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ANTIPLASMODIAL AND DPPH RADICAL SCAVENGING EFFECTS IN EXTRACTS FROM *ACACIA MACROSTACHYA* (MIMOSACEAE) DC

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ABSTRACT

Background: *Acacia macrostachya* is a medicinal plant used in the traditional treatment of snakebites and pathologies accompanied with oxidative stress such as malaria, inflammation, and painful spasms. **Objective:** The purpose is to study the antioxidant and antiplasmodial effects in extracts of leaves and bark of twigs from Acacia macrostachya. **Methodology:** The extracts were prepared by exhaustion using dichloromethane, dichloromethane / methanol (1:1), methanol, methanol / water (1:1) and water. The antioxidant effects were assessed by the 1, 1 diphenyl-2-diphenyl picrylhydrazyl (DPPH) test. In order to prove the antiplasmodial effect in extracts using the optical microtest, wild clinical strains of *Plasmodium falciparum* have grown according to the method of **Trager and Jensen**. Sterols / triterpenes, flavonoids, tannins, alkaloids, and saponins were subsequently sought for. **Results:** All leaf extracts were strongly antioxidant often comparable to quercetin (IC_{50}-Quercetin: 2. 63µg/mL *versus* IC_{50}-MeOH-leave: 1. 11µg/mL). Extracts from bark in dichloromethane (IC_{50}: 14. 92µg/mL) and methanol (IC_{50}: 11. 40µg/mL) were also active. The best antiplasmodial activities were found

in the hydro-methanolic extracts of leaves (IC_{50}: 5. 06µg/mL) and bark (IC_{50}: 4. 92µg/mL). Extracts of *A. machrostachya* have proved very rich in saponins, rich in flavonoids, tannins and alkaloids

KEY WORDS: *A. machrostachya*, antioxidant, antiplasmodial, Burkina Faso.

1. INTRODUCTION

Malaria is an endemic epidemic parasitic tropical disease. It is a real public health concern worldwide with greater predominance in the world poverty belt. According to WHO, there were 214 million new cases of malaria worldwide in 2015. The African Region accounted for most global cases of malaria (88%), followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%). In 2015, there were an estimated 438 000 malaria deaths worldwide, 90% in the African Region, followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%).[1]

The presence of the parasite in the body is associated with oxidative stress. [2] For this purpose, one of the defenses used by the body against the parasite is the generation of free radical oxygen species. This oxidative stress will aggravate the pathogenesis of malaria and manifest In the form of fatigue, pain and muscle weakness.

Since the advent of chloroquine resistance, WHO recommends combination therapy artemisinin (ACTs) for the treatment of uncomplicated malaria.[1] But cases of resistance of *Plasmodium falciparum* to artemisinin and its derivatives was confirmed in 2012 in Cambodia.[1,3] The serious side effects of certain acts are the source of therapeutic noncompliance.

Affected population mainly poor, use traditional herbal medication, less expensive and still promising healing. Plants are a major source of drugs, especially malaria. Of course, quinine and artemisinin used therapeutically are derived from plants.

Acacia macrostachya is a medicinal plant used in the traditional treatment of snakebites and pathologies accompanied with oxidative stress such as malaria, inflammation, and painful spasms.

The purpose of this work is to show the radical scavenging and antiplasmodial effects in leaves and bark extracts of *Acacia macrostachya*.

2. METHODOLOGY

2.1. Plant material

The branches and leaves of Acacia macrostachya were collected in January 2010 in the southern part of Ouagadougou (Burkina Faso, West Africa). The specimen has been certified by Dr. Souleymane GANABA, Department of forestry at the National Centre for Scientific Research and Technology, Ouagadougou (CNRST). A voucher specimen was deposited at Burkina National Herbarium (HNBU) and given No. 8721.

Collected plant materials were dried at room temperature under shade to prevent the direct sun effect. The resultant dried plant parts were individually reduced to powder with mortar and pestle, sieved and kept in a clean dried cupboard before use.

2.2. Extraction of plant materials

The extraction was done by successive maceration of the herbal drug with solvents of increasing polarity during 24 hours: dichloromethane (Cl_2CH_2), dichloromethane + methanol (Cl_2CH_2-MeOH, 1: 1), methanol (MeOH), methanol + distilled water (MeOH-W, 1: 1) and distilled water (W). The solution of the extract collected after filtration, was placed in a rotavapor for evaporation under reduced pressure. The extract obtained was dried in the open air, and the resulting solids were preserved in vials labeled to protect them from light.

Dried plant drugs powder was weighed (M). After a complete exhaustion, the dry extract obtained from the rotavapor was also weighed (m).

Extraction r (%) performance is given by the formula

$r = [m / M] \times 100$

2.3. Phytochemical screening

The main chemical groups have been highlighted by color reactions as described by Ciulei 1982.[4] We looked for alkaloids (Meyer and Dragendorff tests), flavonoids (Shibata tests), saponins (foam tests), tannins (FeCl3 test) and sterols & triterpens (Libermann test).

2.4. DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity

The radical scavenging activity of the extracts was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) as described by **Kim *et al.* 2003** [5] with a slight modification. The wells test (T) contained the extracts (100, 30, 10, 3 and 1 µg/ mL) or Quercetin (reference) prepared in DMSO + methanolic solution of DPPH (101µM, Sigma Germany). However, the wells

control (C) contained the methanol solution of DPPH to which was added DMSO, under the same conditions as above. After an incubation at 37 °C for 30 minutes away from the light, the absorbances (OD) were read on a spectrophotometer at 520 nm against a blank (B) containing extracts prepared in DMSO + methanol. The percentages of inhibition (effect) were calculated using the formula **$(1 - OD_T / OD_C) \times 100$**.

Where: OD_T is the absorbance of the test and OD_C the absorbance of the control.
The display of the dose-response curve was used to determine the IC_{50} using the GrapPad Prism software version 5.0.

2.5. *Plasmodium falciparum* cultivation

The parasites culture was performed according to the technique of Trager and Jensen (1976).[6] It involves putting sedimentation red blood cells in a flat-bottomed container under a continuous flow of a gas mixture of 92% N_2, 5% CO_2 and 3% O_2 in an incubator. The culture medium consisted of RPMI 1640 (sigma, Germany) containing 25 mM HEPES buffer, 4 mM L-glutamine and 25 µg / ml gentamycin. This medium was supplemented with 10% foetal bovine serum. Cultures were made from isolates of *Plasmodium falciparum* obtained by collecting blood from people with *Plasmodium falciparum* malaria before intake. Red cells were first washed 3 times with RPMI 1640 medium and parasitaemia adjusted to 2% by dilution of parasitized red blood cells with healthy red blood cells washed from healthy donors of O rhesus positive group to limit the risk of system incompatibility ABO red blood cells within culture. The whole was suspended in the culture medium with a hematocrit maintained at 5%. The suspension was then distributed in cell culture flasks and placed in the CO2 incubator. The culture medium was renewed daily and parasitaemia was controlled by achieving Giemsa stained smears and if necessary adjusted to 2% by the addition of healthy red blood cells.

2.6. Synchronized stage of parasites in culture

To assess a antiplasmodial activity, it is necessary to have the same evolutionary stage parasites. The method to the Gelofusine® was used. It separates the trophozoïtes older forms of *Plasmodium falciparum*.[7,8] Red blood cells obtained by centrifugation (2500 RPM) were resuspended in RPMI 1640 incomplete at 50% hematocrit, and then we added an equal volume of Gelofusine®. The whole was homogenized and placed at 37 ° C until the appearance of a line between the two phases: a supernatant and a pellet. The supernatant containing about 90% of young trophozoites culture while older trophozoites, schizonts and

healthy red blood cells are found in the pellet. The supernatant was then carefully aspirated, placed in another tube and washed 3 times with RPMI. Trophozoites thus obtained were diluted with healthy red blood cells and returned to culture. After 24 hours culture, the culture medium was renewed and we made blood smear which was observed after Giemsa staining. At this stage trophozoites and schizonts were found. After 48 hours, a further treatment with the Gelofusine® was applied to culture. Blood smears were carried out regularly to ensure synchrony between cultures.

2.7. Optical microtest

The doses to be tested were prepared by serial dilution of half from 10mg / mL of the extract, in RPMI 1640 with DMSO (0.5%) after vortexing for at least 15 minutes. The solutions were filtered before carrying out the tests. Each dose (50 µL) was transferred to 200 µL of the suspension of parasitized erythrocytes to 10% hematocrit and 5% parasitaemia shared per well; the whole placed in CO_2 incubator. A witness (complete RPMI medium + erythrocytes parasitized suspension) and blank (complete RPMI medium + 10% DMSO + erythrocytes parasitized suspension) were carried out under the same conditions. After 24 hours, the supernatant was removed and two smears were performed for each well. To test wells, 200 µL of complete media and 50 µL of the extract solution counterpart wells are added. For the control and the blank is the withdrawn volume of medium that is simply added. The plate is again covered and replaced in the incubator. The operation is repeated 48 and 72 hours after. Parasite densities were determined after fixation with methanol and Giemsa 10% different then smears a percentage of parasitized erythrocytes. Witnesses representing 100% of growth.

The inhibition percentage (effect) under these conditions is determined by the formula

% Inhibition = [1 - (average parasite density tests / average parasite density of witnesses)] x 100

The representation of the dose-response curve was used to determine the IC50 using the GrapPad Prism version 5.0 software.

2.8. Treatment of data

All the experiments were performed in quadruplicate (n = 4). The results are presented in the form of mean (m) ± standard error of the mean (SEM). Values of 50% inhibitory

concentration (IC_{50}) were determined from the dose curves / effects obtained using the PRISM version 5.0 software.

The ANOVA test one factor was used to compare means, the difference was considered statistically significant if the p-value <0.05.

3. RESULTS

3.1. Extraction and phytochemistry: Leaves and barks of *Acacia macrostachya* have been successively exhausted with solvents of increasing polarity: dichloromethane (Cl_2CH_2), dichloromethane / methanol (Cl_2CH_2-MeOH, 1: 1), methanol (MeOH), hydro-methanol (MeOH-W, 1: 1) and water (W). The extraction of drugs *A. macrostachya* methanol has given the best returns: 15.44% for leaves and bark to 19.3% (**Table 1**).

Table 1: Extraction yield of *A. macrostachya*

Solvents	**% of extraction**	
	Leave	**Stem bark**
CH_2Cl_2	7.30	5.04
CH_2Cl_2-MeOH (1 :1)	5.30	1.60
MeOH	**15.44**	**19.30**
MeOH-W (1 :1)	8.00	1.50
W	nd	5.00

nd : not determined

The extracts of *A. macrostachya* contain saponins, flavonoids, tannins and alkaloids salts. Alkaloids were more concentrated in the aqueous extracts of the leaves in salt form (**Figure 1 and Table 2**).

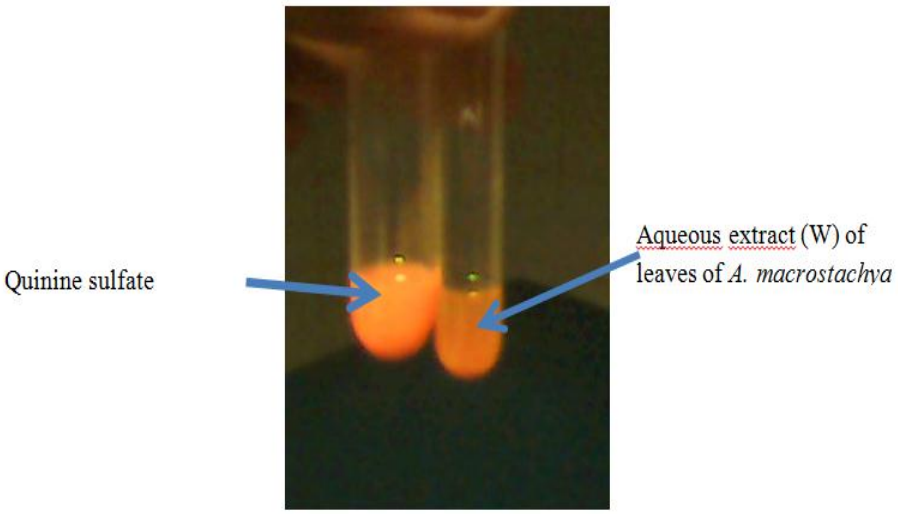

Figure 1 : orange-red precipitate characterizing the presence of salts alkaloids.

Whatever the herbal drug (leaf or bark), the methanol extracts and hydro-methanolic were very rich in saponins but moderately concentrated alkaloids, flavonoids and tannins (**Table 2 and 3**).

Table 2: Phytochemicals and activities in extracts from leaves of *A. macrostachya*

Solvents	Detected phytochemicals	Activities expressed as IC_{50} (µg/mL)	
		Antioxidant	Antiplasmodial
CH_2Cl_2	Stérols/triterpens (+) Flavonoids (+)	**2.04 ± 1.08**	39.02 ± 1.41
CH_2Cl_2-MeOH	Flavonoids (+)	3.47 ± 1.2	16.23 ± 1.43
MeOH	Saponins (+++) Flavonoids (+) Tannins (+)	**1.11 ± 0.81**	**7.13 ± 3.57**
MeOH-W	Saponins (+++) Flavonoids (+) Tannins (+) Alcaloids (+)	**4.62 ± 2.17**	**5.06 ± 2.84**
W	Saponins (+++) Flavonoids (+) Tannins (+) Alcaloids (+++)	5.49 ± 1.53	10.5 ± 1.43
Quercetin		2.63 ± 1.26	-

(+) : present ; (++) : abundant ; (+++) : very abundant

3.2. Radical scavenging activity in extracts of *A. macrostachya*

The radical scavenging activity was assessed by the DPPH test.

The radical scavenging effects varied from one sample to another and from one drug to another (**Tables 2 and 3**). The best results (Table 2) were observed with the leaf extracts with dichloromethane (IC_{50}: 2.04 µg / mL) and methanol (IC_{50}: 1.11 µg / mL).

Quercetin, our reference antioxidant substance had an IC_{50} equal to 2.63 µg / mL.

Table 3 : Phytochemicals and activities in extracts from *A. macrostachya* stem bark

Solvents	Detected phytochemicals	Activities expressed as IC_{50} (µg/mL)	
		Antioxidant	Antiplasmodial
CH_2Cl_2	Stérols/triterpènes (+)	**14.92 ± 1.39**	**9.71 ± 2.07**
CH_2Cl_2-MeOH	No phytochemical group detected	20.11 ± 1.29	27.7 ± 1.49
MeOH	Saponins (+++) Flavonoids (+) Tannins (+)	**11.40 ± 2.08**	51.71 ± 1.27
MeOH-W	Saponins (+++)	25.48 ± 1.7	**4.92 ± 1.95**

	Flavonoids (+) Tannins (+) Alcaloïdes (+)		
W	Saponins (+++) Flavonoids (+) Tannins (+) Alcaloids (++)	**n.d**	**n.d**
Quercetin		2.63 ± 1.26	-

(+) : present ; (++) : abundant ; (+++) : very abundant

3.3. Antiplasmodial activity in extracts of A. macrostachya

Extracts of *A. macrostachya* brought into contact for 72 hours with the *Plasmodium falciparum* strains showed inhibitory effects of their overall growth. The effects were dependent doses (**Figure 2 and 3**). On the whole, the leaf extracts have the best potential antiplasmodial (**Table 2**). The hydro-methanol extracts (**Tables 2 and 3**) were the most active with IC_{50} of 5.06 µg / mL (leaf) and 4.92 µg / mL (bark).

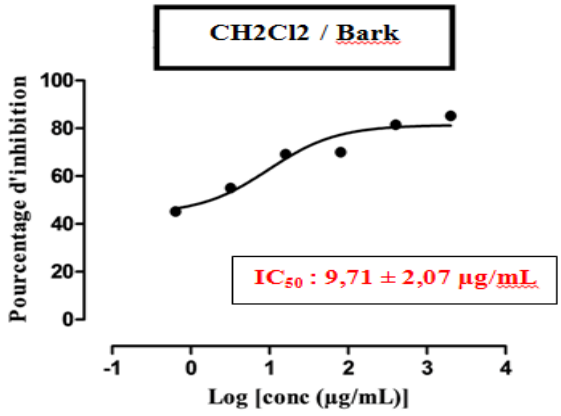

Fig.4a

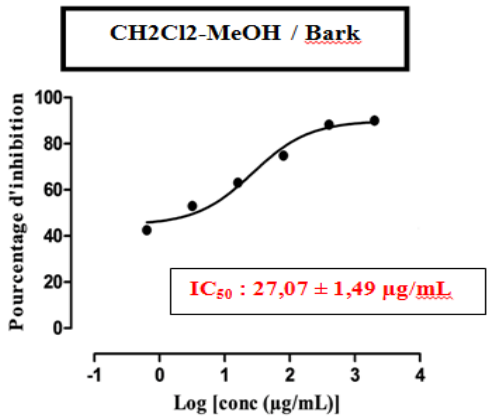

Fig.4b

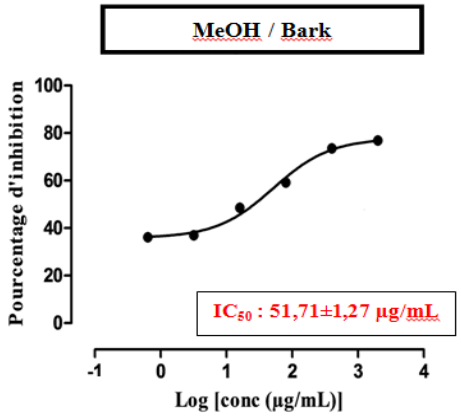

Fig.4c

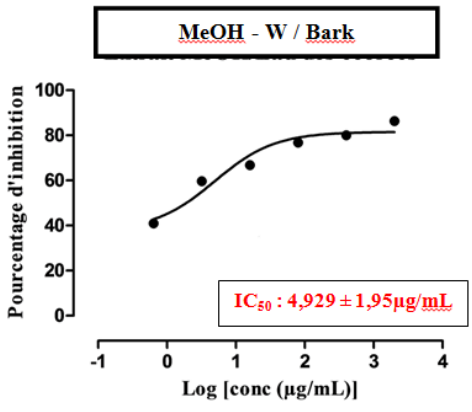

Fig.4d

Figure 2 : Curve dose – antiplasmodial effect in extracts from bark of *A. macrostachya*

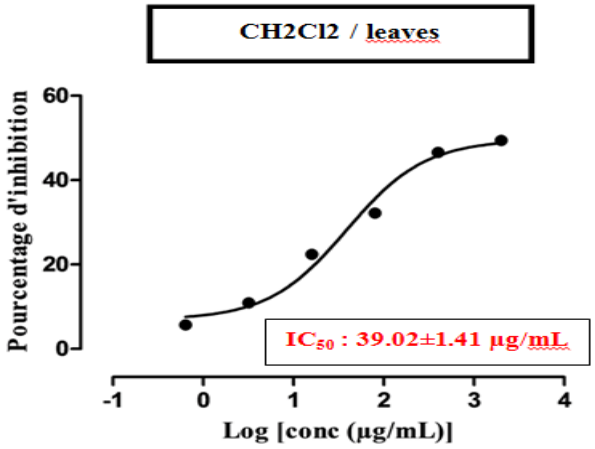

Fig.5a

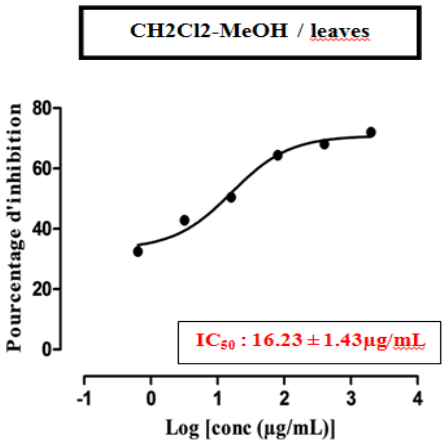

Fig.5b

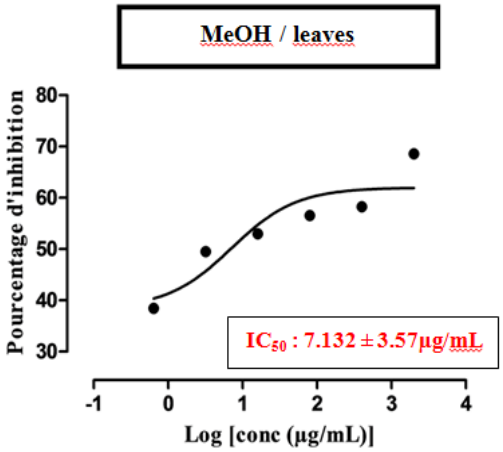

Fig.5c

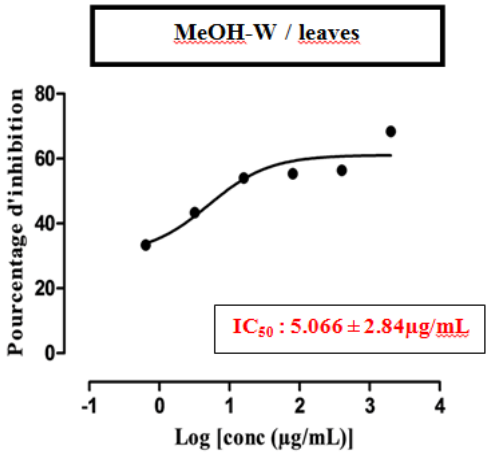

Fig.5d

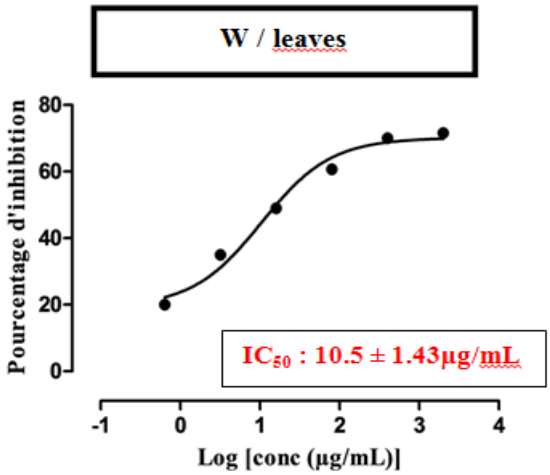

Fig.5e

Figure 3: Curve dose – antiplasmodial effect in extracts from leaves of de *A. macrostachya*

4. DISCUSSION

4.1. Phytochemistry of extracts

Leaves and barks of A. macrostachya were exhausted successively with dichloromethane (Cl_2CH_2), dichloromethane / methanol (Cl_2CH_2-MeOH, 1: 1), methanol (MeOH), hydro-methanol (MeOH-W, 1: 1) and water (W). The best extraction yields were observed with methanol, 15.44% (leaves) and 19.3% (bark). The second largest yields were obtained with dichloromethane, 5.04% and 7.3% respectively for the barks and leaves. These results suggest that the studied drugs concentrate more polar compounds. The methanol extracts and hydro-methanolic A. macrostachya contain saponins in abundance and low alkaloids, flavonoids and tannins. Salts alkaloids were mainly concentrated in the aqueous extracts of leaves.

Sawadogo *et al.* (2011)[9] When working on the methanol extracts had also highlighted the strong presence of saponins and tannins and low presence of alkaloids. However, the author had not detected flavonoids in its extracts. This difference could be explained by the fact that our two (2) plant materials did not come from the same geographical area: the southern Sahelian zone (Ouagadougou) for ours and the southern Sudan region (Dinderesso) for theirs. Indeed the change in the chemical composition of the extracts could be a response of plants to factors such as weather conditions[10], the geological environment of the harvest sites[11] and the harvest period (maturation).[12]

4.2. Antioxidant activity

The antioxidant activity was assessed by the DPPH test. The extracts of *A. macrostachya* presented antioxidant dose-dependent effects (Figure 2-3). The antioxidant effects of extracts (Cl_2CH_2, Cl_2CH_2 -MeOH, MeOH, MeOH-W and W) were assessed and compared to quercetin whose IC_{50} was determined to be equal to 2.63 ± 1.26µg / mL. If the 50% inhibitory concentration (IC_{50}) is lower, the extract is more active.

The best radical scavenging effects were observed with leaf extracts (Table II). Only the leaf aqueous extract (W-fe) was less active than quercetin (p 0.034). Otherwise, the other leaf extracts had their antioxidant effects statistically comparable to that of quercetin (*p 0.11*).

In the bark extracts, antioxidant effect varied from one sample to another (*p 0.000*).

The analysis of IC_{50} shows that the best antioxidant effects were found in the methanol extracts (MeOH-bark, IC_{50}: 11.40 ± 2.08 µg / mL) and dichloromethane (Cl_2CH_2-bark, IC_{50}:

14.92 ± 1.39µg / mL) which otherwise remained largely lower than that of quercetin (*p 0.0031* and *p 0.0002*, respectively).

In this study, all the extracts containing saponins were radical scavengers (Cl_2CH_2, MeOH, MeOH-W and W). The radical scavenging effect was even more marked when, in addition to saponin, the extract contained flavonoids and tannins (MeOH) or did not contain alkaloids (Cl_2CH_2, MeOH and Cl_2CH_2-MeOH). The antioxidant effect was even less marked in the extracts where alkaloids were abundantly detected (MeOH-W and W). These results show that extracts of *A. macrostachya* have undoubtedly radical scavenging effects but also suggest that the presence of alkaloids in these extracts little or not influenced the display of these effects from the presence of polyphenols and saponins. This is consistent with numerous studies that reported weak radical scavenging activity of some alkaloids. [13, 14, 15] Saponins radical scavengers have been described. [16, 17] It is well known that polyphenols (flavonoids and tannins) are excellent antioxidants.[18, 19] As they are not only able to inhibit oxidative enzymes [20] but also trap free radicals. [21] Of course, their aromatic structure with conjugated double bonds (electronic resonance), the configuration and the high number of "hydroxyl" groups (OH) promote greater proton (H) lability, essential for a radical reaction.

Polyphenols and saponins detected in theses extracts could probably act synergistically to increase the potential anti-radical extracts.

4.3. Antiplasmodial activity in extracts of *A. macrostachya*

A. macrostachya extracts brought into contact during 72 hours with the *Plasmodium falciparum* strains showed inhibitory effects of their overall growth. The effects were dose-dependent. Over the IC_{50}, the lower the extract is active. On the whole, the leaf extracts have the best potential antiplasmodial.

Statistical analysis IC_{50} shows that methanol extracts of leaves (IC_{50}: 7.13 ± 3.57µg / mL) and hydro-methanolic (leaf: 5.06 ± 2.17µg / mL and barks: 4.92 ± 1.95µg / mL), the most active, and showed no antiplasmodial effects significantly different (*p 0.506*). The bark extract with Cl_2CH_2 in which sterols / triterpens have been characterized, was also active (IC_{50}: 9.71 ± 2.07 µg / mL). The best antiplasmodial activity was observed with extracts containing both saponins, alkaloids, flavonoids and tannins (MeOH-W). When the extract did not contain alkaloids (Cl_2CH_2, Cl_2CH_2-MeOH) antiplasmodial activity was low. Antiplasmodial lowest activity was observed in the dichloromethane extract which only contains flavonoids.

Nevertheless, all these compounds are capable of antiplasmodial effect. Quinine currently used therapeutically as potent antimalarial drug (antiplasmodial schizonticide) is a cinchona alkaloid. Baghdikian *et al.* (2013) have demonstrated the antiplasmodial effect of 7 alkaloids isolated *Stephania rotunda*.[22] Nasrullah *et al.* (2013), isolated from the bark of *Cryptocaryanigra* 4 antiplasmodial and antioxidant alkaloids (FRAP and DPPH). [23] In many studies, the authors attribute the antiplasmodial activity to flavonoids.[24, 25, 26] Builders *et al.* 2014 reported the antiplasmodial effect of isolated phenolic compounds of *Parkia biglobosa* stem bark.[27] Chokchaisiri *et al.* (2015) bound the antiplasmodial effect to the alkaloids and flavonoids.[28]

Analysis of IC_{50} shows that extracts of *A. macrostachya* have a powerful effect antiplasmodial which opens a significant path for the search for potentially antimalarial substances.

CONCLUSION

Our work has focused on various extracts of *A. macrostachya* that showed excellent inhibitors of the whole growth of wild clinical strains of *Plasmodium falciparum* and effective free radical scavengers (DPPH) better than quercetin (a flavonoid reference antioxidant). These scientific findings could justify the use of extracts from certain African populations in malaria treatment, either because of their antiplasmodial action or to reduce oxidative stress that accompanies and worsens malaria. We link these pharmacological effects to the presence of saponins, sterols / triterpens, polyphenols and alkaloids that we characterized in the extracts.

Other studies about toxicity and the comparison of the antiplasmodial effect of the most active extracts with an antimalarial reference are needed to open a line of research of antimalarial drugs.

CONFLICT OF INTEREST DECLARATION

We declare that we have no conflict of interest

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