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Perceptions of the Traditional Medical Practitioners of North-Western Nigeria on Malaria Treatment and the Potential Antiplasmodial Properties of *Plumeria rubra* Stem-Bark

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Authors' contributions

This work was carried out in collaboration between all authors. Author UAK conceived the idea of the study, participated in field studies and data collection, provided input on methods and manuscript drafting. Author MSA participated in field studies, data collection and provided input on results interpretation. Author AA contributed in manuscript drafting and editing. Author EMA provided the laboratory facilities and guidance in all aspects of this work. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The apparent lack of scientific proof of efficacies claimed by the traditional medical practitioners (TMPs) (locally known as Magori/'Yan-ganye, in Hausa language) of North-Western Nigeria with respect to malaria and the many drawbacks of the current antimalarial drugs stimulated this study. The study was carried out to evaluate the perception of the TMPs on the causes, diagnosis and treatment of malaria and evaluate the potential antiplasmodial properties

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(*in-vivo* in Albino mice) of *Plumeria rubra* Linn. (Apocynaceae) commonly used in traditional treatment of malaria in North-Western Nigeria. The study was aimed at providing scientific basis for use of traditional health knowledge and use of medicinal plant resources in the treatment of malaria.

Study Design: Using an ethno-medical survey, information was obtained from the TMPs relating to identification of plants, their medicinal uses and the mode of preparations of remedies on traditional treatment of malaria.

Place and Duration of Study: The ethno-medicinal survey was carried out at the premises of TMPs from December, 2005 to May, 2008. The laboratory work was carried out at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria from July, 2008 to February, 2010.

Methodology: An ethno-medical survey was conducted in twenty Local Government Areas across four States (Kaduna, Kano, Katsina and Jigawa) in North-Western Nigeria. The communities covered in the survey were selected on the basis of their reputation for being homes to a number of TMPs. The plant used was selected on the basis of ethno-medical information obtained from the TMPs using an exclusion criterion based on claim for activity score. The preferable solvent used by the local people was found to be mostly water and/or alcohol, the plant material was therefore extracted by maceration technique using 70% v/v aqueous-ethanol. The metabolites profiles of the extracts were determined using thin layer chromatographic (TLC) technique on commercially prepared silica gel pre-coated flexible plates.

Results: The TMPs were able to define, diagnose and presumably treat malaria using the indigenous medicines. Median lethal dose (LD_{50}) was established to be greater than 5 gkg^{-1} for the aqueous extract and 3.8 gkg^{-1} for the chloroform extract orally in mice respectively. Antiplasmodial evaluation of the two extracts revealed that the two extracts exhibited dose-dependent *in-vivo* suppressive, curative and prophylactive properties on the development of parasitaemia in Albino mice using a chloroquine sensitive strain of *Plasmodium berghei* (NK-65). TLC profile fingerprints of the aqueous extract revealed three distinct spots with R_f values of 0.23, 0.39 and 0.75 whereas that the chloroform extract revealed three distinct spots with R_f values of 0.33, 0.42 and 0.55 when it was developed in ethyl acetate: ethanol: water: ammonia (65:25:9:1).

Conclusion: These results represented the first conducted evaluation of the perception of TMPs of North-Western Nigeria on the causes, diagnosis and treatment of malaria, antiplasmodial and thin layer chromatographic profile fingerprinting studies on *Plumeria rubra* bark found in North-Western Nigeria. The findings are therefore expected to provide the necessary scientific basis for rational use of traditional health knowledge and use of medicinal plant resources of North-Western Nigeria in the treatment of malaria.

Keywords: Perception; malaria; antimalarial; medicinal-plants; Cissampelos mucronata; north-western Nigeria.

1. INTRODUCTION

New analysis reveals that the prevalence of malaria parasite infection has decreased significantly globally since 2000, and by the year 2013 only a range of 124 to 283 million cases of malaria and a range of 367, 000 to 755, 000 deaths caused by malaria compared with the previous ranges [1]. Although 90% of these cases and deaths occurred in Africa, these ranges indicate a reduction of malaria incidence by 30% globally and by 34% in Africa, which is a direct result of an expansion of malaria interventions between 2000 and 2013 [1]. However, despite these long sustained interventions the disease remains a major health problem in Africa and is responsible for major childhood mortality and maternal mortality as well as for major outpatient visiting in African countries [1]. The need for more interventions and newer antimalarial agents therefore remains. One possible approach for the identification of new antimalarial candidate is to search for compounds from plants empirically used to treat malaria [2]. *Plumeria rubra* Linn. (Apocynaceae) is a fragrant partially deciduous tree commonly found in moist gardens, in lawns and in open plantations growing up to 5 meters high. It has a single trunk and multiple branches of a similar length that support an open spreading canopy. The leaves are 10 to 42 cm long and 4 to 14 cm wide with widest spot at the center of the blade

[3]. The latex of Plumeria has been utilized traditionally in tropical regions for treatment of itches, swellings, and fevers [4]. Ethanolic and chloroform extracts of leaves of the plant were reported to exhibit good inhibitory activities at a concentration of 1500 µg/ml against *Escherischia coli* and *Staphylococcus epidermidis* respectively [5]. The presence of iridoids compounds such as plumericin and isoplumericin in Plumeria rubra were confirmed by Stephens et al. [6]. The bark of the plant enjoyed the confidence of the rural people of Northern Nigeria where they commonly utilized it traditionally in form of decoction for treatment of typhoid fever and malaria related illnesses. Thus, the present study was carried out to evaluate the perception of the traditional medical practitioners (TMPs) on the causes and treatment of malaria and evaluate the potential antiplasmodial properties (*in-vivo* in Albino mice) of *Plumeria rubra* bark. The aim of the study was to provide scientific basis for use of traditional health knowledge and use of medicinal plant resources of North-Western Nigeria in treatment of malaria to open up another possibility for the development of yet another new antimalarial agent from medicinal plants as an additional malaria intervention.

2. MATERIALS AND METHODS

2.1 Study Area and Ethnobotanical Survey

The present study covered twenty distinct local government areas within five States in the north western Nigeria. Drawing from the experiences of Milijaona et al. [7] and Abubakar et al. [8] an ethno-medical survey was carried out to identify plants and methods used for treatment of malaria by the local people, in the period from December, 2005 to May, 2008. Traditional medical practitioners across different localities within the selected local government areas were identified and considered in the study. Such traditional medical practitioners (TMPs) known locally as Magori/'Yan-ganye, in Hausa language, were considered to be the best source of information regarding the existing knowledge, attitudes and practices related to traditional malaria recognition, control and treatment in North-Western Nigeria. The healers were interviewed by means of informal and unstructured conversations (using field notebook), in order to cover different perceptions and behaviors amongst the TMPs about the mode of diagnosis and treatment of malaria-related illnesses.

2.2 Collection and Identification of Plant Material

Information was obtained from the TMPs relating to identification of plants, their medicinal uses and the mode of preparations of remedies. We focused particularly on the information provided concerning such plants used to treat *Zazzabi/Masassara* (malaria sickness in Hausa language). With the help of a field guide, the plants were identified from the given vernacular names, using pharmacognostic techniques and using taxonomic keys and descriptions as outlined by Gill [9], Ghazanfar [10] and Dutta [11]. Samples were collected for all the plants listed, which were then compared with herbarium specimens from the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

2.3 Selection of Potential Antimalarial Plants

Based on the ethno-medical survey conducted, we used inclusive criteria for selecting plants with potential antimalarial activity. The inclusive criteria used were based on claims for activity score as follows:

1. that the plant is available in the locality of the study (+);
2. that the plant is claimed to treat all symptoms of malaria (headache, fever etc) (+);
3. that the plant is mentioned by not less than 80 % of the respondents (+);
4. that the plant recipe is mono-component (+); and
5. that usage of the plant has little or no existing scientific justification (+).

Based on the above criteria *Plumeria rubra* Linn. (Apocynaceae) was selected and considered for scoring 5 points (1+2+3+4+5) on the claims for activity score.

2.4 Experimental Animals

Swiss albino mice (*Mus musculus*) of both sexes, weighing between 20.5 g – 25.5 g obtained from Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, were used as experimental animals for this study. The animals were kept in plastic cages at room temperature (20°C) and moisture, in a naturally

illuminated environment of 12:12 – hour day and night cycle. They were allowed access to clean drinking water and standard livestock feed (Vital Feed Growers) obtained from Brand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria *ad libitum*. Their experimental usage was according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals [12]. These guidelines are consistent with guidelines of Ahmadu Bello University for animal handling.

2.5 Test Parasites

Sample of chloroquine sensitive *Plasmodium berghei* (NK–65 strain), obtained from National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria was used to infect the experimental animals in this study. The parasites were maintained in mice by continuous passage on every 5^{th} days.

2.6 Determination of Macroscopic Properties of the Plant Materials

The macroscopic properties of the *P. rubra* bark, such as the shape, size, surface characters and organoleptic properties were determined following standard procedures [13,11].

2.7 Determination of Microscopic Properties of the Plant Materials

The microscopic properties of the *P. rubra* bark, such as the anatomy of the bark and its powdered properties were also determined following the methods described by Dutta [11] and Evans [13]. The microscopical features were examined using a compound microscope, from Fisher Scientific Company, United Kingdom, and diagnostic features were recorded using Photosmart digital camera (SNPRB-0503 Model) from *hp* Company, China.

2.8 Determination of Physical Constants of the Plant Materials

The physical constants, such as the moisture content, ash value and extractive value of the *P. rubra* bark were determined following standard procedures [13,14]. The spread of values from the mean were expressed in terms of standard error of mean (S.E.M.).

2.9 Preparation and Extraction of the Plant Materials

Sample of the plant material was dusted, cleaned and all visible impurities (foreign matters) such as grasses; other parts of the plant; and sand were removed and air-dried at room temperature until thoroughly dried and pulverized using wooden mortar and pestle as described by African Pharmacopoeia [15]. The preferable solvent used by the local people is mostly water and/or local gin; therefore, the extraction design was based on that fact, whereby 500 g of the plant material was exhaustively extracted with 70% v/v aqueous ethanol using maceration technique at room temperature. The alcoholic solutions were combined and concentrated at 45°C under reduced pressure, suspended in water, and partitioned with chloroform to obtain polar (aqueous) and non-polar (chloroform) portions. The two portions were then concentrated *in-vacuo* to obtain residues, which are subsequently referred to as aqueous extract and chloroform extract.

2.10 Determination of Metabolites' Fingerprint of the Plant Materials

Two extracts (aqueous and chloroform) were prepared for this study and subjected to thin layer chromatographic (TLC) technique to fingerprint the metabolites present. The experiments were carried out following standard procedures [16-18]. The procedure was carried out at room temperature. Vanillin-sulphuric acid reagent (6 g vanillin, 100 ml ethanol and 1 ml H_2SO_4) was used to spray the plates after development and detection of the spots was done after the plates were heated in an oven at 120°C until maximum colouration was observed [19].

2.11 Determination of Safety Profile of Extracts of the Plant Materials

The two extracts (aqueous and chloroform) were subjected to acute toxicity evaluation to determine their median lethal dose (LD_{50}) orally in mice using the method described by Lorke [20] at different geometrical doses (10 – 5000 mg kg^{-1}) in two phases. The animals were kept and observed under the same conditions for 72 hours within which signs of toxicity and mortality were observed and recorded. The experiments were

conducted between the hours of 9: 00 am and 12: 00 (noon) daily.

2.12 Determination of Antimalarial Properties of the Plant Materials

The two extracts (aqueous and chloroform) prepared for fingerprinting of the metabolites were subjected to standard *in-vivo* antiplasmodial test in mice using suppressive, curative and prophylactive procedures. The experiments were carried out according to the procedure described by Bulus et al. [21], Elufioye and Agbedahunsi [22] and Jude et al. [23] to evaluate the efficacy of the traditionally claimed antimalarial properties of the plant material.

2.13 Statistical Analysis

The results generated from this study were statistically expressed as mean ± SEM values. Student t-*test* was used to analyse the data between groups and one-way analysis of variance (ANOVA) among groups with Dunnett's post-test. This was performed using GraphPad Prism software, version 4.00 for Windows, from GraphPad Software Company, San Diego California, USA [24]. The statistical significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Study Area and Ethnobotanical Survey

The study area is situated between latitude 8° 08' and 13° 03' North, and longitude 6° 02' and 10° 35' East in the map of the Federal Republic of Nigeria. The area covered 112, 788 km^2 with population of 25, 601, 471 people according to the 2006 population census [25]. Farming and petty trading are the main occupations of the local people of this region with Islam as their major religion. The weather varies according to the seasons of the year; it is generally cool in the morning, hot in the afternoon and cool again in the evening. Temperature ranges between 18° to 38°C, harmattan season ranges from the month of November to February with general temperature from 18° to 27°C (Fig. 1). The wind is dry from the month of December to March, signalling the arrival of the raining season, which lasts from April to October with mean annual rainfall of 400 to 1300 mm [26]. Recently, research in malaria treatment strategies has focused on investigating folkloric medical treatments in search of potent new antimalarial drugs. For example, the now prominent artemisinin was isolated from the herb *Artemisia annua*, which has been in use in traditional Chinese medicine as remedy for chills and fevers for more than 2000 years [27]. Evaluation of perceptions of traditional medical practitioners on causes, diagnosis and traditional treatment of malaria using medicinal plants is essential for further evaluation of the safety and efficacy of such traditional antimalarial remedies towards discovery and development of new drugs for malaria. There is also the need to generate reliable scientific data to determine whether the plants traditionally used to treat the disease could actually be a potential source of developing safe and effective antimalarial drugs. It is for this reason that in the present study an ethno-medical survey was first carried out to evaluate the perceptions of the TMPs with the hope of generating useful resources and bridging the gaps between empirical treatments and realism in relation to malaria causes, diagnosis and treatment.

The ethno-medical survey to evaluate the perceptions on causes, diagnosis and treatment of malaria generated information from 200 (140 males and 60 females) TMPs from across the different local communities within the study area who are between the ages of 40 and 70 years old. All the respondents spoke Hausa language freely and were observed to have a common approach regarding their practices in relation to causes, diagnosis and treatment of malaria using traditional methods. The TMPs were able to define, identify and presumably treat malaria using indigenous medicines, which we termed herbal antimalarial drugs (Table 1).

3.2 Identification of the Plant Material

Based on the information obtained from the TMPs regarding the causes, diagnosis and treatment of malaria and the kind of medicinal plants traditionally used to treat malaria, 10 plants were identified in the field and their samples collected. The plant samples were transported to Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria for proper identification and authentication and where a voucher specimen of each plant was deposited (Table 2).

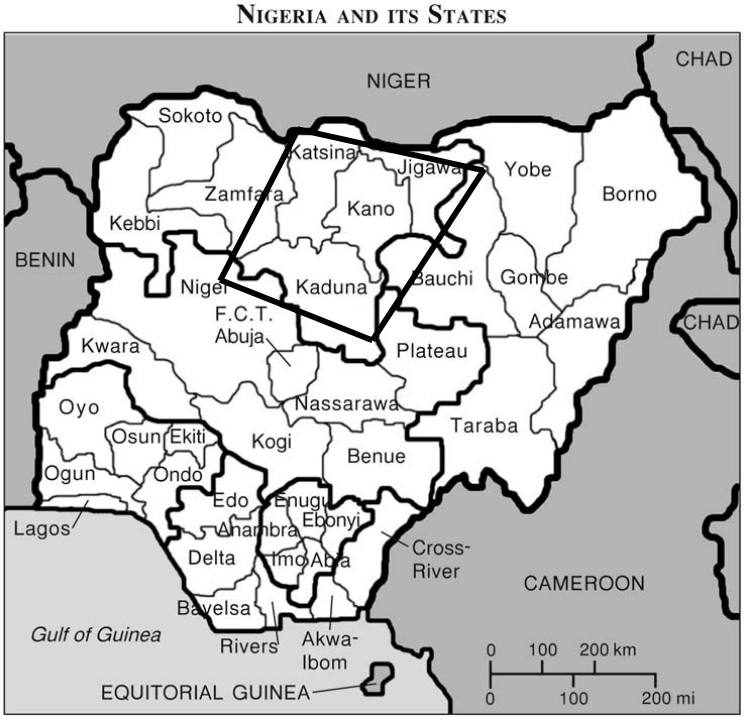

Fig. 1. Study area for the ethnobotanical survey of plants
Note that this map was reproduced from the Wilberforce Conference on Nigerian Federalism, 1997

Table 1. Perception TMPs of north-western Nigeria on causes and treatment of malaria

Parameters	Perception	Frequency
Cause of malaria	Mosquito bites	165 respondents
	Evil causes/misfortunes	35 respondents
Symptoms of malaria	Fever, shivering, muscle and joint pain, fatigue and headache	170 respondents
	Stomach pains and vomiting	30 respondents
Plant remedies	Decoction/or infusion	All the respondents
Mode of preparation	Mixtures of plant components	180 respondents
	Single plant	20 respondents
Duration of treatment	Until symptoms are cleared	All the respondents

3.3 Potential Antimalarial Plants

Based on the ethno-medical survey conducted and the inclusive criteria employed, which were based on claims for activity score (as described in the methodology) for selecting plants with potential antimalarial activity, *Plumeria rubra* Linn. (Apocynaceae) was found to score 5 points (i.e. i + ii + iii + iv + v) on the claims for activity score and was therefore selected. Samples of Plumeria rubra were obtained from Area BZ Staff Quarters, Ahmadu Bello University, Zaria, Nigeria, in the month of July 2005 (Fig. 2).

3.4 Experimental Animals

Swiss albino mice (*Mus musculus*) of both sexes, weighing between 20.5 g and 25.5 g, obtained from Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, were used as experimental animals in this study as described in the methodology section.

3.5 Test Parasites

Chloroquine sensitive *Plasmodium berghei* (NK-65 strain), obtained from National Institute of

Medical Research (NIMR), Yaba, Lagos, Nigeria was used to infect the experimental animals in this study as described in the methodology section.

3.6 Macroscopic Properties of *Plumeria rubra* Bark

The *P. rubra* bark was found to exude latex from the lactiferous cells when cut fresh; it is slightly bent or concave towards inner side when dried with short fracture when broken transversely. The outer surface is light-brown in colour and slightly rough when touched, whereas the inner surface is white and smooth when touched (Fig. 3). The powdered bark has a dusty odour, with coarse texture and light-brown colouration.

3.7 Microscopic Properties of *Plumeria rubra* Bark

The microscopical analysis of the *P. rubra* powdered plant material revealed the presence of polygonal calcium oxalate crystals, starch grains, lignified fibres and heavily lignified sclerenchymatous cells. Worthy of note is the presence of unicellular group of covering trichomes. Another distinguishing feature is the presence of latiferous ducts (latex cells), which are peculiar characteristic features of Apocynaceae members (Fig. 4).

3.8 Physical Constants for *Plumeria rubra* Bark

The moisture content of the powdered plant material was determined to be 11.0±1.5% (W/W), the ash value was determined to be 13.0±0.5% (W/W) and the acid-insoluble ash value was determined to be 7.0±0.5% (W/V). Values for the water-soluble and alcohol-soluble extractives were calculated to be 9.5+1.5% (W/V) and 4.0+0.2% (W/V), respectively. The moisture content of the material is within the limit of not more than 14% as recommended in the African Pharmacopoeia [14], which indicated that any marked difference to such values may indicate a change in the quality of samples.

3.9 Extracts of the *Plumeria rubra* Bark

The ethanolic solutions obtained through repeated maceration of the plant material with 70% v/v aqueous ethanol when combined and dried yielded 110 g dark-brown extract, referred to as *P. rubra* crude ethanolic extract (PlrCEE). 50 g of this dried extract was suspended in water and partitioned with chloroform to produced two portions, polar (aqueous) and non-polar (chloroform). These two portions was concentrated *in-vacuo* to yield dark-brown (28 g) and light-brown (20 g) residues, referred to as *P. rubra* aqueous extract (PlrAE) and *P. rubra* chloroform extract (PlrCE) respectively.

Fig. 2. Example of *Plumeria rubra* Linn. (Apocynaceae) obtained from area BZ, Ahmadu Bello University, Zaria, Nigeria (Mag.: X1)
Note: the bark has been accessibly scrapped for medicinal uses

Table 2. Commonly used plants by TMPs of north-western Nigeria for treatment of malaria

Plant families	Binomial names	Voucher No.	Hausa names	Uses	Parts used
Anarcardiaceae	*Sclerocarya birrea*	ABU-900205	*anya*	Dysentery, malaria, fever	Bark
Apocynaceae	*Plumeria rubra*	ABU-131	*Parangipani*	Malaria, body pains	Bark
Asteraceae	*Aspilia africana*	ABU-900084	*Biraana*	Malaria, coughs	Aerial pats
Bignoniaceace	*Newbouldia laevis*	ABU-2881	*Aduruku*	Malaria, fever, haemorrhages,	Bark, leaves
Caricaceae	*Carica papaya*	ABU-1203	*Gwandar- gida*	Malaria, antihelmintic,	Leaves
Meliaceae	*Azadirachta indica*	ABU-900151	*Dogon- yaro/ Dalbejia*	Malaria, inflammation, rheumatism, skin diseases	Bark, leaves
Meliaceae	*Khaya senegalensis*	ABU-900181	*Madachi*	Malaria, stomachic, bitter tonic, syphilis	Bark
Menispermaceae	*Cissampelos mucronata*	ABU-22396	*Jubdar-kasa*	Malaria, ulcers, stomach pains	Roots
Mimosaceae	*Parkia biglobosa*	ABU-2846	*Dorawa*	Malaria, asthma, toothache	Bark, leaves
Moraceae	*Ficus thonningii*	ABU-1885	*Cediyaa*	Malaria, pains,	Bark, leaves

Fig. 3. Macroscopical features of *Plumeria rubra* bark (Mag.: X 1)

3.10 Metabolites' Profile of Extracts of *Plumeria rubra* Bark

The metabolites profile of the aqueous extract (PlrAE) were carried out on commercially prepared silica gel pre-coated flexible plates (Whatman Ltd) and developed in chloroform: Methanol (4:1) revealing three distinct spots.

The thin layer chromatographic profile of the chloroform extract (PlrCE) was also carried out on commercially prepared silica gel pre-coated flexible plates (Whatman Ltd) and developed in ethyl acetate: ethanol: water: ammonia (65:25:9:1) revealing three distinct spots (Table 3).

3.11 Safety Profile of Extracts of *Plumeria rubra* Bark

The median lethal dose (LD_{50}) for the PlrAE was estimated to be greater than 5 gkg^{-1} while that of the PlrCE was estimated to be 3.8 gkg^{-1}. Mortality was recorded in the mice administered with the 5 gkg^{-1} of the chloroform extracts. The observed signs of toxicity were depression, decreased limb tone and watery stool, which preceded the death.

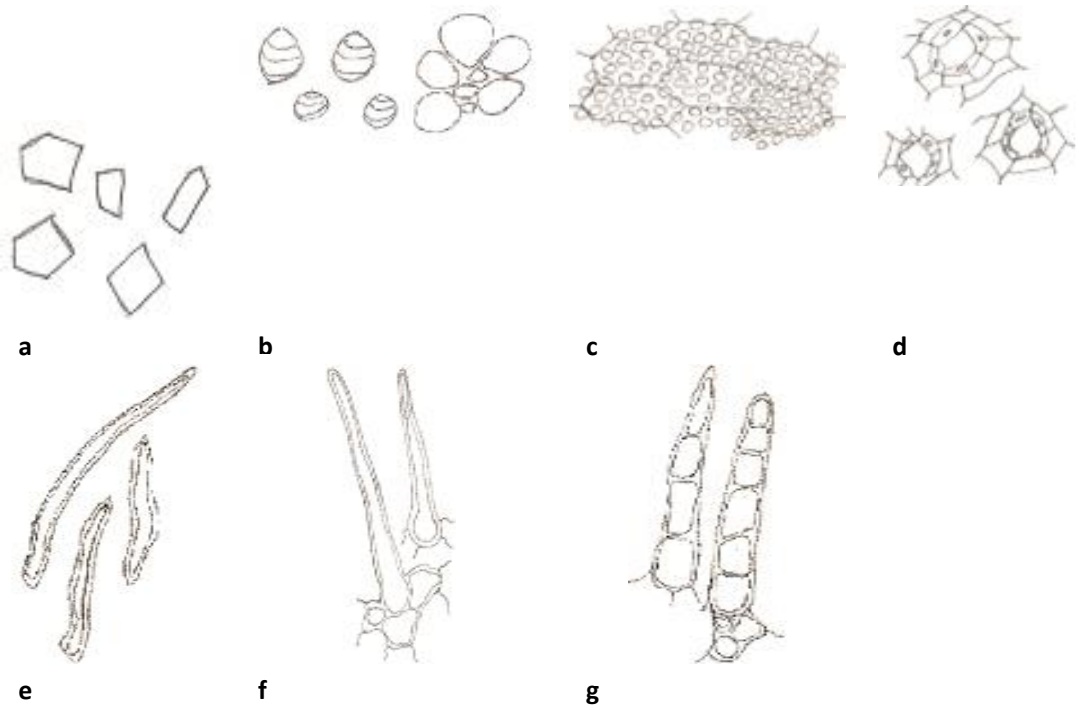

Fig. 4. Microscopical features of powdered *P. rubra* bark (Mag.: X100)
a: polygonal calcium oxalate crystals; b: starch grains;
c: parenchymatous cells with palisade cells underneath;
d: latex cells with suspended globules; e: fibres with lignified walls;
f: unicellular covering trichomes; g:multicellular covering trichomes

3.12 Antimalarial Properties of Extracts of *Plumeria rubra* Bark

3.12.1 Suppressive activity of the extracts

The results of the suppressive test of the *P. rubra* aqueous extract (PlrAE) and *P. rubra* chloroform extract (PlrCE) on the early growth of *P. berghei* at the doses of 50 $mgkg^{-1}day^{-1}$, 100 $mgkg^{-1}day^{-1}$ and 200 $mgkg^{-1}day^{-1}$ (orally in mice) demonstrated significant dose-dependent suppressive effects (P = .05) by inhibiting the growth of the parasites for up to 76.0% and 60.0% respectively at the doses of 200 mg $kg^{-1}day^{-1}$ by both the two extracts, when compared with the reference drug, chloroquine (5 mg $kg^{-1}day^{-1}$) which produced 96% inhibition of the parasite growth (Table 4).

3.12.2 Curative activity of the extracts

The results of the curative test of the *P. rubra* aqueous extract (PlrAE) and *P rubra* chloroform extract (PlrCE) on the established infection of *P. berghei* at the doses of 50 $mgkg^{-1}day^{-1}$, 100 $mgkg^{-1}day^{-1}$ and 200 $mgkg^{-1}day^{-1}$ (orally in mice) demonstrated significant dose dependent curative effects (P = .05) by inhibiting the growth of the parasites for up to 74.1% and 62.7% respectively at the doses of 200 $mgkg^{-1}day^{-1}$ by both the two extracts, when compared with the reference drug, chloroquine (5 $mgkg^{-1}day^{-1}$) which produced 99% inhibition of the parasite growth (Table 5).

3.12.3 Prophylactive activity of the extracts

The two extracts of *P. rubra* (PlrAE and PlrCE) exerted a dose dependent repository activity at the three doses employed (50 $mgkg^{-1}day^{-1}$, 100 $mgkg^{-1}day^{-1}$ and 200 $mgkg^{-1}day^{-1}$) causing significant (P = .05) prophylactive effects (80.3% and 64.1%) at the dose of 200 $mgkg^{-1}day^{-1}$ for both the two extracts, similar to the reference drug, chloroquine (5 $mgkg^{-1}day^{-1}$) which exerted a 100% prophylactive activity (Table 6).

Table 3. Thin layer chromatographic profiles of *Plumeria rubra* bark

Extracts	Solvent system	R_f value	Development time (Min)
PlrAE	$CHCl_3$: CH_3OH (4:1)	0.23, 0.39, 0.75	45
PlrCE	$CH_3.COOC_2H_5$: C_2H_5OH: H_2O: Ammonia (65:25:9:1)	0.33, 0.42, 0.55	60

Table 4. Suppressive activity of *P. rubra* aqueous and chloroform extracts (PlrAE and PlrCE) on early infection of *P. berghei* in mice with chloroquine (CQ) as positive control and normal saline (NmS) as negative control

Treatment (extracts/drug)	Dose ($mgkg^{-}1day^{-}1$, p. o.)	Mean parasitaemial level	Percentage inhibition
PlrAE	50	20.8±2.9	21.1*
PlrAE	100	19.6±1.7	39.2*
PlrAE	200	06.2±1.6	76.0
PlrCE	50	27.0±1.2	18.2*
PlrCE	100	20.0±1.6	36.4*
PlrCE	200	10.8±0.5	60.0
CQ	5	00.0±0.0	96.0
Nms	0.2 $mLday^{-}1$	33.4±2.0	00.0

**= Indicates significant difference (P = .05) between the treated and the control groups*

Table 5. Curative activity of *P. rubra* aqueous and chloroform extracts (PlrAE and PlrCE) on established infection of *P. berghei* in mice with chloroquine (CQ) as positive control and normal saline (Nms) as negative control

Treatment (extracts/drug)	Dose ($mgkg^{-}1day^{-}1$, p. o.)	Mean parasitaemial level	Percentage inhibition
PlrAE	50	25.2±3.1	20.5*
PlrAE	100	18.8±0.5	36.7*
PlrAE	200	07.2±0.7	74.1
PlrCE	50	24.0±1.0	15.2*
PlrCE	100	19.0±1.0	34.2*
PlrCE	200	11.2±1.0	62.7
CQ	5	00.0±0.0	99.0
Nms	0.2 $mLday^{-}1$	32.4±2.0	00.0

**= Indicates significant difference (P = .05) between the treated and the control groups*

Table 6. Prophylactive activity of *P. rubra* aqueous and chloroform extracts (PlrAE and PlrCE) on the residual infection of *P. berghei* in mice with chloroquine (CQ) as positive control and normal saline (Nms) as negative control

Treatment (Extracts/drug)	Dose ($mgkg^{-}1day^{-}1$, p.o.)	Mean parasitaemial level	Percentage inhibition
PlrAE	50	31.8±0.9	16.5*
PlrAE	100	25.4±1.6	25.3*
PlrAE	200	07.4±0.8	80.3
PlrCE	50	31.4±1.1	10.3*
PlrCE	100	21.4±0.8	36.0*
PlrCE	200	11.0±1.9	64.1
CQ	5	00.0±0.0	100.0
Nms	0.2 $mLday^{-}1$	33.4±2.0	00.0

**= Indicates significant difference (P = .05) between the treated and the control groups*

4. CONCLUSION

Evaluation of perceptions of traditional medical practitioners on causes, diagnosis and traditional treatment of malaria using medicinal plants is essential for further evaluation of the safety and efficacy of such traditional antimalarial remedies towards discovery and development of new drugs for malaria. There is also the need to generate reliable scientific data to determine whether the plants traditionally used to treat the disease could actually be a potential source of developing safe and effective antimalarial drugs. It is for this reason that in the present study an ethno-medical survey was first carried out to evaluate the perceptions of the TMPs with the hope of generating useful resources and bridging the gaps between empirical treatments and realism in relation to malaria causes, diagnosis and treatment. Pharmacognostically, the method of choice for identification of herbal drugs is mainly intended to obtain characteristic fingerprints of specific plant materials. For such purposes, thin layer chromatographic technique was employed for chromatographic profiling of the metabolites present in the extracts of the plant material. Throughout the history of drug discovery and development, plant metabolites have provided a fundamental source of drugs for fighting various infectious diseases including malaria. Initial *in-vivo* efficacy screening of new antimalarial agents is usually performed in mice using the rodent malaria parasites like *Plasmodium berghei, P. vinkei, P. chaboudi* or *P. yoelii,* thus we have also chosen to use this model. The observed antiplasmodial properties exhibited by the extracts of the plant material could be as a result of the metabolites present in the plant. The results of the metabolite profiling presented could also be found useful in predicting other plant materials with potential usefulness in the control and treatment of malaria. In general the findings of the study are expected to provide the necessary scientific basis for use of traditional health knowledge and medicinal plant resources in the treatment of malaria as an additional malaria intervention.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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