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Evaluation of the Antimalarial and Liver Function Potentials of Methanol Extract of *Chrysophyllum albidum* Stem Bark in *Plasmodium berghei* - Infected Mice

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Abstract

Chrysophyllum albidum (*C. albidum*) has reputation in Nigeria as remedy for different ailments. This study evaluated the antimalarial potentials of methanol extract of the stem bark of *C. albidum* and specific Alkaline phosphatase (ALP), Alanine transaminase (ALT) and Aspartate transaminase (AST) activities in both sera and livers of *Plasmodium berghei* infected mice treated with methanol extract of *C. albidum* stem bark. The result of the phytochemical screening indicated the presence of anthraquinones, steroids, tannins, alkaloids, glycosides, terpenoides, flavonoids, saponins and phlobatannins. Percentage parasite inhibition was calculated to be 56.97%, 74.10%, 85.26% and 92.83% for groups treated with 200 mg/kg, 400 mg/kg, 600 mg/kg body weight of the methanol extract of *C. albidum* stem bark and 5 mg/kg body weight of Chloroquine (standard drug) respectively. It was observed that, the specific ALP, ALT and AST activities in both sera and liver samples of the group infected but not treated were significantly higher ($p<0.05$) than the infected and treated groups. There was significant difference ($p<0.05$) in the specific ALP, ALT and AST activities between groups treated with 5 mg/kg body weight of Chloroquine and 600mg/kg body weight of the methanol extract of *C. albidum* stem bark. However, there was no significant difference ($p>0.05$) in the specific ALP, ALT and AST activities between the groups treated with 200 mg/kg and 400 mg/kg body weight of methanol extract of *C. albidum* stem bark. The ability of the methanol extract of *C. albidum* stem bark to inhibit parasite multiplication/progression may be attributed to the presence of various bioactive constituents such as flavonoids, tannins, alkaloid or saponins and further support the ethno medicinal claim of the use of other parts of the plant in the treatment of malaria.

Keywords: *Chrysophyllum albidum*; Stem bark; Antimalaria; Alkaline phosphatase; Alanine transaminase; Aspartate

Introduction

One disease which has caused a major public health concern in the world is malaria disease. It has been estimated to cause about 0.7-1 million deaths per year. Approximately, half of the world's population is at risk of malaria, of which about 78% occur in the African region, 15% occurring in Southeast Asia and 5% in Eastern Mediterranean regions [1]. Though malaria disease is preventable and curable, it is still one of the greatest global public health concern especially in sub-Saharan Africa [2]. Owing to the increasing resistance of the parasite to available agents, there is the need for continuous search for new antimalarial agents. It is believed that the use of plant-derived active principles will offer people access to safe and effective products for the prevention and treatment of Malaria, as some medicinal plants have been used locally and found to be effective. According to WHO, more than 80% of world's population, are thought to depend chiefly on traditional medicine, which is largely of plant origin, for their primary health care needs [1]. However, it is widely thought that these valuable medicinal agents in plants are largely untapped because of inadequate scientific technical and commercial infrastructures in developing countries.

Phytochemicals are chemical compounds that are naturally found in plants and are responsible for the colour and organoleptic properties of the plant [3]. Phytochemical components are responsible for both pharmacological and toxic activities of plant. Therefore, the major and overriding criterion in the selection of herbal medicines for use in health services is safety. Plants extracts should not only be efficacious but safe for consumption. There is therefore the need that while screening plants' extracts for their antimalarial activities, their toxic potentials [4] should also be investigated.

Chrysophyllum albidium, commonly called African star Apple, belongs to the family *Sapotaceae* and are used in traditional folklore medicine to cure various diseases. It is widespread in tropical and subtropical regions around the World. Additionally, it is used traditionally as an antiseptic, anthelmintic, mosquito bite repellent, for stomach ailments, tonic, antiscorbutic, astringent, diuretic, headache, arthritis, and digestive and appetite stimulant, an anti-oxidant and for colds, coughs and sore throats [5].

In the last two decades, *Chrysophyllum albidium* has been subjected to extensive phytochemical, pharmacological and clinical investigations and many interesting outcomes in the areas of insecticidal activity [6], anthelmintic [7], anti-osteoporosis, radical scavenging, anti-cholinesterase, cardiac diseases, anticancer [8], antimicrobial [9], eye conditions, inflammatory bowel disease and improved lung function [10] have been reported. However, there is paucity of information on antimalarial efficacy of this plant.

Plant extracts are one of the most attracted sources of new drugs for the treatment of liver disease. Evidences have shown that antioxidants derived from plant sources may be useful in preventing the deleterious

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consequences of oxidative stress and there is increasing interest in the hepatoprotective potentials of natural antioxidants contained in spices, herbs and medicinal plants. A considerable amount of natural antioxidant agent including alkaloid, saponin, xanthones, triterpenes and tannins has been isolated from medicinal plant. On the array of medicinal plant with hepatoprotective potential, is *Chrysophyllum albidum*.

Justification of the Study

There has been an increasing interest world wild on therapeutic value of natural product. Plant based medicine has served from time memorial, as the most therapeutic weapon available to man to fight various human and animal disease. According to WHO, above 80% of the world population depend on traditional based medicine for their primary health care need, and have recommend and encourage such practice especially where assess to conventional treatment is inadequate. It however emphasizes the fact that safety should be overriding criteria for selecting herbal medicine for use in health care. Since *Crysophyllum albidum* bark preparations have been cited in scientific literatures as having medicinal values which communities take advantage of, it is therefore necessary to evaluate the antimalarial and toxicological profile of this plant on various organs and tissues of the body in order to scientifically validate their claimed medicinal potentials and establish their safety for consumption.

Hepatic damage to the liver is gradually becoming a problem of public health concern due to constant exposure to malaria parasites, also the changing lifestyle of people, and increasing exposure to industrial hazardous chemicals. Nigerians even African at large still use herbal remedies in the treatment of diseases. However, the specific role of *Chrysophyllum albidum* bark extract in the management of liver diseases has however not been validated scientifically. The effects of methanol extract of *Chrysophyllum albidum* when established will justify the use and contribution of *Chrysophyllum albidum* in the management of liver damage.

Aim and Objectives

Aim

To evaluate the antimalarial and liver function potentials of methanol extract of *Chrysophyllum albidum* stem bark in *Plasmodium berghei* - infected mice.

Specific objectives

- To determine the phytochemical constituents of methanol extract of *Crysophyllum albidum* stem bark.
- To determine the curative effect of methanol extract of *Crysophyllum albidium* stem bark against *P. berghei* infection in mice.
- To evaluate the specific AST, ALP, and ALP activities in *Plasmodium berghei* infected mice treated with methanol extract of *Chrysophyllum albidum* stem bark.

Materials and Methods

Materials

Plant sample: The stem bark of *Chrysophyllum albidum* was collected in April, 2014 from a cash crop farm in Rore, irepodun local government area, Omu-Aran Kwara State. It was identified by a Botanist in the Department of Biological Sciences, Federal University of Technology Minna, Niger State.

Reagents and chemicals: All chemicals and reagents used were of analytical grade.

Experimental animals: Swiss albino mice weighing between 20-25 g were used in this study. The animals were obtained from animal breeding unit, University of Jos, plateau State. They were housed in plastic cages with saw dust bed and given standard laboratory diet and water ad-libitum. They were then allowed to acclimatize for two weeks to their new environment before the initiation of the experiments.

Parasite: A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. Parasitized erythrocytes were obtained from a donor- infected mouse by cardiac puncture in heparin and made up to 20 mL with normal saline. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing about 1×10^7 parasitized erythrocytes.

Methodology

Sample preparation and extraction procedure: The collected fresh stem bark of *Chrysophyllum albidum* was washed with clean water and air dried at room temperature. The dried sample was grounded using a grinder mill. Extraction of plant materials was performed by reflux extraction using methanol. The resulting methanol extract was concentrated in a water bath (at 45°C) and stored in a refrigerator until required.

Phytochemical screening: Phytochemical analysis to screen the methanol extract of *Chrysophyllum albidum* stem bark for the presence of alkaloids, flavonoids, tannins, saponins, glycosides, anthraquinones, phlobotannins and carbohydrates was carried out according to the method described by Odebiyi and Sofowora and Trease and Evans [11,12].

***In vivo* antiplasmodial test (curative test):** A total of twenty mice were used for the study. On the first day (D_0), standard inoculums of 1×10^7 *P. berghei* infected red blood cells were injected into the mice intraperitoneally. Seventy-two hours later, the mice were checked to ascertain they were infected with the parasite and then divided into five groups of four mice each. Different doses of the extract (200, 400 and 600 mg/kg/day) were administered orally to three groups. Chloroquine phosphate (5 mg/kg/day) was given to the positive control group and 0.2 mL of normal saline to the negative control group. The extract was given once daily for 5 days. Thin blood smears were prepared from tail of each mouse for 5 days to monitor the parasitaemia level. Variation in weight and packed cell volume (PCV) was monitored in the course of the study. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group over a period of 28 days (D_0-D_{27}) [13,14].

Assay of hepatic enzymes: This was done to evaluate the specific activities of the liver enzymes, since the liver is the site of drug metabolism, and is the major organ affected by the malaria parasite. The serum and the liver were used for this analysis.

Tissue collection and preparation: Collection of sample for biochemical analyses was carried out, according to the method described by Yakubu et al. [15]. Mice were anaesthetized using cotton wool soaked in chloroform and blood sample collected into a clean, dry centrifuge tubes. The blood sample were allowed to stand for 10 min at room temperature and then centrifuged at 1000 rpm for 15 min.

The supernatant (serum) was carefully removed with Pasteur pipette and stored in the fridge until needed for analysis. The tissues (liver) were excised, weighed, and transferred into 0.25 M sucrose solution. The tissues which were weighed (1 g) and finely cut with clean sterile blade were homogenized in 4 ml of ice cold 0.25 M sucrose solution using small mortar and pestle. These were then transferred into clean centrifuge tubes and centrifuged at 1000 rpm for 10 min. The supernatants were carefully withdrawn and also stored in the fridge, until needed for analyses.

Biochemical analysis: The total protein concentrations of serum and Liver were determined using biuret method [16] as described by Plummer [17]. Serum and liver collection as well as Enzyme assays were carried out using AGAPPE Diagnostic kit, Switzerland GmbH. Alkaline phosphatase (ALP) was determined based on the method of Wright et al. [18], Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) activities were carried out using the method described by Reitman and Frankel [19].

Statistical analysis

Analysis of variance (ANOVA) was used to statistically analyse data obtained from the study and values of $p<0.05$ were considered significant.

Results

Phytochemical screening

Qualitative phytochemical screening of the methanol extract of *Chrysophyllum albidum* stem bark revealed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones, phlobatannins. The result is as shown in Table 1.

Anti-plasmodial test (curative test)

Average prasitaemia count/field: All the groups infected with *Plasmodium berghei* and treated with the crude methanol extract of *Chrysophyllum albidum* and chloroquine, showed an exponential decrease in parasite count, throughout the study period, with chloroquine showing highest parasite inhibition (Figure 1).

Average body weight: Figure 2 shows the average body weight of *Plasmodium berghei* infected mice treated with methanol extract of *Chrysophyllum albidum* stem bark. There was decrease in body weight of mice infected but not treated. However, for groups treated with 600 mg/kg b.w of methanol extract of *Chrysophyllum albidum* stem bark and 5 mg/kg b.w of cholroquine, there was increase in the average body weight.

Phytochemicals	Inference
Alkaloids	+
Saponins	+
Steroids	+
Terpenoids	+
Tannins	+
Flavonoids	+
Phenols	-
Anthraquinones	+
Phlobatanins	+
Reducing sugar	+
Glycosides	-

Key: (+) present, (-) not detected

Table 1: Qualitative phytochemical constituents of methanol extract of *Chrysophyllum albidum* stem bark.

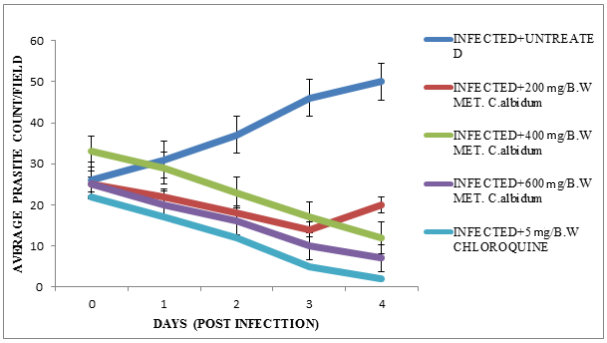

Figure 1: Antiplasmodial effect of the methanol extract of *Chrysophyllum albidum* stem bark on *Plasmodium berghei* infected mice.

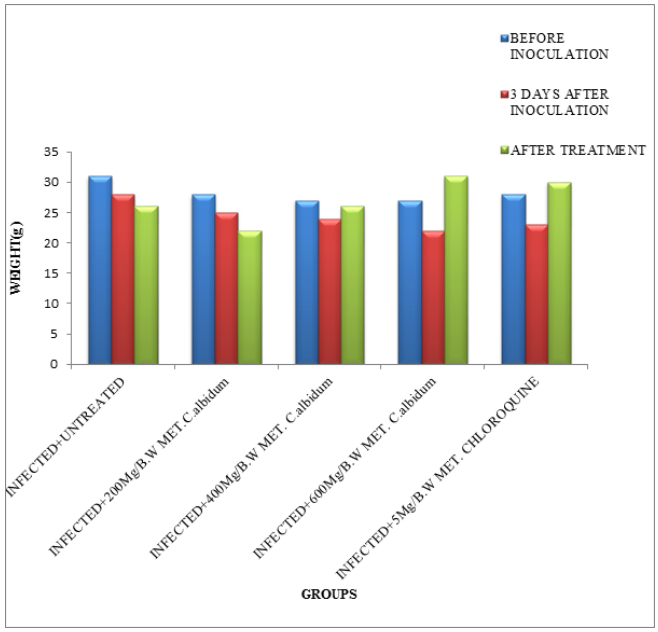

Figure 2: Effect of methanol extract of *Chrysophyllum albidum* bark on the weight of mice infected with *Plasmodium berghei* infected mice.

Percentage packed cell volume (PCV): Figure 3 below shows the percentage packed cell volume of *Plasmodium berghei* infected mice treated with methanol extract of *Chrysophyllum albidum* stem bark.

Specific enzyme activities: Figures 4-6 shows the specific ALP, ALT and AST activities of mice infected with *Plasmodium berghei* and treated with methanol extract of *Chrysophyllum albidum* stem bark. The specific enzyme activity was generally higher (for both liver and serum) in the group infected but not treated for all the enzymes assayed for, when compared to treated groups.

Discussion

It was observed from this study that, methanol extract of *C. albidum* stem bark was most effective at a dose of 600 mg/kg body weight. It has been well established that plants whose phytochemical compounds include anthraquinones, alkaloids and saponins may have antimalarial activities [20]. These established findings are similar to those obtained in this study as methanol extract of *C. albidum* stem bark was found

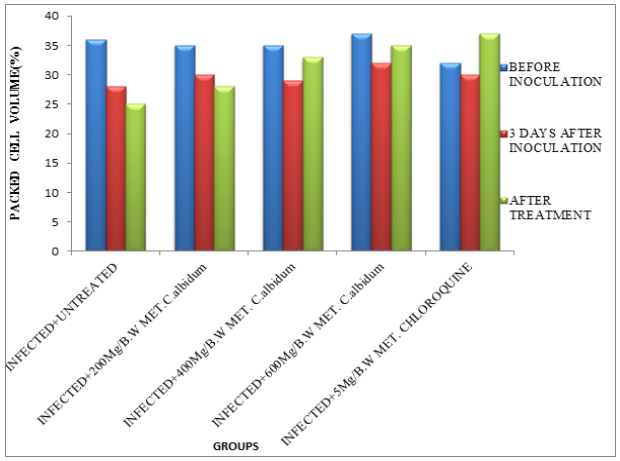

Figure 3: Percentage packed cell volume of mice infected with *Plasmodium berghei* and treated with methanol extract of *Chrysophyllum albidum* stem bark.

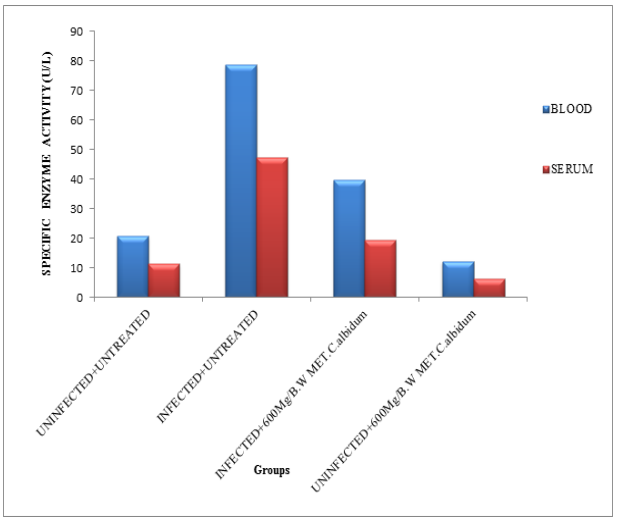

Figure 4: Specific ALP activity of *P. berghei* infected mice treated with methanol extract of *C. albidum* stem bark.

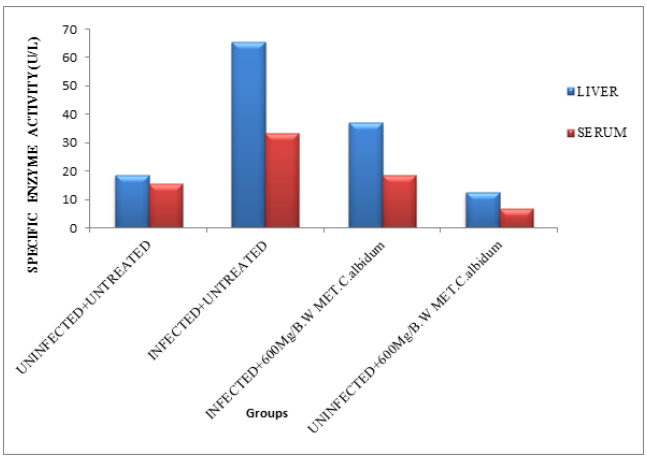

Figure 5: Specific ALT activity of *P. berghei* infected mice treated with methanol extract of *C. albidum* stem bark.

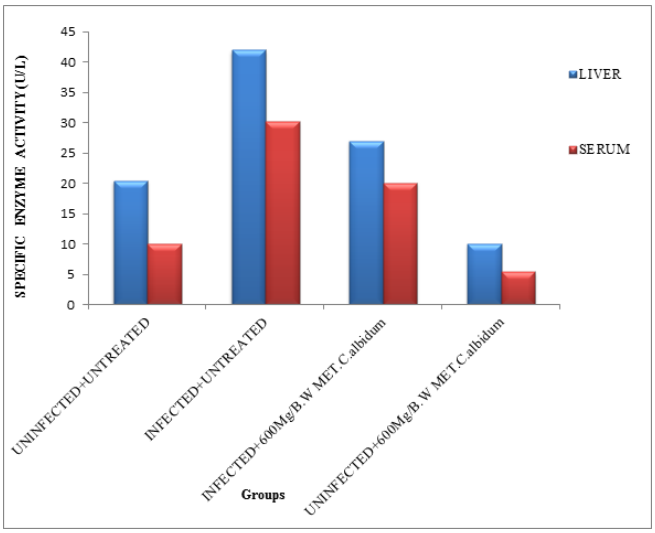

Figure 6: Specific AST activity of *P. berghei* infected mice treated with methanol extract of *C. albidum* stem bark

to contain alkaloids, anthraquinones, saponins and tannins. These phytochemical compounds were also similar to those reportedly found in the leaves and stems of *C. albidum*. Saponins have been found to have antiprotozoan activities and have been found to be detrimental to several infectious protozoans, of which are *P. falciparum* and *P. berghei*. This finding supports what was observed in this study, during established infections. It has been reported by Nobori et al. that the mechanism of action by which saponins work might be through their toxicity to protozoans which may be widespread and non-specific. It might also be as a result of their detergent effect on the cell membranes. *C. albidum* has also been found to contain alkaloids and these have been associated with medicinal uses for ages, though other possible roles have not been examined. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms like bacteria, viruses and protozoans to which malaria parasites belong. These activities have been widely studied for their potential use in the elimination and reduction of liver injuries and human cancer cell lines. Alkaloids also possess anti-inflammatory, antiasthmatic and anti-anaphylactic properties with consequences of altered immunological status *in vivo*.

The significant reduction in parasite count in infected mice treated with methanol extract of *C. albidum* prevented rapid destruction of parasitized red blood cells. The results also show that chloroquine at 5 mg/kg/day is also effective in preventing anaemia due to its anti-protozoan effect in infected mice.

The high specific ALP, ALT and AST activities observed in the livers of infected and untreated mice could be as a result of the destruction of hepatocytes during the hepatic phase of development of the malaria parasites. However, low specific ALP, ALT and AST activities were observed in the uninfected/untreated group, as well as the group not infected, but administered 600 mg/kg b.w of the extract, indicating hepatoprotective activity of the extract. The relative milder changes in the specific ALP, ALT and AST activities observed in the group infected and treated with 600 mg/kg b.w methanol extract of *C. albidum* stem bark, suggest that the extract suppressed the build-up of parasites, in addition to enhanced immune response, in the mice and probably abrogated the hepatic phase of development of the protozoa.

Conclusion

In conclusion, the ability of the methanol extract of *C. albidum* stem bark to inhibit parasite multiplication/progression may be attributed to the presence of various bioactive constituents such as flavonoids, tannins, alkaloid or saponins and further support the ethno medicinal claim of the use of other parts of the plant in the treatment of malaria. It can also be concluded from the results obtained from the enzyme studies that the extract of *Chrysophyllum albidum* possess hepatoprotective property at a dose level 600mg/kg body weight in treated mice. Thus, the extracts may be a potent therapeutic agent for the management of liver damage.

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