

## Evaluation of Antiplasmodial and Antifungal Activity of *Morinda morindoides* (Baker) Milne-Redh (Rubiaceae), an Ivoirian Traditional Medicinal Plant

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### ABSTRACT

*Morinda morindoides* (Rubiaceae) is an indigenous tree of West Africa. This tree is used in Côte d'Ivoire against malaria. In this study, we investigated the *in vitro* antiplasmodial and antifungal activity of the leaves of *M. morindoides*.

*M. morindoides* leaves were collected, air dried and made into a fine powder. Aqueous extracts (Aqe), ethanol (Eeth), ethyl acetate (EAc) and acetate-water (EAc-H<sub>2</sub>O) were performed. EAc extract was tested on *Plasmodium falciparum* and *Aspergillus fumigatus*. IC<sub>50</sub> values of different extracts is ranked in the following order: 6.1 (EAc) < 17.8 (Eeth) < 21.5 (Aqe) < 46.5 (EAc-H<sub>2</sub>O) for *P. falciparum* and 1.3 (EAc) < 6.1 (Eeth) < 12.47 (Aqe) < more than 300 (EAc-H<sub>2</sub>O) for *A. fumigatus*, the ethyl acetate extract being the most active against both pathogens. These results show that *M. morindoides* leaves display significant antiplasmodial and antifungal activity, which justifies its use in traditional medicine against malaria and mycoses.

**Keywords:** *Aspergillus fumigatus*; *Morinda morindoides*; mycoses; malaria; *Plasmodium falciparum*

### INTRODUCTION

In Côte d'Ivoire and elsewhere in Africa, medicinal plants occupy a place of choice in the treatment of various diseases<sup>1</sup>. The survival and intensification of this practice today despite the prodigious development of modern medicine are related to several factors, among which may be mentioned economic constraints and sociocultural factors. Currently, the proportion of the African population uses traditional medicine is estimated at over 80%<sup>2</sup>. This medicine, which often actually proven, relies almost entirely on herbal medicine. Faced with an empirical manipulation by traditional healers in which there is no control of dosing and control of activity, it seems important to involve advantage bodies and scientific structures in the medical and pharmaceutical knowledge and use of plants. It is in this context that we are interested in antimalarial and antifungal activity of *Morinda morindoides*, a plant used against fever and diarrhea in Côte d'Ivoire<sup>3</sup>.

### MATERIALS AND METHODS

*Vegetable material and preparation of extracts*

Vegetable material consisted of leaves of *M. morindoides* collected in the area of the western center of Côte d'Ivoire. The leaves were washed, dried safe from sun and pounded in fine powder with an electric crusher (IKA Labortechnik™, standard MFC). Powder was extracted according to Zihiri and Kra<sup>4</sup> as follows: One hundred grams of powder were macerated in distilled water during 48 hours. The obtained homogenate was filtered successively on cotton then on Whatman paper 3 mm. The filtrate is first reduced using a rotary evaporator BÜCHI type at 60 °C, then collected brown paste is lyophilized<sup>4,5</sup>. We obtained the total aqueous extract (Aqe). Using a magnetic stirrer, five grams of aqueous extract was dissolved in 100 mL of a hydroalcoholic solution (70 % ethanol, 30 % distilled water). The supernatant was collected and lyophilized. We obtained 70 % ethanol extract (Eeth). Five grams of 70 % Eeth was dissolved in 100 mL of a solution composed of a distilled water and ethyl acetate mixture (v/v). The whole, after homogenization, is decanted, two phases are obtained : a supernatant which is ethyl acetate and a lower phase which is distilled water. The two phases are collected, lyophilized

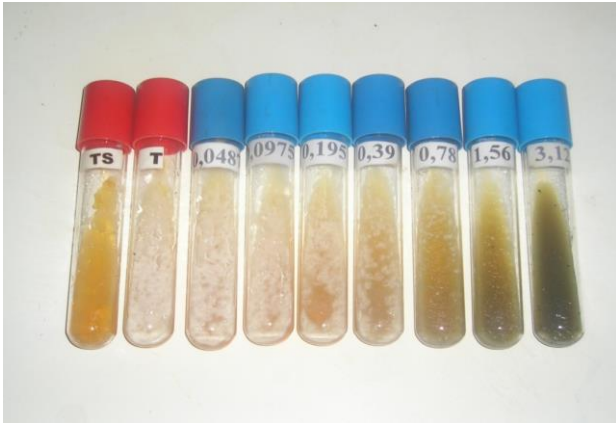


Figure 1: Appearance of *A. fumigatus* cultures after 48 hours of incubation at 30 °C TS : Sabouraud Control T : Growth Control

and reduced. We obtained the ethyl acetate extract (EAc) and the acetate water extract (EAc -H<sub>2</sub>O).

The four extracts Aqe, Eeth, EAc and EAc-H<sub>2</sub>O were tested on *in vitro* growth of the strains of *A. fumigatus* and *P. falciparum*.

#### *In vitro* antifungal assay on *Aspergillus fumigatus*

Assays were realized on a strain of *Aspergillus fumigatus* kindly by Laboratory of Mycology of the UFR of Medical sciences of Félix Houphouët Boigny University (Abidjan, Côte d'Ivoire). The strain was isolated on a patient affected by AIDS. The incorporation of *M. morindoides* to agar Sabouraud (OXOID Ltd) was made according to method of the double dilution, out of leaning tubes. The series comprise, for each extract, 9 test tubes including 7 test tubes containing different extract dilutions and 2 control tubes without extract (one being used as control of growth of germ; the other without germs being used as control of sterility of culture medium). For the 9 test tubes, the extract concentrations vary from 300 to 4.7 mg/mL for Aqe and from 50 to 0.78 mg/mL for the other extracts.

After incorporation of extracts, tubes were sterilized by autoclaving (121°C, 15 min) and let cooled and solidified tilted at temperature of room<sup>5,6</sup>. The antifungal assay was carried out by inoculating the culture of 1000 cells of *A. fumigatus* per tube. The cultures were incubated at 30°C during 48 h. The colonies of *A. fumigatus* were then counted and the growth was expressed as a percentage of survival and, calculated by comparison to the 100% of survival in the tube of control of growth<sup>6,7</sup>.

#### *In vitro* antiplasmodial assay on *P. falciparum*

The *P. falciparum* strain used was the multi-resistant strain K1 isolated in Thailand. It provided us by Team BAMEE (Biodiversity and Adaptation of the Eucaryotes Microorganisms to their Environment). For *in vitro* culture of *P. falciparum*, we used the isotopic alternative of the microphone-test (plate of 96 wells) of Riechman adopted by WHO<sup>8</sup>. This technique measure and quantify the capacity of drug to inhibit the growth of *P. falciparum* at the trophozoites stage.

In this technique, the strains are incubated at 37°C in an impoverished in oxygen and enriched with carbon dioxide with 95% of humidity. After 24 h, plates were removed and added tritiated hypoxanthine (0.5 µCi by well). The

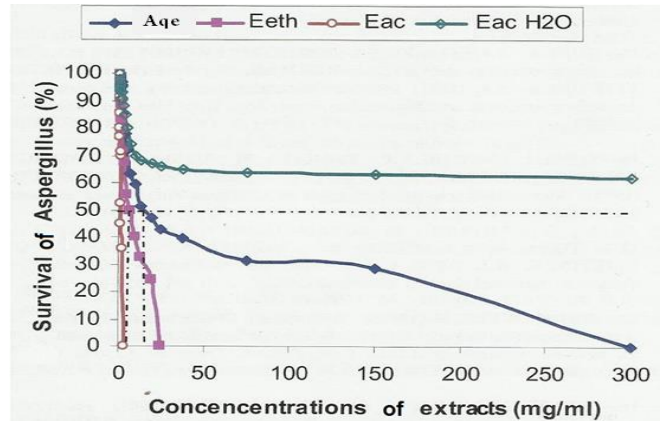


Figure 2: Sensitivity of *A. fumigatus* in the presence of *Morinda morindoides* extracts

plates were again returned to incubator for 24 h. After the incubation, the plates were frozen and thawed. Freezing and thawing of plates free plasmodial DNA radiolabeled by hypoxanthine. The DNA is recovered after washing on a filter paper in a rectangular fiberglass tape with a cell collector. Once the collection is complete, the paper was removed and dried. The radioactivity was measured using a Wallac MicroBeta counter. All results were expressed on a listing.

## RESULTS

### *Antifungal activity of M. morindoides*

After 48 hours incubation at 30°C, we observed compared to the control, a gradual decrease in the number of colonies gradually as the concentration of samples increases in the test tubes, it was observed for all series (See Figure 1). Experimental data translated as curves are represented on the Figure 2. IC<sub>50</sub> and MFC were graphically determined (See Table I). In general, all curves had regularly decreasing form, with more or less strong slopes according to extracts. Except for the curve of the Eac-H<sub>2</sub>O extract, the other curves cut the x-axis at various levels according to the extracts.

### *Antiplasmodiale activity of M. morindoides*

According to the standards of team BAMEE, a plant extract has an antiplasmodial activity when the given IC<sub>50</sub> is lower than 50 µg/mL (IC<sub>50</sub> < 50 µg/mL). The results of the *in vitro* antiplasmodial activity of *M. morindoides* extracts are presented in table II. The extracts of *M. morindoides* have an action on K1 strain. However, the EAc fraction has the best antiplasmodial *in vitro* activity.

## DISCUSSION

In traditional medicine of Côte d'Ivoire, plants are often used to fight malaria and mycoses. One of these plants was tested for its action against *Plasmodium* and *Aspergillus*. The analysis of our results showed that *A. fumigatus* was sensitive to all extracts. Our results showed that there was a progressive reduction amongst colonies as the concentration of the extracts increases in the tubes. After 48 h of incubation at 30°C, we observed a clear and effective inhibition in our series of tubes according to the ranges of concentration. We obtained the following MFC :

Table 1 : Antifungal activity of *M. morindoides* against *Aspergillus fumigatus*

	Antifungal extracts parameters (n = 6 *)	
	IC <sub>50</sub> (mg/mL)	MFC (mg/mL)
Aqueous extract (Aqe)	12,47 ±0,75	300
Ethanol extract 70% (Eeth)	6,1 ±2,1	12,5
ethyl acetate extract (EAc)	1,25 ±0,5	3,125
acetate-water extract (EAc H <sub>2</sub> O)	>300	>300

n: number of experiment repetitions

Table 2 :Antiplasmodial activity of *M. morindoides* against resistant strain K1

Extracts	IC <sub>50</sub> (µg/mL) strain K1 (n*=3)
Aqueous extract (Aqe)	21,50 ± 0,57
Ethanol extract 70% (Eeth)	17,87 ± 0,58
ethyl acetate extract (EAc)	6,12 ± 0,27
acetate-water extract (EAc H <sub>2</sub> O)	46,48 ± 0,48

n: number of experiment repetitions

300mg/mL for aqueous extract, 12.5 mg/mL for the ethanolic 70% extract and 3.1 mg/mL for the ethyl acetate extract. The ethyl acetate extract H<sub>2</sub>O has an unspecified MFC.

The decrease of survival curves illustrated that the extracts were active according to a dose-response relationship (See Figure 2 and Table I).

The results of this study showed that the partition of the aqueous extract in the mixture of solvents ethanol 70%, distilled water 30% then the partition of the alcoholic extract in a second mixture of solvents acetate of ethyl-water, leads to an ethyl acetate extract which improves the anti-aspergillosis activity of the extracts of *M. morindoides*. The report of effectiveness established on the basis as of MFC, watch that:

- $MFC_{Aqe} / MFC_{Eeth} = 300/12.5 = 24$
- $MFC_{Aqe} / MFC_{EAc} = 300/3.1 = 96$
- $MFC_{Eeth} / MFC_{EAc} = 12.5/3.1 = 4$

That means that the ethyl acetate extract is ninety-six (96) times more active than the aqueous extract and four (4) times more active than the ethanolic 70% extract. We thus deduce from this analysis that the ethyl acetate extract is most active, because the values of its antifungal parameters (IC<sub>50</sub>, MFC) are not only lowest but also it improves to a significant degree (96 times) the activity of the basic aqueous extract.

This extract thus concentrates much more active ingredients. We can thus deduce that the method of extraction which implements successive partitions of the extracts in mixtures of solvents allows a better concentration of the active ingredients. These active ingredients which are soluble molecules in ethanol and the ethyl acetate could be either terpenes, or alkaloids and/or vegetable oils. Being given that we worked under the same conditions as Zirihi and al. in 2007<sup>5</sup>, we can deduce that *M. morindoides* is more active on *in vitro* growth of *A. fumigatus* than *Mitracarpus villosus* (Rubiaceae) and *Spermacoce verticillata* (Rubiaceae). Indeed these authors found parameters antifungal higher (MFC = 100 mg/mL

for the ethanolic 70% extract of *S. verticillata*; MFC = 50 mg/mL for the ethanolic extract of *M. villosus*).

After all that we observed, we can say that our approach is acceptable because it enabled us to improve considerably the anti-aspergillosis activity of *M. morindoides* while passing from the aqueous total extract to the ethyl acetate extract (96 times).

The aqueous extract of *M. morindoides* has also an antimalarial activity on the resistant strain K1 (See Table II). This antimalarial action could justify the use of this plant in the treatment of the fevers and/or malaria.

The fractionation of the aqueous total extract enabled us to separate from the groups of molecules having a better schizonticide activity. Thus the fraction of ethyl acetate of *M. morindoides* is the fraction having a better antimalarial activity. The ethyl acetate fraction thus contains a group of molecules which confer on this plant its effectiveness against malaria. This extract of *M. morindoides* could thus be used for stage the resistance of *P. falciparum* to chloroquine.

A series of fractionation of the ethyl acetate fraction would enable us to have an antiplasmodial activity to improve and even insulation of the active molecule of this plant.

## CONCLUSION

Phytotherapy, name erudite of medicine by the plants can occupy an important place in the treatment of several pathologies. Rather than to oppose them to the classical drugs, the remedies containing plants can take a complementary place in the assumption of responsibility of the current affections. The medicinal herbs, in general well tolerated, appropriate to certain diseases, at the same time curative and preventive, must occupy an important place in therapeutic.

The resistance of *P. falciparum* to conventional molecules is currently a problem in the fight against malaria in African countries<sup>9</sup>. This situation requires the taking into consideration of the traditional knowledge in the fight against this endemic disease.

Our study showed that fractions of medicinal herbs used against the fevers and/or malaria have an activity against the resistant blood shape of *P. falciparum*.

In order to develop a better formulation and posology of antimalarial bases of plants, a collaboration should be developed between the guarantors of the ancestral knowledge and the various research laboratories. It is at this price that Africa and particularly Côte d'Ivoire will be able to gain a better profit from its flora.

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