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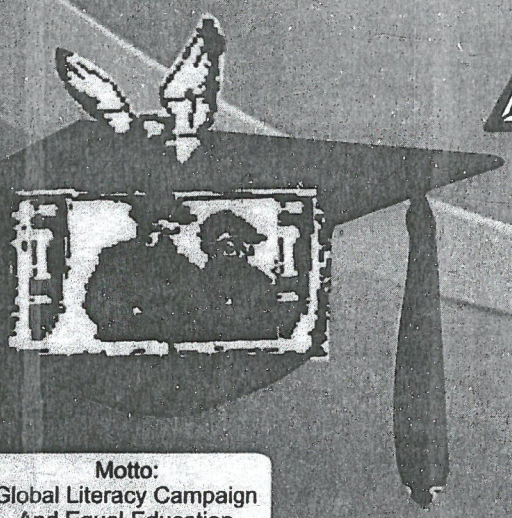

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PHYTOCHEMICAL COMPOSITION AND ANTIMALARIAL ACTIVITY OF METHANOL LEAF EXTRACT OF *Crateva adansonii* in *P.berghei* INFECTED MICE

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ABSTRACT

The need for new compounds active against malaria parasites is made more urgent by the rapid spread of drug-resistance to available antimalarial drugs. The crude methanolic leaf extract of Crateva adansonii was investigated for its antimalarial activity against Plasmodium berghei (NK65) during established infections. A total of 15 mice was intraperitoneally infected with chloroquine sensitive P. berghei strain and divided into 5 equal groups, groups 1 serve as negative control (untreated) group 2,3 and 4 were given 200, 400 and 600mg/kg Crateva adansonii respectively while group 5 serve as positive control and were given 5mglkg chloroquine for five days. The phytochemical constituents of the plant extract were evaluated to elucidate the possibilities of its antimalarial effects. The extract produce a significant dose dependent decrease in the level of parasitarmia compare to infected untreated group. Also,the extract at dose of 400mg/kg and 600mg/kg produce significant increase in body weight and PCV of the infected mice as compare to mice treated with 200mg/kg of extract and infected untreated group. Phytochemical screening showed that the leaf extract contains alkaloids, anthraquinones, tannis, flavonoids, saponins cardiac glycoside and steroids. It is concluded that Crateva adansonii could serve as a possible source of anti malaria compound .

Key word: *Crateva adansonii,* antimalarial, phytochemical, parasitarmia, *P. berghei*

INTRODUCTION

Malaria is a chronic endemic disease that obstruct social and economic development, it is the leading cause of mortality and morbidity around the worl with an estimated 225 million malaria case and 781,000 death being reporte globally in 2009 (USAID, 2011). most of the reported malaria cases occur in tropical and subtropical regions where the atmospheric condition (temperature and rainfall) are favorable for the development of vectors and parasite (Green wood *et al.*, 2008) the mortality occur mostly in young children and pregnant woman (Ogunlana and Ademowo 2009.) also the attack of malaria during pregnancy stage usually result into severe anemia and impairment of fetal nutrition which contribute to the low birth weight, premature delivery mental refardation and 60% miscarriages (WHO 2000) .malaria is caused by protozoan of genus plasmodium transmitted to the vertebrate by female Anopheles mosquitoes

blood suspension (0.2 ml) containing about 1 x 10^7 suspension of *P. berghei* parasitized red blood cells

Sample preparation and Extraction procedure

The collected fresh leaf of *Crateva adansonii* were washed with clean-water and air dried. The dried sample was grounded using a grinder mill. Extraction of plant materials was performed by soxhlet extraction using methanol. The resulting methanolic extract was concentrated in a water bath and stored in a refrigerator until required.

Determination of yield of Extract

The percentage yield of methanolic leaf extract of *Crateva adansonii* was determined by weighing the coarse sample before extraction and the ethanolic leaf extract of *Crateva adansonii* after concentration and then calculated using the formula.

$$\textbf{Percentage yield } (\%) = \frac{\text{Weight (g) of the concentrated extract}}{\text{Weight (g) of the } \textit{Crateva adansonii} \text{ leaf}} \times 100$$

Phytochemical Analysis

methanolic leaf extract of *Crateva adansonii* were characterize for phytochemical composition including alkaloids, anthraquinones, tannis, flavonoids, saponins cardiac glycoside, steroids and phlobatannins according to the methods (Harborne, 1973; Sofowora, 1993).

Anti-Plasmodial Study

Curative test

This is a procedure whereby treatment with the extract is started after three days of inoculation. On the first day (D_0), standard inoculums of 1 x 10^7 *P. berghei* infected red blood cells were injected into mice intraperitoneally. Seventy-two hours later, the mice were divided into five groups of three mice each. All treatment were administerd intraperitoneally for 5 days.

Group I. mice were given 200mg/kg b.w of methanolic leaf extract of *Crateva adansonii*

Group II. mice were given 400 mg/kg b.w of methanolic leaf extract of *Crateva adansonii*

Group III. mice were given 600mg/kg b.w of methanolic leaf extract of *Crateva adansonii*

Group IV mice received 5mg chloroquine /kg body weight

Group V mice were given normal saline/kg body weight.

Daily parasitaemia count

On each day about drops of blood were collected from the tail of each rat, smeared unto a microscopic slide to make a thin films, stained with 10% Glemsa stain and examined microscopically to monitor the parasitaemia level

Determination of Packed Cell Volume (PCV)

The capillary tubes were filled with blood to about 1cm or two-third (2/3) of its length and the vacant end of each of the capillary tubes was sealed by plastic seal or sealer to protect the blood level from spilling. The tubes were placed in haematocrit centrifuge with seal side towards the periphery and then centrifuge for 5-6 minutes. The percentage of packed cell volume or haematocrit was read directly from haematocrit reader (Dacie and Lewis, 2000)

RESULTS

Extract Yield

The percentage yield of leaf extract of *Crateva adansonii* is shown in table 1: The yields of leaf extract of *Crateva adansonii* was 25.09%.

Table 1. The percentage (%) yield of back extract of *Crateva adansonii*

Weight	*Crateva adansonii*
Initial (g)	50.00
Finai (g)	12.547
Extract yield (%)	25.09

Phytochemicals

Table 2: show the result of qualitative phytochemical composition of methanolic leaf extract of *Crateva adansonii*. The results revealed the present of alkaloids, anthraquinones, tannis, flavonoids, saponins cardiac glycoside and steroids. However, phlobatannins were absent.

Table 2: Qualitative phytochemical compositions of methanolic leaf extract of *Crateva adansonii*

Phytochemicals	Inference
1. Alkaloids	++
2. Glycosides	++
3. Anthraquinone	++
4. Steroid	++
5. Tannins	+
6. Saponins	+++
7. Flavonoids	++
8. Reducing sugar	++
9. Phlobatannins	-

Key: (-) absent, (+) slightly present, (++) moderately present, (+++) highly present

Anti-Malaria Study

Parasitaemia count

The average daily parasitaemia level of the *p.bergei* infected mice treated with ethanolic leaf extract of *Crateva adansonii* are shown in figure 1. The average daily parasitaemia of infected mice treated with methanolic leaf extract of *Crateva adansonii* at dose of 400 and 600mg/kg was significantly ($P<0.05$) reduced when compare with the negative control over the period of the experiment. The average daily parasitaemia of infected mice treated chloroquine was significantly ($P<0.05$) reduced when compared with extract treated group. However no significant difference in the level of parasitaemia count of infected mice treated with 200mg/kg leaf extract of *Crateva adansonii* when compares with the control group.

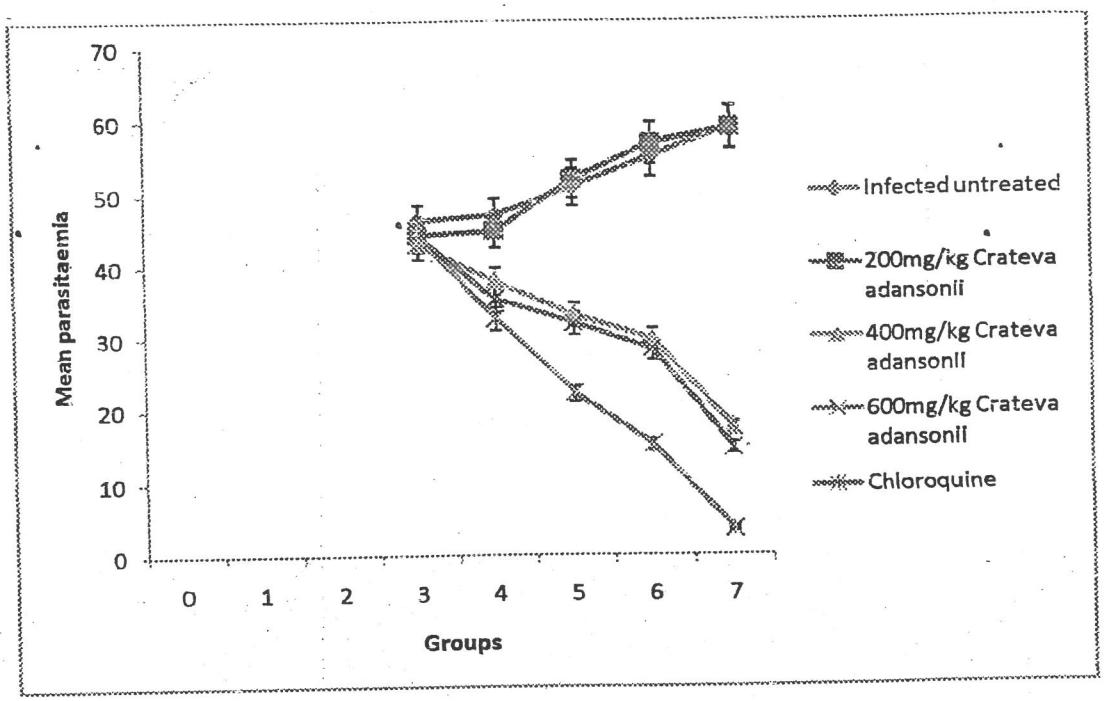

Figure 1: In Vivo Antiplasmodial Activity of methanolic leaf extract *of N. laevis* against *P. berghe infected mice*

Body weight changes

Effect of methanolic leaf extract of *Crateva adansonii* on body weight of P.berghei infected mice are shown in figure 2: the body weight of *P.berghei* infected untreated mice and infected treated with 200mg/kg of *Crateva adansonii* show significant decrease in body weight after 5 days of treatment.however the infected mice treated with 400mg/kg, 600mg/kg of *Crateva adansonii* as well as those treated with 5mg/kg chloroquine show significant increase in body weight after 5 days of treatment

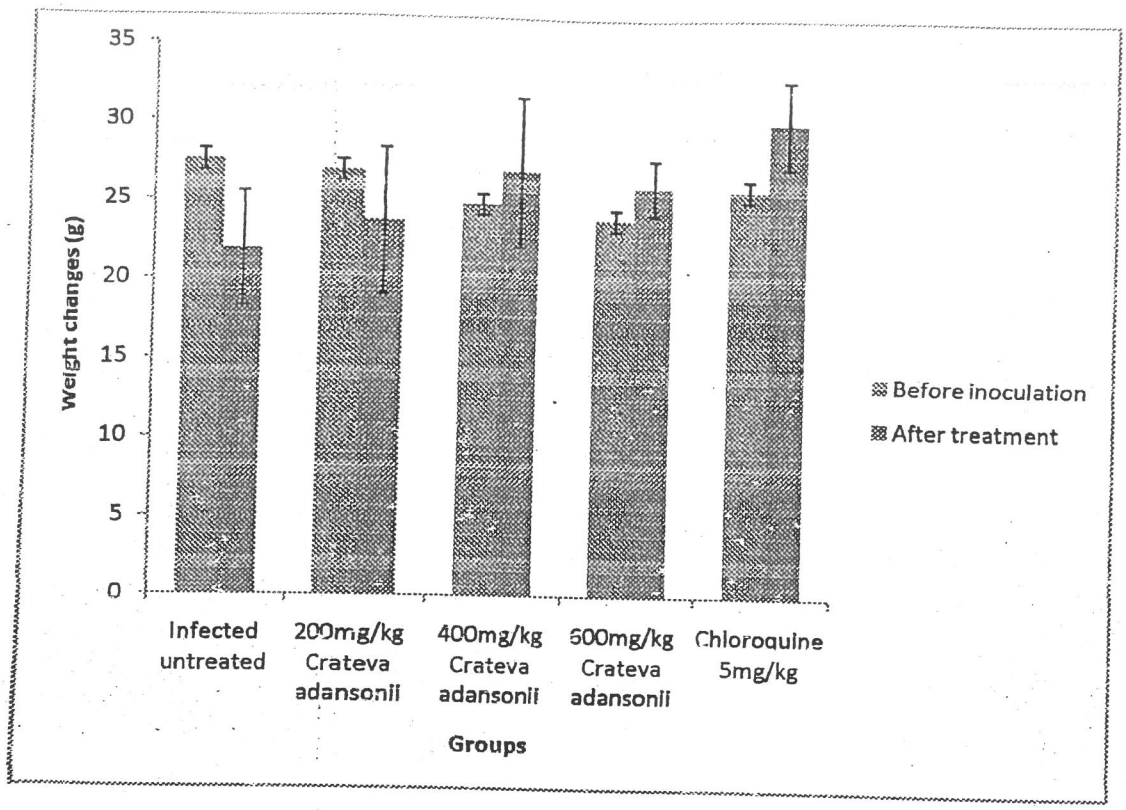

Figure 2: Effect of methanolic leaf extract of *Crateva adansonii* on body weight of P.berghei infected mice

Packed cell volume

Effect of methanolic leaf extract of *Crateva adansonii* on PCV of P.berghei infected mice are shown in figure 3: the PCV of *P.berghei* infected untreated mice and infected treated with 200mg/kg of *Crateva adansonii* show significant decrease in PCV after 5 days of treatment.however the infected mice treated with 400mg/kg, 600mg/kg of *Crateva adansonii* as well as those treated with 5mg/kg chloroquine show significant increase in PCV after 5 days of treatment

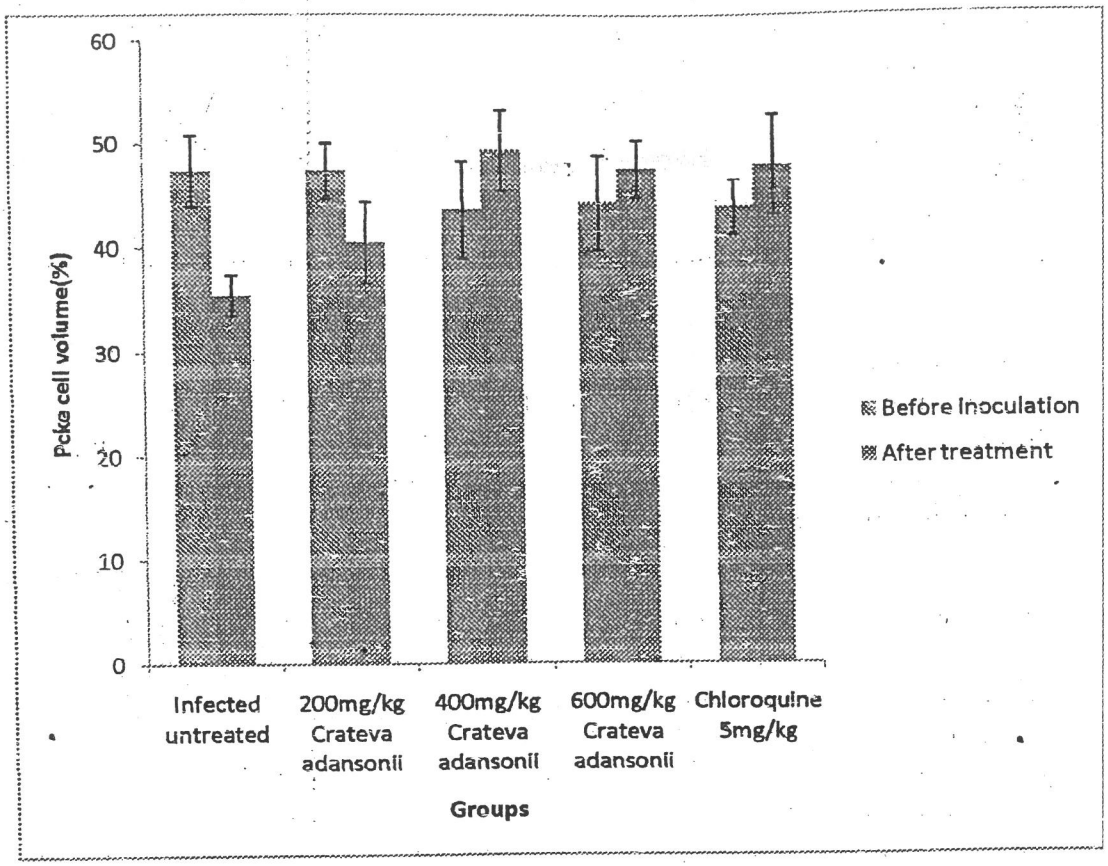

Figure 3: Effect of methanolic leaf extract of *Crateva adansonii* on PCV of *P.berghei* infected mice.

DISCUSSION

Plant use in treatment of disease are said to contain active compounds caled phytochemicals some of which are responsible for the characteristic adours, purgensies and colour of plant while others give a particular plant its culinry medicinal or poisonous virtues (Evans 2002). There is considerable interestby phytochemist to identify the therapeutic agent contained in this plant in orderto establish the basis for their uses in traditional medical practice.

This study revealed the presence of various medicinal important phytochemials including alkaloids, anthraquinones, tannis, flavonoids, saponins cardac glycoside and steroids in methanolic leaf extract of *Crateva adansonii* .

Flavonoid have been reported for their anti-mutagenic anticarcinogenic potentls due to their antioxidant and anti inflamtory properties. Saponin are useas adjuvant in the production of vaccins (ASL and Hossein, 2008) Steroids are ued in the stimulation of bone marrow and growth. It stimulates lean body mass nd also play vital roles in the prevention of bone loss in elderly men (De-piccolli *etal.*, 1991).Alkaloid has been used as CNS stimulant, topical anesthetic in ophthalmology, powerful painkillers, antipyretic action among other use (Heikes

et al; 1995). The cardiac glycoside has been used for over two centuries as stimulant in cases of cardiac failure and diseases (Trease and Evans, 1978).The presence of tannin in theleaf extract of *Crateva adansonii* suggests the ability of these plants to play major roles as antifungal antidiarrheal, antioxidant and antihemorrhoidal agent (Asquith and Butter, 1986)., tannin also have asfringent property, plant containing tannin has been reported to be used for healing of wounds, varicose ulcers, hemorrhoids, frostbile and burn in herbal medicine (Igboko, 1983).

The presence of all this phytochemcials in the leaf extract of *Crateva adansonii* is an indication that this plant if properly screened could yield a drug of pharmacological significant. However the absence of phlobatannins agree with early studies which also found that not all phytochemicals are present in all plant and those present differ with the solvent use in the extraction process (Tijjani *et al.*, 2009).

The invivo antimalaria effect of *Crateva adansonii* against *P.bergei* parasite was evaluated. The extract showed a significant dose dependent and progressive reduction in parasitaemia with time, this is a very promising features in the potentially of *Crateva adansonii* as an antimalarial drug. However, the antiplasmodial effect demonstrated by *Crateva adansonii* leaf extract was lower compare to Chloroquine. Chloroquine has been used as the standard antimalarial drugs because of its established activities on *P. berghei* (Ajaiyeoba, *et al.*, 2006). The *P. berghei*, a rodent malarial parasite although not able to infect man and other primate has been used because of it sensitivity to chloroquine (Fidock *et al.*, 2007) . also the insignificant difference in the level of paracite count of infected mice treated with 200mg/kg of the extract when compared with the control group reflect the inactivity of the extract which could be attributed to low concentration of bioactive agents at that dose.

Blood parameters including packed cell volume (PCV) is used to assess anemia, erythrocytosis, hemodilution and hemoconcentration due to disease condition (Dacei and lewis, 2000). One of the major reason for the development of anemia is oxidative stress (Kremsner *et al.*, 2000) the immune system of the body is activated by malarial infection thereby causing the release of free radicals. In addition to this the malaria parasite also stimulates certain cells to produce R.O.S their by resulting in haemoglobin degradation (Loria *et al.*, 2000) thus the significant decrease level of PCV and body weight of the diabetic untreated is an indication of anemic condition caused by the malarial infection.The significant increase in level of PCV and body weight in mice treated with *Crateva adansonii* at 400 and 600mg/kg when compare with the control group is an indication of ameliorating potentials of the plant extract on the anaemia induced by the malarial infection. Though the rodent malaria model, *P. Berghei* is not similar to that of human plasmodium parasite the anti parasitic activities demonstrated by the extract especially at dose of 600mg/kg against *P. Berghei* mice in this study

could be an indication that the extract possible are effective against human malarial parasite (Audualem, 2010) .

CONCLUSION AND RECOMMENDATIONS

The study suggests that C. *adansonii* leaf contain impotant phytoconstituent that could be implicated in the observed antimalaria effect of the plants. The use C. *adansonii* in herbal medicine to treat malaria has been validated by the present study. It is therefore recommended that further research be carried out on these extract so as to isolate and characterize the bioactive compounds for clinical use in the prevention and treatment of malaria. It is also important that fractions of the crude extracts of C. *Adansonii* be done and tested on *P. berghei*.

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