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Full Length Article

Antiplasmodial activities of ethanol extracts of *Euphorbia hirta* whole plant and *Vernonia amygdalina* leaves in *Plasmodium berghei*-infected mice

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Abstract

The African continent is rich in both flora and fauna resources, which have been extensively used locally in the treatment or cure of a wide variety of ailments. Enquiries into traditional folklore revealed that *Euphorbia hirta* and *Vernonia amygdalina* possess antiplasmodial properties among others for the treatment of febrile conditions. We therefore investigated the phytochemistry, antimalarial potencies, hepatic toxicities and renal toxicities of the crude ethanol extract of the whole plant of *Euphorbia hirta* (CEEH) and leaves of *Vernonia amygdalina* (CEVA) using standard procedures. The obtained results showed that CEEH and CEVA contain flavonoids, alkaloids, tannins and saponins, but not phlobatannins. CEEH and CEVA mildly inhibited *P. berghei* schizont maturation (44.36% and 37.85%, respectively), while Artesunate Combination Therapy (ACT) was only slightly potent (>50%) against chloroquine-sensitive *P. berghei*. CEEH significantly decreased ALP, but significantly increased bilirubin. Both extracts significantly increased the albumin and total protein levels compared to infected, untreated animals. Compared to Camosunate[®], CEVA caused further significant increases in creatinine and urea. However, these increases did not indicate organ damage.

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Keywords: *Euphorbia hirta*; *Vernonia amygdalina*; *Plasmodium berghei*; Malaria

1. Introduction

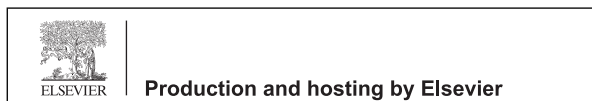
Malaria is an increasing worldwide threat mostly in Africa and sub-Saharan Africa. In 2012, it was reported

that an estimated 482,000 children under five years of age were killed as a result of malarial infection (i.e., 1, 300 children every day or one child almost every minute) [1]. The disease is caused by *Plasmodium* species and is transmitted by female *Anopheles* mosquitoes [2]. Malaria is one of the most important tropical diseases and the greatest cause of hospitalization and death among children aged 6 months to 5 years [3,4]. In Nigeria, malaria transmission occurs year round in the South but seasonal in the North. Nigeria accounts for a quarter of all malaria cases in the WHO-African region [1,5]. The alarming rate at which *Plasmodium falciparum* has developed resistance to chloroquine and

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other synthetic antimalarial drugs makes it necessary to search for more effective antimalarial compounds [6]. The seemingly untameable incidence of malaria in Africa is increasingly alarming. In 2001, the World Health Organization recommended Artemisinin Combination Therapies (ACTs) as the first line of treatment for uncomplicated malaria [7] caused by human *Plasmodium falciparum*. Like quinine, artemisinin is derived from a plant but is a structurally distinct compound. The pharmacophore consists of a peroxide within a 1, 2, 4-trioxane configuration, which leads to several suggestions of how these antimalarials might work [8]. In Africa and other countries where malaria is endemic, traditional medicinal plants are frequently used to treat or cure malaria [9,10]. Antimalarials, such as chloroquine, artesunate, antifolates and tetracyclines, were identified either by their chemical relationship to natural products or from their activity against other infectious pathogens [11]. It is therefore imperative to continue the search of new chemical entities that can qualify as candidate antimalarial drugs as remedies with little or no side effects. As such, adequate knowledge and scientific validation of the antimalarial efficacy of these herbal medicines are necessary to guide policy makers in their formulation of guidelines for the use of herbal medicines in the treatment of malaria in Nigeria [12]. In this study, we evaluated the phytochemistry, antiplasmodial efficacies, hepatic toxicities and renal toxicities of the crude ethanol extracts of the whole plant of *Euphorbia hirta* and the leaves of *Vernonia amygdalina* in *Plasmodium berghei*-infected mice.

2. Materials and methods

2.1. Plant materials

Fresh whole plants of *Euphorbia hirta* and leaves of *Vernonia amygdalina* were collected from a notable organic farm in the premises of Water Works, Ede, Osun State, Nigeria in April 2014 and were identified at the Herbarium of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria where the sample of the plant was deposited. The plants and leaves were air-dried over 14 days under shade at room temperature and then pulverized using Diaka[®] milling machine. Each plant powder (250 g) was soaked in 1.5 L of 95% ethanol. The cold extraction lasted 72 hrs after which the mixture was filtered through Whatmann filter paper No 1. The solvent was removed under pressure using a rotary evaporator (LIDA XMT-J7000/RE52-3 iDNA). The crude ethanol extracts of *Euphorbia hirta* whole

plant and *Vernonia amygdalina* leaves weighed 62 g and 46 g, respectively.

2.2. Phytochemical screening

The crude ethanol extracts were analysed for the presence of important phytochemicals following the protocol previously described by Sofowora [13]. Briefly, tannins and flavonoids were determined by boiling 0.5 g of extract with 20 ml of distilled water for 5 minutes in a water bath. Several drops of 0.1% ferric chloride and hydroxide solutions were added to 2 ml of the filtrate, respectively. Brownish green or blue-black colouration indicated the presence of tannins, while yellow colouration showed the presence of flavonoids. Presence of saponins was determined by frothing upon boiling of 2 g of crude extract with 20 ml of distilled water in a water bath for 10 minutes. The sample was filtered and allowed to cool. The filtrate (10 ml) was diluted with distilled water (1:1 v/v) and shaken vigorously until formation of froth, which was stable for a few minutes. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion. Alkaloids were determined by boiling 1 g of extract with distilled water and acidified with 5 ml of 1% HCl in a water bath. Several drops of Meyer's reagent were added to 2 ml of the filtrate. The formation of creamy white/turbid precipitate indicated the presence of alkaloids. Terpenoids were determined by mixing 5 ml of extract solution with 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was then carefully added to form layers. A reddish-brown precipitate at the interface indicated the presence of terpenoids. Presence of cardiac glycosides was determined by dissolving 5 mg of extract in 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. The mixture was then layered with 1 ml of concentrated H₂SO₄. A brown ring at the interface indicated the presence of cardiac glycosides. Presence of phlobatannins was determined by deposition of a red precipitate when several drops of dilute HCl were added to 2 ml of extract.

2.3. Antiplasmodial screening

The mice and *Plasmodium berghei* used for this study were obtained from the Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. Parasitised erythrocytes obtained from donor mice by ocular bleeding were diluted with PBS (1 × 10⁷ parasites/ml) and were intraperitoneally administered to the mice, which were randomly allocated into 6 groups of 6 animals each.

2.4. Parasitemia determination

Thick smears of blood films were prepared 3 days after inoculation of the mice and 5 days after the completion of treatment using the peripheral blood collected from the mice by tail vein puncture. The films were stained with Giemsa, and the number of parasitised erythrocytes was scored accordingly. The pre- and post-treatment levels were compared.

2.5. Treatment regimen

The extracts or drugs were suspended in corn oil for post-parasitemia administration to the *Plasmodium berghei*-infected mice taking note of their LD₅₀ as reported in literature. The extracts or drugs were administered to the animals by oral gavage over 5 days.

Group I (CEEH) – 400 mg/kg *Euphorbia hirta* whole plant

Group II (CEVA) – 1000 mg/kg *Vernonia amygdalina* leaves

Group III (CAM) – 600 mg/kg Camosunate[®], twice daily

Group IV (NI/NT) – Not infected and not treated

Group V (I/NT) – Infected but not treated

2.6. Kidney function, liver function and total protein quantification

Following sacrifice of the mice by decapitation, sera were prepared from blood collected into plain tubes that were allowed to stand at room temperature for 5 minutes followed by centrifugation at 12 000 rpm. Sera were stored at –50°C until further use. Biochemical markers of kidney and liver function as well as total protein were determined in the sera of the mice. Levels of creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALP), bilirubin, albumin and total protein were determined upon sacrifice after 5 days of treatment by standard methods using Randox[®] kits (England) following manufacturer's instructions. Absorbance (nm) was measured at the respective wavelengths using a CamSpec[®] 105 UV–Vis spectrophotometer (UK).

2.7. Statistical analyses

The results are presented as the mean ± SD and were analysed with ANOVA (SPSS vs. 20.0).

Table 1
Phytochemical screening of whole plant of *E. hirta* and *V. amygdalina* leaves.

Phytochemical Constituents	Ethanol extract of whole plant of <i>E. hirta</i>	Ethanol extract of <i>V. amygdalina</i> leaves
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Cardiac glycoside	+	–
Terpenoids	–	+
Phlobatannins	–	–

+ = Present; – = Absent.

3. Results

The results of preliminary qualitative phytochemical screening of the crude ethanol extracts showed that the whole plant of *E. hirta* contained flavonoids, alkaloids, tannins, saponins and cardiac glycoside and that the leaves of *V. amygdalina* contained flavonoids, alkaloids, tannins, saponins and terpenoids (Table 1).

3.1. Antiplasmodial efficacies

The crude ethanol extracts and Camosunate[®] significantly decreased parasitic load in *P. berghei*-infected mice: Camosunate[®] > *E. hirta* > *V. amygdalina* (8.17 ± 0.50 > 14.36 ± 1.96 > 16.04 ± 1.94), compared to infected and untreated group (25.81 ± 8.70). Thus, 600 mg/kg Camosunate[®] exhibited an average chemosuppression of 68.35%, while average chemosuppressions of 44.36% and 37.85% were observed for 400 mg/kg CEEH and 1000 mg/kg CEVA, respectively. The CEEH and CEVA mildly inhibited *P. berghei* schizont maturation at their tested doses.

3.2. Liver and kidney functions

There was a significant reduction (p < 0.05) in the concentrations of AST in mice treated with CEEH and CEVA, which was similar to the reduction resulting from Camosunate[®], compared to the infected, untreated group. ALT levels significantly increased across all groups. Meanwhile, CEEH and CEVA had no significant effect on ALP levels compared to uninfected group, while CEE significantly decreased ALP levels compared to infected animals. Bilirubin level was significantly increased by CEEH treatment, while CEVA significantly reduced bilirubin level compared to other groups. Albumin and total protein levels were significantly reduced by

Table 2

Pre- and post-treatment effect of crude ethanol extracts of *E. hirta* whole plant and *V. amygdalina* leaves on mean parasitemia in *P. berghei*-infected mice.

Treatment groups	Crude ethanol extract dose	Pre-treatment	Post-treatment
CEEH	400 mg/kg <i>E. hirta</i>	29.89 ± 0.76	14.12 ± 1.72 ^a
CEVA	1000 mg/kg <i>V. amygdalina</i>	29.47 ± 0.52	16.04 ± 1.94 ^b
CAM	600 mg/kg Camosunate [®]	28.33 ± 1.30	8.17 ± 0.50 ^c
NI/NT	Not Infected/Not Treated	00	00
I/NT	Infected/Not Treated	27.41 ± 2.27	25.81 ± 8.70

The alphabets indicate that there are statistical significant differences within or across groups upon comparison of results values.

Table 3

Effects of crude ethanol extract of whole plant of *E. hirta* and leaves of *V. amygdalina* on liver function.

Groups/Biomarkers	AST (μmol/L)	ALT (μmol/L)	ALP (μmol/L)	BIL (mg/dl)	ALB (mg/dl)	TP (mg/dl)
CEEH	37.50 ± 1.91	34.50 ± 1.29	31.58 ± 1.28	0.57 ± 0.11	17.90 ± 0.51	29.24 ± 0.85
CEVA	36.75 ± 0.96	32.35 ± 1.71	34.80 ± 0.96 ^a	0.20 ± 0.12 ^a	17.57 ± 1.23	24.27 ± 1.97 ^a
CAM	36.50 ± 1.29	32.75 ± 0.96 ^a	26.63 ± 1.90 ^{a,b}	0.28 ± 0.05 ^{a,b}	5.40 ± 0.48 ^{a,b}	10.27 ± 0.62 ^{a,b}
NI/NT	35.29 ± 1.60 ^a	22.43 ± 1.72 ^{a,b,c}	32.86 ± 1.15 ^c	0.27 ± 0.05 ^{a,b,c}	16.28 ± 1.10 ^{b,c}	25.70 ± 0.80 ^{a,c}
I/NT	43.29 ± 1.38 ^a	32.00 ± 0.82 ^d	34.81 ± 1.05 ^{a,c}	0.26 ± 0.71 ^{a,b}	10.22 ± 0.79 ^{a,b,c,d}	16.08 ± 1.32 ^{a,b,c,d}

The alphabets indicate that there are statistical significant differences within or across groups upon comparison of results values.

Table 4

Effects of crude ethanol extract of whole plant of *E. hirta* and leaves of *V. amygdalina* on kidney function.

Groups/Biomarkers	Creatinine (mg/dl)	Urea (μmol/L)
CEEH	116.50 ± 1.29	13.92 ± 0.67
CEVA	136.25 ± 1.71 ^a	13.34 ± 1.24
CAM	123.00 ± 4.54 ^b	10.43 ± 0.67 ^{a,b}
NI/NT	68.71 ± 5.77 ^{a,b,c}	7.94 ± 0.91 ^{a,b,c}
I/NT	110.86 ± 7.93 ^d	9.68 ± 0.68 ^{a,b,d}

The alphabets indicate that there are statistical significant differences within or across groups upon comparison of results values.

Camosunate[®] treatment. CEEH and CEVA significantly increased albumin and total protein levels compared to infected, untreated animals (Table 2–4).

The kidney function assay revealed increases in the levels of creatinine and urea in the parasitized, untreated animals compared to normal animals. However, CEVA and Camosunate[®] caused further significant increases in creatinine levels compared to normal animals. Urea levels were slightly increased following CEEH and CEVA treatment. However, these increases did not indicate organ damage.

4. Discussion

Tannins, saponins, flavonoids, phlobatannin, cardiac glycoside, alkaloids and terpenoids are widely distributed in many species of plants as are found in CEEH and CEVA [14]. Saponins are known to improve feeding

in animals, which is necessary in the condition of loss of appetite that occurs with malaria [15]. These classes of phytochemicals have been found to have a wide range of medicinal functions, such as anticancer, antioxidant, anti-allergic, antidiabetic, anti-inflammatory and anti-viral activities [16]. Therefore, in this regard, ethnobotanical medicine offers a rich and relatively untapped potential source of new drugs that could be derived from natural products [17].

Reduced bilirubin levels in parasitized mice administered CEVA and Camosunate[®] suggest that the extracts positively influenced haematological recovery. The reduction observed in the levels of albumin and total protein in the Camosunate[®] group may be a result of clearance of parasitic infestation by the ACT, which may be due to the cleavage of the endoperoxide bridge of the artesunate (a hemi-succinate derivative of artemisinin) component. This mechanism leads to the generation of carbon-centred free radicals and oxidative stress by which artesunate and artesunate-containing drugs are known to act as antimalarial [18]. Moreover, the extracts increased albumin and total protein levels back to normal levels recorded for uninfected, untreated animals, thus attesting to their antiplasmodial potency in red blood cells.

The ACT drug was only slightly potent (>50%) against chloroquine-sensitive *P. berghei* as results from past studies in our group revealed that using chloroquine (65 mg/kg, *i.p.*, twice daily for 5 days) achieved total chemosuppression (100%) [12].

5. Conclusion

This study reports for the first time the poor antiplasmodial efficacy of the crude ethanol extracts of CEEH and CEVA. The extracts did not significantly suppress chloroquine-sensitive strains of *Plasmodium in vivo* despite containing certain promising phytochemical compounds, which are commonly implicated in the antiplasmodial activities of many plants [19,20,12]. The extracts only slightly reduced parasitemia levels (CEEH at 44.36% and CEVA at 37.85%, respectively) compared to Camosunate® (68.35%). Thus, it can be concluded that though the search of new chemical entities from the vast rainforest flora of Africa is increasing in pharmaceutical and medicinal sciences, the extracts do not elicit effective chemosuppression against chloroquine-sensitive *P. berghei* in mice *in vivo* when administered alone. Whether their combinations with other promising herbs will give rise to a potent therapeutic intervention is yet to be studied.

Indiscriminate use of herbs by self-medication should be discouraged among rural dwellers and urban low-income earners as herbs such as those reported in this study do not possess curative potencies when used alone, rather they show mild chemosuppressive activities.

Competing interest

The authors declare that they do not have any conflict of interest or competing financial interests.

Consent

Not Applicable.

Author contributions

Conceived and co-ordinated the experiment: AMA. Designed and supervised the experiments: AEIO. Performed the experiments: ATY and AAA. Analysed the data: AEIO, ATY and AAA. Wrote the paper: AMA and AEIO.

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