be noted that, often, traditional healers do not distinguish fever from malaria. Some medicinal plants used in the treatment of malaria, in combination with others, may have antipyretic activity or may reduce joint pain and therefore impact on the patient's recovery. *Aristolochia elegans* did not show any antiplasmodial activity and this plant contains aristolochic acid (Jou et al., 2004), which has already been reported to be nephrotoxic (Vanherweghem et al., 1993, Nortier et al., 2000). The seed of this plant is used by some traditional healers in the prevention of malaria and this use should be avoided.

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Table 2. In vitro *antiplasmodial and cytotoxic activities, and selectivity index of the selected samples.*

Species	Plant part[a]	Extract	Yield	3D7 IC_{50} (μg/ml)[b]	W2 IC_{50} (μg/ml)[b,c,d]	WI-38 IC_{50} (μg/ml)[c,d]	SI[c,d]
Aristolochia elegans	Seed	MeOH	5.4	>50	nd	nd	nd
		CH_2Cl_2	7.6	>50			
Conyza aegyptiaca	L	MeOH	5.4	22.7 ± 4.2	24.66 ± 2.4	81.9 ± 1.6	3.6
		CH_2Cl_2	7.6	36.8 ± 6.0			
		H_2O	31.7	>50			
Markhamia lutea	L	MeOH	10.7	>50	nd	nd	nd
		CH_2Cl_2	1.7	29.0 ± 0.8			
Microglossa pyrifolia	L	MeOH	18.7	**4.2 ± 1.9**			
		CH_2Cl_2	7.4	**1.5 ± 0.1**	**2.4 ± 0.1**	4.7 ± 0.8	3.2
		H_2O	25.2	**14.3 ± 2.1**			
Mitragyna rubrostipulata	SB	MeOH	16.1	>50	nd	nd	nd
		CH_2Cl_2	0.9	39.9 ± 2.8			
	L	MeOH	29.9	>50	nd		
		CH_2Cl_2	2.4	>50			
Fuerstia africana	L&S	MeOH	13.3	**6.9 ± 2.3**	**4.1 ± 1.6**	13.0 ± 2.3	1.9
		CH_2Cl_2	2.8	40.2			
		H_2O	5.3	>50			
Rumex abyssinicus	R	MeOH CH_2Cl_2	16.2	>50			
		H_2O	7.4	**4.3 ± 2.0**	**3.1 ± 1.2**	13.3 ± 0.9	3.1
			45.4	>50			
Rumex bequaertii	R	MeOH	19.1	>50	nd	nd	nd
		CH_2Cl_2	0.3	>50			
Solanecio mannii	L	MeOH	10.6	21.6 ± 3.4	26.2 ± 0.1		
		CH_2Cl_2	4.2	18.2 ± 6.0	**12.7 ± 2.8**	122.3 ± 3.8	6.7
		H_2O	40.7	>50			
Terminalia mollis	L	MeOH	10.6	>50	nd		nd
		CH_2Cl_2	4.2	> 50			
	SB	MeOH	16.9	> 50	18.9 ± 1.6	77.2 ± 8.9	6.6
		CH_2Cl_2	7.4	> 50			
	RB	MeOH	26.3	**11.7 ± .9**			
		CH_2Cl_2	0.4	> 50			
		H_2O	34.23	33.5 ± 0.3			
Tithonia diversifolia	F	MeOH	14.9	**8.1 ± 3.3**	**6.5 ± 1.9**		
		CH_2Cl_2	48.0	**1.1 ± 0.3**	**1.0 ± 0.3**	5.3 ± 1.3	4.7
	L	H_2O	28.6	24.5 ± 3.9			
		MeOH	5.7	1.2 ± 0.4	**1.5 ± 0.4**	2.5 ± 0.2	4.2
		CH_2Cl_2	49.1	0.6 ± 0.1	**0.7 ± 0.2**		
		H_2O	42.1	15.6 ± 1.8			
Trimeria grandifolia	L	MeOH	23.0	> 50	nd	nd	nd
		CH_2Cl_2	4.3	> 50			
Zanthoxylum chalybeum	SB	MeOH	12.3	42.5 ± 0.4			
		CH_2Cl_2	4.4	41.5 ± 0.9			
	RB	MeOH	16.8	**4.2 ± 2.7**	**1.9 ± 0.5**	40.0 ± 8.5	9.5
		CH_2Cl_2	3.7	**6.2 ± 0.6**			
	RB[e]	H_2O	13.4	> 50			
		MeOH	15.2	38.34 ± 2.0			
Artemisinin				0.0063 ± 0.0009	0.0024 ± 0.0005	nd	nd
Chloroquine				0.0016 ± 0.003	0.329 ± 0.110	nd	nd
Camptothecin						0.0197 ± 0.025	nd

[a] F, flower; L, leaves; R, root; RB, root bark; S, stem; SB, stem bark.
[b] IC_{50} values shown in bold express promising antiplasmodial activity.
[c] For chloroquine, $n = 11$.
[d] nd = not determined.
[e] Root bark collected in August.

3.1. *MITRAGYNA RUBROSTIPULATA*

The only active extract from this plant was its dichloromethane stem bark extract, which presented a weak antiplasmodial activity (IC_{50} = 39.9 μg/ml). Further studies are needed to confirm the use of the root and stem bark of the plant in the treatment of malaria by Rwandan traditional healers, as a metabolization step may be necessary to obtain a good activity. The stem bark is also used to treat intestinal worms, especially amoebiasis and it also has antibacterial and antifungal activities (Rwangabo, 1993). Another species of *Mitragyna*, known as *Mitragyna inermis*, has been found to be more active against *Plasmodium falciparum* (Fiot, 2005) and has proved to also have antibacterial activity (Zongo et al., 2009).

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3.2. *MARKHAMIA LUTEA*

Dichloromethane leaf extract showed a weak antiplasmodial activity (IC_{50} = 29 µg/ml) but in another recent study (Lacroix et al., 2009), the ethyl acetate extract was found to be three times more active (IC_{50} = 10.2 µg/ml). This difference may be explained by the fact that a different solvent was used, by the fact that plant sample was collected in a different region and by the use of a chloroquine-resistant plasmodial strain. The methanolic leaf extract of the plant has been reported also to be active *in vivo* (Hakizamungu and Weri, 1988). Our results, together with the results of these studies may confirm the use of the plant in traditional medicine. The plant has other therapeutic properties and has been identified as a potential treatment for viral respiratory infections (Kernan et al., 1998).

3.3. *CONYZA AEGYPTIACA*

Conyza aegyptiaca is one of the most used plants to treat malaria in Rwandan traditional medicine. It is also used in the treatment of haematuria (Rwangabo, 1993). In our study, the methanolic leaf extract of the plant showed a moderate antiplasmodial activity (IC_{50} = 22.7 µg/ml) and was slightly more active than the dichloromethane leaf extract (IC_{50} = 36.8 µg/ml). The methanolic leaf extract showed a low cytotoxicity (IC_{50} = 80.9 µg/ml) but presented a low selectivity index (3.6). Further study *in vivo* is needed to confirm the use of the plant in traditional medicine and its absence of toxicity.

3.4. *SOLANECIO MANNII*

This plant, also called *Senecio mannii*, is used by Rwandan traditional healers to treat not only malaria but also fever, burns, abscesses, leprosy, anthrax and poisoning (Hakizamungu and Weri, 1988). The dichloromethane leaf extract of the plant showed promising antiplasmodial activity (IC_{50} = 18.2 and 12.9 µg/ml, respectively, on 3D7 and W2) with a quite low level of cytotoxicity (IC_{50} = 122.3 µg/ml) and a significant selectivity index of 6.7. Our results were able to confirm the use of the plant in Rwanda traditional medicine. This plant is then a potential source of a new anti-malarial drug.

3.5. *TERMINALIA MOLLIS*

The methanolic and aqueous root bark extract of the plant are active against *Plasmodium falciparum* with IC_{50} values of 11.7 and 33.5, respectively. This justifies the traditional use of the plant in the treatment of malaria, as water is used as the solvent in the traditional preparation. Subsequently, the cytotoxicity level of the plant was quite low (IC_{50} = 77.2 µg/ml) and its selectivity index was significant (6.6). However, further investigation *in vivo* is needed to confirm its safety. The plant also has antifungal, antibacterial and antiviral activities (Maregesi et al., 2008). A recent study (Maregesi et al., 2009) reported nevertheless that 80% methanol root extract has a very weak activity (IC_{50} = 125–150 µg/ml) against *Plasmodium falciparum*. The difference in the results of both studies may be explained by the fact that the plant samples were collected in two different regions and the *Plasmodium* strains used were different. The antiplasmodial activity is already known in other species of Combretaceae; the species *Terminalia bentzoe* has already been reported to have a promising effect on *Plasmodium falciparum* strains (Jonville et al., 2008) and *Terminalia bellirica* on the 3D7 strain (Valsaraj et al., 1997).

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3.6. *FUERSTIA AFRICANA*

The whole plant without its root is used to treat malaria and fever in Rwandan traditional medicine and the leaf is used to treat gonorrhoea (Rwangabo, 1993). The methanolic leaf and stem extract of the plant showed a very high antiplasmodial activity towards both strains of *Plasmodium falciparum*, 3D7 and W2 (IC_{50} = 6.9 and 4.1 µg/ml, respectively), but the plant was quite cytotoxic (IC_{50} = 13.0 µg/ml) and therefore the selectivity index was low (1.9). The same antiplasmodial activity was already reported (Muthaura et al., 2007) but selectivity index was quite different. The high selectivity indexes found in the paper from Muthaura et al. could be explained by the fact that they calculated the selectivity indexes by using CI_{50} for Plasmodium and CC_{50} for cells (and not CI_{50} in the two cases, as usual). One compound, known as Ferruginol, has been previously isolated from this plant and this substance presented a strong anti-malarial activity, with an IC_{50} of 1.95 µg/ml, but also a cytotoxic activity. Therefore, Ferruginol is not a desirable anti-malarial candidate (Koch et al., 2006).

3.7. *RUMEX ABYSSINICUS*

The root of the plant is traditionally used to treat malaria, gonorrhoea, constipation, poisoning, hepatitis, constipation and sciatic neuralgia, and the leaf is used to treat coughs and gastric ulcers (Rwangabo, 1993). The dichloromethane root extract showed a very high antiplasmodial activity (IC_{50} = 4.3 and 3.1 µg/ml on 3D7 and W2, respectively). To date, there has not been any scientific proof of the antiplasmodial activity of this plant. The cytotoxicity level of the same extract was not negligible (IC_{50} = 13.3 g/ml) but there was a low to moderate selectivity index (3.1). The cytotoxicity of this plant could nevertheless compromise its medicinal use and more clarification regarding this toxicity is needed.

3.8. *MICROGLOSSA PYRIFOLIA*

The leaf of the plant is used traditionally against malaria, and for fever, pain relief, intestinal worms, rheumatism, diarrhoea, gonorrhoea, etc. in Rwanda and in many other African countries (Rwangabo, 1993, Neuwinger, 1996). Aqueous, methanolic and dichloromethane leaf extracts of the plant presented a promising very high effect on 3D7 strain, with IC_{50} values of 14.2, 4.2 and 1.5 µg/ml, respectively. A previous study had shown that the lipophilic extract of the plant was active against malaria with an IC_{50} = 10.5 and 13.1 µg/ml, respectively, on the chloroquine-sensitive strain PoW and the chloroquine-resistant clone Dd2. Two diterpenes, E-Phytol and 6E-geranylgeraniol-19-oic acid, which have also been found to be detectable in aqueous extract, have been found to be responsible for the antiprotozoal activity of the plant (Köhler et al., 2002). The level of antiplasmodial activity obtained in our study was higher than the one already reported. This slight difference could be explained by the fact that we used different strains of *Plasmodium falciparum* on the one hand and, on the other hand, by the fact that the plant samples used came from different countries: Rwanda and Ghana. Although the plant has a good effect on *Plasmodium falciparum*, its cytotoxicity level is relatively high (IC_{50} = 4.7 µg/ml) and the selectivity index moderate (3.2). The cytotoxicity and hepatotoxicity of the plant is already described in the literature (Zirihi et al., 2005, Mukazayire et al., 2009).

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3.9. *TITHONIA DIVERSIFOLIA*

The best antiplasmodial activities were found in leaf and flower, especially when extracted with dichloromethane (1.0 and 0.7 µg/ml). The methanolic leaf and flower extract presented an interesting level of activity (1.2 and 8.1 µg/ml) and a moderate level of activity was found with aqueous extracts (15.6 and 24.5 µg/ml, respectively, for leaf and flower extract). An artemisinic acid analogue was isolated from the mature stem of the plant and may contribute to the antiplasmodial effect (Bordoloi et al., 1996). The aerial parts of the plant also contain Tagitinin C, a lactone sesquitene with a very promising antiplasmodial activity, but this component is also cytotoxic (Goffin et al., 2002). Recently, the toxicity of the aerial parts has also been shown in rats and this compromises the use of the plant in the treatment of malaria (Elufioye et al., 2009).

3.10. *ZANTHOXYLUM CHALYBEUM*

The plant is commonly used in traditional medicine of Eastern African countries (Tabuti, 2008, Gessler et al., 1995). Different extracts of the plant were analysed and the best results were obtained with the methanolic root bark extract [IC_{50} = 4.2 µg/ml (3D7) and 1.9 µg/ml (W2)]. *In vitro* antiplasmodial activity of the plant has been previously reported (Gessler et al., 1994, Rukunga et al., 2009). Our results were quite similar to other IC_{50} values already reported for Kenyan and Tanzanian samples, except in the case of aqueous extracts (Gessler et al., 1995, Rukunga et al., 2009). The aqueous root bark extract in the present study showed a negligible activity towards 3D7, but previous studies have reported that this extract was really active with IC_{50} < 6 µg/ml (Gessler et al., 1994, Rukunga et al., 2009). This noticeable difference may be explained by the fact that the sample used in this study was not fresh. Indeed, plant samples used in this study were collected in Rwanda and then analysed in Belgium some months later. We found that the plant sample collected in August, during the summer period, was less active (IC_{50} = 38.3 g/ml) than the one taken in November during the rainy season, suggesting that the rainy season is appropriate for the synthesis of the active ingredients of our interest. It is known that the time of collection and the locality of plant material may play a major role in its beneficial properties (Capasso, 1985, Gessler et al., 1994). In addition, the same methanolic extract presented the best selectivity index from all plant samples analysed (9.5). The level of cytotoxicity of the methanolic root bark extract shown in this study (IC_{50} = 40.0 µg/ml) is similar to that already reported (Kamuhabwa et al., 2000). This plant has a very promising antiplasmodial activity and a good selectivity index, and it could be very interesting in further anti-malarial studies if the problem of differences in activity between batches is resolved.

4. Conclusions

The majority of the plants analysed in this study presented an antiplasmodial activity, which could justify their use in Rwandan traditional medicine. This preliminary study confirmed the interesting antiplasmodial activities of some plants already used and studied in other countries and revealed for the first time the antiplasmodial activity of *Terminalia mollis* and *Rumex abyssinicus*. Some plant samples, such as *Fuerstia africana*, *Rumex abyssinicus* and *Microglossa pyrifolia*, showed a very

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promising antiplasmodial activity against two plasmodial strains, but they were also cytotoxic and further investigations are therefore needed to clarify which compound is responsible for one or both activities. Some extracts were more active against the chloroquine-resistant strain than against the chloroquine-sensitive one (i.e. *Zanthoxylum chalybeum* and *Fuerstia africana*). This could be an indication of a specific mode of action of these extracts against *P. falciparum.* This study indicated particularly that three plants, *Terminalia mollis*, *Solanecio mannii* and *Zanthoxylum chalybeum*, have an important antiplasmodial activity and significant selectivity index, making them good candidates for further pharmacological study.

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