



SJMLS - 4 (3) - 010

***In-vivo* Antiplasmodial Activity of Hydroethanol Crude Extract of the Roots and Leaves of *Acanthospermum hispidum***

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**Abstract**

This study aimed at assessing the antiplasmodial activity of extracts from *Acanthospermum hispidum* used in Nigeria to treat malaria. The plant leaves and roots were extracted with hydroethanol (water: ethanol 50:50 v/v) and the study was done using the Peters' 4-day suppressive test against *Plasmodium berghei* ANKA in mice. The extracts were given orally to 8 groups (A-H) of five mice infected with the *Plasmodium*. The control group (A) received distilled water while groups B- D received daily 100, 250, and 500mg extract/kg b.wt. of leaves extract, groups F-G received 100, 250, and 500mg extract/kg b.wt. of roots extract respectively, and the group H animals received 10 mg/kg b.wt. of quinine. Qualitative phytochemical screening of the extracts was also done. The phytochemical screening indicated the presence of all tested constituents (alkaloids, glycosides, saponins, steroids, phenols, flavonoids and tannins) in the leaves extracts and the presence of all constituents except saponins in the roots extract. The *in vivo* results for both leaf and root extract showed that, extract of *A. hispidum* demonstrated high parasitaemia inhibition. The test groups caused significant ( $p < 0.05$ ) dose-dependent reduction of schizonticidal, on the parasitaemia, with the maximum level of reduction seen at the uppermost dose. Comparatively, the leaves extract displayed higher activity than the roots extract. Therefore, the results revealed good antiplasmodial activity of *A. hispidum* leaves and roots at the doses used, which also correlated with the phytoconstituents, thereby lending support to the traditional use of this plant against malaria.

**Keywords:** *Antiplasmodial, Acanthospermum hispidum, Plasmodium berghei, Phytochemical screening, Hydroethanol.*

**Introduction**

Malaria is among the most dangerous pathogenic diseases common in endemic regions of the world, predominantly in Africa, Latin America and Asia (Ravikumar *et al.*, 2012; Sha'a *et al.*, 2011). It is a potentially deadly tropical disease caused by protozoan parasites: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae* (Ong'echa *et al.*, 2006), *Plasmodium knowlesi* (Singh and Daneshvar, 2013) and *Plasmodium cynomolgi* (Ta *et al.*, 2014). The most virulent among them, being the *Plasmodium falciparum*. An estimated 216 million malaria cases and 445,000 deaths worldwide were reported by World Health Organization (WHO) in 2017, with 90% of the cases affecting the African region (WHO, 2017). In Africa only, more than 300 million people are projected to be infected by the parasite (*Plasmodium falciparum*) annually, with over one million deaths, recorded in under five years children (Zofou *et al.*, 2011).

The use of artemisinin-based combinations therapies (ACTs) has been recommended by WHO for first-line treatment of uncomplicated malaria (WHO, 2015). The control of malaria has become a tough task for endemic countries because of the spread of drug resistant parasites coupled with inadequacy of effective drugs for malarial treatment, thereby warranting the search for new antimalarial drugs (Sha'a *et al.*, 2011; Zofou *et al.*, 2011). Thus, the continuous search for new and more active antimalarial

compounds specifically from plants extracts is significant in preventing malaria infection (Ravikumar *et al.*, 2012).

In Africa, the traditional use of plants has a vital role in the customary methods of treating malaria by offering good sources for the discovery of new antiplasmodial compounds (Chukwujekwe *et al.*, 2009). The therapeutic activities credited to most medicinal plants are correlated to phytochemical compounds present in them. Phytochemicals like alkaloids, saponins, glycosides, phenols, flavonoids, triterpenoids, etc. have been said to have antimalarial property (Ravikumar *et al.*, 2012; Sha'a *et al.*, 2011; Zofou *et al.*, 2011; Valdes *et al.*, 2010).

*Acanthospermum hispidum* DC (Bristly starbur), is of the Asteraceae family with dichotomous branching, 50 – 80 cm high and thorny in nature (Burkill, 1985). The plant is found in tropical America, tropical Africa, amongst other places. The literature review shows that several parts of *A. hispidum* have medicinal and curative values for diverse ailments. Thus, its antimicrobial (Fleischer *et al.*, 2003), anthelmintic and immunostimulant (Summerfield and Saalmüller, 1998; Adjanohoum *et al.*, 1988) and antiviral (Summerfield *et al.*, 1997) activities have been reported. The plant is also used traditionally for the treatment of jaundice, constipation, fever, stomach ache and malaria (Mshana *et al.*, 2000). Similarly, its antidiarrheal (Agunu *et al.*, 2005), antidiabetic (Roy, 2013), antiplasmodial (Sanon *et al.*, 2003), antitumor (Deepa and Rajendran, 2007) and hepatoprotective (Edewor *et al.*, 2007) activities have been reported as well. In Nigeria, Ghana, Benin and Cameroon, the leaves/ whole plant is used to treat malaria (Pierre *et al.*, 2011; Ganfon *et al.*, 2008; Odugbemi *et al.*, 2007; Asase *et al.*, 2005; Sanon *et al.*, 2003). However, there are insufficient accounts on the antiplasmodial activities of the leaves and roots extracts of the plant, as most of the reports are centered on the leaves. Hence this study was designed to comparatively assess the *in vivo* antiplasmodial activity of hydroethanolic extracts of the leaves and roots of the plant against *Plasmodium berghei* ANKA. Also, the phytochemical screening of the extracts was done.

## Materials and Methods

### The Plant Material

The plant, *Acanthospermum hispidum* was collected from a farm land in Otukpo, Benue State, Nigeria and was authenticated at the Department of Biological Science, Ahmadu Bello University, Zaria. The roots and leaves were washed and air dried in the shade for two weeks and milled. For each sample, the ground plant (50g), was cold macerated for 72 h using hydroethanol (ethanol: water 50: 50 v/v), and filtered through Whatman no.1 filter paper, then the resulting filtrates were rotary evaporated under reduced pressure (40°C). The crude extracts were thereafter stored at -4°C, and reconstituted with distilled water prior to usage. All solvents used in this work were of analytical grade.

### Phytochemical analysis

Preliminary phytochemical analysis of the plant extracts was made to identify the presence of chosen phytochemical constituents (alkaloids, steroids, cardiac glycosides, flavonoids, tannins and saponins) using standard methods as formerly described (Trease and Evans 1989; Sofowora, 1993).

### The Biological Material

#### Experimental animals and parasite

Forty Swiss albino mice (Male and Female) aged 7–8 weeks (16–32 g) used for this experiment, were obtained from the Animal Breeding Unit of the Department of Anatomy, Delta State University. Before commencing the work, the mice were acclimatized at 25°C ± 2°C, with 12:12 light/dark cycle for ten days. Throughout the experiment, the animals were given standard diet (Vita Feeds grower's mash) and water *ad libitum*.

#### Parasites

The *Plasmodium berghei*, ANKA (chloroquine sensitive strain) used was gotten from the National Institute of Medical Research (NIMER), Yaba Lagos, Nigeria, and the parasite was sustained by weekly serial passage of blood taken from infected mice to non-infected ones.

#### Parasite inoculation and In vivo suppressive test of *Plasmodium berghei*

Donor albino mice that were earlier infected with

*P. berghei* with parasitaemia level of 20–30% were used following the procedure discussed previously (Odetola and Basir, 1980).

The *in vivo* antiplasmodial activity test was done according to the 4-day suppressive test (Peters, 1965). The animals (40 mice) were grouped into eight groups of five mice, then treatment (drug or extract) was administered orally as follows.

- Group A = Infected Mice + normal saline
- Group B = Infected Mice + 100 mg/kg of *A. hispidum* roots extract
- Group C = Infected Mice + 250 mg/kg of *A. hispidum* roots extract
- Group D = Infected Mice + 500 mg/kg of *A. hispidum* roots extract
- Group E = Infected Mice + 100 mg/kg of *A. hispidum* leaves extract
- Group F = Infected Mice + 250 mg/kg of *A. hispidum* leaves extract
- Group G = Infected Mice + 500 mg/kg of *A. hispidum* leaves extract
- Group H = Infected Mice + 10 mg/kg of quinine.

On day five of the experiment, thin blood smears were made from blood taken from each mouse, and the slides were fixed for microscopic examination. The percentage parasitemia was calculated:

$$\% \text{ Parasitemia} = \left[ \frac{\text{Number of infected RBCs}}{\text{Total Number of RBCs}} \right] \times 100$$

The extract activity was evaluated by estimating the percentage reduction of parasitemia using:

$$\% \text{ reduction} = \left[ \frac{\% \text{ parasitemia of Negative control} - \% \text{ parasitemia of Treated group}}{\% \text{ parasitemia of Negative control}} \right] \times 100$$

### Statistical analysis

Data obtained were analyzed statistically using ANOVA (two-way) followed by a post test (Sidak's multiple comparisons test) and the results were expressed as means  $\pm$  standard deviation (SD). Mean differences were considered significant at 95% level of significance ( $p < 0.05$ ).

### Results

#### Phytochemical Analyses

Seven qualitative phytochemical tests were made using hydroethanolic on the leaf and roots extracts of the plant to identify the presence of alkaloids, steroids, cardiac glycosides, flavonoids, tannins and saponins. The results indicated the presence of all tested constituents in the leaves extracts and the presence of all constituents except saponins in the roots extract (Table 1).

**Table 1: Preliminary phytochemical studies of ethanol extracts of *Acanthospermum hispidum* DC.**

Phytoconstituent	Ethanol leaf extract	Ethanol roots extract
Alkaloids	++	+
Glycosides	+	+
Saponins	++	-
Steroids	+	+
Phenols	+	+
Flavonoids	++	+
Tannins	++	+

**Key:** +++: highly present, ++: moderately present, +: Low, -: absent.

#### Effect on suppressive activity of hydroethanolic extracts of *Acanthospermum hispidum*

The extract showed a dose-dependent chemosuppressive effect on the parasitaemia. These effects were statistically significant relative to the control ( $p < 0.05$ ). The reduction percentages ranged from 37.36 $\pm$ 1.58 to 61.24 $\pm$ 3.68 % for the leaves extract and from 29.80 $\pm$ 4.22 to 46.34 $\pm$ 1.79 % for the roots extract (Table 2). However, the effect of both extracts was relatively weak when compared to standard drug, quinine, with a reduction of 95.71 $\pm$ 0.09 % (Table 2).

The results for both leave and roots extract showed that, all extracts of *A. hispidum* demonstrated high parasitemia inhibition. In comparing the test groups with the control group, the extracts caused significant ( $p < 0.05$ ) reduction in the parasitaemia level at all doses used, with the maximum level of parasitemia reduction seen at the uppermost dose. Thus, the extracts displayed a dose-dependent effect of schizonticidal, on the parasitaemia, with the leaves extract displaying higher activity than the roots extract. Therefore, the results revealed good antiplasmodial activity of *A. hispidum* leaves and roots at the doses used.

**Table 2: Antiplasmodial activity of *A. hispidum* roots and leaves extracts.**

Drug/extract	Doses (mg/kg)	Parasitemia		Percentage of reduction	
		Leaves extract	Roots extract	Leaves extract	Roots extract
Normal saline	Control	48.14±1.68 <sup>a</sup>	48.14±1.68 <sup>f</sup>	0.00	0.00
Crude extract	100mg/kg	30.14±0.86 <sup>b</sup>	33.76±1.55 <sup>g</sup>	37.36±1.58 <sup>o</sup>	29.80±4.22 <sup>r</sup>
	250mg/kg	25.35±1.72 <sup>c</sup>	29.40±1.26 <sup>h</sup>	47.38±4.33 <sup>p</sup>	38.92±3.53 <sup>s</sup>
	500mg/kg	18.67±1.30 <sup>d</sup>	25.82±0.79 <sup>i</sup>	61.24±3.68 <sup>q</sup>	46.34±1.79 <sup>t</sup>
Quinine	Drug (10mg/kg)	2.07±0.06 <sup>e</sup>	2.07±0.06 <sup>j</sup>	95.71±0.09 <sup>r</sup>	95.71±0.09 <sup>u</sup>

**Key:** \*The results are expressed as mean of parasitemia/% reduction ± standard deviation.

### Discussion

In Africa, medicinal plants play an essential role in the conventional approaches of malaria treatment through which novel antiplasmodial compounds are discovered (Chukwujekwe *et al.*, 2009). Medicinal plant research as a way of discovering promising herbs and new chemical compounds against diseases including malaria has increased over time (Tilburt and Kaptchuk, 2008). In Nigeria and some other African Countries, *A. hispidum* plant is used to treat malaria (Pierre *et al.*, 2011; Ganfon *et al.*, 2008; Odugbemi *et al.*, 2007; Asase *et al.*, 2005; Sanon *et al.*, 2003). However, there are few accounts on the antiplasmodial activities of the plant, hence this study was designed to comparatively assess the *in vivo* antiplasmodial activity of hydroethanolic extracts of the leaves and roots of the plant against Plasmodium berghei ANKA. Also, the phytochemical screening of the extracts was done. The *in vivo* activity was evaluated by the decrease in the parasitemia caused by each extract (dose), comparing to the control.

The results of this study for both leaves and roots extract showed that, extract of *A. hispidum* demonstrated high parasitemia inhibition. In comparing the test groups with the control group, the extracts caused significant reduction in the

parasitemia level at all doses used, with the maximum level of parasitaemia reduction seen at the uppermost dose. Therefore, the extracts displayed a dose-dependent effect of schizonticidal, on the parasitaemia, with the leaves extract displaying higher activity than the roots extract. Thus, the results revealed good antiplasmodial activity of *A. hispidum* leaves and roots at the doses used, thereby justifying the traditional use of this plant against malaria (Pierre *et al.*, 2011; Ganfon *et al.*, 2008; Odugbemi *et al.*, 2007; Asase *et al.*, 2005; Sanon *et al.*, 2003).

The results of phytochemical screening indicated the presence of all tested constituents (alkaloids, glycosides, saponins, steroids, phenols, flavonoids and tannins) in the leaves extracts and the presence of all constituents except saponins in the roots extract. Thus, the results partly agree with earlier findings on *A. hispidum* leaves which indicated that they contained cardiac glycoside, saponins, alkaloids, triterpene, steroids, anthraquinones, tannins and flavonoids (Chika *et al.*, 2018; Okoro *et al.*, 2017; Roy *et al.*, 2010). These chemical classes have been reported as having efficient antioxidant/ antimalarial activities (Sha'a *et al.*, 2011; Zofou *et al.*, 2011; Valdes *et al.*, 2010).



Flavonoids are said to exert antiplasmodial activity through chelating (with nucleic acid base pairing) of the parasite (Lui *et al.*, 1992), whilst the terpenes/ sesquiterpenes plasmodicidal activities have been associated to endoperoxidation (Hatzakis *et al.*, 2007). The plasmodicidal activity of *A. hispidum* may be due to the presence of these compounds and could be used in part to explain the possible antiplasmodial mechanism of the extract, as the presence of phytochemicals, have been said to confer protective and antioxidative effect against the malaria parasite stress induced oxidation in the parasitized red blood cells (RBCs) (Nethengwe *et al.*, 2012).

Furthermore, antioxidant potentials of certain plants and products (e.g. flavonoids) have been reported to promote schizonticidal activity in moderating the cell signaling pathway which has been said to account for antiplasmodial activity of some compounds, like quercetin (Ganesh *et al.*, 2012; Cimanga *et al.*, 2009). Severe malaria complications are caused by elevated free radicals levels and these are normal characteristics of malaria disease. This may well be one of the modes of action of *A. hispidum* extract, which contains flavonoids and phenolics with reported antioxidant activity (Okoro *et al.*, 2015).

The extracts may have also inhibited free oxidized haem (FP – Fe III) detoxification, by intercalating with the iron-carboxylate bond which connects the haem units of the malaria pigment (hemozoin), so inhibiting their polymerization (Ravikumar *et al.*, 2012; Becker *et al.*, 2004). The extracts may also contain constituents capable of inhibiting Plasmodium growth through hindering the parasite choline intracellular transport needed for the biosynthesis of the phosphatidylcholines that are important molecules for Plasmodium (Hilou *et al.*, 2006). Consequently, the phytochemical principles in the extracts could have a relative association with antiplasmodial activity (Ravikumar *et al.*, 2012).

Therefore, the *in vivo* study results showed good activity of *A. hispidum* with the plant extracts considerably reducing parasitaemia dose

independently in the suppressive model, thus corroborating the antimalarial ability of the plant. However, the antiplasmodial effect was weak when equated to the standard drug (quinine). These observations were further buttressed by the phytochemical screening results. Comparatively, the leaves extract of *A. hispidum* showed better antiplasmodial activity than the roots extracts and the leaves extract also exhibited higher presence of the tested phytochemicals relative to the roots extract.

### Conclusions

The results from this study provide support to past reports that *A. hispidum* has antiplasmodial activity. Antiplasmodial activity was dose dependently exhibited by both leaves and roots extract of *A. hispidum* with leaves extract showing better activity than the roots extract. These activities were correlated with the phytochemical screening results. Thus, the phytochemicals present may be responsible for the observed antiplasmodial properties.

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**Citation:** Okoro, I.O. *In-vivo* Antiplasmodial Activity of Hydroethanol Crude Extract of the Roots and Leaves of *Acanthospermum hispidum*. *Sokoto Journal of Medical Laboratory Science*; 4(3): 77- 83.

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