ANTIPLASMODIAL ACTIVITY OF CRUDE EXTRACTS AND ALKALOID FRACTIONS OF *ACANTHOSPERMUM HISPIDUM*

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ABSTRACT - The crude extracts and alkaloids fractions of *Acanthospermum hispidum* were tested at four different concentrations against *Plasmodium falciparum* parasites using Artemetmer/Lumefantrine as control drug. It was found that the chloroform crude extract and ethyl acetate alkaloids fraction have eliminated the parasites by 63% and 54% respectively.

Keywords: *Acanthospermum hispidum*, crude extracts, alkaloid fractions, antiplasmodial activity

INTRODUCTION

Despite the successes being recorded in the fight against malaria, the burden of the disease still remains of concern to humanity. The recent WHO report revealed that, in 2018 about 228 million cases and 405,000 deaths had been estimated worldwide. *Plasmodium falciparum* and *Plasmodium vivax* remains the two most prevalent malaria parasites with the former being dominant in Africa and other endemic regions of the world [1]. Since antimalarial drugs proved to be the mainstay for rolling back this disease, the search for new, effective, and affordable drugs continues to be of paramount importance especially from natural products [2-7]. *Acanthospermum hispidum* DC (Asteraceae) is an annual herbaceous plant widely used for malaria treatment in West and Central Africa [8, 9]. Previous studies have supported the traditional uses of this plant. For instance the *in vitro* antiplasmodial activity of the aqueous, methanolic and ethanolic extracts of *A. hispidum* had been investigated against chloroquine-sensitive (3D7) and chloroquine-resistant (D2d) strains of *P. falciparum*. The methanolic extract was found to exhibit a moderate to high inhibitory activity against both strains [9]. This study affirmed the result of a similar work which demonstrated a weak activity for the aqueous extract of the plant [10]. But in another work, the ethanolic extract was found to exhibit a significant antimalarial activity against chloroquine-resistant (FcB1) srain of *P. falciparum* [11]. In a related study, the crude alkaloid extract obtained from *A. hispidum* was evaluated against chloroquine-resistant (W2), chloroquine-sensitive (D6) strains as well as six isolates of *P. falciparum*. The results demonstrated a significant inhibitory activity against all the tested strains [12]. Notwithstanding the aforementioned studies, we noted that different approach of bioactivity studies would further provide more guide on the possible isolation of the active compounds in this plant. We are particularly interested in renewing a hope for the potentiality of alkaloids to offer new antimalarial agents. This is convincing considering the historical use of chloroquine in malaria treatment, and the current WHO recommended medication — Artemisinin combination treatments, ACTs — where six of the eight approved drugs constitute alkaloids [13]. Thus, in this communication, we report the antiplasmodial activities of the methanol, ethylacetate, and chloroform crude extracts as well as the alkaloids fractions of *A. hispidum*.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The fresh leaves of *Acanthospermum hispidum* were collected in Batagarawa town, Katsina State. It was identified at the Biology Department, Umaru Musa Yar'adua University, Katsina. The herbarium sample specimen with voucher number UMYU 0306, was deposited at the Botanical Museum of the University. Subsequently, the distilled water washed leaves were air dried and finely ground into powder.

Extracts Preparation

The extracts were prepared using maceration for cycle 24 h. Thus the powdered plant material (150g) was treated with methanol, ethylacetate, and chloroform (500 mL each). The mixtures were filtered and the solvents removed at 40°C using a rotavap to afford the corresponding extracts.

Extraction of Crude Alkaloids

The methanol extract of the plant material was treated with 5% aqueous acetic acid and filtered. The resulting filtrate was then extracted with chloroform and the biphasic layer separated. The organic layer was evaporated to afford neutral and acidic materials, while the aqueous layer was treated with sodium carbonate (10%) until it reached pH 10. This mixture was then extracted with chloroform. The chloroform extract was evaporated to afford the crude alkaloids mixture while the aqueous layer contains water soluble materials [12].

Fractionation of Crude alkaloids

Distilled water (10 ml) was used to dissolve 5g of crude alkaloids by sonication. The solution was then transferred into a separating funnel and chloroform (10 ml) was added. After vigorous shaking, a biphasic layer was obtained which was then collected separately. More volumes of chloroform were added, separated until there was no observable difference between the chloroform phase and the pure chloroform. The same process was performed but using ethyl acetate as the solvent. But in order to obtain methanol fraction, a portion of the crude alkaloids was loaded onto a glass column packed with silica, and then washed with methanol to afford the corresponding fraction.

Antiplasmodial Assay

This was carried out according to the procedure adopted by us in a related study [14]. Accordingly, the antiplasmodial activity was determined based on the percentage elimination of *P. falciparum* parasites after 24 h and 48 hr of incubation.

RESULTS AND DISCUSSION

The antiplasmodial activity was basically determined by assessing the number of the parasitized cells in the test cultures after 24 and 48 hours incubation period. Among the three crude extracts of *A. hispidum* (Table 1), the chloroform extract recorded 63% elimination of the parasites. This was followed by the methanol and ethyl acetate extracts with percentage activities of 54% and 43% respectively. Unlike in the previous study where methanol extract was shown to be the most active, [9] here the less polar solvent appeared contain the most active constituents.

Table 1. Antiplasmodial activity of crude extracts of *Acanthospermum hispidum*

Extract	Average number of parasites per field before incubation	Concentrations of extracts (mg/ml)	Average number of dead parasites per field at the end of incubation	Percentage of elimination at the end of incubation (%)
Chloroform	52	10.5, 5.0, 2.5, 1.25	33	63
Ethyl acetate	52	10.5, 5.0, 2.5, 1.25	22	43
Methanol	52	10.5, 5.0, 2.5, 1.25	28	54
Artemether/ Lumefantrine	52	10.5, 5.0, 2.5, 1.25	49	94

Evaluation of the antiplasmodial activity of the crude alkaloids fractions (Table 2) revealed that the ethyl acetate fraction exhibited the best activity (54%) while the chloroform has the least (37%). Comparing the two different approaches, it could be seen that generally the crude extracts surpasses the alkaloid fractions. But the margin between the antiplasmodial activity of the crude chloroform extract (63%) and ethyl acetate alkaloid fraction (54%) was not too wide. It could therefore be envisioned that the alkaloid mixture may contain some promising compounds. This finding further elaborated on the earlier work which only investigated the crude alkaloids mixture [12].

Table 2. Antiplasmodial activity of Alkaloids fractions of *Acanthospermum hispidum*

Fractions	Average number of parasite per field before incubation	Concentrations of fraction (mg/ml)	Average number of dead parasites per field at the end of incubation	Percentage of elimination at the end of incubation (%)
Chloroform	52	10.5, 5.0, 2.5, 1.25	19	37%
Ethyl acetate	52	10.5, 5.0, 2.5, 1.25	28	54%
Methanol	52	10.5, 5.0, 2.5, 1.25	23	44%
Artemether/ Lumefantrine	52	10.5, 5.0, 2.5, 1.25	49	94%

CONCLUSION

The approach explored for the antiplasmodial investigation of *Acanthospermum hispidum* in this work has provided more insight into the possible extracts that could hold the antimalarial lead compounds buried in this plant. At the very least, it demonstrated that the chloroform crude extract and ethyl acetate alkaloids fraction of *A. hispidum* holds a treasure of promising compounds waiting to be unlocked. This task is currently underway in our laboratory.

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