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Antimalarial activity of some extracts and isolated constituents from *Morinda morindoides* leaves

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Abstract

Objective: The study deals with the evaluation of the *in vitro* antimalarial activity of three crude extracts (ethanol and 80% methanol) from the leaves of *Morinda morindoides* (Baker) Milne-Redh. (Rubiaceae), that of their respective soluble fractions and isolated compounds, and that of the dichloromethane extract. It also reports the *in vivo* antimalarial activity of the three crude extracts and the petroleum ether soluble fraction from the ethanol extract as well as the toxicity of crude extracts in mice. Materials and methods: The ethanol, 80% methanol and dichloromethane extracts were obtained by maceration and percolation of powdered dried *M. morindoides* leaves. The ethanol extract was dissolved in 100 ml distilled water and extracted with petroleum ether, and then acidified with HCl 2N (pH 2-3) and successively and exhaustively treated with chloroform and isoamylic alcohol. On the other hand the 80% methanol extract was dissolved in 100 ml distilled water and successively and exhaustively extracted with chloroform, ethyl acetate and n-butanol. A series of flavonoids, anthraquinones and iridoids were isolated from the 80% methanol extract. All dried samples were tested for their potential *in vitro* antiplasmodial activity against Congolese the chloroquine-sensitive or the chloroquine-sensitive NF54/64, clone A19 strains of *Plasmodium falciparum* according to the case. The three extracts and one soluble fraction were tested *in vivo* against *Plasmodium berghei berghei* in a classical 4-suppressive test. Results: The petroleum ether, isoamylic alcohol and chloroform soluble fractions from the partition of ethanol extract showed an *in vitro* antiplasmodial activity against Congolese the chloroquine-sensitive strain of *P. falciparum* with IC_{50} values of 1.8 ± 0.2, 15.3 ± 3.6 and 8.8 ± 2.5 µg/ml respectively. Only the chloroform soluble fraction from the partition of the 80% methanol exhibited good antiplasmodial activity with IC_{50} value of 8.3 ± 1.6 µg/ml against the chloroquine-sensitive NF54/64, clone A1A9 strain of *P. falciparum*. Among isolated compounds, quercetin exhibited good antiplasmodial activity with IC_{50} value of 5.5. ± 1.8 µg/ml, alizarin and chrysarin showed a moderate activity with IC_{50} ranging from 14 to 26 µg/ml. *In vivo* test, at a daily oral dose of 200 mg/kg, ethanol, 80% methanol and dichloromethane extracts, and the petroleum ether soluble fraction produced 33%, 54% and 73%, and 75% chemosuppression respectively. Conclusion: These results may partly justify and support the traditional use of *Morinda morinoides* leaves for the treatment of malaria.

Keywords: *Morinda morindoides*, extracts, anthraquinones, favonoids, antimalarial activity, toxicity.

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1. Introduction

Malaria is a problem of global importance, responsible for 1-2 million deaths per year mainly in sub-saharan Africa. A high proportion of the world's population remains at risk of malaria, which presents increasing problems because of breakdown of traditional control methods, civil disturbance, mobile populations, resistance to insecticides and resistance to cheap and safe antimalarial drugs [1]. In many endemic areas of the world, drug resistance of *Plasmodium falciparum* have been found and majority of conventional antimalarial drugs have been associated with treatment failure [2].

Due to limited access to known antimalarial drugs in sub-saharan Africa, more than 80% of the population relies on traditional medicine and healers as the primary source of health care using different preparations of medicinal plants as remedies. However, few data are available on the efficiency and safety of several medicinal plants, used by healers to treat malaria in their daily practices. The validation of traditional practices could lead to involve new strategies particularly in the control and treatment of malaria. Several studies have been undertaken to assess the potential inhibitory effects of various plant extracts or isolated constituents against different *P. falciparum* strains growth *in vitro* and *in vivo* tests to prove their effectiveness as well as their cytotoxic effects against different cell-lines *in vitro* [3-10].

In Democratic Republic of Congo (DRCongo) a project was intiated since 1998 to evaluate the *in vitro* and *in vivo* antimalarial potential of several plants claimed to be effective against malaria by traditional healers in Kinshasa and some other Congolese regions. The obtained results were very interesting [5, 11-13]. Some medicinal plants needed extensive phytochemical studies leading to the isolation and structure elucidation of active constistituents. Among these plants, *Morinda morindoides* commonly named Nkonga bululu in Tshiluba, Nkongo bololo or Nkama mesu in Lingala and Kikongo in this country has long been used in villages and in towns for the treatment of some parasitic diseases such as malaria and amoebiasis with success. An aquoeus decoction from fresh leaves is employed for this purpose. Other medical indications include stomach pain, rheumatism, diarrhea, constipation associated with intestinal worms, scabies, hemorrhoids, mycosis, fatigue tonic for rachitic children and adults [14, 15]. The present study deals with the *in vitro* and *in vivo* evaluation of the potential antimalarial activity of some extracts and isolated constituents from *M. morindoides* leaves.

2. Materials and methods

2.1. Reagents

Ethanol absolute with analytical grade (purity: 99.99%) was purchased from Fisher Scientific (UK), n-butanol (purity: 99% extra pure), chloroform (purity: 99.97%), ethyl acetate (purity: 99.80%), methanol (purity: 99.80%) and petroleum ether 40-60°C (purity: 99.96%) were obtained from Acros organics (USA) and were of HPLC grade. Hydrochloric acid (purity: 25%) and isoamylic alcohol (purity: min. 99. 00%) were obtained from Merck (Germany) and were of analytical grade. Distilled water was used for the dilution of hydrochloric acid and the preparation of solvents for extraction (80% methanol).

2.2. Plant material

The leaves of *Morinda morindoides* (Baker) Milne-Redh. (Synoym: Gaertnera morindoides Baker (Rubiaceae) were collected in Kinshasa, DR Congo in Kinshasa in March 2004. The plant was idenfied by M. N. Nlandu of the Institute National d'Etudes et de Recherches en Agronomie (INERA of the University of

Kinshasa. A voucher specimen had been deposited at the herbarium of this institute. The leaves were dried at room temperature and reduced to powder.

2.3. Preparation of extracts and isolation

In the first time, 50 g of powdered dried leaves were macerated for 24 h and exhaustively percolated with ethanol. The macerate and percolate were combined and evaporated in vacuo to give a dried extract denoted as extract A (22.15g, 44.30 %). An amount of extract A (20g) was dissolved in 100 ml distilled water and filtered. The filtrate was exhaustively extracted with petroleum ether. The organic phase was treated as described above to give a semi solid dried extract denoted as fraction A1 (2.45g 12.25%). The remaining aqueous phase was acidified (HCl 0.2 N, pH 2-3) and heated for 30 min. After cooling, it was successively and exhaustively extracted with chloroform and isoamylic alcohol. The organic phases were evaporated in vacuo to give corresponding dried extracts denoted as fractions A2 (3.18g, 15.90%) and A3 (5.27g, 26.35%) respectively. The acid aqueous phase was treated as described above to give a dried extract denoted as fraction A4 (8.64g, 43.20%). On the other hand, 20 g of powdered dried leaves was macerated with 200 ml dichloromethane for 24 h. The mixture was filtered and the filtrate evaporated in vacuo to give a dried extract denoted as extract B (6.34g, 31.70%).

In the second time, another batch of powdered dried plant material (50g) was macerated for 24 h and exhaustively percolated with 80% methanol. The macerate and percolate were combined and treated as described above, yielding a dried extract denoted as extract C.(31.45g, 62.90%). An amount of extract A (20g) was dissolved in 100 ml distilled water and filtered. The filtrate was successively and exhaustively extracted with chloroform, ethyl acetate and n-butanol which were treated as described above to give corresponding dried extracts denoted as fractions C1(3.34g, 15.70%), C2 (4.17g, 20.85%) and C3 (5.27, 25.35%) respectively. The remaining aqueous phase was also evaporated in vacuo yielding a dried extract denoted as fraction C4(7.36g, 36.80%). A series of flavonoids including quercetin, quercetin 7, 4'-dimethylether, quercetin-3-*O*-rutinoside, quercetin-3-*O*-rhamnoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-rhamnoside, kaempferol-7-*O*-rhamnosylsophoroside, chrysoeriol-7-*O*-neohesperidoside, apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside; anthraquinones including alizarin and chrysarin, iridoids including gaertneroside, gaertneric acid, methoxygaertneroside, acetylgaertneroside, dehydrogaertneroside, dehydromethoxy-gaertneroside, epoxygaertne-roside and epoxymethoxygaertneroside were isolated from the 80% methanol extract by different chromatographic methods and identified by various conventional spectroscopic methods as previously described [16-18].

2.4. In vitro antiplasmodial activity

The *in vitro* antiplasmodial activity of a dichloromethane extract and an ethanol extract from *M. morindoides* leaves and the soluble fractions from the ethanol extract were evaluated against a Congolese the chloroquine-sensitive strain of *P. falciparum* from Institute of Tropical Medicine, Faculty of Medicine, University of Kinshasa, according to the method previously described in detail [11]. Briefly, 2 mg of each test sample (extracts and fractions) was dissolved in 2 ml ethanol 80% and diluted in two fold with culture medium (RPMI 1640) to give a series of test concentrations ranging from 1 to 100 µg/ml which were tested in triplicate against Congolese the chloroquine-sensitive strain of *P. falciparum* strain infected human blood. Quinine 2 HCl from Laboratoire

d'Analyse et de Contrôle des Médicaments et des Denrées Alimentaires (LACOMEDA), Faculty of Pharmacy, University of Kinshasa, was used as an antimalarial reference product and applied in six fold 4-fold dilutions (final concentrations from 0.1 to 0.005 µg/ml). IC_{50} values were derived from dose-response curves.

On the other hand, the *in vitro* semi-automated micro-dilution assay that measures the ability of extracts, fractions and isolated compounds to inhibit the incorporation of [1_H]-hypoxanthine into the malaria parasite was also used. By this technique, the *in vitro* antiplasmodial activity of an 80% methanol extract from *M. morindoides* leaves, its soluble fractions and isolated compounds was evaluated against the chloroquine-sensitive NF 54/64, clone A1A9 strain of *P. falciparum* from the Institute of Tropical Medicine, Antwerp, Belgium, according to the procedure previously described in detail [19]. Briefly, 2 mg of each sample was dissolved in 2 ml DMSO to give respective stock solutions of 1 mg/ml. Infected human red blood cells were exposed to serial sample dilutions (100 to 0.01 µg/l) from respective stock solutions of 1 mg/ml in microtiter plates for 72 h. Viability was assessed by measuring the incorporation of 25 µl [^{1}H]-hypoxanthine during the final 24 h of incubation by liquid scintillation counting. Chloroquine (Nivaquine, Rhône-Poulenc) was used in the same experiment as a reference and applied in six fold 4-fold dilutions (final concentrations from 0.1 to 0.005 µg/ml). The mean results obtained as counts per min were expressed as percentage of incorporation or growth inhibition compared to the negative control. IC_{50} values were derived from the sigmoid dose-response curve. Each sample was tested in triplicate.

2.5. In vivo antimalarial activity

The three crude extracts (ethanol, 80% methanol and dichloromethane) from *M. morindoides* leaves and the petroleum ether soluble fraction from the partition of the ethanol extract were assessed for their potential *in vivo* activity in a classical 4-day suppressive test against *Plasmodium berghei berghei* infections in mice according to the procedure previously described [12]. The ANKA strain of *P. berghei berghei* was obtained from the Institute of Tropical Medicine of Antwerp, Belgium. A standard innoculum of 1¯ x ¯107 of parasitized erythrocytes from a donor mouse was used to infect each mouse intraperitoneally. The samples were dissolved in Tween 80 and then given as a daily oral dose of 200 mg, 400 mg or 800 mg dry matter/kg body weight (0.2 ml of solution/mouse). Quinine 2HCl (10 mg/kg in water) was used as positive control. Fifteen mice (25 g body weight) were divided into groups of five (three mice for each dose of extract or fraction). The test mice were treated daily from day 0 (immediately after infection) to day 3. From day 0 to day 4, a thin smear was made from a tail-blood sample from each mouse and stained with Giemsa so that the level of parasitaemia (as the % of erythrocytes infected) could be evaluated (50% reduction of the number of schizonts with at least three nuclei, counted in 200 erythrocytes). On day 4, the mean level of the parasitaemia in each group of mice was determined so that the percentage chemosuppression could be calculated as : $[(A - B)/A] \times 100$ where A is the mean parasitaemia in the negative-control group and *B* the parasitaemia in the test group (n=3).

2.6. Acute toxicology

20 white, adult Swiss mice with a mean (S.D) body weight of 22 (2) g, from the Institute National de Recherches Biomédicales (INRB) in Kinshasa, were acclimatized to laboratory conditions and randomly divided into 4 groups of mice (one group of 4 mice for each extract

plus one control group given water). Each mouse was starved for 24 h prior to treatment by gavage with a single oral dose of dissolved extract in distilled water (5g/ Kg body weight) or diluent for 7 days. The mice were given food 30 min after the gavage. Each mouse was weighed and checked for signs of toxicity including death daily for 7 days.

2.7. Subacute toxicity

The procedure used was similar to that used to test acute toxicology, but all mice were treated with a daily oral dose of 5 g/kg bodyweight for 4 weeks. Each mouse was again starved for 24 h prior to each treatment and offered food 30 min later. Each mouse was weighed and checked for signs of toxicity and mortality daily until 30 days after treatment.

2.8. Histology

On day 30 after treatment, the animals used in the toxicology test were all killed by decapitation if no death was observed. The brains, hearts, large intestines, livers, lungs, kidneys and spleens were carefully dissected out and fixed in Boun's liquid, embedded in paraffin wax, cut into 3-5-µm sections and safranin. The histology so revealed in the organs of mice treated with extracts was compared with that seen in the organs of the control mice which had only been given water.

3. Statistical analysis

The statistical significance of difference in activity between control and test samples was assessed using Student's *t*-tests. p-values of 0.05 or less were considered significant.

Table 1. Antiplasmodial activity of some extracts and isolated compounds from *M. morindoides* leaves

Samples	Congolese strain of *P. falciparum* (IC_{50}, µg/ml)	NF 54/64 strain of *P. falciparum* (IC_{50}, µg/ml)
Ethanol extract	94.2 ± 3.4	
Petroleum ether	1.8 ± 0.2	
Isoamylic alcohol	15.3 ± 3.6	
Chloroform	8.8 ± 2.5	
Water phase	> 100	
Dichloromethane extract	> 100	
Quinine 2 HCl	0.039 ± 0.01	
Methanol extract		> 50
Chloroform		8.3 ± 1.6
Ethyl acetate		> 50
n-butanol		> 50
Water phase		> 50
Quercetin		5.5 ± 1.8
Alizarin		25.3 ± 3.2
Chryzarin		14.5 ± 2.7
Chloroquine		0.03 ± 0.01

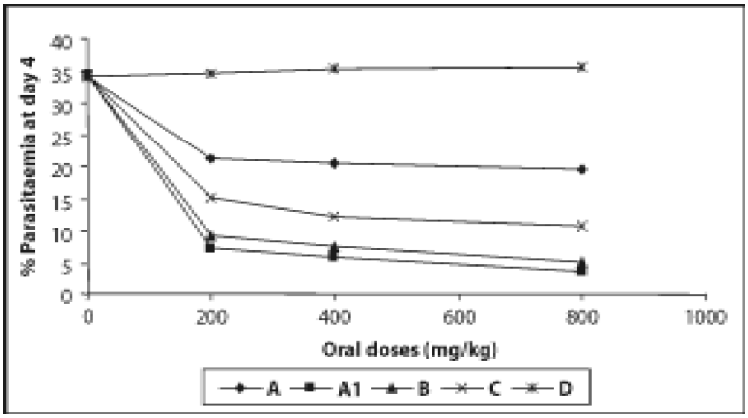

Fig. 1. Dose-dependent reduction of parasiteamia in infected mice with *P. berghei berghei* different extracts from *M. morindoides* leaves at day 4.

Legend: A: Ethanol extract, A1: petroleum ether soluble fraction from the partition of A extract, B: dichloromethane extract, C: 80% methanol extract, D: negative control. At an oral dose of 10 mg/Kg, quinine dihydrochloride used as a positive control produced 100% chemosuppression.

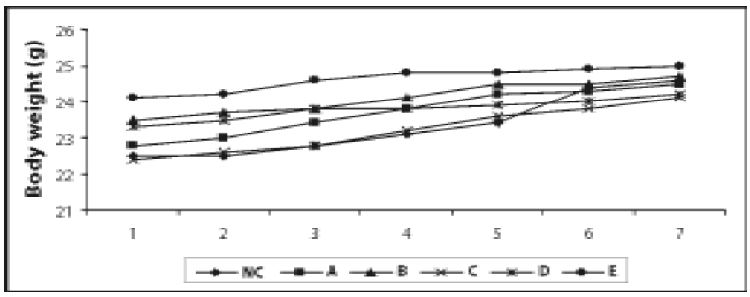

Fig. 2. Acute toxicity: comparison of the weight gain in the treatment mice with those in the corresponding untreated control mice. In the experiments, mice were treated with different dried extracts of *M. morindoides* leaves as a single oral dose of 5g/Kg.

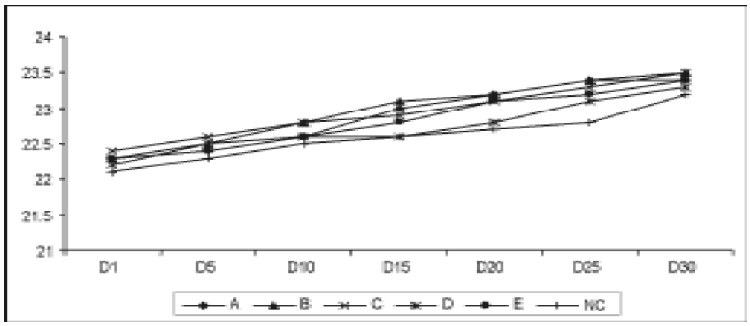

Fig. 3. Sub-acute toxicity: comparison of the weight gain in the treatment mice with those in the corresponding untreated control mice. In the experiments, mice were treated with different dried extracts of *M. morindoides* leaves as a daily oral dose of 5g/Kg.

Legend: A: aqueous, B: ethanol, C: 80% methanol, D: dichloromethane extracts respectively, E: petroleum ether soluble fraction from extract B. NC: negative control.

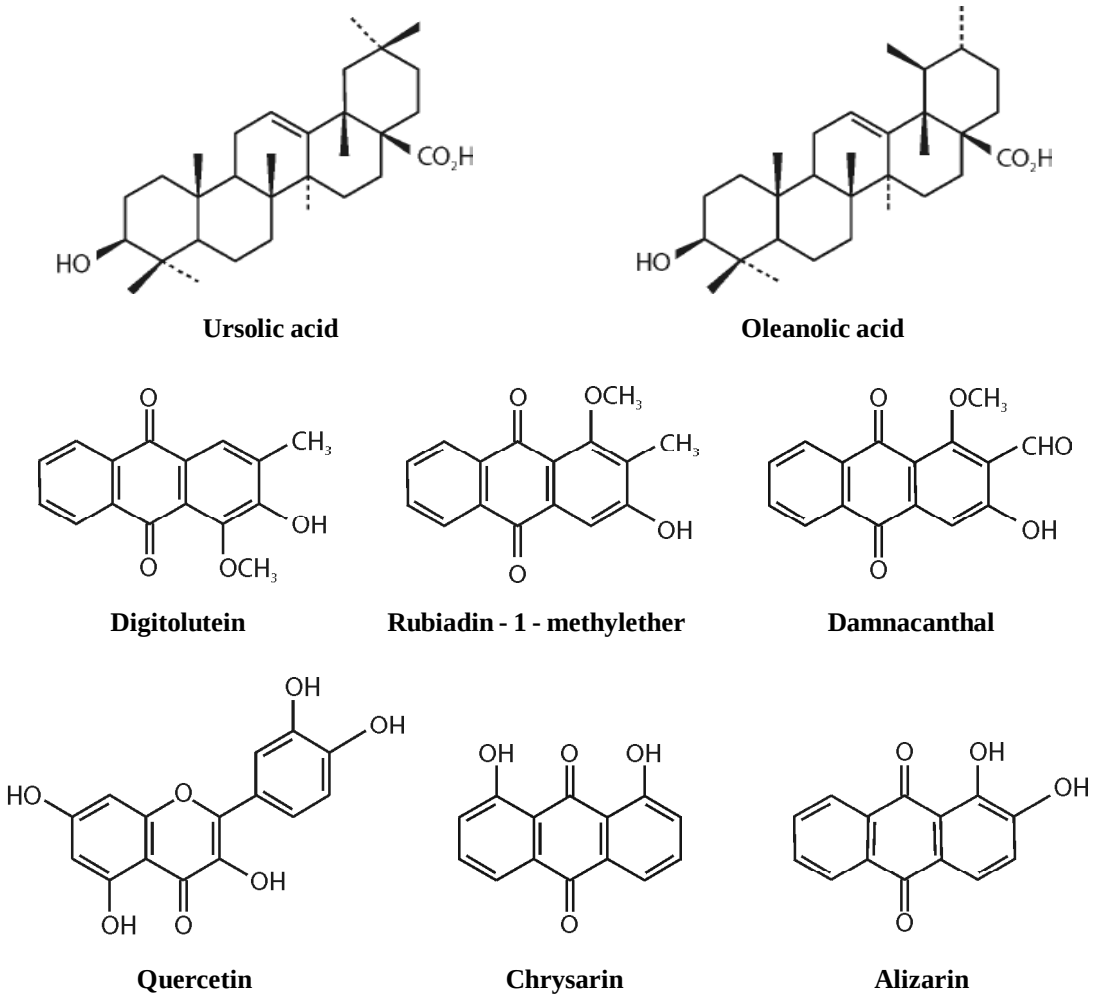

Fig. 4. Structures of some antimalarial compounds isolated from *M. morindoides* or *M. lucida* leaves

4. Results and discussion

The *in vitro* antiplasmodial activity of different tested samples form *M. morindoides* leaves are presented in Table 1. From these results, it was observed that the ethanol extract and its soluble fractions exhibited an antiplasmodial activity against the Congolese chloroquine-sensitive strain of *P. falciparum* at differents extents. The most active sample was the petroleum ether soluble fraction showing an IC_{50} value of 1.8 ± 0.2 µg/ml (very high activity), followed by good activity showed by the chloroform soluble fraction with IC_{50} value of 8.8 ± 2.5 ($p < 0.001$). TLC analysis of these fractions revealed the presence of terpenes and steroids as the major constituents, but were not the same as indicated by different Rf values of detected spots with Liebermann-Burchard's reagent (chromatogram not presented). The isoamylic alcohol soluble fraction exhibited a moderate antiplasmodial activity with IC_{50} value of 15.3 ± 3.6 µg/ml, but higher than that of the parent extract ($p < 0.001$).

The chemical composition of this fraction obtained by TLC analysis indicated that it contains flavonoid aglycones obtained after acid hydrolysis as described above, as major constituents, detected with Neu's reagent (1% methanolic diphenylboric acid ethanolamine complex). The ethanol extract showed a poor activity (IC_{50} = 94.2 ± 3.4 µg/ml), but significant compared to the negative control ($p < 0.001$) while the water soluble fraction and the dichloromethane extract were found to be inactive at the highest test concentration of 100 µg/ml.

On the other hand, only the chloroform soluble fraction from the partition of an 80% methanol extract of *M. morindoides* leaves exhibited a good antiplasmodial activity with IC_{50} value of 8.8 ± 2.5 µ/ml against the chloroquine-sensitive NF 54 strain of *P. falciparum*. The 80% methanol extract, its ethyl acetate, n-butanol and water soluble fractions were found to be inactive against this *P. falciparum* strain at the highest concentration of 50 µg/ml. This observation is also valid for all isolated flavonoid glycosides and iridoids from *M. morindoides* leaves. Quercetin isolated from the 80% methanol extract showed a good antiplasmodial activity with IC_{50} value of 5.5 ± 1.8 µg/ml while alizarin and chrysarin isolated from the chloroform soluble fraction displayed a moderate activity with IC_{50} values of 25.3 ± 3.2 and 14.5 ± 2.7 µg/ml respectively. The chloroform soluble fraction showed a higher activity than the two isolated anthraquinones ($p < 0.001$). This finding could not suspect a possible existence of synergetic effect between different constituents present in this active fraction since the possiblity to find minor constituents with a higher activity than the parent fraction could not be excluded. Other anthraquinones extracted from other *Morinda* species such as *M. lucida* root had been reported to exhibit an *in vitro* antiplasmodial activity [20]. Recently, it had been shown that the *in vitro* and *in vivo* antimalarial activity exhibited by the petroleum extract from *M. lucida* leaves was related to the presence of ursolic acid showing a IC_{50} value of 3.1 ± 1.3 µg/ml against the Congolese chloroquine sensitive strain of *P. falciparum* and producing more than 95% chemosuppression in mice infected with *P. berghei berghei*. Oleanolic acid which was also isolated from this plant species showed a moderate *in vitro* antiplasmodial activity (IC_{50} = 15.2 ± 3.4 µg/ml and produced at least 40% chemosuppression *in vivo* [7]. Other studies have also reported the *in vitro* antiplasmodial activity of extracts from *M. morindoides* leaves collected in other African countries against various *P. falciparum* strains. From these investigations, it had been shown that an 80% ethanol extract from the sample collected in Ivory Coast exhibited a very good activity against the chloroquine-resistant K1 strain of *P. falciparum* with IC_{50} value of 3.54 µg/ml [3]. The ethanol extract from the same plant material collected in Colombia inhibited the Colombian chloroquine-resistant FCB1 strain of *P. falciparum* growth with IC_{50} value of 11.6 ± 3.7 µg/ml and showed a low cytotoxic effect against mammalian cell lines MRC-5 (CC_{50} = 42.2 ± 3.2 µg/ml) [6]. However, the aqueous and ethanol extracts from the sample collected in Congo Brazzaville were found to be inactive against the Cameroon chloroquine-resistant FCM29 strain of *P. falciparum* (IC_{50} > 100 µg/ml) [8]. Although the tested *P. falciparum* strains are differents, this last result seems to be in good agreement of our observations for the activity of ethanol and 80% methanol extracts reported in the present investigation. Results from these different reports clearly show that the *in vitro* antiplasmodial activity of extracts of this medicinal plant seems to be mainly dependent on the type of *P. falciparum* strain used for

the testing. To our opinion, the chemical composition of these different samples collected in different areas seems not to play an important role for the manifestation of the activity because of different *P. falciparum* strains used for the testing. The contrary could be accepted and justified when these samples were tested against the same *P. falciparum* strain leading to obtaining of different results.

In vivo using a classical 4-day suppressive model, results reported in the present study show that at a daily oral dose of 200 mg/Kg, the ethanol extract only produced 33.3 ± 1.40 chemosuppression on day 4 less than 50%, but significant as compared to the negative control ($p < 0.001$). Parasitaemias in all the mice given the petroleum ether soluble fraction from the partition of the ethanol extract at the same oral dose fell with time, always to the value of 75.45 ± 2.71 by day 4 which was higher than that of the parent extract ($p < 0.001$). In contract with the antiplasmodial activity showed by the dichloromethane extract *in vitro* test, this extract produced good reduction of parasitamia of 73.2 ± 2.43% at day 4 at the same daily oral dose. TLC analysis of this extract revealed the presence of terpenes and seteroids as the major constituents. These two lipophylic samples were found to produce similar levels of chemosuppression ($p > 0.05$). Our results are in good agreement with those previously reported [12]. Curiously, the chemosuppression observed in the present study for the dichloromethane extract (73%) from *M. moridoides* leaves collected in March was markedly higher than that seen with the same extract obtained in the same manner of the leaves collected in August (33%). The same conclusion was also previously deduced suggesting the influence of the timing of plant material collection and the locality on the concentration level of antimalarial constituents [12]. In the present study, an 80% methanol extract produced a significant chemosuppression of parasiteamia of 54.3 ± 3.4% compared to the negative control ($p < 0.001$) and was higher than that seen with the ethanol extract ($p < 0.01$). Apolar extracts showed a higher activity in both test than the polar one ($p < 0.05$), activity which may be due to the presence of terpenes and steroids because these phytochemical groups extracted from other plant species had been reported to exhibit an antiplasmodial activity at different extents [21, 22]. All extracts and the petroleum ether soluble fraction from the partition of the ethanol extract showed a significant dose-dependent reduction of parasitaemia in infected mice by day 4 when tested at different daily oral doses from 200 to 800 mg/Kg (Fig. 1). For more tested *M. morindoides* samples, a good relation between the *in vitro* and *in vivo* antimalarial activity was observed.

Figures 2 and 3 show the acute and subacute toxic effects of different extracts from *M. morindoides* leaves. Results indicate that mice treated with an oral single dose of 5 g/kg of each extract continued to gain bodyweight at a similar rate to that seen in their respective untreated control groups within 7 and 30 days ($p > 0.05$). The LD_{50} for these extracts were assumed to be > 5 g/Kg body weight. None of the vital organs such as heart, lungs, kidney, liver, large intestines of both controlled and treated groups showed any unusual signs of lesions and all were found to be normal by a routine histology.

Other interesting antiprotozoal studies conducted on *M. morindoides* leaves have been recently reported on the antiamoebic activity of extracts, flavonoids and iridoids against *Entamoeba histolytica* and the effect of an ethanol extract against *Trypanosoma brucei rhodeseinse*. In the first study, it had

been shown that an aqueous extract (decoction) which is the typical traditional remedy and an 80% methanol extract from *M. morindoides* leaves exhibited a very good antiamoebic activity with IC_{50} values ranging from 1 to 4 µg/ml. The same level of activity was also found in the n-butanol soluble fraction from the partition of the 80% methanol in which some flavonoids and iridoids were isolated as mentioned above. Different iridoids isolated from the leaves of this medicinal plant had been shown to exhibit very good or good antiamoebic activity with IC_{50} values ranging between 1 to 8 µg/ml and flavonoid glycosides showed moderate and weak activity ($10 < IC_{50} < 90$ µg/ml) or were inactive ($IC_{50} > 100$ µg/ml) according to their chemical structures. All samples were also found to be devoid of cytotoxic effects against MTT-4 cell lines ($CC_{50} > 250$ µg/ml) [23, 24]. In the second study, a 90% ethanol extract from the same plant material was found to exhibit an antiprotozoal activity against *T. brucei rhodesiense* with IC_{50} value of 10 µg/ml [3]. These antiprotozoal activities highlight the potentiality of this medicinal plant as a new source of natural antiprotozoal agents.

Several other interesting biological activities of this medicinal plant had been previously reported in relation to its traditional uses. They include the anticomplement activity of iridoids isolated from the leaves [18], the anticomplement activity and the inhibitory effects of xanthine oxidase and scavenging of superoxide anions activity of isolated flavonoids isolated from the leaves which partly justify its use for the treatment of rheumatism [25, 26, 27], the spasmolytic and antibacterial activities of an aqueous extract (decoction) from the leaves supporting its traditional use to treat diarrhea of various origins [28], the contractile effect on isolated heart of mice of different fractions from ethanol extract of the leaves showing a cardioinhibition by a negative inotropic effect along with a negative chronotropic effect, effect antagonized by atropin [29] and gastrointestinal activity of an aqueous extract of the leaves producing a significant decrease in the frequency of defacation, severity of diarrhea in mice treated with castor oil. The extract also significantly inhibited the castor oil-induced intraluminal fluid accumulation with no effect on the weight of intestinal content indicating that the extract possesses significant antidiarrheal activity due to its inhibitory effects on gastrointestinal propulsion, fluid secretion and mediated through α_2-adrenergic system or nitric oxide pathway [30].

In conclusion, different traditional uses of *M. morindoides* leaves seem to be supported by various appropriated pharmacological tests previously reported. The present study clearly show that some polar and apolar extracts as well as isolated compounds from the leaves exhibited antiplasmodial activity at different extents that can partly justify and support its use for the treatment of malaria in traditional medicine. There is a need to pursue an extensive chemical investigation on the dichloromethane extract which showed an interesting chemosuppression and the petroleum ether soluble fraction which were the most active samples in both tests, leading to the characterisation of active components.

References.

1. Brown GV, Beck HP, Molyneux M, Marsh K. (2000) *Parasitol*. Today. 10: 448 - 451.

2. Olliaro PL, Yuthavong Y. (1999) *Pharmacol. Ther*. 81: 91-110.

3. Atindehou KK, Schmid C, Brun R, Koné, MW, Traore D. (2004) *J. Ethnopharmacol*. 90: 221-227.

4. Waako PJ, Gumed B, Smille P, Folb PJ. (2005) *J. Ethnopharmacol*. 99: 137-143.

5. Mesia,KG, Tona LG, Penge O, Lusakibanza M, Nanga TM, Cimanga KR, Apers S, Van Miert S, Totté J, Pieters L, Vlietinck AJ. (2005) *Ann. Trop. Med. Parasitol*. 99: 345-357.

6. Zirihi N, Mambu L, Guéde-Guina F, Bodo B, Grellier P. (2005) *J. Ethnopharmacol*. 98: 281-285

7. Cimanga KR, Tona LG, Mesia KG, Kambu KO, Bakana PD, Kalenda DTP, Penge OA, Muyembe TJJ, Totté J, Pieters L, Vlietinck AJ. (2006) *Pharm. Biology*. 44: 677-681.

8. Mbatchi SF, Mbatchi B, Banzouzi JT, Bansimba T, Nsonde Ntandou GF, Ouamba JM, Berry A, Benoît Vical F. (2006) *J. Ethnopharmacol*. 104: 168 - 174.

9. Okonon JE, Antia BS, Igbossoiyi AC, Essein EE, Mbanguru HOC. (2007) *J. Ethnopharmacol*. 111: 464-467.

10. Irungu BN, Rukunga GM, Mungai GM, Muthanna GN. (2007) *Afr. J. Bot*. 73: 204-207.

11. Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S, de Bruyne T, Pieters L, Totté J, Vlietinck AJ. (1999) *J. Ethnopharmacol*. 68: 193-203.

12. Tona L, Mesia K, Ngimbi NP, Chirimwami B, Akond'Akoka A, Cimanga K, de Bruyne T, Apers S., Hermans N, Totté J, Pieters L, Vlietinck AJ. (2001) *Ann. Trop. Med. Parasitol*. 65: 47-57.

13. Tona L, Cimanga KR, Mesia K, Musuamba TC, de Bruyne T, Apers S, Hermans N, Van Miert S, Pieters L, Totté J, Vlietinck AJ. (2004) *J. Ethnopharmacol*. 93: 27-32.

14. Kambu, K. (1990) *Eléments de Phytothérapie Comparés. Plantes Médicinales Africaines*. Centre de Recherches Pédagogiques, Kinshasa: 61-62.

15. Neuwinger HD. (2000) *African Traditional Medicine. A Dictionary of Plant Use and Applications*. Medpharm Scientific Publishers, Stuttgart: 343-344.

16. Cimanga K, de Bruyne T, Lasure A, Li Q, Pieters L, Claeys M, Vanden Berghe D, Kambu K, Tona L, Vlietinck AJ. (1995) *Phytochemistry*. 38: 1301-1303.

17. Cimanga K. (1997) Biologically active constituents from two African medicinal plants: *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Periplocaceae) and *Morinda morindoides* (Baker) Milne-Redhead (Rubiaceae). PhD Thesis. University of Antwerp, Belgium.

18. Cimanga K, Hermans N, Apers S, Van Miert S, Van den Heuvel H, Claeys M, Kambu K, Tona L, Pieters L, Totté J, Vlietinck AJ. (2003) *J. Nat. Prod*. 66: 97-102.

19. Desjardins RH, Canfied ME, Hayness CJ, Chubby D. (1979) *Antimicrob. Agents Chemother*. 16: 710-718.

20. Koumaglo K, Gbseassor M, Nikabu O, De Souza C, Werne W. (1992) *Planta Med*. 58: 533-534.

21. Christensen SB, Kharazmi A. (2001) In: Tringali, C (Ed.). *Bioactive Compounds from Natural Sources. Isolation, Characterisation and Biological Properties*.Taylor & Francis: London and New-York: 433-472

22. Caniota R, Puricelli L. (2003) *Critical Rev. Plant Sci*. 22: 79-105.

23. Cimanga KR, Kambu K, Tona L, Hermans N, Apers S, Totté J, Pieters L. Vlietinck AJ. (2006) *J. Ethnopharmacol*. 107: 83-90.

24. Cimanga K, Kambu K, Tona L, Hermans N, Apers S, Totté J, Pieters L, Vlietinck AJ. (2006) *Planta Med*. 72: 751-753.

25 .Cimanga K, de Bruyne T, Lasure A, van Poel B, Pieters L, Kambu K, Tona L, Vanden Berghe D, Vlietinck AJ. (1995) *J. Nat. Prod*. 58: 372-378.

26. Cimanga K, de Bruyne T, Van Poel B, Claeys M, Pieters L, Kambu K, Tona L, Bakana P, Vanden Berghe D, Vlietinck AJ. (1997) *Planta Med*. 63: 220-223.

27. Cimanga K, de Bruyne T, Hu T, Cos P, Aper S, Pieters L, Totté J, Kambu K, Tona L, Vlietinck AJ. (1999) *Pharm. Pharmacol. Comm*. 5: 419-424.

28. Tona L, Kambu K, Mesia K, Cimanga K, Apers S, de Bruyne T, Pieters, L, Totté J, Vlietinck AJ. (1999) *Phytomedicine* 6: 59-68.

29. N'Guessan J.D, Trébisson JND, Bahi C, Guede-Guina F. (2001) *Rev. Iv. Sci. Tech*. 03: 129- 141.

30. Adeyemi OG, Okpo SO, Adesanga, AA. (2003) *West Afr. J. Pharmacol. Drug Res*. 19: 22-27.