

# ***In Vitro* Assessment Of The Antiplasmodial Activity Of Three Plants Extracts Used In Local Traditional Medicine In Saloum (Senegal)**

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## **Abstract**

Against the scourge of malaria in Africa and the development of chemo-resistance, discovering new natural cures is a major concern for many researchers. As a consequence, the present study was carried out in order, first, to identify the phytochemical compounds, and second, to assess the antiplasmodial activity, of three medicinal plants extracts, selected from an ethno-botany survey conducted in Senegal. The parts of the plants used were *Strychnos spinosa* (Strychnaceae) leaves and stems, *Combretum glutinosum* (Combretaceae) barks, and the whole aerial part of *Pennisetum polystachion* (Poaceae). Among these plants, the hydromethanolic extracts of *Strychnos spinosa* stems showed the most important activity against *Plasmodium falciparum* strains, with a  $IC_{50}$  of 15  $\mu\text{g/ml}$ , followed by the leaves chlorophormic extracts and the ethyl acetate extracts of *Pennisetum polystachion*, with respective  $IC_{50}$  of 20  $\mu\text{g/ml}$  and 21  $\mu\text{g/ml}$ . *Combretum glutinosum* extracts showed the least important activity, in all tests.

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**Keywords:** *Plasmodium*, activity, extracts, medicinal plants.

## Introduction

Malaria remains the most important parasitic disease in the world, and is caused by protozoa of which the most dreadful species is *Plasmodium falciparum*. The development and spreading of plasmodial strains, resistant to anti-malaria drugs available today, are a threat to public health, hence the necessity to try to find new anti-malaria drugs. Prior works carried out on traditional medicinal plants allowed the discovery of efficient anti-malaria molecules such as quinine and artemisinin. Several African traditional plants were also tested and used in the prevention and treatment of malaria, including mango leaves (Madu, 2007), *Picralimanitida* various organs (Erharuyi & al, 2014), *Caricapapaya*, *Azadirachta indica*, *Icacinasenegalensis* leaves (Sarret & al, 2011). Some studies have indicated that lemon grass was successfully used to treat drugs resistant malarial fever and typhoid (Akininyi, 2006). This encourages exploring plants for the discovery of new natural anti-malarial drugs (Schwikkard, 2002).

In such a context, an ethno-botany survey with traditional healers in Senegal allowed selecting three medicinal plants, of which, *Strychnos spinosa* (Strychnaceae), *Combretum glutinosum* (Combretaceae) and *Pennisetum polystachion* (Poaceae). These plants are used against malaria (*Strychnos spinosa*) but also against some bacterial infections (*Combretum glutinosum* and *Pennisetum polystachion*).

The objective of the present study is to identify, first, the phytochemical compounds and, second, to evaluate *in vitro* the antiplasmodial activity of these plants used in traditional medicine in Senegal.

## Materials and Methodology

### Plant material

The plant material is composed of *Strychnos spinosa* leaves and stems, *Combretum glutinosum* barks, and all the aerial part of *Pennisetum polystachion*.

### Blood samples preparation

The blood used was taken from subjects suffering from simple *P. falciparum* malaria confirmed by thick drop and thin smear. Once in the laboratory, the blood was transferred in a 15 ml tube, then a thin smear was done to assess the parasite load, and the tube is centrifuged at 2000 revolutions/mn for ten minutes, so as to separate the plasma and the blood corpuscles. Two other centrifugations, in the presence of non-supplemented RPMI, was carried out to rid the latter of any immunity components.

### **Parasites culture**

The cleaned samples are put in a culture medium, if the parasite load were contained between the interval of 0.4 - 1%. If the parasite load were higher than 1%, it is adjusted with O<sup>+</sup> blood; if by contrast it is lower than 0.4%, blood will not be used in this study.

For culture purpose, the hematocrit were adjusted at 2% with complete RPMI; and with a multi-ducts pipette, 180 µl of parasitized blood was distributed in the plates filled, beforehand, with 20 µl of plants extracts at different levels of concentrations. The culture medium thus prepared was blended, placed in a tightly closed container, exposed to a gaseous atmosphere (1% O<sub>2</sub>, 5% CO<sub>2</sub>, and 94% N<sub>2</sub>), then incubated at 37<sup>o</sup> C for 48 hours. After a while, a thin smear was done to check parasites growth (reinvasion) and the absence of contamination.

### **Plant material preparation**

Fresh parts of plants were collected and after botanic identification, these various parts were dried in the shade at room temperature (25<sup>o</sup> C), at Cheikh Anta Diop University of Dakar, Laboratory of Analytical Chemistry and Bromatology, and then reduced to fine powder with a grinder.

### **Extraction**

A mass of 20g of powder was macerated in 200 ml of a mixture of methanol/water in a 50/50 (v/v) proportion, for 24 hours. That mixture of solvent and plant powder was filtered through filter-paper and then concentrated with a rotating evaporator. The concentrated extract was dried in an incubator between 37 and 45<sup>o</sup> C.

30g of powder from the various plants parts was macerated in 300 ml of solvents (methanol, chloroform, diethyl ether, ethyl acetate). The mixtures was filtered through filter-paper and then concentrated with a rotating evaporator.

For *Strychnos*, 100 g of plant power were put in 500 ml of water at 90<sup>o</sup> C (Atsushi and *al*, 2004); after cooling an equal volume of methanol was added.

### **Determination of the phytochemical compounds**

The phytochemical compounds was determined according to the methodology described by Trease and Evans (1997), with methanol/water based extracts (50/50; v/v).

### **Preparing extracts solutions**

With 1 mg/ml of base concentration prepared as follows: 10 mg of extracts was dissolved in 1ml of DMSO, and then completed with RPMI at 10ml, before being submitted to Ultrason for 15 mn.

It was from that base solution that the following concentrations were prepared, that is to say: 6 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml. To comply with the plasmodium cells culture conditions, supplemented RPMI was used to compose these concentrations. The total DMSO percentage is lower than 0.1%, for all tests.

### ***In vitro* testing of antiplasmodial activity**

The antiplasmodial activity was tested on parasites according to the DAPI *ex vivo* dosage methodology (Ndiaye and *al*, 2010). Briefly, 180 µl of supplemented RPMI containing parasitized red blood in 2% of hematocrit were distributed in plates of 96 holes preloaded with 20 µl of solutions diluted in series of plants extracts. Extract concentrations vary from 6-50 µg/ml. Besides, in each plate, there are 6-8 negative control holes, with the culture medium alone.

Parasites were cultured for 48-72 hours at 37° C in standard air conditions (1% O<sub>2</sub>, 5% CO<sub>2</sub>, 94% N<sub>2</sub>), before adding the solution of 4', 6-diamidino-2-phenylindol (DAPI) (Banieki and *al*, 2007; Ndiaye and *al*, 2010).

Data were collected by measuring the fluorescence relative units (RFU), using a Fluoroskan plate reader (Thermo Scientific; ex 358 nm, em 461 nm).

### **Data statistical analysis**

The dynamic variation of the DAPI trial *ex vivo* was assessed by calculating the signal-to-noise ratio (SNR) and the Z' factor of each test. The SNR was measured by dividing the fluorescence signal (RFU) from holes without extract, by the maximum fluorescence signal of holes with extracts.

Statistical analysis was carried out with GraphPadPrism software programme (v5.0d, San Diego, CA), which allowed calculating some IC<sub>50</sub> values, according to a non linear regression model.

### **Results**

Table I below represents the throughput in mass percentage, of extractions carried out with various solvents.

Table I: Extractions throughput in mass percentage

Extractions throughput in %					
Extraction solvents	Methanol/Water	Ethyl acetate	Chloroform	Diethyl ether	methanol
<i>C. glutinosum</i>	8.0	1.2	0.8	1.0	6.2
<i>P. polystachion</i>	9.6	2.4	1.8	1.7	6.5
<i>S. spinosa</i> (leaves)	11.6	3.8	2.0	3.2	10.9
<i>S. spinosa</i> (stems)	8.2	2.8	3.1	1.7	8.1

The figures represent the throughputs values

Table II below shows the phytochemical compounds of selected plants hydromethanolic extracts.

Table II: Phytochemical compounds of hydromethanolic extracts.

Plants	flavonoids	Tannins	Anthracene heterosides	Cardiotonic heterosides	Saponosides	Alcaloids
P. polystachion	+	+	-	+	-	-
S. spinosa	+	+	-	+	-	-
C. glutinosum						

+: present \_ -: absent

Figures 1-5 below represent the *in vitro* tests results of the antiplasmodial activity of the various plants used.

Figure 1: Antiplasmodial activity of *Combretum glutinosum* extracts

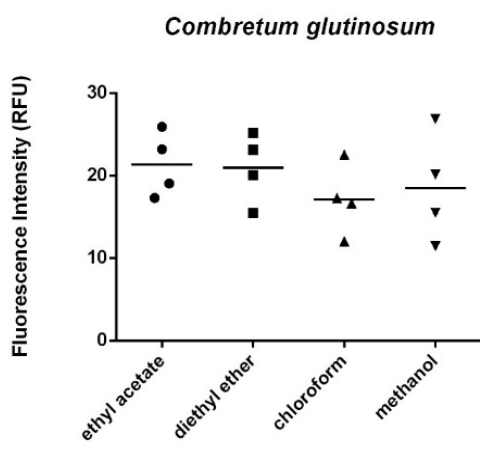


Figure 2: Antiplasmodial activity of Hydromethanolic extracts

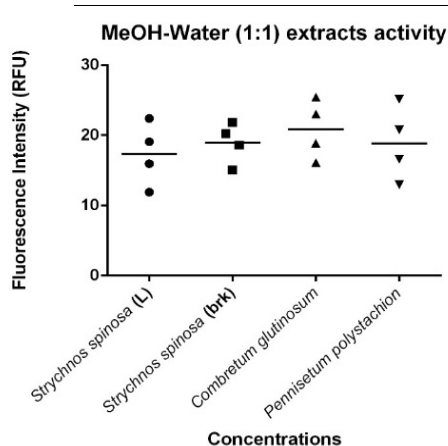


Figure 3: Antiplasmodial activity of *Strychnos spinosa* stems extracts

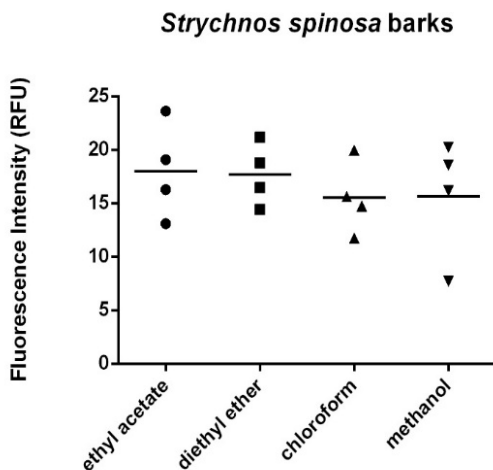


Figure 4: Antiplasmodial activity of *Strychnos spinosa*

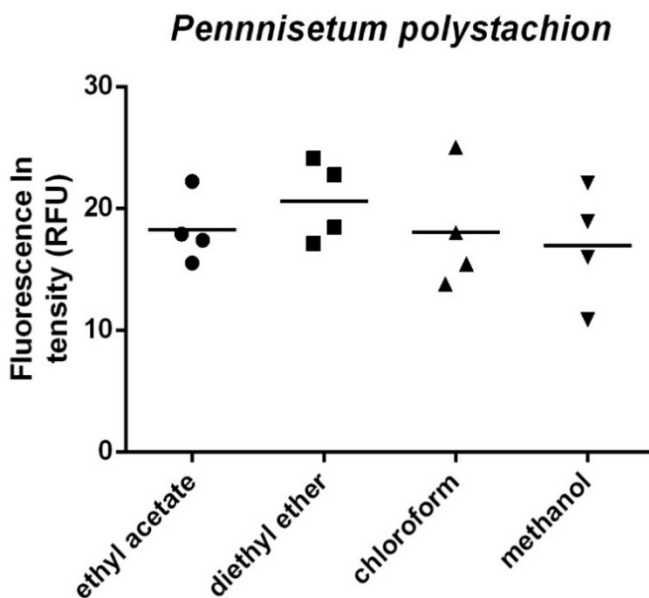
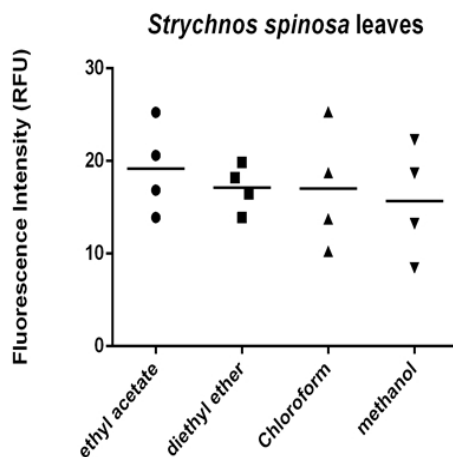


Figure 5: Antiplasmodial activity *Pennisetum polystachion* extracts

Table III under, represents the concentrations values which cause 50% inhibition of parasites growth.

Table III: IC<sub>50</sub> in absolute value of the various plants extracts studied

Extraction solvents	IC <sub>50</sub> in µg/ml				
	Ethyl acetate	Diethyl ether	Chloroform	Methanol	Methanol/Water
<i>C. glutinosum</i>	41	65	38	45	35
<i>P. Polystachion</i>	28	48	53	33	28
<i>S. spinosa</i> (leaves)	55	40	20	38	33
<i>S. spinosa</i> (stems)	30	36	16	25	15

## Discussion

The potential of higher plants as a source of new drugs is still widely unexplored. Among the plants species estimated between 250 000-500 000, only a small percentage were studied, and the phytochemical fractions submitted to biological or pharmacological test are even smaller (Ravikumar and *al*, 2012).

Plants great potential as drug sources remains as yet underexploited. For example, among the species that yielded 939 approved molecules, 369 drugs are at clinical trials stage, of which 119 in preclinical trials. About 19800 bioactive natural products belong to only 114 of the 6763 species family known in nature. Moreover, 80% of approved drugs and 67% of drugs in clinical trials are concentrated respectively in 17 and 30 prolific drugs families (Zhu and *al*, 2011).

The medicinal importance of these plants is due to the presence of secondary metabolites products, which are accountable for the plants therapeutic effects observed on the human body (Savithamma and *al*, 2011). A great number of studies showed that the different chemical clusters identified (Table II), namely flavonoids (Ramazana and *al*, 2010), tannins, anthracene heterosides, and cardiotoxic heterosides (Ravikumar and *al*, 2011), may have antiplasmodial activities.

The *Strychnos spinosa* extracts turned out to be the most active, among the extracts tested (Table III). The stems hydromethanolic extract is the most active of all, with a IC<sub>50</sub> of 15 µg/ml, followed by the chloroformic extracts with a IC<sub>50</sub> of 16 µg/ml (Table III) (figure 3). The *Pennisetum polystachion* leaves chloroformic extracts and ethyl acetate extracts (Table III and Figure 5) follow, with respective IC<sub>50</sub> of 20 and 21 µg/ml.

Of all the extracts tested, the *Combretum glutinosum* extracts was the less active (Figure I and Table III).

On the whole, the hydromethanolic blend was the most active among the extraction solvents, followed by chloroform and methanol. Of all tests, ether extracts are the less sensitive (Table III). This hydroalcoholic extract activity is proportional to its extraction yields, followed by those of methanol and ethyl acetate (Table I). These solvents activity was in agreement with the

studies carried out by (Katuural and *al*, 2007) and (Sattar and *al*, 2008) on plant antiplasmodial activity.

These results are interesting according to the following classification: an extract is very active if  $IC_{50} < 5 \mu\text{g/ml}$ , active when  $5 \mu\text{g/ml} < IC_{50} < 50 \mu\text{g/ml}$ , weakly active if  $50 \mu\text{g/ml} < IC_{50} < 100 \mu\text{g/ml}$ , and inactive if  $IC_{50} > 100 \mu\text{g/ml}$  (Bickii and *al*, 2007). All  $IC_{50}$  obtained are lower than  $50 \mu\text{g/ml}$ .

## Conclusion

These encouraging results show again the importance of traditional knowledge about medicinal plants, which are worth exploiting for the purpose of bringing solutions to present day health issues.

However, it is necessary to carry on with efforts to discover new antimalaria matrix molecules from natural plants.

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