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Ethnobotanical Survey and Antiplasmodial Screening of Commonly used Plants in the Treatment of Malaria in Ilorin Metropolis

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Abstract

The development of resistance to currently known conventional antimalarial drugs has necessitated search for more potent and less toxic antimalarial agents of plants origin. This study aim to document plants commonly used to treat malaria in Ilorin metropolis, Kwara State, Nigeria and also validate the antiplasmodial activity of ten commonly used plants. Basic information was collected by interviewing indigenous people, using a semi-structured mode of interveiw. Collected plant samples were identified and authenticated at the Department of plant Biology Herbarium, University of Ilorin. The antiplasmodium potential of aqueous extracts of ten commonly used plants at 200mg/kg body weight against *Plasmodium berghei* NK 65 chloroquine sensitive strain in mice were also evaluated. A total of forty three species belonging to 28 plant families were recorded as antimalarial plants in the study area. *Cymbopogon citrates* (17.1%), *Azadirachta indica* (12.9%),

Prosopis africana (12.9%), *Vernonia amygdalina* (11.4%), *Khaya senagalesis* (10%), *Terminalia glaucescens* (10%), *Zingiber officinale* (7.1%), *Citrus grandis* (7.1%), *Parquetina nigrescens* (7.1%) and *Psidium guajava* (7.1%) were the 10 most and frequently used for the treatment of malaria. The families of Rubiaceae and Asteraceae were the most represented species used as antimalarial. The leaves were the most common plant part used in the preparation of herbal remedies. The aqueous extracts of all the most commonly used plants showed significant ($p < 0.05$) parasite inhibition at 200 mg/kg body weight. The antiplasmodial activities also correlated well with the activity of the standard drug (chloroquine). *Prosopis africana* extract demonstrated higher parasite inhibition effect (90.02%) and mean survival time of 23 days when compared with the standard (chloroquine, 60.70%) on day 8. This study provides plant species and validate the folklore usage of the plants for the treatment of malaria in Ilorin metropolis which revealed a potential source of antimalarial agents in antimalarial drug production.

Key words: Ethnobotanical survey, Ilorin metropolis, *Plasmodium berghei*, antiplasmodium screening, malaria.

Introduction

The World Health Organization (WHO) estimated malaria mortality rate for children under five in Nigeria at 729 per 100,000. The constant evolution of the malaria parasite has rendered the cheapest and most widely available antimalarial treatments ineffective.

In addition, the recent reports about the increasing resistance of *Plasmodium falciparum* to artemisinin-based compounds is a barrier to effective treatment (Htut, 2009 and Cui *et al.*, 2012). Consequently, there is an urgent need to explore and use the naturally endowed rich biodiversity of

indigenous communities through research that could be translated to benefit mankind (Malik *et al.*, 2010). Such investigations on medicinal and beneficial plants could provide useful leads for the synthesis of important active compounds for treatment of malaria.

Plant materials have been a major source of natural therapeutic remedies and are used to treat various infectious diseases in many developing countries (Ody, 1993). Nowadays, natural products of plant sources have been the centre of focus as the main source of new, safer and more effective bioactive compounds with medicinal properties (Nitta *et al.*, 2002). The use of medicinal plants in the treatment of various human diseases including malaria is common among people in local community in different parts of Africa. Ethno botanical approaches are significant in highlighting locally important plant species, particularly for new crude drugs. They are harvested for

local use and also as ingredients of herbal medicine for use in pharmaceutical industries both locally and internationally (Gakuya *et al.*, 2013). About 80% of the people of developing countries are still dependent on traditional indigenous medicines for their basic healthcare (Anonymous, 2002; Malik *et al.*, 2010).

Over 1200 plant species have been documented from various studies out of which about 160 of families were used in the treatment of malaria or fever (Willcox and Bodeker, 2004). Similar investigations have been carried out in many African nations like Ethiopia (Bekalo *et al.*, 2009), Kenya (Bussmann, 2006 and Njoroge and Bussmann, 2006), Ghana (Asase *et al.*, 2005), Cameroon (Titanji *et al.*, 2008) and Nigeria (Odugbemi *et al.*, 2007; Ajibesin *et al.*, 2008; Olowokudejo *et al.*, 2008; Idowu *et al.*, 2009; Kayode *et al.*, 2009; Adebayo and

Krettli, 2011). The present study, however, covers some areas in Ilorin metropolis, Kwara State of Nigeria with the intent of identifying newer plants that are traditionally employed in the treatment of malaria across the metropolis and to validate the antimalarial potential of some of the plants.

Materials and methods

Study area

The study area covers three Local Government Areas, namely Ilorin East, Ilorin West, and Ilorin South of Kwara State, Nigeria. Ilorin, the capital city of Kwara State, Nigeria is located on latitude $8^{\circ} 24'N$ and $8^{\circ} 36'N$ and longitude $4^{\circ} 10'E$ and $4^{\circ} 36'E$ with an area of about 100Km^2 (Kwara State Diary, 1997). It is situated at a strategic point between the densely populated South Western and the sparsely populated Middle Belt of Nigeria. Ilorin is located in traditional zone between the deciduous woodland of the

South and dry Savanna of North of Nigeria (Jimoh, 2003). The climate of Ilorin is characterized by both wet and dry seasons. The major occupation in the area are weaving cloth, farming and hunting.

Ethnobotanical survey for data collection

The interview was carried out using semi-structured method. The questionnaire was designed according to the method described by Olorunnisola *et al.* (2013) with little modification, so as to meet all the criteria for an ethno botanical survey and to obtain a good ethno botanical score. The plants encountered were photographed. The survey was carried out from July to September 2013. The respondents included, herbalists, traditional herb sellers, house wives and elderly member of the communities from 60 years and above, the female respondents were 60% while male 40%. The participants were questioned on parts of plants employed in the treatment of

malaria, mode of preparation, method of extraction, administration and accessibility of the plant materials. The plants that were singly and most frequently used by the people were also sought for during the survey.

Plants material and authentication

The plants were obtained from the herbalist and traditional herb sellers at Oja-oba and Oja- tuntun markets, Ilorin, Nigeria. The plants were identified at the Herbarium of Department of Plant Biology, University of Ilorin.

Plant Preparation

The plant materials were washed separately, air dried and then milled with blender (Marlex excella, Model no: L 31454) into powder. 1,500 ml of cool distilled water was used to extract 150 g of the powdered plants sample for 48 hours. The filtered extracts were concentrated by freeze drying and percentage yield determined.

Malaria parasite and animal grouping

NK65 strain of *Plasmodium berghei* was obtained from the Institute for Advance Malaria Research and Training (IMRAT), College of Medicine, University of Ibadan and sustained in the laboratory by continuous passage of parasitized blood into mice. Fifty five of the sixty albino mice of six weeks old were inoculated intra-peritoneally with 1×10^7 infected erythrocytes. The animals were divided into four groups of seven animals each. Group A served as control- uninfected. Group B were untreated infected *P. berghei* mice, group C were chloroquine-treated (5 mg/kg body weight) mice, while groups D to M were extracts treated mice at 200 mg/kg body weight. The mice were treated for four days with the different extracts and standard

drug accordingly. Handling of animals conformed to the guidelines of National Institute of Health publication 1985 for laboratory animal care and use.

Curative antimalarial test

The design adopted was modified method of Rayley and Peters (1970). The experimental mice were inoculated with *P.berghei* and confirmation of malaria infection after 72 hours post infection by determining the percentage parasitaemia level, before the commencement of 4 days treatments with plant extracts and chloroquine.

Percentage parasitaemia determination

Thin blood films were made from the tail of each infected mouse, stained with Giemsa and examined under light microscope at 100 magnifications with oil immersion, to assess

the level of parasitaemia. The percentage parasitaemia was calculated using the formula:

$$\text{Parasitized RBC} \div (\text{Parasitized RBC} + \text{Non-parasitized RBC}) \times 100$$

Percentage of Chemo-suppressive of Parasite Multiplications

The mean percentage chemo-suppression of parasite multiplication was determined using the formula of Obih and Makinde (1985):

$$\text{Mean\% chemo-suppression (A)} = \frac{B - C}{B} \times 100$$

A = mean % chemo-suppression, B = mean parasitaemia in control, C = mean parasitaemia in test

Statistical analysis

All data were presented as mean of seven determinations $\pm$ standard error of mean (SEM). The significance of difference among groups was determined. The means were separated using Duncan's Test (or Duncans

multiple range test) for the Post Hoc analyses and $p < 0.05$ was accepted as significant (Mahajan, 1997). The graph pad prism 5 was used for the analysis.

Results

Ethno botanical survey

Based on the frequency of citation, members of Rubiaceae, Asteraceae, Fabaceae, Apocynaceae and Meliaceae were commonly used plant families Rubiaceae provided the highest proportion of antimalarial plant with five species followed by Asteraceae, with three species each (Table 1).

Table 1: List of the plants commonly used for the treatment of malaria in Ilorin metropolis

S/N	SCIENTIFIC NAMES	V/N	FAMILY NAME	COMMON NAME	PART US
1.	<i>Alstonia boonei</i> De wild	UIH 960	Apocynaceae	Ahun (Y) Alstonia (E)	Bark
2.	<i>Azadirachta indica</i> A. Juss.	UIH 1095	Meliaceae	Dongoyaro (Y), Neem (E)	Bark, leav
3.	<i>Anacardium occidentale</i> Linn.	UIH 835	Anacardaceae	Kasu (Y), Cashew nut tree (E)	Bark, leav
4.	<i>Ananas comosus</i> Linn. Aeus	UIH 1084	Bromeliaceae	Ope-oyinbo (Y), Pineapple (E)	Fruit, leav
5.	<i>Bauhinia monandra</i> Kurz.	UIH 647	Fabaceae	Abafe (Y), Orchid tree (E)	Barks, lea
6.	<i>Bridelia ferruginea</i> Benth.	UIH 313	Euphorbiaceae	Odan (Y), Mitzeerie (E)	Leaves
7.	<i>Canna indica</i> Linn.	UIH 026	Cannaceae	Ido (Y), Indian shot (E)	Leaves
8.	<i>Capsicum frutescens</i> Linn.	UIH 751	Solanaceae	Ata-ijosi (Y), Cayenne (E)	Fruit
9.	<i>Carica papaya</i> Linn.	UIH 945	Caricaceae	Ibepe (Y), Pawpaw (E)	Fruit, leav
10.	<i>Chromolaena odorata</i> Linn.	UIH 862	Compositae	Akintola (Y), Siam weed (E)	Leaves
11.	<i>Citrus aurantifolia</i> Christm.	UIH 1059	Rutaceae	Osan wewe (Y), Lime (E)	Roots, b fruit
12.	<i>Citrus aurantium</i> Peti.	UIH 997	Rutaceae	Oronbo (Y), Lemon (E)	Roots, bai
13.	<i>Citrus grandis</i> Merr.	UIH 995	Rutaceae	Osan gerepu (Y), Grape (E)	Fruit, leav
14.	<i>Curcuma longa</i> Linn.	UIH 855	Zingiberaceae	Laali-pupa (Y), Turmeric (E)	Rhizomes
15.	<i>Cymbopogon citrates</i> (Dc) Stapf.	UIH 800	Poeceae	Ewe tea (Y), Lemon grass (E)	Leaves
16.	<i>Dalbergieua welwitschii</i> Taub.	UIH 929	Fabaceae	Paran (Y), West African black wood (E)	Bark
17.	<i>Diospyros mespiliformis</i> Hochst.	UIH 937	Ebeneceae	Igi dudu (Y), Ebony tree (E)	Bark, leav
18.	<i>Enantia chlorantia</i> Linn.	UIH 1013	Annonaceae	Awopa (Y), African yellow wood (E)	Bark
19.	<i>Funtumia africana</i> Benth.	UIH 990	Apocynaceae	Ako-ire (Y), Funtumia (E)	Roots
20.	<i>Gossypium barbadense</i> Linn.	UIH 977	Malvaceae	Owu (Y),	Leaves

				Cotton (E)	
21.	<i>Harungana madagascariensis</i> Lam.	UIH 120	Hypericeae	Asunje (Y), Drangon blood (E)	Bark, leav
22.	<i>Hyptis suaveolens</i> (L.) Poit.	UIH 114	Lamiaceae	Jogbo (Y), Pignut (E)	Leaves
23.	<i>Khaya senegalesis</i> (Desr.) A. Juss	UIH 852	Meliaceae	Agaahoo (Y), African mahogany (E)	Bark
24.	<i>Lawsonia inermis</i> (Linn.) Henna	UIH 101	Lythraceae	Laali (Y), Mignonett tree (E)	Leaves
25.	<i>Lecaniodiscus cupanioides</i> Benth.	UIH 1057	Sapindaceae	Akika (Y), Lecaniodiscus (E)	Roots
26.	<i>Mangiferan indica</i> Linn.	UIH 1080	Anacardiaceae	Mangoro (Y), Mango (E)	Bark, leav
27.	<i>Morinda lucida</i> Benth.	UIH 1085	Rubiaceae	Oruwo (Y), Brimstone tree (E)	Roots, lea
28.	<i>Moringa stemopetala</i> Baker.	UIH 1066	Moringaceae	Agun maniye (Y), Cabbage-tree (E)	Leaves, b
29.	<i>Nauclea latifolia</i> Sm.	UIH 506	Rubiaceae	Egbesi (Y), African peach (E)	Roots, lea
30.	<i>Ocimum gratissium</i> Linn.	UIH 984	Labiatae	Efinrin (Y), Tea bush (E)	Leaves
31.	<i>Parquetina nigrescens</i> (Afzel.) Bullock	UIH 876	Periplocaceae	Ogbo (Y), Parquetina (E)	Leaves, w
32.	<i>Physalis angulata</i> Linn.	UIH 1029	Solanaceae	Koropo (Y), Cutleaf ground cherry (E)	Leaves
33.	<i>Prosopis africana</i> Guill & Perr.)	UIH 473	Fabaceae	Ayan (Y), Iron tree (E)	Bark
34.	<i>Psidium guajava</i> Linn.	UIH 973	Myrtaceae	Gilofa (Y), Guava (E)	Leaves, b
35.	<i>Rauvolfia vomitoria</i> Afzel.	UIH 880	Apocynaceae	Asofeyeje (Y), Serpent wood (E)	Roots, lea
36.	<i>Senna siamea</i> Lam.	UIH 980	Fabaceae	Kasia (Y), Senna (E)	Bark
37.	<i>Sphenocentrum jollyanum</i> Pierre.	UIH 480	Menispermaceae	Akerejupon (Y), Child nodes (E)	Roots, lea
38.	<i>Terminalia glaucescens</i> (Planch.) ex. Benth	UIH 1039	Combretaceae	Idi (Y), Terminalia (E)	Bark
39.	<i>Tetracera alnifolia</i> Wild.	UIH 920	Dilliniaceae	Opon (Y), Tetracera (E)	Roots, bai
40.	<i>Tithonia diversifolia</i> (Heml.) A. Gray	UIH 1129	Asteraceae	Tree marigold (E)	Leaves
41.	<i>Vernonia amygdalina</i> Delile.	UIH 972	Asteraceae	Ewuro (Y), Bitter leaf (E)	Leaves, st
42.	<i>Wissadula ampissima</i> Linn.	UIH 1092	Malvaceae	Furu (Y), Wissadula (E)	Roots, lea
43.	<i>Zingiber officinale</i> Rosc.	UIH 1083	Zingiberaceae	Ata-ile (Y), Ginger (E)	Rhizome

Legend: Y: Yoruba

E: English

The leave (67.4%) were the most common parts used in the preparation of herbal remedies followed by barks (51.2%), roots (25.6%), fruits (11.6%) and whole plant (2.3%). According to the respondents thirteen plant species namely, *Enantia chlorantia* (14.3%), *Mangiferan indica* (12.9%), *Prosopis africana* (12.9%), *Azadirachta indica* (12.9%), *Alstonia boonei* (11.4%), *Vernonia amygdalina* (11.4%), *Morinda lucida* (10%), *Terminalia glaucescens* (10%), *Parguetina nigrescens* (7.1%), *Zingiber officinale* (7.1%), *Rauvolfia vomiloria* (7.1%), *Gossypium barbadense* (5.7%), *Lawsonia inermis* (2.9%), were found to be more effective when used singly in the treatment of malaria in Ilorin metropolis. Six out of the ten most commonly mentioned plants can also be used singly in malarial treatment in the study area.

Preliminary Antiplasmodial screening

The aqueous leaves and barks extracts of the ten most cited plants showed significant ($p < 0.05$) increase in *P. berghei* inhibition potential at 200 mg/kg body weight when compared with the untreated group. Fig. 4 showed the rate of decrease in the level of parasitaemia in the extracts and standard drug

treated *P. berghei* parasitized mice significantly ($P < 0.05$) rise from day four to day eight when compared with the negative control. The result also showed that the activity of the extract is dose dependent. *Prosopis africana* treated mice at 200 mg/kg body weight had significantly ($p < 0.05$) high rate of reduction in parasitaemia level in *P. berghei* parasitized mice when compared with chloroquine treated and other extracts at 200 mg/kg body weight on day eight (Fig. 4).

Fig. 5 showed the percentage chemo-suppression (inhibition) of parasite multiplication in the extract and standard drug treated. All the extracts exhibited significant parasite inhibition at 200 mg/kg body weight. The inhibitory effect of the extracts were more pronounced on the day eight when compared with negative control (not treated *P. berghei* mice). However, treatment with extract of *P. africana* at 200 mg/kg body weight appeared to demonstrate higher significant ($P < 0.05$) inhibitory effect (90.01%) when compared with chloroquine (61.99%) treated group.

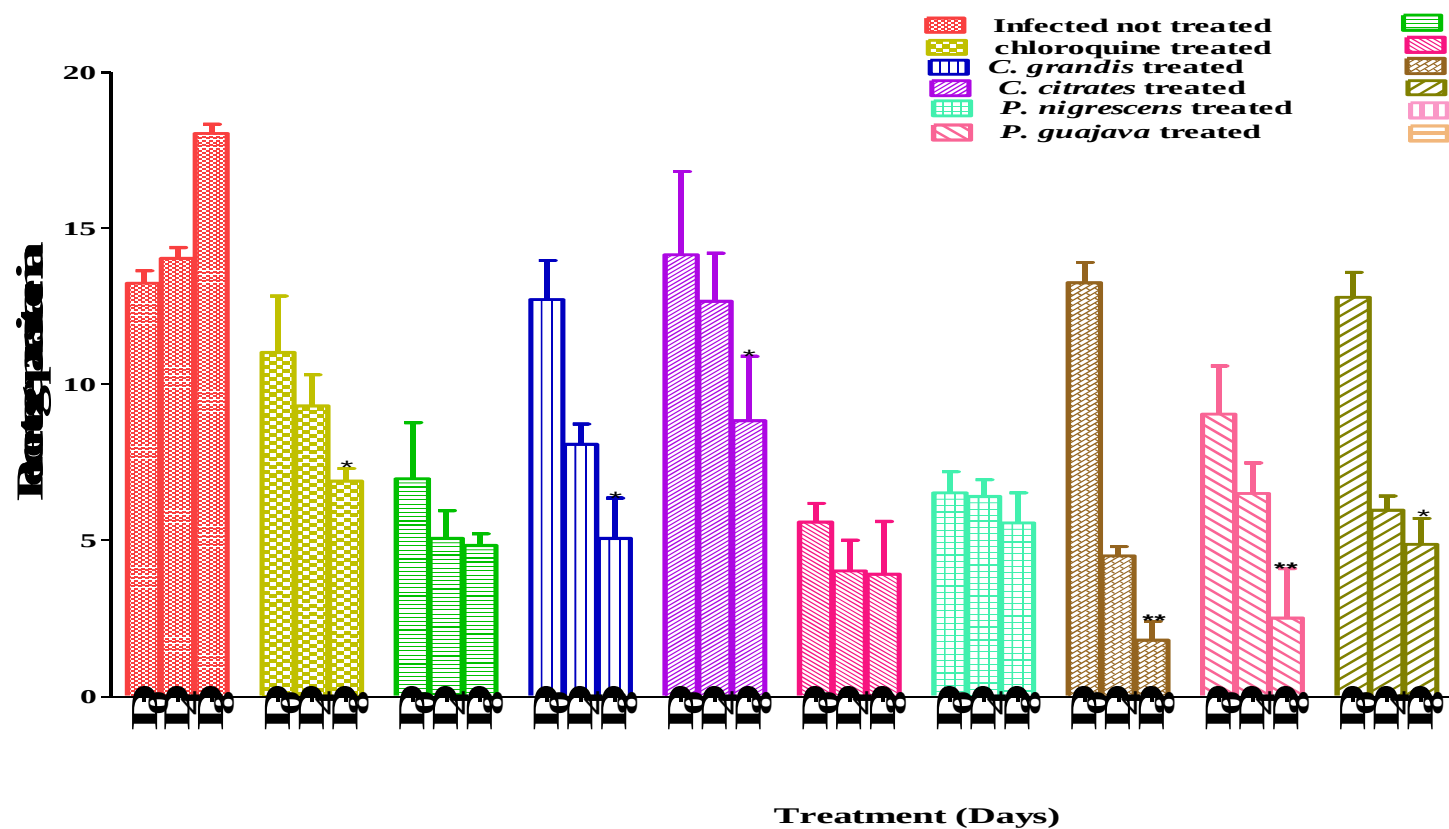


Figure 4: Percentage parasitaemia in extracts (200mg/kg bwt) and standard drug (chloroquine 5 mg/kg bwt) treated *P. berghei* parasitized mice. Legend: D0, D4 and D8 are first, fourth and eighth day after infection with *P. berghei* and with different treatments. The bars are mean $\pm$ standard error of mean, ** show the statistical different at $P < 0.01$ and * at $P < 0.05$.

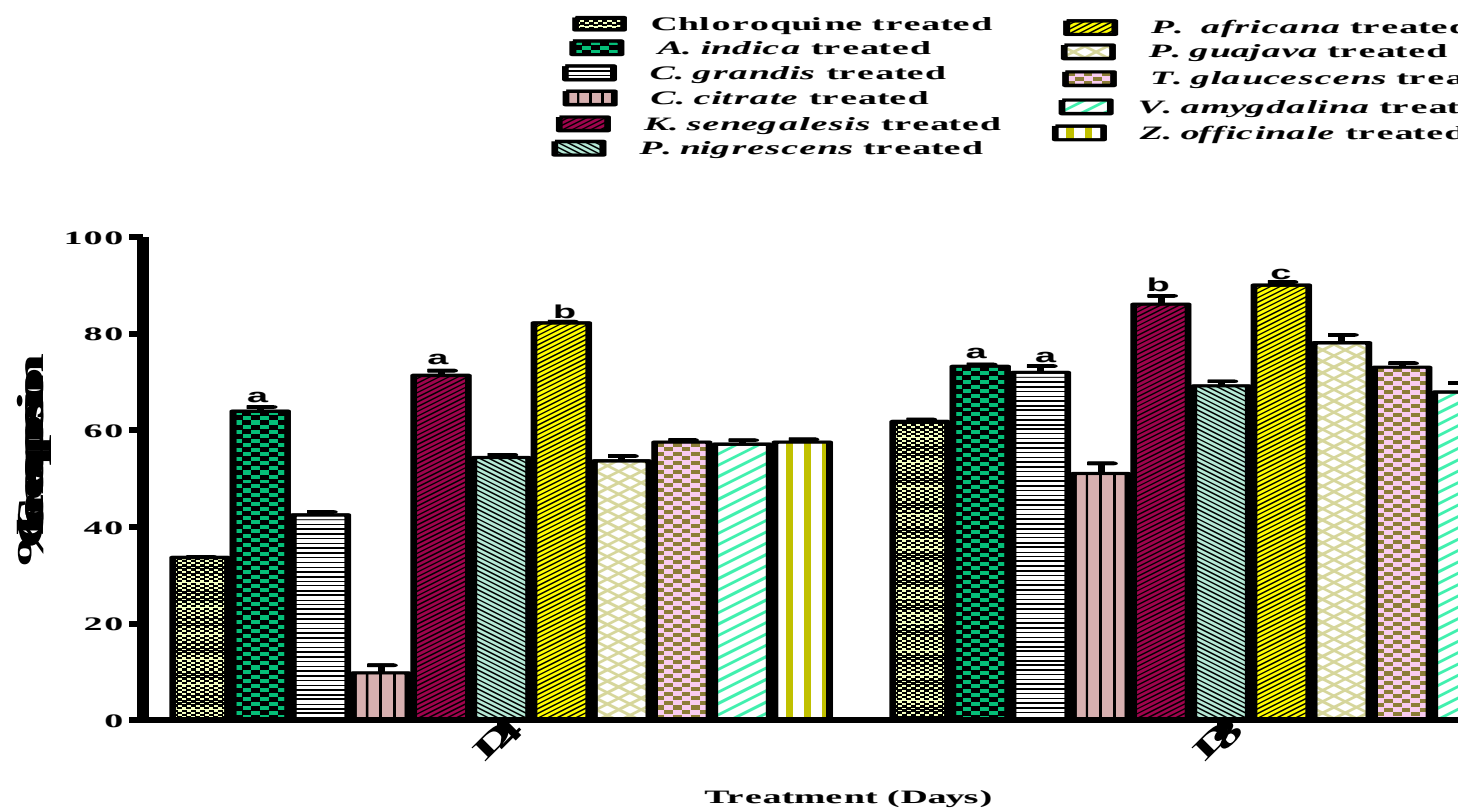


Figure 5: The percentage chemo- suppression (inhibition) of parasite in mice by chloroquine (5 mg/kg bwt) and different plant extracts (20 bwt) (curative) Legend: D4 and D8 are fourth and eighth day after infection with *P. berghei* and with different treatments. The bars are mean standard error of mean, the alphabets show the statistical difference at $P < 0.05$

Discussion

Ethnobotanical survey

Ethno botanical survey is one of the methods employed in the discovery of indigenous plants with effective pharmacological properties for the treatment of various human diseases (Malik *et al.*, 2010). The present study revealed that 43 plant species belonging to 28 different families were commonly used in the treatment and management of malaria in Ilorin metropolis (Table 3). The most frequently utilized plant families are Asteraeae (11%), Rutaceae (11.1%), Meliaceae (7.4%), Zingiberaceae (7.4%), Poaceae (3.7%), Periplocaceae (3.7%), Fabaceae (3.7%), Myrtaceae (3.7%). The families of Asteraeae (three species) and Rutaceae (five species) has the highest number of species implored in the treatment of malaria. Some of the plants species identify in this study have previously been reported to demonstrate significant antimalarial property. For instance, *A. indica* has be reported to demonstrate significant antimalarial activities (Singh *et al.*, 2006; Imam *et al.*, 2012; Ibrahim *et al.*, 2012). Also *V. amygdalina* is been used by local people of Uganda and Nigeria to treat

malaria (Katuura *et al.*, 2007; Namukobe *et al.*, 2011; Stangeland *et al.*, 2011; Tabuti, 2008). A number of studies have also shown that members of the *Anacardiaceae*, *Meliaceae*, *Celastraceae*, *Rutaceae*, *Asteraceae* and *Combretaceae* are commonly used for the treatment of malaria (Muthaura *et al.*, 2007; Asase and Oppong-Mensah, 2009).

It was observed from this study that majority of the respondents used the leaves to make their herbal preparation and to less extent the roots, barks, while few people use fruit, rhizome, whole plant and stem. The preference towards the used of leaves may be linked to the fact that plants leaves are the main photosynthetic organs in plant and also act as the reservoirs for photosynthesis or exudates which contain more bioactive secondary compound for protection against devourers (Balick and Cox, 1996). These secondary metabolites are reported to have pharmacological functions, which are said to be of medicinal value to human body (Balick and Cox, 1996; Bhattarai *et al.*, 2006). Most of the plants used in these study are also been reported in other studies to demonstrate antidiabetes, anti-inflammatory

and antimicrobial properties (Kokwaro, 1993; Gakuya *et al.*, 2012; Dike *et al.*, 2012; Olorunnisola *et al.*, 2013).

Preliminary antiplasmodial screening

The results from the preliminary screening showed that all the studied extracts (*A. indica*, *C. citrate*, *C. grandis*, *K. senegalesis*, *P. nigrescens*, *P. guajava*, *V. amygdalina*, *T. glaucescens* and *Z. officinale*) demonstrate a significant ($p < 0.05$) increase in *P. berghei* inhibition on the day 8 thereby reducing parasite growth. The aqueous extracts of *P. africana*, *C. citrates*, *P. guajava*, and *T. glaucescence* exhibited higher parasite growth inhibition (90.02%, 80.02%, 78.08% and 72.08% respectively) at dose of 200 mg/kg body weight on the day 8 (Fig. 5). The four plant extracts are said to be active according to Isha *et al.* (2003) who reported that extract are considered active if they demonstrated 50% growth inhibition of parasite. The growth inhibitions of all the plants extracts were significantly higher than the chloroquine, with aqueous extract of *P. africana* demonstrating highest growth inhibition than other plant extracts and standard drug (chloroquine). The observed

antimalarial properties of these plants suggested that the plants may contain antimalarial agents.

Previous studies on some of the plants assayed in this study have be reported to be antioxidant, anti inflammatory and anagestic effect (Ojewole, 2006; Singh *et al.*, 2008; Wrigh *et al.*, 2009; Sasidharan and Menon, 2010; Takahashi *et al.*, 2011; Bellik *et al.*, 2013). Malaria parasite are known to generate free radicals as a result of heamolysis of the red blood cell, the antimalarial potential exhibited by most of the plants in this study could be linked to the reported properties of antioxidant, antimicrobial, antianaemic, anagestic and anti inflammatory potentials of most of the plant extracts. The reported anti-inflammatory, antioxidant and anagestic activities of the plant extracts might assist in mopping up the free radical that might have been generated by the parasite during infection.

Conclusion

The ethno-botanical survey and preliminary screening of the most plant used in the treatment of malaria in Ilorin metropolis revealed that some of the plants possess antimalarial properties with *Prosopis*

africana stem bark demonstrating high antimalarial activity among the other plant implored in the treatment of malaria in the study area.

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