# Antiplasmodial and Hepatoprotective Potentials of Certain Traditional Antimalarial Remedies Used in Nigeria

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**Abstract:** This study was performed to evaluate the antiplasmodial and hepatoprotective activities of aqueous leaves and stem bark extract of selected medicinal plants (*Azadirachta indica*, *Khaya senegalensis*, *Parquetina nigrescens*, and *Citrus paradisi*) in *Plasmodium berghei* infected mice and to document the components of these plants. The selected plants were marcerated in distilled water. Mice were inoculated with chloroquine sensitive NK 65 *P. berghei* for each test. Mice were treated with 100 mg/kg of extracts and 5 mg/kg chloroquine (positive control) and distilled water only. Blood films were prepared on slides, stained in Giemsa and examined for number of parasiteamia using microscope. Alkaline phosphatase (ALP), Gamma-glutamyltransferase (GGT), and Alanine aminotransferase (ALT) in serum were determined using standard laboratory procedure. Photomicrographs of liver sections were also determined to examine the histoarchitecture of this organ of biotransformation. Gas Chromatography system was used to determine the chemical profile of the extracts. The aqueous crude extracts of *A. indica*, *K. senegalensis*, *C. paradisi* leaves and stem bark at 100 mg/kg significantly ($p<0.05$) inhibited the parasite with varying percentage in the curative, suppressive, and prophylactic tests. However there was no significant ($p<0.05$) reduction in parasitaemia load in the prophylactic test for *P. nigrescens*. The histopathological studies of the liver sections showed that some of the extracts had significant ($p<0.05$) hepatoprotective activity which was in close correlation with the activities of the liver enzymes results. The Gas Chromatographical analyses also revealed many phytochemicals, lipids and essential oils which could be responsible for the hepatoprotective effect of the extracts. These findings provide some justification for the use of the selected plants in the treatment of malaria in Nigeria. Therefore, further studies are ongoing to study the mechanism of action of some of the identified components.

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**Keywords**: Antiplasmmodial; *Plasmodium berghei*; Hepatoprotective; Medicinal plant; GC

## 1. Introduction

Malaria is a major parasitic disease that is responsible for 2 to 3 million deaths every year, mostly among children under five years and pregnant women (WHO, 2008). In the last decades resistance to several antimalarial remedies became widely spread, while the cost of effective treatment is exorbitant for the large majority of the populace in most of the Asian and African countries. Consequently, there is an urgent need to search for new and cheap chemotherapeutic compounds locally in these countries. One possible source for such affordable treatments lies in the use of traditional herbal remedies. The use of plants for the treatment of malaria extends over at least three continents, including several countries in Africa, in the Americas and in Asia (Phillipson *et al*., 1987). However, few data are available to assess the extent to which these plant remedies are used in the health care systems of tropical countries. The recognition and validation of traditional medical practices and the search for plant-derived drugs could lead to new strategies in malaria control. Since many modern drugs such as quinine and artemisinin originate from plants, it is essential that other medicinal plants which have folklore reputation for antimalarial properties are investigated, in order to establish their safety and efficacy, and to determine their potential as sources of new antimalarial drugs (Gessler *et al*., 1994). To overcome some of the most common problems in this field, this study evaluated four selected plants (*K. senegalensis, A. indica, P. nigrescens* and *Citrus paradisi*) used frequently by the traditional healers in Nigeria to treat malaria.

The stem-bark and leaves of *K. senegalensis* have been used in Adamawa State in Northern Nigeria in forms of decoction and concoctions for the cure of malarial fever, mucous diarrhea, syphilis and pyrexia (Olayinka *et al*., 1997). *A. indica* is traditionally used for the treatment of fever reduction, malaria, urinary diseases, piles, leprosy, intestinal worms, contraceptive, and psoriasis (Kuashik *et al*., 2007). The decoction of *P. nigrescens* plant is taken to treat

serious kidney problems, severe constipation and to induce abortion. The root bark and stem bark of *C. paradisi* and other seleted plants are rich in secondary metabolites hence, there popularity as a source of traditional medicine (Olayinka *et al*., 1997 and Kuashik *et al*., 2007; Sathe *et al*., 2010).

The investigation into the antimalarial components of the four selected frequently utilized antimalarial plant species might be an important lead to detecting effective antimalarial compounds. Therefore, this study was performed to evaluate the antiplasmodial activities of extracts of selected medicinal plants (*A. indica*, *K. senegalensis*, *P. nigrescens*, and *C. paradisi*) in *P. berghei* infected mice and document the possible antiplasmodial components of these plants.

## 2. Material and Methods

**Collection, Identification of plant materials and extract preparation**

Plant materials were collected from the Research Farm, Faculty of Agricultural Sciences, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso, Oyo State., Nigeria in September, 2014. Samples of *A. indica, K. senegalensis, P. nigrescens,* and *C. paradisi* were identified and authenticated by Prof. A.J. Ogunkunle of the Department of Pure and Applied Biology and a voucher number was assigned to each plant as UIH 979, UIH 852, UIH 876, and UIH 1081 respectively. The plant materials were washed and air-dried at room temperature. They were then pulverised using electric blender and kept in airtight containers. One hundred grams of the dried pulverized leaves or stem bark were mercerated in 1000 mL distilled water for 72 hr. The liquid extracts were concentrated by freeze drying.

**Parasite inoculation and Assay protocol**

Swiss Albino mice of weight range of 19-25g were obtained from the Animal House of Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso. The animals were kept in cages and allowed to acclimatize for a period of one week before the commencement of the experiment. Albino mice previously infected with *P. berghei* having different levels of parasitaemia were used as donor. A drop of blood from a donor mouse was collected on a microscopic slide by cutting the tail of the mouse with a sterile pair of scissors, and thin smear was prepared on the microscopic slide, dried and fixed with methanol. The blood film was stained with Giemsa and examined under the microscope using X 40 magnification to determine the parasitaemia levels. The donor mice were then sacrificed and blood was collected by cardiac puncture. The parasitized blood was subsequently diluted with normal saline. 0.3 mL donor blood was collected with needle and syringe and made up to 4 ml with normal physiological saline. Then, 0.2 ml of the diluted blood which contained about $1 \times 10^7$ *P. berghei* infected erythrocytes was administered intra-peritoneally to each mouse.

The mice used for the test were treated as follows: Group 1 was neither inoculated nor treatment and served as negative control. Groups 2 was inoculated and not treated and served as the positive control. Group 3 was inoculated and treated with 5 mg/kg chloroquine. Group 4-8 each was inoculated and treated with 100 mg/kg. The mice were left untreated for four days and the treatment then commenced and was continued for four days. The percentage parasitaemia was checked on day 4, day 5, day 6, day 7, day 8, day 12, day 17, day 22 and day 27. All the assays were performed in triplicate.

Percent parasitaemia and percent parasitaemia inhibition (% PI) were calculated using the modified Peters and Robinson formula (1992):

$$\% \text{ Parasitaemia} = \frac{\text{Number of parasitized RBC} \times 100}{\text{Total number of RBC}}$$

And

$$\% \text{ PI} = \frac{\text{Mean \% Parasitaemia of untreated} - \text{Mean \% Parasitaemia of treated}}{\text{Mean \% Parasitaemia of untreated}} \times 100$$

(Saidu *et al*., 2000)

**Antiplasmodial studies**

A 4-day earlier infection method (Peters *et al*., 1993; Okokon *et al*., 2006) was used for the suppressive test. The prophylaxis activity was assessed using the method described by Peters (1965). The curative activity was assessed using the Ryley and Peters method (1970). Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period.

**Enzyme assay**

Mice were weighed, anaesthetized (in diethylether) and sacrificed by jugular puncture. Blood was obtained through their jugular vein into plain sterile bottles. The blood samples were

centrifuged at 4000 rpm for 5 minutes at room temperature to obtain the sera. The sera samples were collected into plain sterile sample bottles and stored in a freezer until required for analyses. Alkaline phosphatase (ALP), Gamma glutamyltransferase (GGT), Alanine aminotransferase (ALT), Urea and Creatinine in serum were determined in mice serum using enzymatic kits (RANDOX, United Kingdom) according to the manufacturer's instructions.

**Histopathological analysis.**

The liver sample of each mouse were excised, weighed and fixed in 10% formalin. The samples of the liver were sectioned at 5–7 µm and stained with hemotoxylen. and analysed for histopathological examination using microscope.

**Gas Chromatography analyses**

1 mg of the crude aqueous extract of the plants were re-dissolved in 10 mL methanol and concentrated into 1 mL, which was injected in the GC system (Hewlett Packard HP series II) in an injection volume of 1 µL. The operating conditions of the column are as follows: oven temperature is at 250°C, the final temperature was kept for 20 minutes. The injector temperature was kept for 20 minutes. The injector temperature was maintained at 250°C, the carrier gas is nitrogen which works at 22 mL/min, and the air compress at 400 mL/min, the flow rate is at 40 mL/min, total GC running time is between 15 minutes to 20 minutes. Phytochemical constituents, Lipid contents and Essential oils of plant extracts were identified using software-peak simple Essential oils and Phytochemical Standard Libraries.

**Statistical analysis**

Graph Pad prism version 5.00 was used to analyze the data obtained and these were expressed as mean ± standard error of mean. The differences between means were compared using one way analysis of variance (ANOVA) followed by Bonferrini's multiple comparison tests. $P < 0.05$ was considered significant.

## 3. Results

**Percentage Parasitaemia analyses**

There is a significant ($p<0.05$) increase in the parasite load of the mice that were not treated (positive control) whereas there is a significant decrease in the parasite load of the treated groups. The chloroquine and the 100 mg/kg extract treated groups showed the most significant ($p<0.05$) chemosuppression of the parasite (Fig. 1).

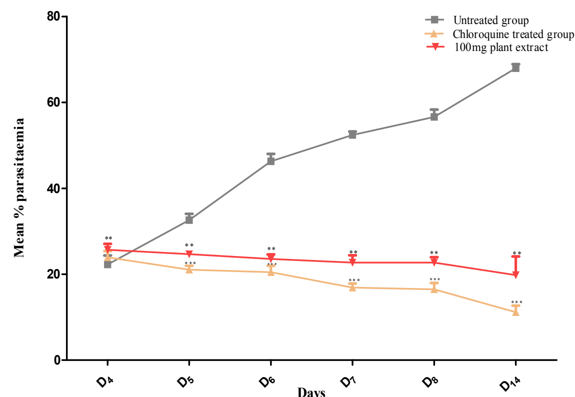


**Figure 1: Percentage parasitaemia of mice treated with aqueous extract of *K. senegalensis* and *P. berghei***

Group 1: Negative control (Not infected and not treated)

Group 2: Positive control (Infected but not treated)

Group 3: Infected and treated with chloroquine (5 mg/kg)

Group 4: Infected and treated with 100 mg/kg of plant extract

*= significantly high at ($p<0.05$) in comparison with the control

**= significantly higher at ($p<0.05$) in comparison with the control

***= most highly significant at ($p<0.05$) in comparison with the control

# = not significant, $D_0$-$D_{14}$ = days of treatment

The percentage of parasitized erythrocytes in the infected untreated group was significantly higher ($p<0.05$) compared to the groups treated with either the extract or the standard drug dose (chloroquine). The chloroquine and the 100 mg/kg treated groups showed the most ($p<0.05$) significant chemosuppression of the parasite (Fig. 2).

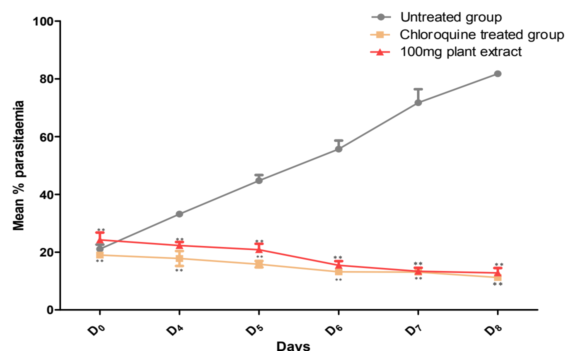


**Figure 2: Percentage parasitaemia of mice treated with aqueous extract of *A. indica* and *P. berghei***
**Legend as indicated above for Fig.1.**

Mice treated with the parasite and 100 mg/kg extract showed insignificant ($p<0.05$) decrease in parasitaemia level when compared to the mice in the control group. The chloroquine treated group also showed a slight chemosuppression of the parasite. The control showed an insignificant ($p<0.05$) decrease in the parasite load (Fig 3).

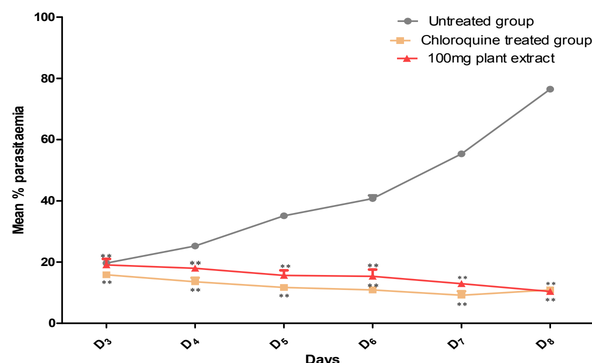


**Figure 3: Percentage parasitaemia of mice treated with aqueous extract of *P. nigrescens* and *P. berghei* . Legend as indicated for Fig 3**

There is a significant ($p<0.05$) increase in the parasite load of the mice that were not treated (positive control) whereas there is a significant ($p<0.05$) reduction in the parasite load of the treated groups. The chloroquine and the 100 mg/kg extract treated groups showed the most significant ($p<0.05$) chemosuppression of the parasite (Fig. 3).

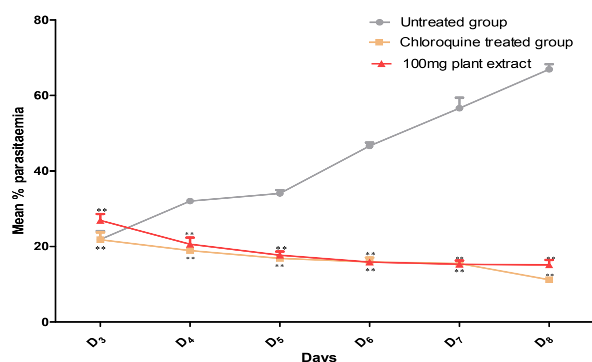


**Fig. 4: Percentage parasitaemia of mice treated with aqueous extract of the leaves of *C. paradisi* and *P. berghei***
**Legend as indicated for Fig. 1.**

The percentage of parasitized erythrocytes in the infected untreated group was significantly higher ($p<0.05$) compared to the groups treated with either the extract or the standard drug dose (chloroquine). The chloroquine and the 100 mg/kg treated groups showed the most significant ($p<0.05$) chemosuppression of the parasite (Fig. 5).

**Liver enzymes assay**

The hepatoprotective effect of extract was evaluated by the assay of liver function biochemical parameters such as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Gamma glutamyl transferase (GGT) according to standard methods. The extract and chloroquine treatment resulted in significant ($p<0.05$) decrease in the level of the serum enzyme, γ-Glutamyl Transferase (GGT) when compared to the control group (Table 1).

**Table 1: Effects of extract of plant extracts on serum enzymes**

| Treatments | Activity (U/I) | | |
|---|---|---|---|
| | GGT | ALP | ALT |
| Distilled water | 30.3±2.3 | 49.6±5.8 | 36.0±0.4 |
| *P.berghei* alone | 94.2±0.0 | 62.8±0.0 | 39.4±1.5 |
| Chloroquine | 32.0±2.3 | 50.4±7.1 | 39.2±0.6 |
| *A. indica* | 34.7±6.0 | 55.4±5.1 | 25.5±6.0 |
| *P. nigrescens* | 34.6±3.0 | 39.1±53.1 | 25.3±2.1 |
| *C. paradisi* leaves | 38.3±2.0 | 60.7±11.8 | 27.5±2.9 |
| *K. senegalensis* | 30.0±2.0 | 64.7±15.8 | 29.5±4.0 |

The control group showed a significant ($p<0.05$) increase in the level of the serum enzyme, alkaline phosphatase (ALP) except for *P. nigrescens*. The uninfected group, chloroquine and extract treated groups showed a significant ($p<0.05$) decrease in the level of the enzyme when compared to the control group (Table 1). The uninfected, chloroquine, and extract treated mice showed a significant ($p<0.05$) decrease in the level of the serum enzyme, alanine aminotransferase (ALT) when compared to the control group. The control group showed a significant ($p<0.05$) increase in the level of the serum enzyme, alkaline phosphatase (ALP) except for *P. nigrescens*.

**Histopathological analyses**

The liver section showed that the sinusoids of all the mice appeared normal without infiltration of inflammatory cells. The central venules and portal tracts of the mice in the negative, chloroquine and the 100 mg/kg of the stem bark *C. paradisi* treated groups, appeared normal and not congested while that of the positive control groups showed peri portal and peri vascular infiltration of inflammatory cells and portal traditis. The hepatocytes of all groups showed normal morphology (Figure 6).

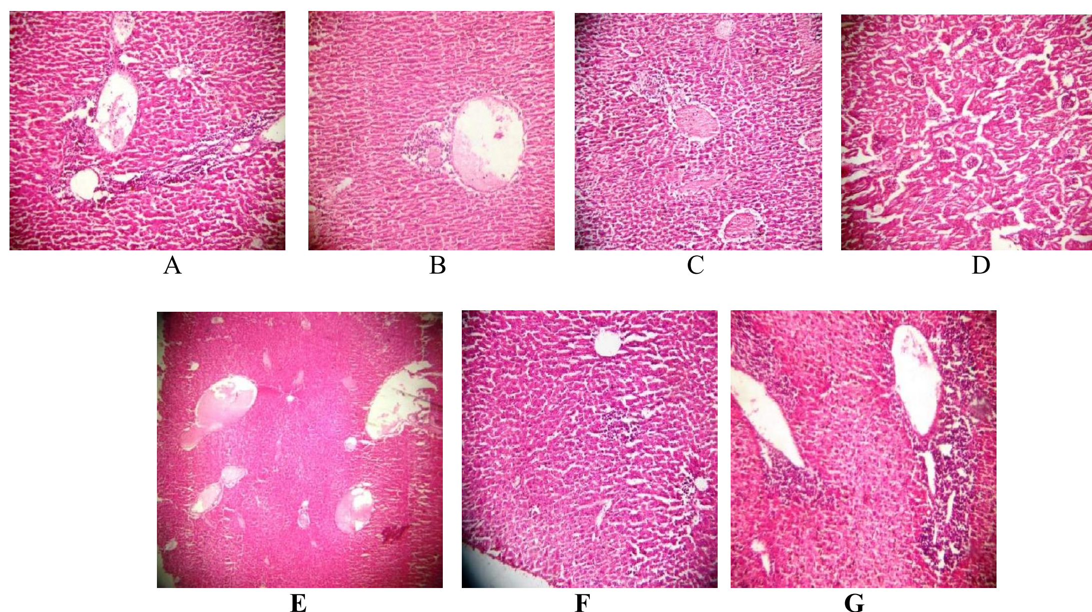


**Figure 6: Photomicrograph of liver section of mice treated with *P. berghei*, chloroquine and *C. paradisi* stem bark. The arrows indicate the portal tract.**
**A = not infected and not treated group (negative control)**
**B = infected and not treated (positive control)**
**C = infected and treated with chloroquine**
**D = infected and treated with *C. paradisi* stem bark**
**E= infected and treated with *P. nigrescens* stem bark**
**F= infected and treated with *K. senegalensis* stem bark**
**G = infected and treated with *A. indica* stem bark**

The liver section showed that the sinusoids of the negative, positive and chloroquine groups appeared normal while that of the 100 mg/kg of the leaves of *C. paradisi* treated group showed scanty infiltration of inflammatory cells. Similarly, that of the 100 mg/kg *P. nigrescens* treated group showed a very mild infiltration of inflammatory cells. In addition, the liver parenchyma of 100 mg/kg *K. senegalensis* and *P. nigrescens* treated group showed very mild lymphocyte aggregates. The hepatocytes of the extract groups showed poor architecture and moderate cytoplasmic vacuolation while others appeared normal.

**GC Analyses**

The selected plants were analysed for essential oil, lipid and other phytochemical constituent using GC. The components of the selected plants identified by GC are reported in Figure 7-9.

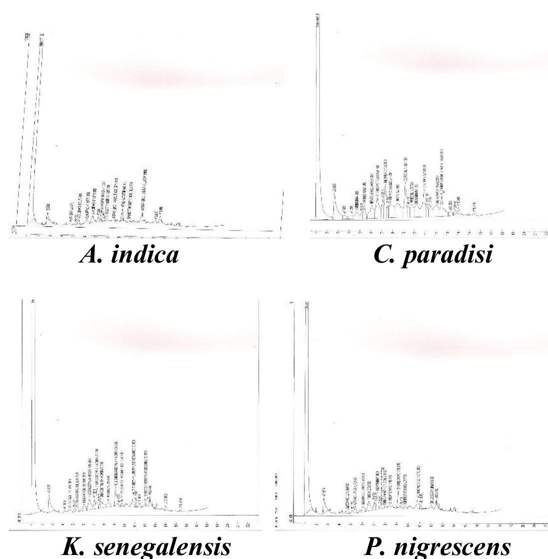


**Figure 7: Gas chromatogram of the phytochemical components the selected plant extract**

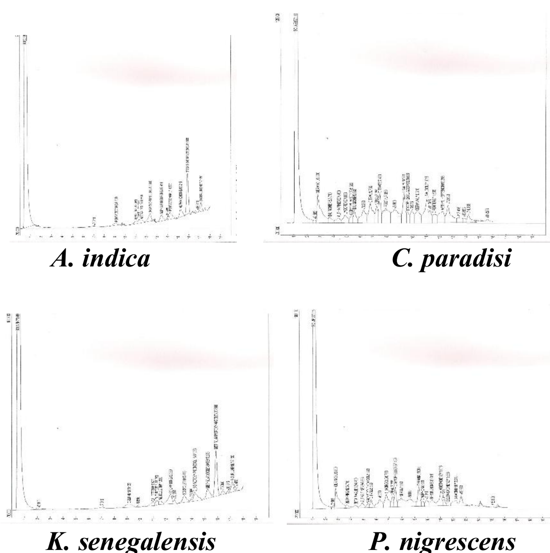


**Figure 8: Gas chromatogram of the essential oil of the selected plants**

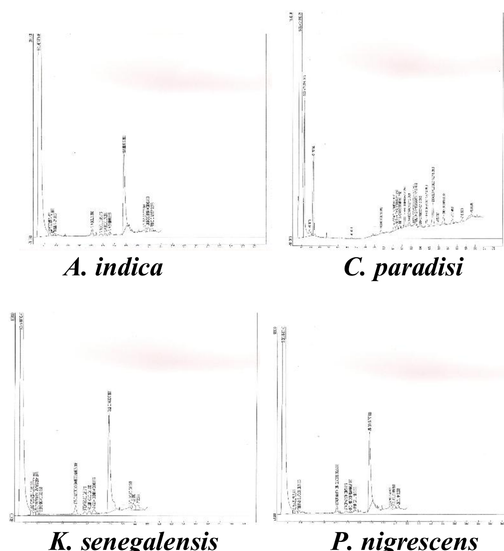


**Figure 9: Gas chromatogram of the lipid components of the selected plants**

**4. Discussions**

The study was carried out to determine the antiplasmodial activity of aqueous extract of the selected plants against chloroquine sensitive strains of the malaria parasite, *P. berghei*. The rodent parasite (*P. berghei*) discovered by Vinckey and Lips (1948) have been used in studying the activity of potential antimalarial agent in mice (Thomas *et al*., 1998) and of recent, in rats (Pedroni *et al*., 2006). On the other hand, plants have proved to be sources of antimalarial agents, especially with the success of quinine isolated from various plants. We opted for this *in vivo* model because it takes into account any pro-drug effect and the likelihood of immune system in controlling infection (Waako *et al*., 2005).

The percentage of parasitized erythrocytes in the infected untreated group was significantly higher ($p<0.05$) compared to the groups treated with the extract or the standard drug dose (chloroquine). The chemosuppressive activity of the standard drug, chloroquine and the plant extract (*A. indica*) at 5 mg/kg and 100 mg/kg was 70.76% and 64.65% respectively. The percentage of parasitized erythrocytes in the infected untreated group was significantly higher ($p<0.05$) compared to the groups treated with the extract or the standard drug dose (chloroquine). The chemosuppressive activity of the standard drug, chloroquine and the plant extract (*K. senegalensis*) at 5 mg/kg and 100 mg/kg respectively was 60.46% and 57.33%. The percentage of parasitized erythrocytes in the infected untreated group was significantly higher ($p<0.05$) compared to the groups treated with the extract (*P. nigrescens*) or the standard drug dose (chloroquine). The chemosuppressive activity of the standard drug, chloroquine at 5 mg/kg was 5.13%. The percentage of parasitized erythrocytes in the infected untreated group was significantly higher ($p<0.05$) compared to the groups treated with the extract (*C. paradisi* leaves) or the standard drug dose (chloroquine). The chemosuppressive activity of the standard drug, chloroquine and the plant extract at 5 mg/kg and 100 mg/kg respectively was 61.19% and 56.80%. The percentage of parasitized erythrocytes in the infected untreated group was significantly higher ($p<0.05$) compared to the groups treated with the extract (*C. paradisi* stem bark) or the standard drug dose (chloroquine). The chemosuppressive activity of the standard drug, chloroquine and the plant extract at 5 mg/kg and 100 mg/kg was 71.44 and 71.92 % respectively. The chemosuppressive effects of the selected plants as compared to chloroquine in P. berghei infection in mice might justify the used of these plants by local populace in Nigeria for malaria treatment. In addition, within a background of rising antimalarial resistance and the challenges for most households in poor and rural areas of Nigeria to have access and meet the expense of effective antimalarial drugs, the use of herbal remedies might possibly be a justifiable way out to malaria treatment in some areas in Nigeria.

Gas Chromatographical analyses showed that the aqueous extract of the selected plants contain alkaloids, flavonoids, sesquiterpenes etc. These constituents have been found in other natural products which exhibited antimalarial activity (Ayoola *et al*., 2008). Saganuwan (Saganuwan *et al*., 2011) reported that plants which contain many phytochemicals with biological activities like alkaloids and flavonoids

could serve as sources of antimalarial drugs. Alkaloids and flavonoids have been implicated in creation of an intracellular environment that is unfavourable to *Plasmodia* growth (Kirby *et al*., 1989; Alli *et al*., 2011).

The positive control (infected and not treated group) had significant ($p<0.05$) rise in the levels of the biochemical enzymes, ALT, ALP, and GGT when compared to the negative control group (not infected group) except for the ALP of *P. nigrescens* which reduced drastically. This abnormal increase in the activities of the enzymes could be an indication of damage to the hepatocytes which caused infiltration of inflammatory cells as evident from the photomicrograph (Figure 3) derived from the histopathological analysis. At ($p<0.05$), the levels of the enzymes were within the normal limit for the groups treated with chloroquine and plant extracts respectively. Moreover, there is a more significant correlation between the activities of the enzymes of the negative control group (not infected group) and the groups treated with chloroquine than when compared to the groups treated with the extracts.

The histopathological result showed that the negative control (not infected group) hepatocytes were not damaged, this is because the central venules, the portal tract, and the sinusoids appeared normal and not congested while the portal tracts and the central venules of the positive control (infected and not treated group) appeared to have peri portal and peri vascular infiltration of inflammatory cells and portal triaditis (Fig 6). This is in correlation with the result of the parasitaemia because the positive control (infected and not treated group) had a high parasite load. Nevertheless, the enzyme result is also in accordance with this result because an abnormal increase in the activities of enzymes that serves as indicators of liver damage was noticed. The liver sections of the chloroquine treated group showed normal architecture, the central venules and portal tract appeared normal and not congested, the sinusoids appeared normal without infiltration of inflammatory cells. The hepatocytes also showed normal morphology. This justifies that the the plant extract had a potent hepatoprotective effect.

The liver sections of the groups treated with the aqueous extract of the leaves of *C. paradisi* showed moderately normal architecture, none of the vessles shows infiltration, the central venules and portal tract appeared normal and not congested, the sinusoids showed scanty infiltration of inflammatory cells (Fig 6). A focal area of aggregates of inflammatory cells was noticed but the hepatocytes showed normal morphology. This mild aggregation of inflammatory cells is not an indication of a damage that could affect the normal functioning of the hepatocytes. Therefore, the aqueous extracts of *C. paradisi* had a significant hepatoprotective effect on *P. berghei* infected mice. The liver sections of the group treated with *P. nigrescens* showed normal architecture, the central venules and portal tract appeared normal and not congested, the sinusoids showed very mild infiltration of inflammatory cells (Fig 6). The liver parenchyma showed very mild lymphocytes aggregates while the hepatocytes showed normal morphology. It can be deduced that the aqueous extract of *P. nigrescens* had hepatoprotection against *P. berghei* infection but this result is not significant in correlation with the enzyme results and parasitaemia results. The liver sections of the group treated with *K. senegalensis* showed poor architecture, the portal tracts and venules showed moderate peri portal and peri vascular infiltration of inflammatory cells, the sinusoids showed very mild infiltration. The hepatocytes showed moderate cytoplasmic vacuolation (Fig. 6). Since the abnormalies are mild, there is a justification that the extract of *K. senegalensis* had hepatoprotective effects against *P. berghei* infection.

The liver sections of the groups treated with *A. indica* showed normal architecture, the central venule and portal tract appeared normal showing no congestion, nor inflammation, the sinusoids appeared normal without infiltration of inflammatory cells. There is a very mild area of lymphocytes aggregate. The hepatocytes showed normal morphology (Fig 6). This result also justifies that the aqueous extract of *A. indica* had hepatoprotective effect on *P. berghei* infection in mice. The liver sections of the stem bark of *C. paradisi* showed normal architecture, the central venules and portal tract appeared normal and not congested, the sinusoids showed scanty inflammatory cells. The hepatocytes showed normal morphology (Fig 6). This result also justifies the fact that the aqeuous stem bark extract of *C. paradisi* had hepatoprotective effect on *P. berghei* infection in mice.

All the plant extracts used in the experiment were screened for the presence or absence of various classes of secondary metabolites through the application of Gas-chromatography mass-spectrometry (GC-MS). Alkaloids, flavonoids and sesquitepenes have been reported to be potent plant secondary metabolites with broad spectrum of bioactivities (Mazid *et al*., 2011). Antimalarial activity is attributed to the presence of various secondary metabolites in plants (Mazid *et al*., 2011). Previous reports have confirmed that one or combinations of compounds may be responsible for the observed antimalarial activities. Consequently, myricetin, naringenin, hesperidin, diosmin, coumarin, bergamottin, aesculetin, azadirachtin, terpenes, geranoil, citral, cadinene, camphor, lycopene, linalool

identified in the extracts of the selected plants may be the active constituents responsible for the observed *in vivo* antimalarial activity (Khalid *et al*., 2007; Wang *et al*., 2010; Mazid *et al*., 2011).

Myricetin, a phytochemical present in *A. indica*, is a member of the flavonoid class of polyphenolic compounds, with antioxidant properties (Ong and Khoo 1997). Myricetin is structurally similar to fisetin, luteolin, and quercetin and is reported to have many of the same functions as these other members of the flavonol class of flavonoids (Ross and Kasum 2002). Myricetin can induce the enzyme glutathione S-transferase (GST). GST has been suggested to protect cells against oxidative stress by protecting cells against free-radicals. In vitro studies have shown that myricetin significantly increased GST activity (Ross and Kasum 2002). In the present study, myricetin could be responsible for the antimalarial activity of *A. indica* by possibly protecting cells against oxidants produced by the parasite invasion in the body. The mechanism involved could be by inducing the enzyme glutathione S-transferase (GST), an enzyme that acts as antioxidant by protecting cells against free radicals (Ross and Kasum 2002).

Azadirachtin, a chemical compound belonging to the limonoid group and identified in *A. indica*, is a secondary metabolite present in neem seeds. It is an antioxidant. It is a highly oxidized tetranortriterpenoid which boasts a plethora of oxygen functionality, comprising an enol, ether acetal, hemiacetal, and tetra substituted oxirane as well as a variety of carboxylic esters. The possible mechanism of action of azadirachtin is to downregulate the hormone, ecdyson receptor (EcR), which regulates the development and reproduction of the parasite (Thummel and Chory 2002).

Bergamottin, a derivative of coumarin identified in the leaves and stem bark of *C. paradisi*, is a potent inhibitor of cytochrome $P_{450}$, an enzyme that is responsible for the metabolism of approximately 60% of clinically relevant drugs (Steven 2014). Bergamottin, a very stable and lipophilic coumarin, reacts with the metabolic enzyme cytochrome P450 found in the liver and the cells that line the intestine (Steven, 2014). It inhibit CYP3A4 resulting in its conjugation with GSH (Lin *et al*., 2004), and therefore acts as an antioxidant by protecting cells against free radicals.

Coumarin, also identified in the leaves and stem bark of *C. paradisi* is a potent inhibitor of cytochrome P450 via the mechanism discussed above for bergamottin (Lin *et al*., 2004). Scoparone, an essential oil with pharmacological and immunosuppressive activity, could be responsible for the antiplasmodial activity of *K. senegalensis*. This compound is known to exhibit an anti-phospholipase A2 activity since Phospholipases A2 (PLA2) build up a class of enzymes which catalyses the release of AA and other unsaturated fatty acids by hydrolysing phospholipids on the sn-2 position (Glaser *et al*., 1993). The inhibited PLA2 may be a calcium-independent PLA2 (Glaser *et al*., 1993). The presence of these antiplasmodial compounds in the selected plants may be responsible for their traditional use in the treatment of malaria and hepatoprotective activity. Moreover, this study gives room for advance studies of the mechanisms of antiplasmodial effects of these compounds and their optimistic clinical importance in the face of multi-drug resistance to known antimalarial remedy.

**Conclusion**

This finding justifies the use of the selected plants in the treatment of malaria in Nigeria and their hepatoprotective activities in *P. berghei* infection. Therefore, the aqueous extracts of the selected plants are potential sources of antiplasmodial compounds.

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