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In-vivo assessment of the antiplasmodial efficacy of *Corchorus catharticus* Blanco whole plant extract

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Abstract
Malaria is a disease of global concern, with a greater threat impact in Sub-Saharan Africa. With the emergence of multidrug-resistant strains of *Plasmodium* species, and the dissemination of resistance within disease vectors to vector control modalities; with a lack of vaccine. The need, therefore, exists for the search and screening of newer and better molecules of promising efficacy in the treatment of malaria. The aim of this study was to assess the in-vivo antiplasmodial efficacy of *Corchorus catharticus* Blanco whole plant extract, an efficacy of 20% and 47% was recorded for suppressive and curative tests respectively at the highest dose of 600mg/kg/b.w. the positive control (Arthemeter/Lumenfantrine) had an efficacy of 80% and 94% for suppressive and curative tests respectively. This study suggests and supports the use of *Corchorus catharticus* Blanco in ethno medicine for malaria treatment, due to its promising antiplasmodial efficacy.

Keywords: Assessment, *in vivo*, antiplasmodial, efficacy, whole plant extract

Introduction
Malaria is a disease condition caused by the species of the parasite of the genus *Plasmodium*, which is transmitted by an infected Anopheles mosquito during a blood meal [1]. With the incidence of resistance of the insect vector [2], and parasite, especially with the emergence of multidrug-resistant strains of *Plasmodium falciparum* [3-4] to treatment, control and prevention modalities, with no effective vaccine in sight [5]. Malaria has become a global health problem due to increased rates of morbidity, mortality, and economic loss [6].
According to WHO (2018), Africa is faced with the largest malaria burden, most especially sub-Saharan Africa which accounts for the highest case of malaria morbidity; with pregnant women and children under the age of 5 years at risk. 3.3 billion People are at risk of malaria globally, with 1.6 billion being at high risk [7].
The threat posed by malaria is aggravated in developing countries by poverty [8], the ability of some of the species of the parasite *Plasmodium* to infect non-human primates increasing the risk of zoonotic transmission [9], and the coinfection of malaria with other infections such as HIV [10-11] and Typhoid [12] which increases the morbidity rates of malaria infection and plays an important role in the development and occurrence of drug resistance, leading to severe infections in infected individuals. Disease importation also increases the threat posed by malaria infection [13]. Plants have been known to possess phytochemical constituents which have been investigated for their antimicrobial activity [14] and antiplasmodial activity [15-17]. The affordability of herbs over expensive pharmaceutical drugs to treat diseases among non-industrialized societies is fast becoming revolutionized [18], most especially in Africa and other developing countries of the world which have been endowed with assorted species of plant flora and fauna. The use of plants for medicinal purposes are not only restricted to developing nations but has attained global status [19]. Herbal medicine or medicinal practice is of great importance to man and his health [20].
Corchorus (Family: Malvaceae) is a genus of annual herbs, of which more than 40 species are known to occur in nature and are distributed in both the tropics of both hemispheres. *Corchorus* is a leafy green plant, of which most species are used as foods while different parts of the plant are used in folk medicine [21].
C. longicarpus and *C. catharticus* Blanco are synonyms of *C. olitorius* L. [22]. Anti-malarial traditional remedies still offer new tracks for identifying promising antiplasmodial molecules, and a way to ensure that all people have access to care [23]. Previous studies revealed that other *Corchorus* plant species possessed cardiac, antioxidant, anti-inflammatory, analgesic, antipyretic, antimicrobial, insecticidal, and many other pharmacological effects [24].

Medicinally, *Corchorus spp.* are used as a demulcent, diuretic, purgative, bitter tonic, laxative, refrigerant, carminative and lactagogue [19]. Several parts of the plant are used in the preparation of phytomedicines: Leafy stems, leaves, seeds, flowers and roots. The leaves and the whole plant are mainly solicited. Ethno-medicinally, it is used in the treatment of several pathologies such as malaria, typhoid fever, heart disease, infantile malnutrition [25].

The aim of this study is to assess the antiplasmodial efficacy of *Corchorus catharticus* Blanco plant extract. With a specific objective of; determining the suppressive and curative efficacy of *Corchorus catharticus* Blanco plant extract against malarial parasite.

2. Methodology

2.1 Plant Source and Identification

The whole plant *Corchorus catharticus* Blanco was obtained from the University of Jos, senior staff quarters, Bauchi road and was identified at the Federal College of Forestry, Jos, Plateau state, and assigned the voucher number FHJ268.

2.2 Preparation of Plant Material

The plant material obtained was dried indoors for a period of time and protected from direct sunlight, to allow for proper drying. The dried plant material was then grounded to powder using a laboratory mortar and pestle.

Two hundred grams (200 g) of the grounded plant material was weighed and soaked in the hydro-alcoholic extraction solvent consisting of water and 95% ethanol in the ratio of 30:70, respectively. A quantity of the crushed plant fiber was then macerated in 1.0 L of solvent for 72 hours then filtered and, the filtrate was exposed to normal room conditions allowing for the evaporation of the solvent. When the solvent was evaporated to a satisfactory level the mixture was freeze-dried. The freeze dryer used was Labconco Freeze Zone. The extract in solvent was frozen at 25 °C in a freezer for 24 hrs.

2.3 Phytochemical Screening

Determination of the phytochemical constituents of the plant was carried out using the method described by [26-27].

2.4 Animals and Parasite

The study was conformed to the principles for laboratory animal use and care as declared in the European Community guidelines (EEC Directive of 1986:86/609/EEC). Swiss albino mice (11–23 g, 4–8 weeks old) of both sexes used for the study were obtained from the animal house, University of Jos, Plateau State, Nigeria.

The Swiss albino mice were housed under standard environmental conditions of temperature (25.5±4 °C) and 12 h dark-light cycle and allowed free access to drinking water and a standard pellet diet.

The parasite *Plasmodium berghei* was obtained from the animal house of the University of Jos.

2.5 Standard Control Drug

The standard control drug was bought from MaCray Pharmaceuticals Ltd, No. 9 Shendam Street old Bukuru Park Jos, Plateau State. The standard drug with the trade name Havax® was manufactured by Laborate Pharmaceutical in India.

The standard drug is an Artesimin in combination therapy containing Artemether/Lumefantrine (80/480 mg). The standard drug used had a Manufacturer's license number; 861-OSP (H), Batch no. 18HX236, Manufacturing date; 10/2018, Expiry date; 09/2021.

2.6 Experimental Design

2.6.1 Parasite Inoculation

All animals used in this study were quarantined 7 days prior to infection. Blood from mice infected with *Plasmodium berghei* was used to infect the animals used in this study. Standard inoculums of 1×10^7 infected erythrocytes in 0.2 mL was prepared by diluting infected blood with 0.9% normal saline. Each mouse was inoculated by intraperitoneal injection with a blood suspension (0.2 mL) containing 1×10^7 parasitized erythrocytes. The parasite was maintained by serial passage of blood from infected to non-infected mice on a weekly basis [28].

2.6.2 Determination of the suppressive and curative efficacy of *Corchorus catharticus* Blanco Plant Extract on *Plasmodium berghei* infected Swiss Albino mice

The evaluation of the suppressive and curative potential of the extract in experimental animals was carried out using the methods described by Ryley and Peters, (1970) as used by [4]. Thirty mice were used for the study. Infected animals were divided into 6 groups (n = 5) when the level of parasitemia is observed to be > 4%. A stock solution of the extract was prepared at 30mg/ml from which the plant extract was administered to the experimental animals according to their body weight. The extract was administered at 3 different dose levels (200, 400, and 600 mg/kg/b.w./day). Three control groups were used namely, normal (uninfected and untreated), positive (infected and treated with control drugs; Arthemeter-Lumefantrine 8mg/kg/b.w/day), and negative (infected and treated with distilled water). The experimental animals were observed for suppressive effect after four days, while the curative effect was monitored after seven days. Blood samples were collected from the tip of the tails of the animals on day 4 and day 7 post-treatment.

2.6.3 Parasitemia Monitoring in Experimental animal

Parasitemia was monitored using the method described by [4], in which blood samples were collected from the tip of the tails of the animals on days four and seven. Thin blood films were prepared and fixed (for 15 minutes) using methanol, and subsequently stained with 10% Giemsa for 25 minutes. The stained film was washed off using phosphate buffer, pH 7.2, and allowed to dry. The film was immersed in oil and viewed at x100 magnification. The parasitemia level was determined by counting the number of parasitized erythrocytes out of 100 erythrocytes in random fields of the microscope [29]. Average percentage parasitemia was calculated using the formula (1) [4]:

$$\%\text{Parasitemia}=\frac{\text{Total number of parasitized erythrocytes}}{\text{Total number of erythrocytes counted}}\times100 \quad (1)$$

The average percentage of suppressive and curative efficacy of the plant extract was calculated using the formula (2) [4]:

$$\%\text{Suppression}=\frac{\text{Parasitemia in negative control-Parasitemia in test group}}{\text{Parasitemia in negative control}}\times100 \quad (2)$$

2.6.4 Determination of Hematological Index (Packed cell volume)

Packed cell volume (PCV) was determined using the Wintro be method as described by [30]. The packed cell volume is that

proportion of whole blood occupied by red cells, expressed as a ratio (liter/liter).
Anticoagulated blood in a glass capillary of specified length bore size, and wall-thickness is centrifuged in a microhaematocrit centrifuge at RCF 12000-15000 xg for 3-5 minutes to obtain constant packing of the red cells. A small amount of plasma remains trapped between the packed red cells. The PCV value is read from the scale of a microhaematocrit reader or calculated by dividing the height of the red cell column by the height of the total column of blood.

2.6.5 Data Analysis
The results were presented as the mean ± SEM (standard error of the mean) for each group of experiments. The test groups were compared with the negative control group using the Chi-square method. All data was analyzed at a 95% confidence interval. P-values less than 0.05 were considered statistically significant. The data obtained from this research was analyzed using IBM SPSS statistics tool software version 23.0.0.0

2.6.6 Ethical Approval
Ethical approval was obtained for the use of experimental animal models and parasite for this study, from the Department of Pharmacology, Faculty of Pharmaceutical studies, University of Jos, Jos, Plateau State.

3. Results
3.1 Suppressive and Curative Efficacy of the *Corchorus catharticus* Blanco whole plant extract on *Plasmodium berghei* infected Swiss Albino mice.
On day four, *Corchorus catharticus* Blanco whole plant extract suppressed parasitemia by 10.00%, 15.00%, and 20.00% at 200, 400, and 600 mg/kg/b.w doses, respectively as shown in Table 1. Similarly, on day 7 *Corchorus_catharticus*_Blanco whole plant extract suppressed the parasite load by 21.00%, 44.00%, and 47.00% at 200, 400, and 600 mg/kg/b.w doses, respectively as shown in Table 2.

3.2 Hematological indices (Packed cell Volume) of experimental animals
The values obtained for the evaluation of packed cell volume of the Swiss albino mice used for this study are 15.00±4.08, 14.00±4.24, 15.00±6.00, 18.33±3.51, 37.00±9.8, and 33.80±19.23 in the negative control, 200, 400, 600mg/kg/b.w, positive control (Arthemeter/Lumenfantrine 8mg/kg/b.w) and uninfected, untreated group respectively as shown in Table 3.

3.2 Phytochemical Constituents of *Corchorus catharticus* Blanco whole plant extract
The qualitative phytochemical screening detects the presence of alkaloids, tannins, saponin, reducing sugars, glycoside, terpenoids, steroids, and phenols while flavonoids were not detected as indicated in Table 4.

Table 1: Suppressive effect of hydroethanolic whole plant extract of *Corchorus catharticus* Blanco in *Plasmodium berghei* infected mice on day four.

Drug/Extract	Dose (mg/kg/b.w)	Parasitemia Control	
		Parasitemia	% Suppresion
Negative control	H_2O	19.75±3.09	0.00
Extract	200	18.00±3.00	10
Extract	400	17.33±6.66	15
Extract	600	16.00±1.00	20
Positive control (Arthemeter/Lumefantrine)	8	3.67±1.16	80
Uninfected/untreated	-	0	0

Values are expressed as mean ± S. E. M

Table 2: Curative effect of hydroethanolic whole plant extract of *Corchorus_cartharticus* Blanco in *Plasmodium berghei* infected mice on day seven.

Drug/Extract	Dose (mg/kg/b.w)	Parasitemia	
		Control Parasitemia	% Suppresion
Negative control	H_2O	33.50±5.51	0.00
Extract	200	27.00±1.41	21
Extract	400	19.33±10.01	44
Extract	600	18.33±3.05	47
Positive control (Arthemeter/Lumefantrine)	8	2.0±1.41	94
Uninfected/untreated	-	-	-

Values are expressed as mean ± S. E. M

Table 3: Packed cell volume of experimental animal models on day seven

Drug/Extract	Dose (mg/kg/b.w)	Packed cell volume
Negative control	H_2O	15.00±4.08
Extract	200	14.00±4.24
Extract	400	15.00±6.00
Extract	600	18.33±3.51
Positive control (Arthemeter/Lumefantrine)	8	37.00±9.8
Uninfected/untreated	-	33.80±19.23

Values are expressed as mean ± S. E. M

Table 4: Phytochemical constituents of *Corchorus catharticus* Blanco whole plant extract

Phytochemical constituents	Presence (+) / absence (-)
Alkaloid	+
Flavonoid	-
Tannins	+
Saponin	+
Reducing sugar	+
Glycoside	+
Terpenoids	+
Steroids	+
Phenol	+

KEY; + == present - == absent

4. Discussion

This study was designed and carried out to assess the antiplasmodial efficacy of *Corchorus catharticus* Blanco whole plant extract, in *Plasmodium berghei* infected mice. The findings of this study show that the antimicrobial activity of the plant extracts was dose-dependent [16] and probably associated with the group of the phytochemical constituents of the plant [31]. A number of groups of phytochemicals constituents are responsible for the observed antiplasmodial activity, alkaloids are recognized for their toxic effects on bacteria, viruses, and protozoans; while, saponins are recognized for antiprotozoal activity [4]. Antiplasmodial activity of triterpenoids has been reported by [16]. Treatment of metabolic and vascular diseases is the clinical significance of saponins [32].

The highest observed antiplasmodial efficacy of the *Corchorus catharticus* Blanco whole plant extract is less when compared to the standard drug for both suppressive and curative effects. The observed low antiplasmodial efficacy of *Corchorus catharticus* Blanco whole plant extract maybe associated with the absence of flavonoids, which have been reported to have strong antiplasmodial activity [15, 17]. The efficacy of plant extracts and drugs are dose-dependent [16, 4], therefore the dose administered might be low in relation to the observed antiplasmodial efficacy of the *Corchorus Catharticus* Blanco whole plant extract in this study.

Statistical analysis showed that the antiplasmodial efficacy was statistically insignificant ($p>0.05$) for *Corchorus Catharticus* Blanco whole plant extract.

5. Conclusion

The plant demonstrated both suppressive and curative effects on the experimental animals, the experimental animals also showed improved pack cell volume. The plant phytoconstituents include alkaloids, tannins, saponin, reducing sugars, glycoside, terpenoids, steroids, and phenols while flavonoids were not detected. The results suggest the need for the purification of the plant extract and toxicity test.

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