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Antimalarial effect and phytochemical Thin-layer chromatography analysis of freeze-dried extracts of *Psychotria calceata* leaves in *Plasmodium berghei* infected Mouse

Effet antipaludique et analyse phytochimique par chromatographie sur couche mince d'extraits lyophilisés des feuilles de *Psychotria calceata* chez des souris infectées par *Plasmodium berghei*

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Article original

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ABSTRACT

Background: Throughout the world, and particularly in Africa, many organic disorders are treated using extracts of natural products derived from so called medicinal plants. These plants are often used empirically, without any scientific proof. Such is the case with *Psychotria calceata*, a plant belonging to the Rubiaceae family with real pharmacological potential that has yet to be demonstrated. The aim of this study was to provide scientific evidence to justify the use of its leaves in the treatment of malaria.
Methods: The composition in bioactive substances of lyophilized extracts was determined by Thin-Layer Chromatography (TLC). The inhibitory power (%I) of lyophilizates on 3D7 and Dd2 *Plasmodium falciparum* strains, followed by determination of the inhibitory concentration 50 (IC_{50}) of the most active lyophilizates, were carried out using the method of Desjardins *et al.* (1979). The parasite suppression rate (%S) was determined using the technique described by Peters (1970).
Results: TLC revealed the presence of alkaloids, flavonoids, terpenes, sterols and saponosides in *Psychotria calceata* leaf lyophilizates. Aqueous infused lyophilizate (AIL) at 10 mg/mL had a %I of 69.505±3.091 and 84.96±2.103 on strains 3D7 and Dd2 respectively. IC_{50} values were 6.35±3.21 and 19.98±0.28 µg/mL on 3D7 and Dd2 respectively. The %S for AIL was 97.50% at dose of 62.5 mg/kg.
Conclusions: *Psychotria calceata* has proven antimalarial activity. This could justify its use in traditional medicine to treat malaria, and enable research into new anti-malarial molecules.

RESUME

Introduction : Les plantes médicinales sont souvent utilisées sans aucune preuve scientifique. C'est le cas de *Psychotria calceata*, une plante de la famille des Rubiaceae. L'objectif de cette étude était d'apporter des preuves scientifiques justifiant l'utilisation de ses feuilles dans le traitement du paludisme.
Méthodes : La composition en substances bioactives des extraits lyophilisés a été déterminée par la chromatographie sur couche mince (CCM). Le pouvoir inhibiteur des lyophilisats sur les souches 3D7 et Dd2 du *Plasmodium falciparum* a été déterminé, puis la concentration inhibitrice 50 (CI_{50}) du lyophilisat le plus actif a été évaluée selon la méthode de Desjardins *et al.* (1979). Le taux de suppression des parasites (%S) a été déterminé selon la technique décrite par Peters (1970).
Résultats : La CCM a révélé la présence d'alcaloïdes, de flavonoïdes, de terpènes, de stérols et de saponosides. Le lyophilisat aqueux (LA) à 10 mg/mL avait un %I de 69,505±3,091 et 84,96±2,103 sur les souches 3D7 et Dd2 respectivement. Les valeurs CI_{50} étaient de 6,35 ± 3,21 et 19,98 ± 0,28 µg/mL pour les souches 3D7 et Dd2 respectivement. Le %S du LA était de 97,50 % à la dose de 62,5 mg/kg.
Conclusion : *Psychotria calceata* présente une activité antipaludique démontrée. Cela pourrait justifier son utilisation en médecine traditionnelle dans le traitement du paludisme et permettre la recherche de nouvelles molécules antipaludiques.

Introduction

Biological activity is the ability of a specific molecular entity to produce a defined biological effect on a target. It is the result of certain effects observed after exposure of an organism to a molecule, and is always the basis of a metabolic or physiological response. It is applied to both simple and complex molecular and reaction systems. There are several types of biological activity, which are studied *in vivo*, i*n vitro* and/or *ex vivo*. Biological activity is always dose dependent on the molecule or compound administered to the living organism. It is therefore logical and more interesting to show the effects (beneficial or undesirable) of a molecule [1]. However, molecules with effects on a living organism with disorders are among the most sought-after with a view to correcting, restoring or modifying these organic disorders. One source where they are found in abundance is the plant world, through medicinal plants. Medicinal plants are plants used in traditional medicine and having at least one medicinal value [2]. Their effects derive from their composition in primary and secondary metabolites, or from the synergy between the various existing compounds [3]. Medicinal plants are used for their properties, which are beneficial to human health. One or more parts of them may be used.

According to the World Health Organization (WHO), of the more than 20.000 plant species used worldwide for their medicinal properties, only 2.000 to 3.000 have been scientifically studied. This poses the problem of the lack of scientific evidence justifying the use of these medicinal plants in the treatment of organic disorders in humans. With a view to helping solve this problem, a medicinal plant from the Rubiaceae family with several therapeutic properties was the subject of the present study. Belonging to the pantropical genus Psychotria, *Psychotria calceata* was chosen on the basis of a literature review. It was chosen for its pharmacological properties (anti-inflammatory, antioxidant, analgesic, antiviral, antimalarial, anticancer, hallucinogenic, antimicrobial, antipyretic, uterotonic, antimitotic, anticonvulsant) [4–7] and for its endangered status due to excessive logging in the tropical forest of southern Cameroon [8].

With a view to valorizing its medicinal use, its antimalarial activity was evaluated as part of this study. The choice of antimalarial activity is based on the damage that malaria continues to cause throughout the world, and particularly in sub-Saharan Africa.

Materials and Methods

Materials

- ***Identification and collection plant***

Psychotria calceata was identified by looking for characteristic features such as the dorsal and ventral color of the leaves and their general shape, the more or less marked veins, the edges of the leaf blade, the presence or absence of down, the presence of petioles and the position of the leaves on the stem. After identification of the species, the leaves were collected from *Afane Essokié,* a small village 25 km from the borough of Campo 'ô, in the South Cameroon region, 60 km from Kribi, capital of the Ocean department. After harvesting, the species was identified (figure 1) by comparison with MIKIO's botanical collection number C5 registered at the Cameroon National Herbarium under number 54000/HNC.

Figure 1: *Fruit-bearing leaves of Psychotria calceata*

- ***Experimental animals***

Male mice of the NMRI strain, aged between 10 and 12 weeks and weighing 25.33 ± 5.21 g, were used in this study.

These animals were obtained from CIRDES (Centre International de Recherche - Développement sur l' Elevage en zone Sub-humide, Bobo-Dioulasso, Burkina-Faso) and acclimatized at the CNRFP (Centre National de Recherche et de formation sur le Paludisme) animal house (Ouagadougou, Burkina-Faso) for one week in polycarbonate cages with wood shavings as bedding before being used. They were fed with food pellets from CIRDES and watered with running water under normal temperature conditions (25 ˚C) followed by a 12 hours light/dark cycle. All experiments were performed in accordance with the procedures of the Helsinki Declaration's Guide to Good Practice in Animal Experimentation.

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- ***Malaria parasite***

Inoculums of chloroquino-sensitive (3D7) and chloroquino-resistant (Dd2) strains and *Plasmodium berghei* donated by the Centre National de Recherche et de Formation sur le Paludisme (CNRFP) under the auspices of the Centre National de Recherche Scientifique et Technologique (CNRST), Ouagadougou, Burkina-Faso, were used to evaluate antimalarial activity *in vitro* and *in vivo* respectively.

Methods

- ***Psychotria calceata leaf powder production***

After harvesting, *P. calceata* leaves were cleaned of foreign matter and fungi, then dried at laboratory temperature (25 °C) in the shade for 14 days. During the drying process, the leaves were turned daily to facilitate air circulation and ensure even drying. After drying, the drug was pulverized into powder (figure 2) using a homemade blade grinder.

Figure 2: *Psychotria calceata leaf powder*

- ***Preparation of Psychotria calceata leaf extract***

Infusion and maceration were the two extraction methods applied to the powder.

✓ **Infusion**

Infusion involved first placing 20 g of leaf powder in 200 mL of distilled water, then in the 75/25 (v/v) water / ethanol 96° (Sigma-Aldrich, France). The mixtures were then kept in contact for 10 min at a temperature of 90 °C [9].

✓ **Maceration**

Powder-solvent mixtures (aqueous and organic 25%, 50%, 75%) were respectively maintained under magnetic stirring (H20 series, Ibx instruments, France) for 24 h at room temperature [9]. Preparations were filtered, centrifuged (Centrifuge 5430, eppendorf, Germany) at 3000 rpm for 10 min and then concentrated (for organic solvents) in a vacuum rotary evaporator (Waterbath B-48, Rotavapor R-114, Büchi, Germany) at 50 °C before being stored in a refrigerator at 4-8 °C [9,10]. Trials were carried out in triplicate with the aim of depleting the drug.

✓ **Freeze-drying**

The resulting infusions and macerates were then freeze-dried using a freeze-dryer (Alpha 1-4 LSCbasic, Christ, Germany). For the aqueous extract, the freeze-drying temperature, pressure and time were -55.2 °C, 0.0633 mbar and 62 h respectively. For the hydroethanolic macerate, they were -55.6 °C, 0.305 mbar and 69 h 38 min. The powders obtained after freeze-drying were weighed, placed in vacuum-sealed food-grade plastics, sealed in polystyrene jars and stored in a dry place to avoid moisture uptake [11]. The extraction yield was determined [10].

Identifying phytochemicals in lyophilized extracts using TLC

The infused and freeze-dried macerates of *Psychotria calceata* leaves were subjected to protocols for detecting the various families of secondary metabolites (alkaloids, terpenes, flavonoids, sterols and saponosides) by thin layer chromatography (**Table I**) [12].

I*nvestigation of the antimalarial activity of lyophilizates of Psychotria calceata leaves*

✓ ***In vitro* inhibitory potency and concentration 50 on *Plasmodium falciparum***

The measurement of hypoxanthine uptake by parasites during short-term cultures described by Desjardins RE et *al*. (1979) was the method used [13]. The antimalarial activity of lyophilizates was assessed by inhibiting the growth of *P. falciparum* in culture, compared with control cultures. Serial dilutions were used to obtain sigmoidal dose-response curves.

✓ **Thawing Plasmodium strains**

Following the method described by Witkowski et *al*. (2010) [14], cryotubes containing parasites (reference strains of *Plasmodium falciparum* sensitive (3D7) and resistant (Dd2) to chloroquine) were removed from liquid nitrogen, thawed in a water bath at 37 °C for 5 min and their contents transferred under a laminar flow hood into 15 mL Falcons tubes. These tubes were then centrifuged (eppendorf, Germany) at 3000 rpm for 5 min and the supernatant removed. An equal volume of 3.5% NaCl solution was added dropwise to the

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blood pellet, and the whole set was slowly shaken. The tubes were left to stand for 1 min, then incomplete medium (RPMI 1640) (LM011-01, Welgene, South Korea) pre-warmed to 37 °C was added (approximately three times the volume of the blood pellet).

The whole was centrifuged at 3000 rpm for 5 min and the supernatant was removed. This washing operation was performed 3 times.

Table I: *TLC identification conditions for chemical groups in freeze-dried extracts of P. calceata leaves*

Chemical groups	Extraction solvent	Elution systems	Revealers	Color	
				To the naked eye	365 nm
Sterols	Hexane	n-hexane-ethyl acetate (20 : 4 ; v/v)	Vanillin sulfuric acid	Blue and blue violet	Blue violet ; red brown
Terpenes	Hexane	n-hexane-ethyl acetate (20 : 4 ; v/v)	Vanillin sulfuric acid	Violet ; pink and orange	Blue violet ; red brown
Flavonoids	Ethyl acetate	Ethyl acetate-glacial acetic acid-formic acid-water (100 : 11 : 11 : 26 ; v/v/v/v)	Neu	Yellow	Blue violet ; red brown
Saponosides	Dichloromethane	Ethyl acetate-petroleum ether (2 : 1 ; v/v)	Sulfuric anisaldehyde	Blue; blue violet and yellow	Green ; red
Alkaloids	Methanol	Toluene-ethyl acetate diethylamine (70 : 20 : 10 ; v/v/v)	Dragendorff	Orange	Light blue ; yellow ; green

Preparation of Plasmodium falciparum parasitized red blood cells

A 10 mL volume of blood from a healthy, uninfected donor with blood group O and rhesus (+) was collected and introduced into two 5 mL collection tubes. These were centrifuged at 3000 rpm for 5 min, and the blood serum and buffy coat were gently removed. The blood pellet was washed three times in incomplete medium consisting of 12.6 mL 25 mM HEPES pH 7.4, 100 mL hypoxanthine, 312.5 µL gentamycin (40 mg/mL) and glucose (20 g/L), and maintained in a 15 mL Falcon tube. Both pellets were exposed *Plasmodium falciparum* strains 3D7 and Dd2 for cell infestation.

Cells culture

After dilution, the parasites present in the mixture were cultured using the method described by Trager and Jensen in 1976 [15] until 2% hematocrit was obtained in RPMI 1640 complete culture medium.

The cultures were then incubated at 37 °C in a special atmosphere (crystal gas mixture 5% CO_2; 5% O_2 and 90% N_2; liquid air).

Media changes and daily parasitemia checks using May Grünwald-Giemsa (RenyLab, Italy) stained smears were carried out to dilute and maintain the culture at 2% hematocrit.

Exposing test substances to *Plasmodium falciparum*-infected cells and incubating specimens

Aqueous solutions of 10 mg/mL *Psychotria calceata* leaf lyophilizates, 25 µg/mL chloroquine (CQ) and 10 ng/mL Dihydroartemisinin (DHA) were prepared and dispensed into 96-well plates (determination of inhibitory potency).

On the other hand, news solutions were obtained by successive dilutions of the stock solutions in complete medium, directly into the 96-well microplates (determination of IC_{50}). Only extracts with an inhibitory power of over 50% were used for IC_{50}) determination. A volume of 100 µL of inoculum (parasitized erythrocytes) and cultures was added to each well to reach a final volume of 200 µL using a multichannel pipette. Wells containing no drug substances were used as negative controls (healthy erythrocytes), while those treated with CQ or DHA were used as positive controls. Microplates were incubated at in an incubator for 72 h. All experiments were performed in triplicate.

Measurement of the inhibitory potency of the samples tested and determination of the IC_{50} values

After incubation, 20 µL of each well was transferred to a new 96-well microplate after homogenization and 100 µL of MALSAT (distilled water, Triton X-100, L-lactate (Sigma code L2250), Trizma base (code T6066), 3-acetylpiridine adenine

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dinucleotide (APAD)) at pH 9 and 25 mL NTB/PES (distilled water, NTB (Nitro Blue Tetrazolium), PES (phenazine ethosulfate)) were added to each well. Plates were incubated in a darkroom at 37 °C for 10-15 min. The absorbances of each well were measured using a spectrofluorometer at 630 nm. The IC_{50} (concentration inhibiting 50% of parasite growth) was determined by dose-response curve analysis [8] using Gen5 software. Only the substance with the best IC_{50} was retained for further activity.

Determination of antimalarial effect of aqueous infused lyophilizate in mice

Mice were divided into 6 groups (n=6) according to their average weight and placed under the same feeding and acclimatization conditions of the laboratory environment during the experiment. The 4-day suppressive test was conducted according to the protocol of Ryley and Peters (1970) [16] adopted par Iwalokun (2008) [17].

Preparing donor mice

For this purpose, on D0, a batch of 6 mice (donors) was infected intraperitoneally with 200 µL of a *Plasmodium berghei* suspension, and parasitemia was checked 72 h later. As soon as a parasitemia of at least 3% was obtained, blood from the donor mice was collected by cardiac puncture using an insulin syringe, after subjecting said mice to general ether anesthesia.

Test batch processing

The first five (05) groups received approximately 107 erythrocytes parasitized with the *Plasmodium berghei* strain. After determination of the hematocrit of the donor mice, followed by infestation of the others, 3 hours after this infestation, these batches were treated *per os* for four (04) successive days (D3 to D7) with doses of 500, 250, 125, 62 mg/kg of aqueous infused lyophilizate and 25 mg/kg of chloroquine phosphate respectively. Parasitemia was checked during the four days of treatment. Animals were weighed daily and dosed once a day according to their body weight during treatment. The sixth group (negative control) received 10 mg/kg of distilled water, with a maximum volume to be administered of 0.5 mL per day per mouse. Parasitemia levels were monitored daily during the treatment period.

Determination of pest control rate

At D8, after sacrifice and tail blood sampling for smears, the percentage of parasitemia (%P) was determined for each mouse [18] as well as their parasitemia suppression rate (%S).

$$\%P = \frac{NGRP}{NtGR} * 100 \; ; \; \%S = \frac{(A-B)}{A} * 100$$

With NGRP as the number of parasitized red blood cells and NtGR the total number of red blood cells, A and B the mean percentages of parasitemia in the negative control and test groups, and S the suppression rate of the extract tested according to the method of Kalra (2006) [19].

Statistical analysis

Results were expressed as means ± standard deviations. Statistical analyses were performed using the classic ANOVA variance test. Graphical representations of the data were made using Graph Pad Prism 8.0 software.

Results

Physical and chemical characteristics of the leaf powder of Psychotria calceata

The conditions prevailing during extraction of the bioactive compounds and the yield of the lyophilizates are presented in **Table II**.

Phytochemical composition of the lyophilizates

Phytochemical screening of the lyophilizates obtained revealed the presence of major chemical groups. The chromatographic results show the presence of five families of secondary metabolites flavonoids, saponosides, sterols, triterpenes and alkaloids (figure 3) at UV/360 nm.

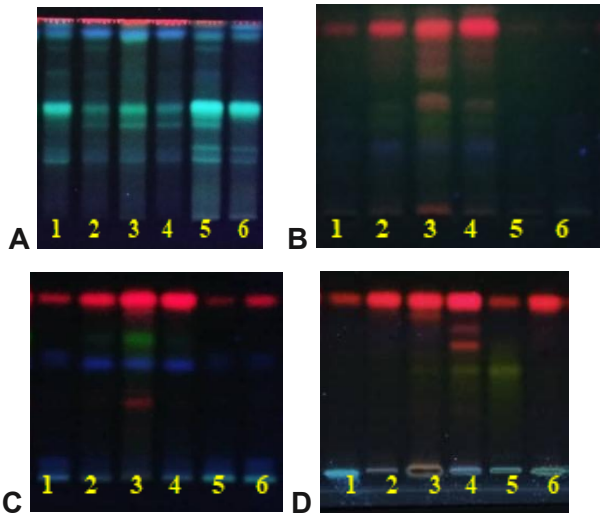

Figure 3: *Chromatographic prints of Flavonoids (A), Saponosides (B), Sterols andTriterpenes (C), and Alkaloids (D) in lyophilizates of hydroethanolic macerates (1(75/25), 2(50/50), 3(25/75)), hydroethanolic infusion (4), aqueous infusion (5), aqueous macerate (6) of P. calceata leaf powder.*

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Table II: *Characteristics of methods for extracting bioactive compounds from Psychotria calceata leaves*

Extraction methods						
Parameters	**Infusion**		**Maceration**			
Solvent used	H_2O	H_2O/ Et OH 96°	H_2O	H_2O/ Et OH 96°	H_2O / Et OH 96°	H_2O / Et OH 96°
Water content (%)	100	75	100	25	50	75
Mass/volume (g/mL)				20/200		
Freeze-drying time (h)	44	48	72	98	72	72
Rd (%) of lyophilizates	35.294	8.844	34.423	07.471	05.158	28.594

Antimalarial activity of Psychotria calceata lyophilizates

- **Inhibiting *Plasmodium falciparum in vitro***

The results of the *in vitro* antimalarial activities of *P. calceata* lyophilizates are presented in Tables III and IV.

Table III: *percentage inhibition of chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) Plasmodium falciparum trains by P.calceata lyophilizates (10 mg/mL) and Dihydroartemisinin (DHA) (10g/mL) used as positive control*

Strains	3D7	Dd2
Sample tested	**% Inhibition**	
Freeze-dried aqueous infusion	69.505±3.091	84.96±2.103
Infused H_2O/EtOH (75/25)	66.766±4.192	75.256±2.408
Aqueous Maceration	62.061±2.202	74.07±3.841
Maceration with H_2O/EtOH (75/25)	69.679±3.132	72.973±5.674
Maceration with H_2O/EtOH (50/50)	66.766±2.175	52.615±5.919
Maceration with H_2O/EtOH (25/75)	71.919±1.341	81.402±0.091
Dihydroartemisinin	76.643±0.504	80.898±1.989

The freeze-dried aqueous infusion showed a better inhibition percentage than the other samples on Dd2 and 3D7. Statistical analysis showed a significant difference (P < 0.05, ANOVA) between the freeze-dried aqueous infusion of *P. calceata* and the Dihydroartemisinin positive control on both *Plasmodium falciparum* strains.

Table IV: I*nhibitory concentration 50 (IC_{50}) of lyophilized aqueous infusion of Psychotria calceata leaves, chloroquine and Dihydroartemisinin on susceptible (3D7) and resistant (Dd2) strains of Plasmodium falciparum.*

Strains	3D7	Dd2
Sample tested	**CI_{50}**	
Aqueous Infused (µg/mL)	6.35±3.21	19.98±0.28
Chloroquine (µg/mL)	11.67±7.54	-
Dihydroartemisinin (µg/mL)	-	6.46±0.17

Statistical analysis shows significant differences (P < 0.05, ANOVA) between the lyophilized aqueous infusion of *P. calceata* and the positive controls Dihydroartemisinin and chloroquine on both *Plasmodium falciparum* strains.

Suppressing parasitemia

Figure 4 shows the results of *in vivo* antimalarial activities, following administration to *Plasmodium berghei*-infected NMRI mice, of doses from 500 mg/kg to 62.5 mg/kg of the lyophilized aqueous infusion of *Psychotria calceata* leaves and chloroquine used as a positive control at 25 mg/kg.

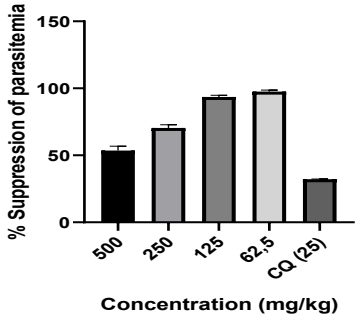

Figure 4*: Percentage suppression of parasite load in mice obtained with freeze-dried aqueous infusion of Psychotria calceata leaves*

Statistical analysis shows a significant difference (P < 0.05, ANOVA) between the different concentrations of lyophilized aqueous infusion of *P. calceata* and Chloroquine used as a positive control on *Plasmodium berghei.*

Discussion

The main results obtained are the qualitative analysis of bioactive compounds and the exploration of the antimalarial activity in mice of the freeze-dried aqueous infusion of *Psychotria calceata* leaf powder.

Two extraction methods (infusion and maceration), followed by freeze-drying, were used in order to make the best choice of extraction method. Freeze-drying times were 44 h and 72 h and extraction yields were 35.29% and 34.42% for

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infusion and maceration respectively. Aqueous infusion, with its faster lyophilization and higher yield, was therefore selected for further work. These results also confirm the fact that extraction yields depend on factors such as the nature and dielectric constant of the solvent, the solvent-drug volume ratio, the granulometry of the drug used, the drying time and the duration of the extraction operation [20–23]. Studies have also reported that extraction with aqueous solvent such as infusion increases that of soluble bioactive compounds as well as cell wall permeability.

Phytochemical screening of *Psychotria calceata* leaf lyophilizates revealed alkaloids, flavonoids, sterols, terpenes and saponosides. Hongmei et *al*. have already demonstrated that alkaloids, terpenoids and phenolic compounds are the major chemical compounds found in plants of the genus Psychotria [4]. Some authors have also reported the presence of alkaloids, flavonoids and tannins in *Psychotria carthagenis, Psychotria leiocarpa, Psychotria capillacea* and *Psychotria deflexa* [24–26]. Variations in secondary metabolites within species of the *Psychotria genus* can be attributed to the climatic conditions in which the species grow, the qualitative and quantitative composition of the constituent elements of the host soils, the part of the plant used and, above all, the time of harvesting and the technique used.

The IC_{50} for CQ on the susceptible strain was 11.67±7.54 µg/mL and DHA was 6.46±0.17 µg/mL for the CQ-resistant strain. The sensitivity test carried out at concentrations of 10 mg/mL gave a percentage inhibition of 69.50% on 3D7 and 84.96% on Dd2 for the aqueous lyophilizate of *Psychotria calceata* infusion. The 50% inhibitory concentrations (IC_{50}) show that the infusion is active (6.35±3.21) and moderately active (19.98±0.28 µg/mL) for the susceptible 3D7 and resistant Dd2 strains of *Plasmodium falciparum.* Infused could therefore be a good candidate for the development of antimalarial drugs. The analysis of the spots in the aqueous infusion shows that the latter appears to be richer in flavonoid compounds, which could be the main bioactive compounds responsible for the antiplasmodial activity of *Psychotria calceata* leaves. However, it cannot be excluded that other classes of compounds whose antimalarial activities have been shown in other studies are associated [27–30].

Several plant extracts have already demonstrated *in vitro* antimalarial effects on *Plasmodium falciparum*. These include *Senna occidentalis* [27], *Argemone mexicana, Securinega virosa, Spondias mombin* and *Opilia celtidifolia*, whose IC50 on the chloroquine resistant strain ranged from 1.00 to 4.01 µg/mL [31]. Zofou et al. (2011) also demonstrated the antimalarial activities of the following plants used in the western region of Cameroon in the management of malaria: *Dacryodes edulis* (leaf IC_{50}: 6.45 and 8.2 µg/mL on 3D7 and Dd2), *Vernonia amygdalina* (leaf I_{C50}: 8.72 and 11.27 µg/mL on 3D7 and Dd2; IC_{50} of roots: 8.72 µg/mL on 3D7), *Coula edulis* (IC_{50} of leaves: 13.80 and 5.79 µg/mL on 3D7 and Dd2), *Eucalyptus globulus* (IC_{50} of leaves: 16.80 and 26.45 µg/mL on 3D7 and Dd2), *Cuviera longiflora* (IC_{50} of trunk bark: 20.24 and 13.91 µg/mL on 3D7 and Dd2) [32]. *Cassia nigricans, Sebastiania chamaelea* and *Euphorbia hirta* also showed good antimalarial activity, with IC_{50} values of 2.8 µg/mL, 3.3 µg/mL and 3.7 µg/mL respectively [33]. Finally, the IC*50* of the methanolic extract of *Landolphia kirkii* leaves was 9.9 µg/mL [30].

The percentages of parasitemia suppression (%S) after 04 days were 53.60%, 70.42%, 93.57% and 97.50% in NMRI mice infected with *Plasmodium berghei* and treated with 500, 250, 125 and 62.5 mg/kg of the freeze-dried aqueous infusion per day respectively, compared with 32.24% in mice treated with 25 mg/kg of Chloroquine. The antimalarial effect thus obtained appears to be inversely proportional to the doses of the infusion administered to the mice. No deaths were observed during the experiment. These results, which confirm the antimalarial activity of the infusion, are comparable to those of *Artemisia annua* and *Alisicarpus ovalifolius* extracts. In fact, at doses of 100, 250 and 500 mg/kg, the percentages of parasitemia reduction were 87.2, 96.8 and 96% respectively for *Artemisia annua* and 61.6, 71.20 and 80.8% for *Alisicarpus ovalifolius* [34]. Contrary to the results obtained in our work, the latter are dose dependent. This difference could be explained, on the one hand, by the fact that the compounds or chemical groups responsible for the antimalarial activity of *P. calceata* infusions act at low doses for optimum effect and, on the other hand, by the phenomena of desensitization of the receptors involved in this activity when concentrations are increased, and competition at the site of action between the molecules of interest (to be determined) and other metabolites that may have an affinity for the same receptors.

Conclusion

This work has shown that freeze-dried aqueous infusions of *Psychotria calceata* leaves have an antimalarial effect *in vivo* in NMRI mice at 62.5 mg/kg. Alkaloids, flavonoids, terpenes, sterols and saponins, which could explain this pharmacological activity, were revealed during

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phytochemical screening. These results, which confirm the traditional uses of *Psychotria calceata*, make it possible to envisage the pharmaceutical development of phytomedicines or improved traditional antimalarial drugs based on powders or standardized extracts of *Psychotria calceata* leaf powders.

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Contributions of Authors

NGOLSOU Françis (Carrying out the various activities and drafting the manuscript), SILUE Gnièngèfèrètien Nounaféri Awa (Participation in antiplasmodial studies and proofreading the manuscript), SORE Harouna (Participation in antiplasmodial activities and analysis of the results), NGO NYOBE Judith Caroline (Proofreading and translation of the document), OUEDRAOGO Salfo (Proofreading of the final document), NNANGA NGA (Activity supervisor), SEMDE Rasmané (Activity supervisor)

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