IN VIVO ANTIPLASMODIAL ACTIVITY COMPARISON OF PHYLLANTHUS ODONTADENIUS Müll. Arg. FROM THREE DIFFERENTS REGIONS IN DRC

Abstract

Aims: *Phyllanthus odontadenius*, one of the genus Phyllanthus species, used for number diseases treatment including malaria. Given the interest in various *P. odontadenius* crude extracts *in vitro* on Plasmodium falciparum, an *in vivo* study seems necessary in order to judge the extracts effectiveness of this plant. This study therefore aims to find justification for *P. odontadenius* secondary metabolites antiplasmodial activity which would have been revealed in the three samples from three different province sites.

Study design: Plants *P. odontadenius* samples from three province harvested and dried separately, plant phytochemistry screening realized, extracts preparation for antiplasmodial test, mice parasitization with *P. berghei* strain, orally administration drug; Parasiteamia determination using an immersion microscope.

Place and Duration of study: Department of Radiobiology, Applied Microbiology section, General Atomic Energy Commission, Regional Nuclear Studies Center of Kinshasa. MPI and pharmacognosy laboratories in the National Biochemical Research Institute (INRB). This work took place over the period from October 11, 2020 to March 12, 2021.

Methodology: Phytochemical screening *P. odontadenius* samples was previously determined by reagent reactions and TLC. Then, *P. odontadenius* methanol extracts from aerial parts harvested in three sites (Kinshasa, Kasangulu and Kwango-bridge) were administered to test mice (12.5 mg/kg and 25 mg//kg bw) after infected mice with *Plasmodium berghei* strain. DMSO 10% and quinine 10 mg/kg bw were also used as controls for comparison with tested *P. odontadenius* extracts. After 5 days, parasitemia of each test and controls mice was determined. Percent of parasitemia, parasite density and percent of inhibition were calculated. Finally, the effect dose 50 of each *P. odontadenius* specimen was determined.

Results: Parasiteamia rates of negative control (DMSO 10%) was high (69.98±15.03%) comparing to positive control (27.43±11.46%) and tested mice with *P. odontadenius extracts* (12.5 and 25 mg/kg bw) which percent's varied from 24.66±15.84% to 59.01±22.44%. Negative control presented high parasite density with 11,342 (±2,436) comparing to the positive control (4,447±1,857) and all *P. odontadenius* methanol extracts which varied from 3,995±2,343 for 25 mg/kg bw to 9,570±3,319 for 12.5 mg/kg bw. Parasiteamia reduction rates followed inversely parasite density, thus, *Po*3 25 mg/kg bw had high parasiteamia reduction rate (65.23%) comparing to positive control with 61.32% and to *P. odontadenius* methanol extracts. *Po*3 presented 2.44 mg/kg bw as effect dose 50 comparing to *Po*1 (2.93 mg/kg bw) and *Po*2 (2.68 mg/kg bw). Males mice were highly affected to *P. berghei* than the females.

Conclusion: This study revealed that all specimens of *P. odontadenius* had good *in vivo* antiplasmodial activities on *P. berghei*. All *P. odontadenius* extracts showed good parasitaemia inhibition compared to negative control, but *P. odontadenius* from Kwango-brigde (*Po*3) presented good behavior concerning *in vivo* antiplasmodial activity in comparison to *P. odontadenius* from Kasangulu (*Po*2) and that from Kinshasa (*Po*1).

Keywords: *Phyllanthus odontadenius*; *In vivo* antiplasmodial activity; Malaria, *Plasmodium berghei;* Parasiteamia, Effect dose 50.

1. Introduction

Malaria is a hemolyzing erythrocytopathy caused by parasite species of Plasmodium genus. It is a major endemic which causes great devastation in almost all developing countries and particularly in Africa. Malaria is easily the world's largest parasitic disease, killing more

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people throughout history than any other communicable disease except tuberculosis. The total number of deaths readily exceeds that from AIDS [1]. Two billion four hundred million people are at risk of malaria worldwide and two million children under five age die from it each year [2].

Malaria today poses a real public health problem for more than hundred countries representing a total of 2.4 billion people, or 40% of the world population. These countries are mainly countries located in Africa, Asia and Latin America. In Democratic Republic of the Congo (DRC), Malaria is one of the three major causes. It is the cause of 77.6% serious anemia requiring blood transfusion and therefore represents nearly 95% of deaths. Last year, malaria afflicted 228 million people and killed an estimated 405 000, mostly in sub-Saharan Africa. In 2018, an estimated 11 million pregnant women were infected with malaria in areas of moderate and high disease transmission in sub-Saharan Africa. As a result, nearly 900 000 children were born with a low birthweight [3]. The malaria impact is on the capacity reduction for work (invalidation and absenteeism) and on the economy [4].

Parasite chemoresistance to modern antimalarial drugs such as chloroquine, an easily accessible antimalarial, is a real calamity for Africa. Replacement products are either expensive, toxic or ineffective. From 1970, the malaria eradication failure was recognized by WHO, even today this parasite chemoresistance continues to spread to other similar antimalarials. It is with this in mind that the new antimalarial molecules search could therefore be undertaken within plant biodiversity using ethnopharmacology [5]; [6].

This approach makes it possible to select the most interesting species for their potential antiplasmodial activity. Traditional antimalarial study remedies used by population indigenous people are therefore essential and could lead to validation of traditional use and/or discovery of new molecules with beneficial antimalarial potential. Previous studies on *in vitro* antiplasmodial activity of *Phyllanthus odontadenius* species have revealed virtues that place it in a good position among many other medicinal plants from the rich plant flora of DRC [7]; [8]; [9]; [10].

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As part of traditional medicine promotion in general and particularly malaria treatment in DRC, better phytochemical knowledge of medicinal plants is essential. It is in this context that *P. odontadenius* samples coming from the city province of Kinshasa, from Kasangulu in Kongo Central province and Kenge in the Kwango province were analyzed. On the one hand for implementation molecules with possible pharmacological activities against *Plasmodium falciparum* and on the other hand, compare the *in vivo* antiplasmodial activity from *P. odontadenius* extracts coming from different aforementioned sites. This study therefore aims to find a justification for the antiplasmodial activity of the secondary metabolites of *P. odontadenius* which would have been revealed in the three samples.

In addition to traditional medicine interest in promoting, this study is also intended to provide researchers guidance in their research work on antimalarial compounds. This would allow researchers to obtain effective and non-toxic phytomedicines which would be used in fight against this endemic disease (malaria) which still continues to cause problems with most children deaths under 5 years old and disability in adults preventing them from properly carrying out their daily tasks.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Study site and period

This work took place over the period from October 11, 2020 to March 12, 2021. First in the Life Sciences laboratory located within the General Atomic Energy Commission/Regional Nuclear Studies Center of Kinshasa (CGEA/CREN-K) then to the Biomedical Research National Institute (INRB) located on Avenue des Huileries in Gombe commune in Kinshasa. Samples were preserved and re-powdered at CGEA/CREN-K. Manipulations on analyzes plant extracts phytochemical and the in vivo antiplasmodial activity of samples were carried out at the INRB respectively at the MPI laboratory in biochemistry and in parasitology.

2.1.2. Biologycal materials

2.1.2.1. Plant material

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Plant material consisted of aerial parts samples (stems, leaves and seeds) of *P. odontadedius* from Cécomaf site in river N'djili valley of Kinshasa's N'djili commune, from Kwango-brigde in Kwango region and from Kasangulu in Kongo Central region. The samples were harvested in October 2020 then identified for confirmation at the INRA Herbarium in Biology Department of Sciences Faculty in Kinshasa University.

2.1.2.2. Methods*Plasmodium berghei* strain

Plasmodium berghei strain used for the *in vivo* antiplasmodial activity was provided to us by the Biomedical National Institute of Research (INRB). It was preserved in nitrogen liquid at very low temperature. *P. berghei* strain is suitable for rodents.

2.1.2.3. *NMRI mouse*

White albino NMRI (for Naval Medical Research Institute) mice of both sexes, weighing 20–25 g were used for the *in vitro* antiplasmodial activity testing which came from the INRB animal facility. Mice were put randomly into test and control groups, each group containing six mice. In this study case, overall 56 NMRI white mice were used. Animals were acclimatized to the laboratory conditions, supplied with food and water for 5 days before being used for the test.

2.2. Methods

2.2.1. Sampling

These are the samples of *P. odontadenius* collected in Kinshasa (*Po*1), Kasangulu (*Po*2) and Kwango-bridge (*Po*3) which were the subject of this study. Their different aerial parts were used to obtain powders that could be used in the preparation of aqueous and methanolic extracts. These different extracts made it possible to carry out the chemical screening and the *in vivo* antiplasmodial activity determination.

2.2.2. *Preparation of crude extracts*

The harvested plant organs were dried in the Biotechnology laboratory from the Radiobiology Department of CGEA/CREN-K away from light, humidity and dust. After approximately ten days, dried plant organs were ground in THOMAS SCIENTIFIC mill to obtain

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powder with 500 µm of diameter. Samples were then sent to the INRB for phytochemical analysis in order to identify and determine secondary metabolites. The *in vivo* antiplasmodial activity evaluation of plant extracts on NMRI mice previously infected with *P. berghei* strain was realized for the three plant extracts comparison.

Aqueous and methanolic extracts preparation of different crude extracts was done as follows:

For Aqueous crude extracts preparation by decoction, five grams of powder were weighed using the KERN analytical balance and mixed with 50 ml distilled water. Mixture was then heated for 15 minutes in the water bath at 100°C. Decoctions obtained were filtered using Wattman No. 1 brand filter paper then collected in the dry and clean bottle.

1 ml of the filtrate is taken and placed in the Eppendorf tube. After drying in the oven at 45°C, dry extract was obtained and which should be used in solution preparation for subsequent analyses. Rest of the filtrate was used for phytochemical screening.

For crude Methanolic extracts preparation, 5 grams of powder were mixed with 50 ml of hydromethanolic solution (80:20). The mixture was stirred well to obtain a homogeneous solution. Resulting solution was kept at room temperature for 48 hours. After filtration, the filtrate obtained was dried in the oven at 45°C until the dry extract was obtained.

2.2.3. ***Phyllanthus phytochemical screening***

The search of different chemical groups of secondary metabolites such as flavonoids, anthocyanins, tannins, quinones, diterpenoids, triterpenoids and steroids, alkaloids and saponins had been carried out on aqueous and methanolic extracts by following different protocols that exist such asthose of Harborne (1998) and Mabry *et al*. (1970) reported by [11].

2.2.4. ***In vivo*** **antiplasmodial activity Evaluation**

2.2.4.1. *Parasite Inoculation*

The *in vivo* antiplasmodial activity was evaluated by the classical 4-day suppressive test (Peters *et al*., 1975) reported by [12]. Four white NMRI albino mice, including two males and two females, were chosen to carry out the first passage (*Plasmodium* multiplication). Each

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mouse had 0.2 ml injection of *Plasmodium berghei* strain kept frozen after thawing and 48 hours were necessary to spread. The blood was taken to carry out double spread (GE: thick drop, FM: thin smear) and slides were read under the Zeiss primo microscope with 100X objective to determine the parasitized mice and pool blood in the glucose buffer.

The NMRI strain mice suffering from malaria were introduced into hermetically closed jars, in which care was taken beforehand to place cotton wool impregnated with ether. A few minutes later, the NMRI mouse maintained in this state fell asleep and was removed from its jar in order to be fixed on the dissection plane. The mouse thorax is opened to access its heart and few EDTA drops are poured into it. Then the blood was taken using the syringe. The infested blood is injected intraperitoneal into healthy mice with 0.2 ml of blood dose per mouse.

Four other mice (two males and two females) were used to carry out the second passage with blood pool from the first four mice. Serial passages modify capacity virulence and development of the parasite. In order to carry out reproducible and reliable *in vivo* tests. After three days, parasitemia was checked using the Zeiss primo microscope with the 100X objective; only mice with 30% to 50% parasitemia of the red blood cells were used for the test. Blood from parasitized strains was collected to reconstitute the blood pool using a glucose buffer which will be used for the test.

48 four-month-old mice weighing between 12 and 25 g were selected and randomly distributed into 8 different cages containing each 6 mice (3 males and 3 females) including two control groups: negative (10% DMSO) and positive (Quinine sulfate 10 mg/kg) controls [13]. Mice were then marked using picric acid on head, back, tail, left side, right side and stomach to differentiate them according to doses and cages.

Mice were previously weighed and malarial blood (0.2 ml) inoculated intraperitoneally to each. Each mouse received approximately intraperitoneally (IP) 10^7 parasitized erythrocytes obtained by parasitized blood dilution from infected mice. *P. odontadenius* extracts doses to be administered to mice (12.5 mg/kg or 25 mg/kg of body weight) were prepared beforehand in the same way as Quinine sulfate (10 mg/kg bw) used as positive control and dimethyl sulfoxide (10%DMSO) used as negative control. Each mouse was administered orally 0.5 ml of extract

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(12.5 mg/kg or 25 mg/kg of body weight) obtained by dissolving *P. odontadenius* methanolic extract in 10% DMSO. Different doses are administered to each test batch of mice orally by gavage using the rounded-tipped syringe.

2.2.4.2. *4e Four-Day Suppressive Test.*

Alongside these test batches, control batches also receive 10^7 parasitized erythrocytes but control mice are treated either with 0.5 ml of Quinine sulfate (10 mg/kg bw) or with 0.5 ml of 10% DMSO. The mice are placed in the same feeding and environmental temperature conditions for the entire 5 days of the experiment. After this time, the parasitemia of each test mouse was determined [12]; [14]. After that, mortality caused by the injection of the extracts is observed daily.

2.2.4.3. *Collection and spreading*

Blood is taken by tip cutting of the infested mouse's tail with the scissor. Gentle pressure along the mouse's tail is exerted to express drops of blood which will be collected on the underside of a glass slide held by its edges and previously numbered. The drop is extended immediately after its deposit with the angle of the second blade using circular movements to obtain a homogeneous disk. Secondly, the smaller drop is collected on the same slide. The Smear is made by applying the edge of another slide in the front of the drop. With a quick and

regular movement, the drop is pushed forward towards the other end of the horizontal blade to obtain a thin film of blood which does not touch the end of the blade. The blade is dried away from flies and other insects.

2.2.4.4. ***Coloring of smears and reading***

The part of the slide containing the smear will be fixed by immersion for 30 seconds in methanol then dried. The entire slide will then be immersed in the May grünwald Giemsa solution which will act for 15 to 20 minutes. The blade is rinsed under the tap then dried horizontally. The reading under an immersion microscope used and the parasite densities applied to the smear calculated.

2.2.4.5. ***4e Four-Day Suppressive test: Antiplasmodial test***

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On D4 (5th day), parasitemia is checked using a thin smear stained with Giemsa to evaluate the progress of the treatment then read under immersion under an optical microscope. The number of parasite-infected RBCs were counted using a light microscope with an oil immersion objective lens at a magnification power of 100×. The survival of the mice is checked twice a day for the duration of the Test. The parasite density and the percentage reduction in parasitemia are calculated from the following formulas, knowing that in mice there are 8.54 million red blood cells/ml of blood ± 0.93 and 527 red blood cells/microscopic field ± 33.85.

The percent of parasitemia and percent of inhibition were calculated by the following Peters–Robinson formula [15]; [16] and the parasite densities by those from [17]:

% parasitaemia = (Number of parasitized RBC/ total number of RBC counted) × 100. (1)

Parasite density (PD) =

A = Number of parasitized red blood cells counted; B = Number of fields read (3 or 5); 8.10^6 = Average number of RBC/mm^3 of blood in mice; 527 = Number of red blood cells per microscopic field considered [17].

The percent of parasite growth suppression (PGS) or the percent of parasitemia reduction was determined by the formula reported by [18], [19], [20], [21] and [17] as following:

where A is the average parasitemia of the negative-control group and B corresponds to the parasitemia of the test group.

The determination of the effect dose 50 of the most active extract is expressed in effect dose fifty (ED_{50}) that is to say the dose of extract which results in the fifty percent reduction of the mice tested parasitemia compared to the control mice. The effect dose 50 (ED_{50}) was

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determined using dose-response curves with the log (doses) on the abscissa and the reduction percentages on the ordinate [19].

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. *Phytochemical screening results*

Phytochemical screening results of the extracts from different *P. odontadenius* samples carried out in tubes and which were confirmed by thin layer chromatography (TLC) are included in the tables and figures which follow.

3.1.1.1. *Phytochemical screening in tubes*

Table 1 presents the different secondary metabolites contained in *P. odontadenius* samples from three sites: Kinshasa (CÉCOMAF) (*Po*1) and Kongo Central (KASANGULU) (*Po*2) and Kwango-bridge (KWANGO) (*Po*3).

Table 1.: Chemical groups of *P. odontadenius* aerial part extracts.

Plants	Chemical groups									
	Extracts	Alc.	Flav.	Anth	G.Tan.	C. Tan.	Ster.	Triterp.	Sap.	Quin.
Po1	Aqueous	+	+	+	+	+	+	+	-	±
	Methanolic	+	+	+	+	+	+	+	+	±
Po2	Aqueous	+	+	+	+	+	+	+	-	±
	Methanolic	+	+	+	+	+	+	+	-	±
Po3	Aqueous	+	+	+	+	+	+	+	-	±
	Methanolic	+	+	+	+	+	+	+	+	+

*Po*1: *P. odontadenius* from Kinshasa, *Po*2: *P. odontadenius* from Kasangulu, *Po*3: *P. odontadenius* from Kwango–bridge; + = presence, - = absence, ± = weak.

Regarding Table 1, it appears that *P. odontadenius* aqueous and methatanolic extracts coming from the Kinshasa city province, Kasangulu in Kongo central and Kwango-bridge in the Kwango province, reveal presence of gallic and catechic tannins, alkaloids, terpenes and flavonoids. Saponins were found to be absent in aqueous extracts but present in *Po*1 and *Po*3 methanolic extracts. Quinones are found in trace form.

3.1.1.2. Thin layer chromatography (TLC)

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Phytochemical screening results of *P. odontadenius* extracts samples from three different regions were confirmed by TLC results regarding alkaloids, flavonoids, Terpenes and steroids. But, these results will be presented in other work.

3.1.2. *In vivo* Antiplasmodial activity

The *in vivo* antiplasmodial activity results of *P. odontadenius* crude extracts from Kinshasa, Kasangulu and Kwango-bridge are shown in the figures which follow.

3.1.2.1. Parasitemia

The parasitemia of mices infected with *P. berghei* and treated with *P. odontadenius* extracts according to provenance sites was illustrated in Figure 1.

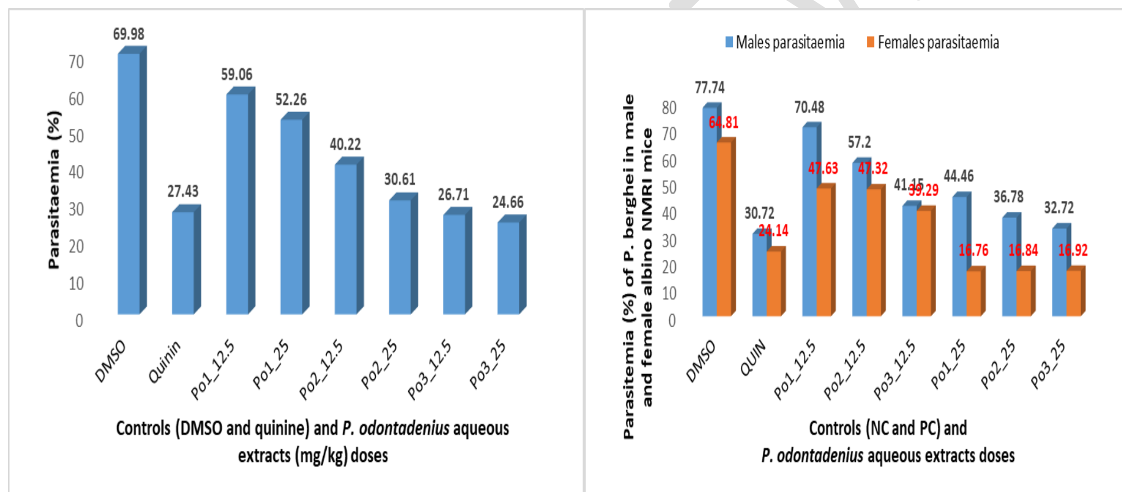

Figure 1: Parasitaemia in NMRI mice depending *P. odontadenius* aqueous extracts dose in comparison negative control (DMSO 10%) and quinine (10 mg/ml) (a); Parasitemia in NMRI mice depending on doses and sex (b).

It appears from Figure 1 that depending on the sex, males presented high parasitemia compared to the females. It appears that the lowest parasitemia is found in the females of the *Po1* specimen at the concentration of 25 mg/kg with 16.76±14.42 % and the high parasitemia showed in the male negative control. Globally, the negative control had high parasitemia compared to the positive control and *P. odontadenius* methanol extracts. The negative control had high parasitemia compared to the positive control and *P. odontadenius* methanol extracts.

It appears here that the parasite depends on the individual's behavior with regard to the drug to which they are subjected. The F test shows that there is a significant difference between the treatments at the 5% confidence threshold because F-cal (3.06) is greater than F-tab (2.96). Statistically, the negative controls parasitemia differs from other parasitemias at the 5% probability threshold. Except in males where the negative control parasitemia does not differ from that of *P. odontadenius* methanol extract dosed at 12.5 mg/kg of body weight.

3.1.2.2. **Parasite density**

The parasite density in NMRI mice infected with *P. berghei* and treated with *P. odontadenius* extracts according to provenance sites was illustrated in Figure 2.

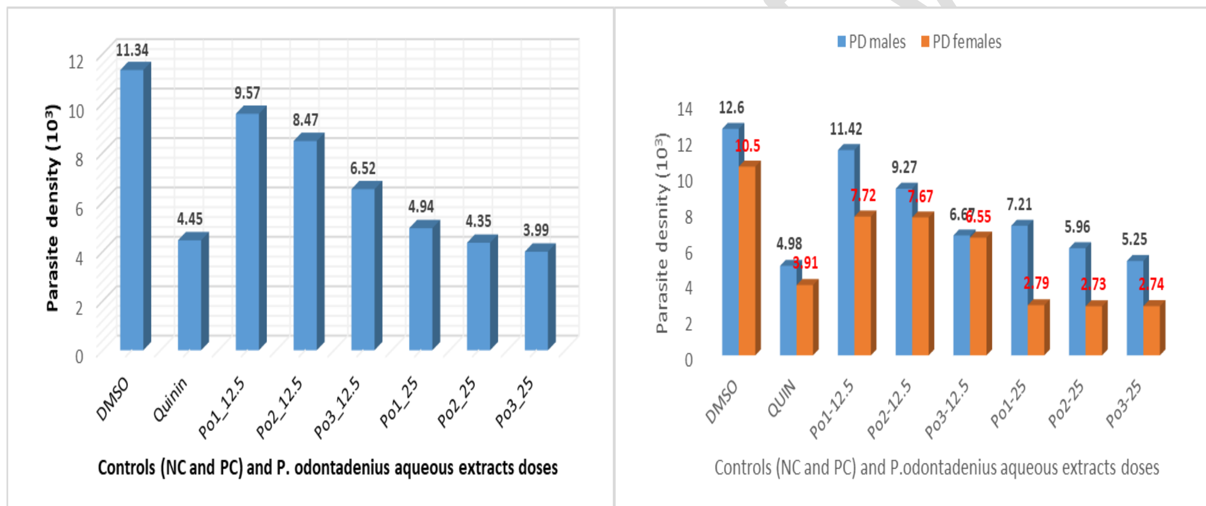

Figure 2: Parasite density in NMRI mice depending *P. odontadenius* methanol extracts dose in comparison negative control (DMSO 10%) and quinine (10 mg/ml bw) (a); Parasite density in NMRI mice depending on doses and sex (b).

It appears from Figure 2, in globally, negative control presented high parasite density with 11342 (±2436) compared to the positive control (4447±1857) and all *P. odontadenius* methanol extracts. It shows that depending on the sex, male's parasite densities showed higher values than theses of females, the lowest parasite density is found in the females of *Po*2 specimen at the concentration of 25 mg/kg bw with 2730 parasites.

It appears here that the parasite density depends on the individual's behavior with regard to the drug to which they are subjected. The F test shows that there is a significant

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difference between the treatments at the 5% confidence threshold because F-cal (5.75) is greater than F-tab (2.03). Statistically, parasite density of negative control differs of all values except theses of *Po*1-12.5 (*P. odontadenius* methanol extract from Kinshasa) and *Po*2-12.5 (*P. odontadenius* aqueous extract from Kasangulu).

3.1.2.3. *Parasitaemia inhibition*

The *P. berghei* parasitic inhibition in mice infected and treated with *P. odontadenius* extracts according to the sites of provenance was illustrated in Figure 3 (Fig.3a and Fig.3b).

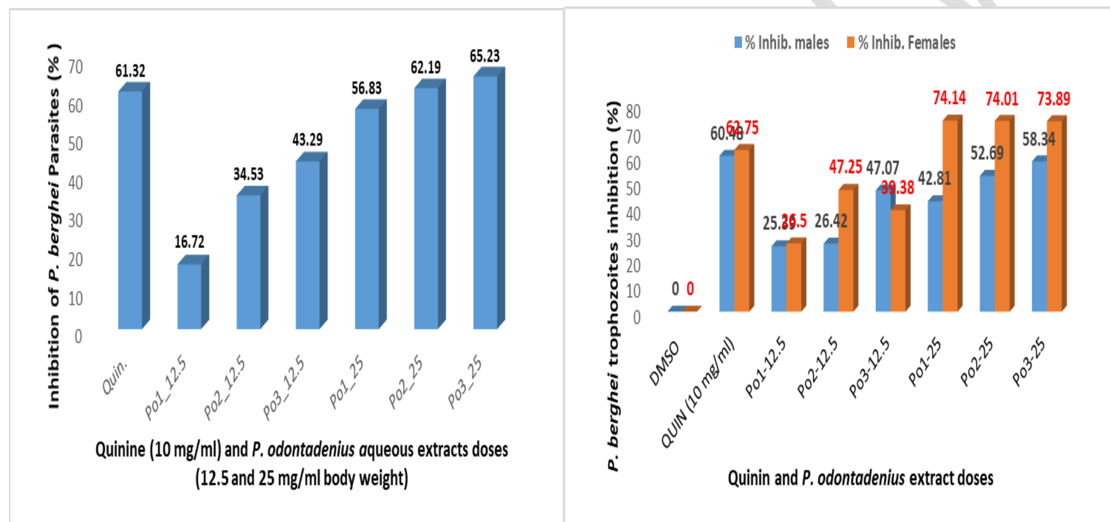

Figure 3: Rates inhibition of *P. berghei* trophozoites in NMRI mice according to *P. odontadenius* methanol extracts concentrations compared to negative and positive control (a) and according to sex (b).

It appears from Figure 3 that the parasite inhibition increases with the doses and changes with sites where *P. odontadenius* harvested (Fig.3a). On the other hand, *P. odontadenius* methanol extracts have high trophozoites inhibition effect on females MNRI comparing to males except for *Po*3-12.5. Females had high rates of parasite inhibition compared to males and also for the positive control (quinine 10 mg/ml bw) except *Po*3 at the dose 12.5 mg/kg bw presented higher average inhibition rate (47.07%) in males than in females with 39.38%.

Looking at Fig., it appears that in general, the parasitemia reduction is more evident in male white mice than in females. However, the greatest parasitemia reduction value in relation

to sex showed in *Po*3 (65.23%) for females. A significant difference emerges between the treatments by the F test.

3.1.2.4. Effect dose 50 (ED_{50})

The *P. odontadenius* methanol extract effect doses in mice infected and treated with *P. odontadenius* extracts according to the sites of provenance was illustrated in figure 4.

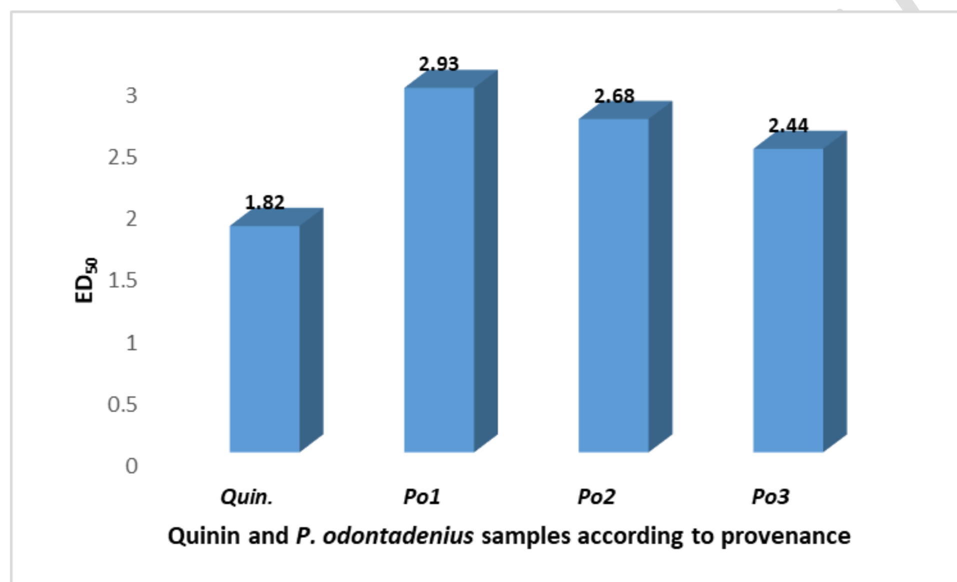

Figure 4: ED_{50} (µg/ml) of *P. odontadenius* extracts on NMRI mice

It appears in figure 4 that the effect doses from *P. odontadenius* methanol extracts varied from 2.44 to2.93 µg/ml. These values were inferior to those of quinines used as positive control with 1.82 mg/ml. Concerning *P. odontadenius* methanol extracts, the high effect dose 50 was *Po*3 (*P. odontadenius* from Kwango-bridge in Kwango region) with 2.44 mg/ml, following those from *Po*2 (*P. odontadenius* from Kasangulu) with 2.68 µg /ml and *Po*1 (*P. odontadenius* from Kinshasa) with 2.93 µg/ml. Quinine, with 1.82 1.82 µg/ml presented a higher effect dose 50 than all P. odontadenius methanol extracts.

3.1.2.5. Survival rate of NMRI mice

The NMRI mice survival rates after seven days are shown in figure 5.

Figure 5 shows that NMRI mice in cage C3 subjected to *Po*2 12.5 mg/kg bw treatment at 83.33% had a high survival rate compared to all other cages. *Po*3 and *Po*2 at 25 mg/kg bw with

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50% survival on day 6 occupied the third position in survival rate. Quinine had shown, with 66.67%, a survival rate in second place compared to treated cages. We believe that these deaths could be explained by the behavior of the NMRI mice towards the aqueous extracts of *P. odontadenius* because the negative control also had a low survival rate, as did the mice treated with the *P. odontadenius* methanol extract (*Po*1_12.5, *Po*3_12.5, *Po*1_25).

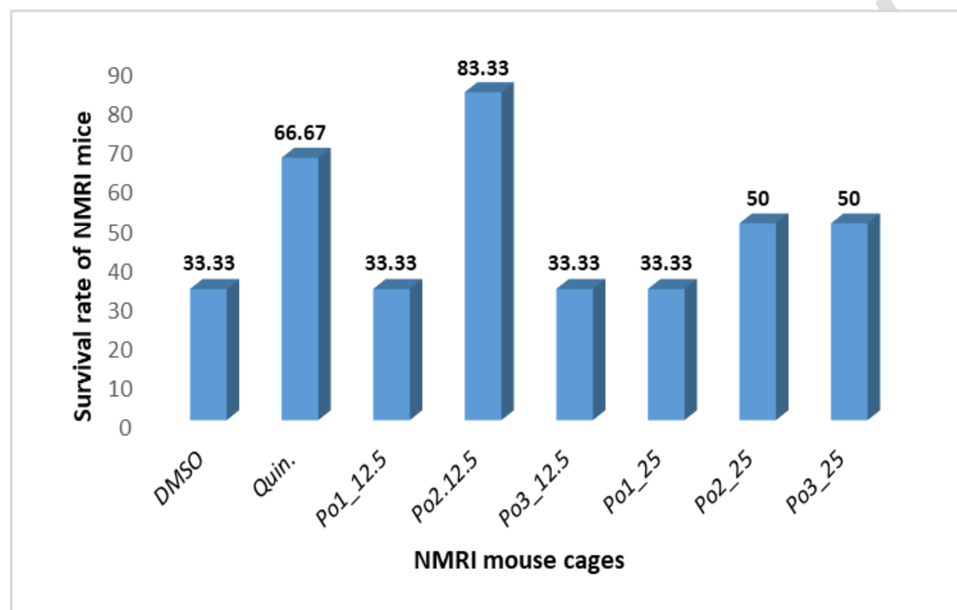

Fig 5. NMRI mice survival rates after seven days

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3.2. Discussion

The seriousness of malaria in the face of the Plasmodium falciparum chemo-resistance against commonly used drugs has pushed us to take an interest in the inexhaustible sources of natural products with therapeutic value which are medicinal plants. Important pathophysiological features of malaria include parasite mediated damage or loss of red blood cells (RBCs) and anaemia [22]. It is in this context that aqueous and methanolic extracts of *P. odontadenius* from Kinshasa, Kasangulu and Kenge were analyzed to determine the different phytochemical groups in this plant and to verify the *in vivo* antiplasmodial activity from this plant collected to three different regions.

Concerning phytochemical analysis of *P. odontadenius*, results obtained in the present study such as the presence of alkaloids, flavonoids, tannins, terpenoids confirm these already proved on Phyllanthus genus against Plasmodium species by others researchers such as [7] who proved the existence on the extracts plants from different species of the genus Phyllanthus main active constituents isolated including alkaloids, flavonoids, lignans, phenols, and terpenes. Others also obtained the same compounds in their research, such as [23], [9], [24]; [20], [25], [26]; [27]; [28]; [29]. But, the saponines almost absence had also been proved by some authors such as [29]; [27] and others cited previously although *Po*2 and *Po*3 hydromethanolic extracts reported their presence in the present work.

The *in vitro* antiplasmodial activies of Phyllanthus species, specially these of *P. odontadenius*, have also proved [29]; [30], but the *in vivo* antiplasmodial of *P. odontadenius* have not yet experimented regarding our knowledge. [10] had previously reported that aqueous and methanolic extracts of *P. odontadenius* exhibited high *in vitro* antiplasmodial activities ($IC_{50}<5$ µg/ml), promising ($5<IC_{50}<15$ µg/ml) or moderate ($15< IC_{50}<50$ µg/ml) *in vitro* antiplasmodial activities of *P. odontadenius* on clinical isolates or on the multi-resistant *P. falciparum* K1 strain.

The results found in this work show that *P. odontadenius* presents good *in vivo* antiplasmodial activity with values expressed as effect dose 50 (ED_{50}) respectively 2.93±0.77 mg/kg for *Po*1, 2.68±0.65 mg/kg for *Po*2 and 2.44±1.05 mg/kg for *Po*3 which are less than 5 mg/kg and which corroborate those reported previously above. [20] had already reported the *in vivo* antiplasmodial activity of *P. emblica* with 14.37±0.17 µg/ml on *P. falciparum* K1 strain, value classed in promising doses ($5<IC_{50}<15$ µg/ml). [31] also reported the Antiplasmodial, antimalarial activities and toxicity from five Phyllanthus species analyzed (*P. amarus*, *P. fraternus*, *P. muellerianus*, *P. niruri* and *P. urinaria*) by some authors on Plasmodium species (*P. falciparum* Dd2, 3d7, W2, Fcb1/Colombia strain and FCB1) which the IC50 varied from 0.44 µg/ml to 11.7 µg/m. [32] previously reported that some aqueous extracts including *P. urinaria* aqueous extract were as active as dichloromethane extracts with IC_{50} values of $<$ or $=$ 4 µg/ml.

These results confirm these obtained on *P. odontadenius* aqueous extracts with ED50's obtained with values less than 4 µg/ml. [20] also report several results including those of [32],

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[8], [12], [33] and [34] showing the aqueous extracts effectiveness of Phyllanthus species including *P. niruri*, *P. urinaria* and *P. acuminatus* on different *P. falciparum* strains such as K1, W2, D-10 and FCR-3 strains. [22] reported that *Phyllanthus amarus* has anti-plasmodial activities and the RBC protective effects because the aqueous and ethanolic extracts of *P. amarus* inhibited growth of the chloroquine-resistant P. falciparum parasites used in their cultures. [35] reported also that the aqueous extracts of P. nummulariifolius exhibited dosedependent inhibition of *P. berghei* ANKA parasites in vivo.

This MeOH extracts effectiveness of Phyllanthus species on plasmodium strains could be explained by the secondary metabolites richness in which they contain such as flavonoids, tannins, alkaloids, terpenoids and many others [30]. These compounds work by either inhibiting the growth of parasites, enhancing the animal organism immunity, or acting as antioxidants to eliminate free radicals released by reactive oxygen species reactions [28]; [36]. As reported by [20], the action mechanism of *P. odontadenius* secondary metabolites was not evaluated in the present study, some of the metabolites exert their antiplasmodial effect either by increasing the oxidation of red blood cells or by inhibiting the synthesis of proteins.

The dose effect values 50 go hand in hand with parasitemia, parasite density and inhibition of parasitemia. It turned on the one hand out that NMRI mice treated with *P. odontadenius* methanol extracts of *Po*3 showed low parasitemia (Fig.1), low parasite density (Fig.2) and therefore strong inhibition of parasitemia (Fig.3) compared to mice treated with methanol extracts of *Po*1 and *Po*2 and compared to controls: positive control (quinine 10 mg/kg body weight) and negative (DMSO 10%). These differences in parasitemia could be explained by the difference of plant content compounds (Tab.1).

On the other hand, *P. odontadenius* methanol extracts act well in females as well in comparison with the males because the females presented high parasitemia inhibition rates compared to the males (Fig. 3b); on the other hand, the latter's were a favorable environment for the parasite (*P. berghei*) multiplication with high parasitemia and parasite density values in comparison to female mice’s (Fig.1b and Fig.3b). These differences in behavior between male and female mice could be explained by the physiology of mice because females could present different periods that help them to resist certain parasites or other attacks compared to males.

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[37] reported that difference in initial parasitemia on the different strains of mice is due to changes in the virulence of *P. berghei* on the one hand, and on the other hand, is due to quite complex genetic influences may explain the resistance to infection of mice by *P. berghei*.

CONCLUSION

The aim of this study was to compare *P. odontadenius in vivo* antiplasmodial activity at three sites located in three different regions (Kinshasa, Kongo-Central and Kwango-bridge). It revealed that all three specimens of *P. odontadenius* had good *in vivo* antiplasmodial activities. All *P. odontadenius* methanol extracts showed good inhibition of parasitemia compared to the negative control, but *P. odontadenius* from Kwango-bridge in the Kwango region presented good behavior in comparison with *P. odontadenius* from Kasangulu and Kinshasa. It is worth noting that male mice were strongly affected by *P. berghei* in comparison to females. Methanol extracts (12.5 mg/kg bw) of *P. odontadenius* from Kasangulu extended the survival rate of NMRI mice after 7 days (D6) since infestation by *P. berghei* and treated with *P. odontadenius* methanol extracts.

The antimalarial activity evaluation of a plant is complex and requires numerous analytical tools. In this study, the extracts effectiveness and the parasite inhibition rate were demonstrated by the in vivo activity of each *P. odontadenius* extract against *Plasmodium berghei* which allows us to validate the use of this plant, but above all we suggest that other studies can be continued particularly on *P. odontadenius* toxicity and also can be carried out the responsible secondary metabolites against Plasmodium strains.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical approval is obtained by the National Institute for Research Biomedicine for works on laboratories animals.

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