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Original Article

IN VITRO BEHAVIOUR OF *PLASMODIUM FALCIPARUM* STRAINS BY ALKALOIDS AND TANNINS EXTRACTED FROM ROOT OF *MITRAGYNA INERMIS*, A MEDICINAL PLANT

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ABSTRACT

Objective: *Mitragyna inermis* (Willd.) O. Ktze is a plant belonging to the *Rubiaceae* family. It is used in West Africa by traditional healers for treatment of accesses febrile generally bound to access malaria, but also to treat several other pathologies. We have collected a medicinal plant, *Mitragyna inermis* root, currently used for malaria treatment in Burkina-Faso.

Methods: Alkaloids and Tannins were extracted by using conventional methods and antimalarial activities were tested.

Results: After extracted Alkaloids and Tannins, the *in vitro* culture of *Plasmodium falciparum* strain isolated from infected patients showed that the alkaloids extracts presented an excellent antiplasmodial activity on *P. falciparum* strain (IC_{50} = 2.36 and 2.56 µg/ml respectively) after 24 and 48h of incubation at 37 °C. On the other hand, the tannins extract no presented antiplasmodial activity (IC_{50}>100 µg/ml) but presented an important maturity of *P. falciparum* strains, letting foretell a possible use of the polyphenolic compounds by *P. falciparum* as a source of cellular carbon and energy.

Conclusion: These results although exploratory are of fundamental importance for the research in biochemistry and medicine for find new antimalarial prototypes.

Keywords: *Mitragyna inermis*, Malaria, Roots, Tannins, Alkaloids, *Plasmodium falciparum*.

INTRODUCTION

Malaria remains a major public health problem in tropical developing countries, particularly in Africa [1]; leading to more than 219 million cases each year and mostly children less than five years [2]. The *Plasmodium falciparum* parasite is becoming resistant to standard antimalarial drugs, which need a continuous effort of research for new antimalarial drugs. The topic of new antimalarial drugs from traditional medicines has been largely reviewed in recent years [3]. In this view, plants resources are potential targets for research and development of alternative malarial drugs, with novel modes of action [4].

Plants are a rich source for new drugs and one of the promising approaches on malaria research is the investigation of plants activity used by traditional healers [5, 6]. In Africa, a considerable portion of the population uses plants for fever or malaria treatment, without any scientific evidence of efficacy and safety.

Mitragyna inermis (Willd.) O. Ktze is a plant belonging to the *Rubiaceae* family, commonly used to treat malaria and several other pathologies [7]. The inhibition effect of those compounds on *Plasmodium falciparum,* in particular, is still demonstrated. Since the appearance of chemio-resistances of *Plasmodium falciparum* to the commonly used anti-malaria drugs, the researchers of the developing countries are showing a particular interest on medicinal plants rich on alkaloids as a source of new anti-malaria drugs.

If the antifungal and antibacterial properties of tannins have been demonstrated, it is not the case for their anti-parasitic properties. Until now, through the few studies achieved to this subject, it comes out again that the tannins have a weak anti-parasitic activity on *Plasmodium falciparum* [8, 9].

Indeed, it remains important to underline that certain microorganisms seems to be nevertheless indifferent or, can degrade the tannins [10]. In particular, the tannins that are able to be hydrolyzed (Gallo tannins) via the enzyme tannase (acyl-tannin hydrolase), this enzyme is produced by a range saprophyte fungi such as *Aspergillus* and *Penicillium* [11], but also the yeast of the gender *Candida*. The condensed tannins (catechic) have been considered as less or non-bio-degradable and the rare work has demonstrated that the *Aspergillus* and *Penicillium* might degrade apple's tannins or the procyanidin like structure, the condensed tannins constituents [12, 13]. Several bacteria of the *Bacillus, Pseudomonas* and *Klebsiella* genera have been shown to use the gallotannins as an organic source of carbon [14].

This article greats the results demonstrating an eventual biodegradability of roots alkaloids and tannins extracts of *Mitragyna inermis* by the *Plasmodium falciparum* strains on *in vitro* culture; although we were looking for the activity of alkaloids and tannins extracts.

MATERIALS AND METHODS

Plant material

The roots of *Mitragyna inermis* were harvested in the surrounding flora of the city of Ouagadougou and dried at ambient temperature (25 to 30 °C). They are carried out in a semi-industrial grinder and are finely pulverized. The obtained powder is preserved in the tightly plastic bags.

Biological materials

The biological material is constituted of the *Plasmodium falciparum* strains isolated from five infected children aged of 2 to 7 y from the Barogo village, situated at seven kilometers from the city of Ouagadougou, after having collected the information concerning a possible malaria treatment or prophylaxis of the children before the exam previously.

The selected subjects showed a blood parasite density ranging from 500 and 10000 trophozoites/µl of blood. For each children, 10 to 15

mL of blood are collected in a vacutainer tube containing an anticoagulant (EDTA 0.025 %), and transported as quick as possible to the laboratory for culture initiation.

The mixture was centrifuged at 2000 g for 7 min; at 25 °C. The serum was collected and heated in a water bath at 56 °C for 30 min. The pellet containing the infected red blood cells was put on RPMI-1640 medium up to an hematocrit of 10%, supplemented with autologous serum, HEPES buffer, sodium bicarbonate, streptomycin and L-glutamine.

150 µl of cellular pellet were introduced in each microplate wells and 50 µl of plant extract added. The microplate was sealed with parafilm, and incubated for 24 to 48 h at 37 °C, under CO_2 enriched atmosphere. After incubation, thin blood smears were colored with Giemsa and read under a photonic microscope. The trophozoites and schizontes were quantified as per µl of blood. The maturation and inhibition percentage (%) of the schizontes growth are given using the following formulas:

$$\%\ \text{maturation} = \frac{\text{average schizontes for a given concentration}}{\text{average schizontes on the control wellw}} \times 100$$

$$\%\ \text{inhibition} = 100 - \%\ \text{of schizontes maturation}$$

Preparation of crude extracts

The air dried plants were powdered using a semi-industrial crusher. Alkaloids were extracted according to method described [15], modified as follows: after humidification with NH_4OH (12.5%), alkaloids were extracted using petroleum ether and treated with H_2SO_4 (5%). The acid aqueous solution was washed with hexane, alkalinized with Na_2CO_3 (10%), and extracted with chloroform. The organic solution was washed with water, dried over Na_2SO4, and evaporated. Alkaloid bases were dissolved in acetone with Hydrochloric acid (HCl) vapour. The presence of hydro chlorate alkaloid residue was confirmed using the Mayer and Dragendorff test.

The powder was de-lipided and tannins were extracted using acetone. After elimination of pigments, the acetone was evaporated, and the tannin precipitates were characterised by $FeCl_3$ in accordance with CEE recommendations [16]. Quantitative studies were conducted using gravimetric analysis. For *in vitro* antiplasmodial activity, stock solutions of alkaloids and tannins at 5.000 µg/ml were prepared after filtration using 0.22 mm Millipore membrane. A chloroquine stock was prepared at 1.000 µg/ml.

Antiplasmodial activity

A chloroquine-sensitive strain (3D7) and chloroquine resistance strain (W2) of *P. falciparum* were continuously cultured according to a modified method [17].

The antimalarial activity was assessed by incubating parasites cultures with four concentrations (1250, 125, 12.5 and 1.25µg/ml) of each plant extracts for 24h and 48h, and all tests were conducted in duplicate in a 96 well flat-bottom culture plate (Costar, UK). Chloroquine diphosphate (Sigma) was used as a positive control and drug-free medium as a negative control. After incubation, thin blood films were prepared and stained with Giemsa; two trained microscopists determined the number of infected red blood cells per well independently. In case of a more than 10% discrepancy, a third reading was done. The IC50 value which represents the amount of the compound required to inhibit 50% of the parasite growth of the drug-free control was then calculated. The curves of % of the inhibition = f(-log[plants extract]) follows a polynomial regression to order 2. According to several authors [17], IC50 were classified as follows: (1) IC50 less than 5 µg/ml, the extract was considered highly active, (2) from 5 to 10 µg/ml, the extract was considered moderately active, and (3) over 10 µg/ml, the extract was considered inactive.

RESULTS AND DISCUSSION

Quantitative and qualitative analyses of plant extracts

The qualitative analysis showed that *Mitragyna inermis* roots are rich in alkaloids and tannins. Quantitative analysis showed that *Mitragyna inermis* roots present 0.4±0.02% of alkaloids and 5±0.03% of tannins. The quantitative study demonstrated that the tannin contents obtained by spectrophotometric method were 0.08% and alkaloids content determined using by gravimetric method was 0.29%. Those results are in line with that obtained using qualitative analysis.

Pharmacodynamic studies of alkaloids and tannins of *Mitragyna inermis*

Inhibitory effect of alkaloids on *in vitro* plasmodium falciparum culture

The results obtained for the inhibitory test on *Plasmodium falciparum* strains growth are presented in table 1. The quantification of the inhibitory effect of plant extracts is based on the IC_{50} (Concentration of 50% inhibitory growth of parasites) value.

The probate analysis of the relationship between the algorithmic dose and the percentage inhibition of the *Plasmodium falciparum* strains (schizonts') growth called, log(curve/probate), gave a regression curve from which the CI_{50} can be calculated.

The alkaloids extracts from *Mitragyna inermis* root, presented after 24 and 48h of incubation respectively 2.35µg/ml and 2.56µg/ml. The curve: % inhibition = f (-log [alkaloids]), after 24 and 48h of incubation is presented in fig. 1A and fig. 1B.

According to [17] work, the inhibitory effect of plant extracts on *Plasmodium falciparum* is excellent if its CI_{50} is less than 0.05 mg/ml. From our study, the alkaloids from *Mitragyna inermis* roots with CI_{50} of 0.027 and 0.013 mg/ml after 24 and 48 h of incubation, respectively; can be considered as highly inhibitory of the growth rate of *Plasmodium falciparum in vitro* [18]. This result is not surprising since the role of alkaloids (quinine, chloroquine, artemisinin) is well established on the prophylactic and curative treatments of malaria.

Activatory effect of tannins on *in vitro* Plasmodium falciparum culture

The results showing the activatory role of tannins on the *Plasmodium falciparum* strains *in vitro* are presented in table 2.

In the absence of a reliable method of quantification of the *Plasmodium falciparum* strains maturation in the presence of tannins, fig. 2 presents the curve:

% inhibition = f (-log [alkaloids]).

Table 2 above and fig. 2 permits let us appreciate the maturation of the schizonts of *Plasmodium falciprum*. This maturation is characterized by the increase of the number of schizonts to the concentrations of 0.05 and 0.005 mg/ml of tannins compare to the control (number of schizonts obtained in the absence of the active compound).

Such a result suggests the hypothesis of a possible biodegradation of the tannins by plasmodium and by way of consequence the use of this polyphenolic compound by the hematozoaire of malaria as a cellular carbon source. It remains important nevertheless to specify that this result has been obtained, whereas we were looking at the schizonticidal effect of the tannins on the *Plasmodium falciparum* culture. From then on, several complementary studies must be done, most important being the kinetics of deterioration of the tannins by the parasite.

The method of biodegradation of the tannins by the loss of the astringent power seems to be the more indicated, since the loss of the astringency of the tannins in the presence of a microorganism can justify its biodegradability [14].

However, a spectrophotometric method can be put to profit to follow the kinetics of deterioration of the tannins, from the moment these compounds absorb at a given wavelength. Finally, we insist once besides, on the preliminary character of these results.

Table 1: Number of *P. falciparum* strains (schizontes) per µl of blood after 24h incubation with various of concentrations of alkaloids from *Mitragyna inermis* roots (% of inhibition is reported as IC50 values)

	***P. falciparum* strains number/µl before Alkaloids using**							
Alkaloids extracts	**Isolate1**	**Isolate2**	**Isolate3**	**Isolate4**	**Isolate5**	**Total**	**%Inhibition**	**IC50 (µg/ml)**
Concentrations in µg/ml (log)	**1480**	**2780**	**1060**	**540**	**9750**	**15610**		
	***P. falciparum* strains number/µl after Alkaloids using**							
1250 (3,09)	0	0	0	0	0	0	100	
125 (2,09)	0	0	8	0	8	16	100	2.35
12.5 (1,09)	820	1006	840	164	3480	6310	60	
1.25 (0,09)	1068	1270	1060	264	7430	11092	29	

Table 2: Number of *P. falciparum* strains (schizontes) per µl of blood after 24h incubation with various of concentrations of tannins from *Mitragyna inermis* roots (% of maturation is reported as IC50 values).

	***P. falciparum* strains number/µl before Tannins using**							
Tannins extracts Concentrations in	**Isolate1**	**Isolate2**	**Isolate3**	**Isolate4**	**Isolate5**	**Total**	**%Maturation**	**IC50 (µg/ml)**
µg/ml (log)	**1480**	**2780**	**1060**	**540**	**9750**	**15610**		
	***P. falciparum* strains number/µl after Tannins using**							
1250 (3,09)	1306	2446	933	475	8730	13890	89	
125 (2,09)	1820	3392	1293	659	11900	19064	122	**>100**
12.5 (1,09)	2312	4337	1654	842	14920	24065	154	
1.25 (0,09)	2850	5238	2035	1037	18440	29600	189	

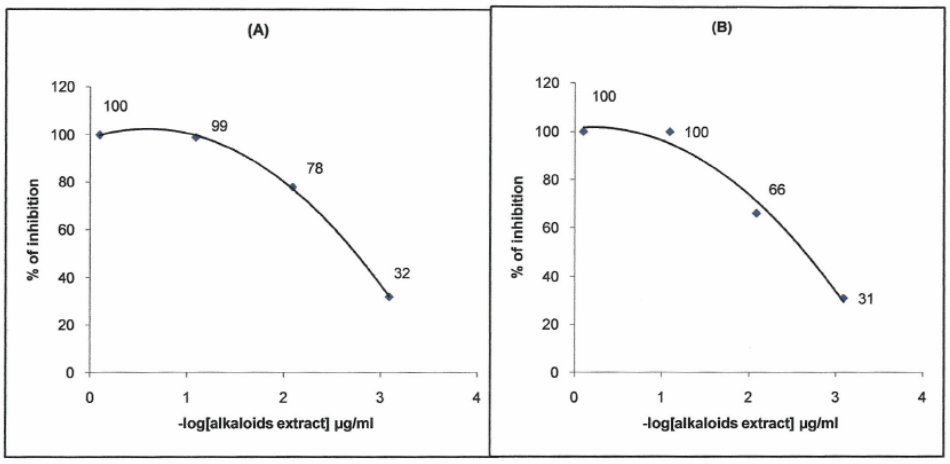

Fig. 1: Antimalarial activity of alkaloids of *Mitragyna inermis* roots after incubation for 24h (A) and 48h (B) at 37 °C

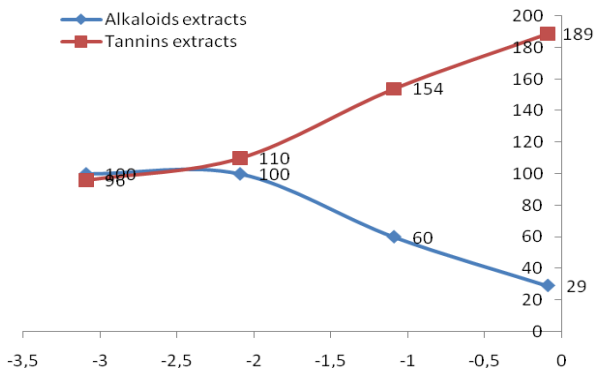

Fig. 2: Cinetic on number of *P. falciparum* strains (schizonts) and percentage (%) maturation as log [alkaloids]

CONCLUSION

The alkaloids are complex enough organic substances of raw formula, containing a tertiary nitrogen atom that confers them an alkali reaction from where the name alkaloid is coming from. Several of those alkaloid compounds are parasitic, the case of quinine isolated from *Cinchona*, is the first anti-malaria of natural origin and it is very poisonous for the malaria hematozoaire.

The study showed the anti-malarial activity of the alkaloids from roots of *Mitragyna inermis*, observed during our survey. It is important to undertake deeper research, especially in knowing the exact nature of the active compounds responsible for the *Plasmodium falciparum* strains activity. Pharmacological and pharmacokinetic studies of this drug will be done, with the goal of clearly administrate a posology, to provide to a human being with efficient product free of secondary effects.

Oppositely, the study presented a biodegradation of the tannins by *P. falciparum* is of fundamental importance, since, if one succeeds in confirming the use of the tannins by Plamodies as a cellular carbon source, it would bring on the fundamental research, a larger knowledge of the pharmacological role of the tannins on the microbial metabolism. On the medical plan, the use of the tannins as source of carbon by *Plasmodium* could enrich the composition of the culture medium of this *in vitro* parasite, regularly used in the study of the chimiosensitivity of *Plasmodium falciparum* with regard to the anti-malaria commonly used: the case of the WHO micro test (World Organization of Health), often used to value the chloroquine-resistance of *Plasmodium falciparum*.

CONFLICT OF INTERESTS

Declared none

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