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Antiplasmodial Activity of the Root Bark of *Strophanthus hispidus* DC.

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ABSTRACT

Malaria is one of the most frequent infectious diseases in the world with an annual mortality rate of more than one million people per year. The parasite responsible, *Plasmodium*, is becoming increasingly resistant to the drugs currently in use. Thus, *Strophanthus hispidus* DC, a medicinal plant widely used in traditional medicine in West Africa and in the Democratic Republic of Congo, was chosen to evaluate the anti-malarial activity of different extracts from the bark of its roots on malaria-infected blood using the microplate method. *In vitro* evaluation of the antiplasmodial activity of crude extracts (petroleum ether, dichloromethane, ethyl acetate, ethanolic, aqueous and methanolic) showed good antiplasmodial activity, with IC_{50} of 6.25 µg/mL, 6.01 µg/mL, 3.10 µg/mL, 3.12 µg/mL, 3.02 µg/mL and 0.39 µg/mL, respectively. This activity could be due to the presence of secondary metabolites found in various extracts including polyphenols and flavonoids. The dichloromethane and ethyl acetate extracts were separated by thin layer chromatography, each of which yielded two fractions that also showed very good antimalarial activities with IC_{50} values of 0.78 µg/mL, 1.02 µg/mL, 1.56 µg/mL, 12.20 µg/mL for the dichloromethane 1, dichloromethane 2, ethyl acetate 1 and ethyl acetate 2 fractions respectively. These results justify the traditional use of *S. hispidus* DC. against malaria.

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INTRODUCTION

The World Health Organization (WHO) reports that malaria is responsible for the death of more than one million people a year worldwide. About 40% of the world population is exposed to this disease and 500 million clinical cases are observed each year worldwide [MÉNARD et *al.*, 2012 ; AGBODEKA et *al.*, 2017]. Throughout the world, traditional pharmacopoeias have played and continued to play a very important role in the discovery of new molecules of therapeutic interest, and more particularly in the fight against parasitic diseases such as malaria [PENCHEV, 2010]. Indeed, plants represent an inexhaustible and renewable source of active ingredients, the traditional and medical use of which has been known for a long time. This is why scientific research is currently being carried out on medicinal plants in malaria-endemic area in order to find new active molecules [MULULA et *al.*, 2017; AGBAJE ET FAGEYINBO, 2014; TABA et *al.*, 2017].

Among the medicinal plants is *Strophanthus hispidus* DC. from the Apocynaceae family, which is used in West Africa and in D. R. Congo in traditional medicine in the form of a decocted of the plant parts (roots, bark of the stem or of the leaves) for the treatment of skin diseases, malaria, dysentery, Buruli ulcer, gonorrhea and rheumatic diseases [MULULA et *al.*, 2017; AGBAJE ET FAGEYINBO, 2014]. *S. hispidus* DC. is now widely exploited due to its various biological activities (antibacterial, anti-nociceptive, antioxidant, anti-inflammatory, anti-hypoglycemic, antidiabetic, etc.) known in the traditional pharmacopoeia [TAOFIK et *al.*, 2015; SAMUEL et *al.*, 2016; AGBAJE and FAGEYINBO, 2012; DURUGO, 2013; OSIBEMHE et *al.*, 2016; YEMOA et *al.*, 2008]. However, to our knowledge, its antimalarial activity has not yet

been the subject of an exhaustive study. It is in this context that we set to evaluate the antimalarial efficacy of organic extracts of *S. hispidus* DC. against *Plasmodium falciparum*.

MATERIAL AND METHODS

Plant material

The roots of *S. hispidus* DC. were collected on February 20, 2016 in the village of Mayala in the province of Kongo central (D.R. Congo). The certification of the plant was made at the herbarium of the National Institute of Study and Agronomic Research (INERA), in the Department of Biology, Faculty of Sciences University of Kinshasa/D. R. Congo. The roots were washed and then the barks were separated from wood. These rinds were air dried at room temperature, and crushed to give a fine powder. Instead of malaria strains, the blood of a 4-year-old child with high parasitaemia was used to perform the targeted biological activity.

Preparation of the crude extracts:

- Maceration extraction with increasing polarity was performed with five organic solvents including petroleum ether (PEt), dichloromethane (DCM), ethyl acetate (EtOAc), ethanol (EtOH) and methanol (MeOH): of which 38 g of powder was macerated in 500 mL of solvent (PEt) in a 1000 mL flask for 48 hrs. The residue of one extraction was used as powder for the next extraction. The filtrate obtained was evaporated in a Heidolph rotative evaporator depending on the boiling temperature of each solvent [PRADINES et *al.,* 1996].
- The extraction by decoction with distilled water: 7 g of fine powder of *S. hispidus* DC. were put in 100 ml of water, the mixture was carried to boiling during 40 minutes at 100˚C and then evaporated under reduced pressure with a Heidolph rotative evaporator, the residue obtained was dried in an oven overnight.

Chemical screening

The various secondary metabolites were identified by conventional methods. Polyphenols by BURTON reagent, tannins by STIASNY reagent, flavonoids by SHINODA reagent, alkaloids, by DRAGENDORFF reagent, steroids and triterpenoids by acetic anhydride [NASSIROU et *al*., 2015].

Biological activity

The antimalarial activity of the extracts was evaluated at the parasitology laboratory at the National Institute for Biomedical Research (INRB) in Kinshasa. The test is said to be Ex vivo since it uses malarious human blood and not an isolated strain of P. falciparum. This is a maturation test for P. falciparum found in malarious blood. The culture medium used is RPMI 1640. Human serum, 4 mL, was incubated together with the malarious blood with strong parasitaemia and the stock solutions of the extracts of S. hispidus DC. at 37° C for 48 hours. Stock solutions were prepared from 0.1 mg/mL extract in 99.9% methanol. The latter were incubated 24 hours before being mixed with the RPMI then 12 cascading dilutions were carried out from a concentration of 100 µg/mL until a concentration of 0.04 µg/mL [BATTEGAY et al., 1991; KAYEMBE et al., 2010; TABA et al., 2012; NASSIROU et al., 2015].

Inhibitory concentration

The IC_{50} value was obtained from software (Origin) by extrapolating on the curve representing the variation of parasite growth inhibition as a function of the concentration of each extract.

Chromatographic methods

The separation and identification of the DCM and EtOAc extracts were done by preparative thin layer chromatography (TLC). The following materials were used: aluminum plate for analytical TLC, silica plate for preparative TLC, chloroform/methanol 9:1 as eluting solvent for DCM extract and ethyl acetate/methanol (9:1) for EtOAc extract, and as developers (UV lamp, iodine vapor, sulfuric vanillin). [STILL et *al*., 1978; COCHARD, 2002; TABA et *al*., 2012; NASSIROU et *al.,* 2015].

RESULTS AND DISCUSSION

Phytochemical screening

Chemical screening results of organic extracts and aqueous extract of the root bark of *S. hispidus* DC. are reported in Table 1. Secondary metabolites such as polyphenols, tannins, flavonoids, alkaloids, steroids and triterpenoids were found. MULULA et *al.* [2017] studied the stem barks of the same plant and found the same secondary metabolites and saponins.

Table I. Chemical screening of extracts of root bark of S. hispidus DC.

Chemical groups	PEt	DCM	EtOAc	EtOH	MeOH	H_2O
Polyphenols	-	+	+	+	+	+
Flavonoids	-	+	+	+	+	+
Tannins	-	-	+	+	-	+
Alkaloids	-	+	-	-	-	+
Steroids	-	-	+	-	-	+
Terpenoids	+	-	-	-	-	-

Legend: + presence – absence; PEt= Petroleum ether; DCM= dichloromethane; EtOAc= Ethyl acetate; EtOH= Ethanol

The different secondary metabolites found in the root barks of this plant roots, polyphenols, flavonoids, tannins, alkaloids and tepenoids could potentially bear anti- malarial molecules. [YEMOA et *al*., 2008; TAOFIK et *al*., 2015]. The nonpolar solvent petroleum ether had only terpenoids as secondary metabolites whereas water extract had all the metabolite except terpenoids. Alkaloids which are often present in many traditional recipes to

treat malaria and known to possess antimalarial activity, was only found in the dichloromethane extract.

Antimalarial activity

Results of *in vitro* evaluation of the antimalarial activity of all the crude extracts of the root bark of *S. hispidus* DC. are shown in the Table 2:

Table 2. Extraction yield and IC_{50} of the different extracts

Extracts	Mass of extract	Extraction yield	IC_{50}
	g	*%*	*µg/mL*
PEt	0.07	0.18	6.25
DCM	0.350	0.92	6.01
EtOAc	0.220	0.57	3.10
EtOH	0.180	0.47	3.12
MeOH	0.05	0.13	0.39
H_2O	0.960	13.7 (not lyophilized)	3.02
Quinine			<0.39

The different yields (%v/v) obtained after extraction with petroleum ether, dichloromethane, ethyl acetate, ethanol, methanol and aqueous were respectively 0.18%, 0.92%, 0.57%, 0.47%, 0.13% and 13.7%. Among organic extracts, dichloromethane extract had the highest yield whereas MeOH extract yield was the lowest. Only the methanol extract with only two secondary metabolites, had an IC_{50} below 1 µg/mL, which was comparable to the standard drug (quinine IC50<0.39 µg/mL). Polyphenols and flavonoids were found to be present in the methanol extract and may be responsible for the observed antiplasmodial activity of the extract [KAYEMBE et *al.*, 2010], though the active principle is yet to be identified. Water, ethyl acetate and ethanol extracts had IC_{50} respectively of 3.02, 3.10 and 3.12 µg/mL, which are excellent antiplasmodial activities according to the scale proposed by WILLCOX et *al.* [2011] and KOPA et *al.* [2016]. The IC_{50} of petroleum ether and dichloromethane extracts were between 5< IC_{50} < 15 which denote active crude extract according to AGBODEKA et *al.* [2017]. The antiplasmodial activities found in the different crude extracts are similar to results reported in the literature for several plants used in traditional pharmacopeia to treat malaria [AGBODEKA et *al.*, 2017; NASSIROU et *al.*, 2015; TABA et *al.*, 2012].

Chromatographic study

Bio-guided separation of DCM and EtOAc crude extracts gave two majors fractions for each extract. The different values of the frontal ratio (Rf) as well as the IC_{50} values of the different fractions obtained from crude extracts are presented in Table 3.

Table 3. Major fractions isolated from DCM and EtOAc extracts

Major fractions	Rf	IC_{50} (µg/mL)
DCM_1	0.81	0.78
DCM_2	0.94	1.02
$EtOAC_1$	0.79	1.56
$EtOAC_2$	0.96	12.20

The IC_{50} values of the two major fractions of dichloromethane crude extract with a value of 0.78 (Rf=0.81) and 1.02 (Rf=0.94) were lower than that of crude extract (IC_{50}= 6.01) and could contain very active anti- malarial molecules according to the scale of WILLCOX et *al.* [2011]. Only one fraction of the ethyl acetate crude extract (RF=0.79) had an IC_{50} below 2, the other fraction showed very moderate antimalarial activity with IC_{50} of 12;20 (RF= 0.96) The DCM crude extract was found to contain only polyphenols and flavonoids which were shown by KAYEMBE et *al.* [2010] to possess remarkable anti- malarial activities; It is therefore very likely that the activities found in those two fractions belong to molecules belonging in the family of those two metabolites.

CONCLUSION

This work aimed at evaluating antiplasmodial activity of organic and aqueous extracts of the root bark of *S. hispidus* D.C. Extraction with increasing solvent polarity of five organic solvents, petroleum ether, dichloromethane, ethyl acetate, ethanol and methanol, led to the characterization of the following secondary metabolites: polyphenols, flavonoids, tannins, terpenoids, alkaloids and steroids.

The methanol crude extract with only polyphenols and flavonoids as secondary metabolites had very active antimalarial activity (IC_{50}<1 µg/mL). The crude extracts of dichloromethane and ethyl acetate were submitted to further separation which gave two major fractions for each. The two fractions from DCM and one of EtOAc had an antimalarial activity with an IC_{50} below 2µg/mL.

Results obtained in this work attest of very interesting antimalarial activity of the root bark of S. *hispidus* and justify its use in traditional medicine to treat malaria. Further work will involve purification of very active antimalarial fractions in order to isolate and characterize antimalarial molecules.

RESUME

Activité Antiplasmodiale des Ecorces des Racines du *Strophanthus hispidus* DC.

Le paludisme est l'une des maladies infectieuses les plus fréquentes dans le monde avec un taux de mortalité annuel qui s'élève à plus d'un million de personnes. Le parasite responsable,

Plasmodium, devient de plus en plus résistant aux médicaments en usage actuel. Les plantes médicinales constituent une grande opportunité pour trouver de nouveaux médicaments actifs plus efficaces. C'est ainsi que le choix a été porté sur *Strophanthus hispidus* DC., une plante médicinale largement utilisée en médecine traditionnelle en Afrique de l'Ouest et en R.D. Congo afin d'évaluer l'activité antipaludique des différents extraits des écorces de ses racines sur le sang impaludé en utilisant la méthode des microplaques. L'évaluation *In vitro* de l'activité antiplasmodiale d'extraits bruts (éther de pétrole, dichlorométhanique, acétate d'éthyle, éthanolique, aqueux et méthanolique) présentent une bonne activité antiplasmodiale, avec des CI_{50} respectivement de 6.25 µg/mL, 6.01 µg/mL, 3.10 µg/mL, 3.12 µg/mL, 3. 02 µg/mL et 0.39 µg/mL. Cette activité pourrait être due à la présence des métabolites secondaires trouvés dans différents extraits, entre autres les polyphénols dont les flavonoïdes. Les extraits dichlorométhanique et acétate d'éthyle ont été séparés par chromatographie sur couche mince et chacun a donné deux fractions qui ont également présenté de très bonnes activités antipaludiques avec comme valeurs des CI_{50} 0.78 µg/mL, 1.02 µg/mL, 1.56 µg/mL, 12.20 µg/mL respectivement pour les fractions dichlorométhane 1, dichlorométhane 2, acétate d'éthyle 1 et acétate d'éthyle 2. Ces résultats justifient l'utilisation traditionnelle du *S. hispidus* DC. contre la malaria.

Mots clés

P. falciparum, Résistance, S. hispidus DC., CI50, chromatographie

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