Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

In vivo antiplasmodial activities of aqueous extract of *Bridelia ferruginea* stem bark against *Plasmodium berghei berghei* in mice

Chukwuemeka C. Mbah, Godwin C. Akuodor, Ngozi A. Anyalewechi, Tom C. Iwuanyanwu & Uche A. Osunkwo

To cite this article: Chukwuemeka C. Mbah, Godwin C. Akuodor, Ngozi A. Anyalewechi, Tom C. Iwuanyanwu & Uche A. Osunkwo (2012) *In vivo* antiplasmodial activities of aqueous extract of *Bridelia ferruginea* stem bark against *Plasmodium berghei berghei* in mice, Pharmaceutical Biology, 50:2, 188-194, DOI: 10.3109/13880209.2011.592537

To link to this article: https://doi.org/10.3109/13880209.2011.592537

Published online: 12 Jan 2012.

Pharmaceutical Biology, 2012; 50(2): 188–194

ISSN 1388-0209 print/ISSN 1744-5116 online
DOI: 10.3109/13880209.2011.592537

informa
healthcare

RESEARCH ARTICLE

In vivo antiplasmodial activities of aqueous extract of *Bridelia ferruginea* stem bark against *Plasmodium berghei berghei* in mice

Chukwuemeka C. Mbah[1], Godwin C. Akuodor[2], Ngozi A. Anyalewechi[2], Tom C. Iwuanyanwu[2], and Uche A. Osunkwo[2]

[1]*Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B 21 Garki, Abuja, Nigeria and* [2]*Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), P. M. B. 21, Garki, Abuja, Nigeria*

Abstract

Context: Bridelia ferruginea Benth (Euphorbiaceae) is an indigenous medicinal plant in Nigeria. It is usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition. Decoctions of parts of this plant have been employed in ethno medicine in many parts of Africa for treatment of many ailments including malaria fever.

Objective: In vivo antiplasmodial activity of aqueous stem bark extract of BF was investigated against *Plasmodium berghei*-infected mice.

Materials and methods: The aqueous stem bark extract of BF (100–400 mg/kg) was administered orally to *P. berghei*-infected mice in both early and established models of antiplasmodial studies.

Results: The extract exhibited significant ($p < 0.05$) antiplasmodial activity in early and established infection tests with a considerable mean survival time comparable to that of chloroquine, 10 mg/kg. The oral LD_{50} obtained was greater than 5000 mg/kg in mice.

Discussion and conclusions: The findings show that aqueous stem bark extract of *Bridelia ferruginea* possesses considerable antiplasmodial activity which can be developed in malaria therapy.

Keywords: Herbal medicine, phytochemical screening, antimalarial activity, suppressive test, curative test

Introduction

Malaria is widespread in many parts of the world mainly in the tropical and subtropical countries and transmission also occurs in many temperate regions. It is estimated that about 300–500 million clinical cases of malaria and approximately 2.5 million deaths occur each year (WHO, 2010). However, it is more wide-spread in African countries especially along the equatorial region that favors the growth of mosquitoes. It had been estimated that Africa carries 80–90% of the world's malaria burden (Okore et al., 1999). Malaria is one of the leading causes of morbidity and mortality in Africa. The alarming rate at which the malaria parasite, particularly *Plasmodium falciparum*, develops resistance to currently used antimalarial drugs such as chloroquine, sulfadoxine-pyrimethamine, and so on, makes it imperative to search for newer, more affordable and accessible antimalarial agents. The first documented case of chloroquine-resistant *P. falciparum* was reported in Columbia in 1961 (Moore & Lanier, 1961; Onoria, 1984; Ekanem, 1988). Natural products have played dominant roles in the discovery of leads for the development of drugs to treat human diseases. This anticipates that new antimalarial leads may certainly emerge from tropical plant sources.

Bridelia ferruginea Benth (Euphorbiaceae) (BF) is an indigenous medicinal plant in Nigeria, commonly found

Address for Correspondence: Chukwuemeka C. Mbah, Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B 21 Garki, Abuja, Nigeria. Phone number: +234 8087716982. E-mail: mbaccc@yahoo.com

(Received 17 August 2010; revised 26 April 2011; accepted 25 May 2011)

in the Savannah. It is also found in other more humid Savannah regions of Africa, especially from Guinea to Zaire and Angola. The plant is usually a gnarled shrub which, in suitable condition, sometimes reaches the size of a tree of height 6–15 m high, up to 1.5 m in girth and bole crooked branching low down. Its common names in Nigeria include Kirni, Kizni (Hausa), Maren (Fulani), Ora (Igede), Iralodan (Yoruba), Ola, Egede, Ede (Igbo). The bark of the mature plant is dark grey, rough, and often marked with scars (Rashidi et al., 2000; Adeboye & Ishola, 2009).

B. ferruginea has been in use in ethnomedicine for treatment of various ailments in many parts of Africa. The bark, roots, fruits and leaves are used mainly as decoctions. In Togo, the roots are used externally for treatment of skin diseases and eruptions (Oliver-Bever, 1986), and also for intestinal- and bladder-disorder remedies (De-Bruyne et al., 1997). Results of studies carried out on the aqueous and methanol extracts of the leaves of BF by Iwu (1980) supported its use in the treatment of diabetes in many parts of the West African subregion. The extracts significantly lowered the fasting blood sugar of local albino rats from 250 mg% to a normal level of <120 mg%, though it failed to protect the animals against alloxan induced diabetes mellitus. Aqueous infusions of the leaves of BF are used for the treatment of chronic diabetes particularly in cases where ketosis has set in (Iwu, 1983; Ngueyem et al., 2009).

The plant is also used as a purgative and a vermifuge (Cimanga et al., 1999). The bark extract is used for milk coagulation and also in lime juice for the formulation of traditional gargle "Ogun Efu" (Orafidiya et al., 1990). The stem bark extract also possesses antimicrobial activities against some microorganisms known to cause enteric and secondary upper respiratory tract infections (Jose & Kayode, 2009). Activities of the methanol extracts of BF stem bark and root against *Salmonella typhi* (Adebayo et al., 1998) may account for the ethnomedicinal use in the treatment of typhoid fever in Nkalagu-Obukpa, Enugu state, Southeast, Nigeria. Antiinflammatory activity and its potential for water treatment were also reported (Olajide, 1999; Kolawole & Olayemi, 2003). Decoctions of the bark and leaves are used in certain parts of Southeast and Southwest Nigeria for malaria therapy (Odugbemi et al., 2007). In the Southeastern Nigeria, some traditional healers often use decoctions of the bark to treat the local inhabitants of fevers suspected to be due to typhoid or malaria with good therapeutic outcome. The material is soaked and squeezed in water and a cupful of the extract obtained taken once daily for a period of 3–7 days.

The present study was aimed at scientifically evaluating the aqueous stem bark extract of BF for antiplasmodial activity. The result is hoped to substantiate or otherwise, the ethnomedicinal use of the plant extracts for treatment of malaria fever and possibly pave the way for its wider acceptability as antimalarial agent.

Materials and methods

Collection and preparation of *Bridelia*

The plant material was collected by Chukwuemeka C. Mbah on 28 November, 2009 from the bush at Odenigbo, Nkalagu-Obukpa in Nsukka District, Enugu state, Nigeria and identified by Jemilat A. Ibrahim at the Department of Medicinal Plant Research and Traditional Medicine, NIPRD (Herbarium Voucher number: NIPRD/H/6414). The international plant name index is Euphorbiaceae *Bridelia ferruginea* Niger Fl. [W. J. Hooker]. 511. 1849[Nov–Dec 1849] (IK). The bark was cleaned and scales removed. The clean bark was then cut into pieces and air-dried at room temperature for 7 days and ground to powder using mortar and pestle. It was then soaked in boiled distilled water overnight and filtered. The filtrate was heated over water bath to recover the extract and the yield calculated to be 50.29% w/w. The bark extract was subsequently reconstituted in water at appropriate concentrations for the experiment.

Phytochemical screening

The phytochemical composition of the aqueous extract of BF stem bark was determined using standard procedures (Sofowora, 1993; Evans, 2005). The following phytochemical tests were carried out:

Alkaloids

The presence of alkaloids was tested using Meyer's, Dragendoff's, Wagner's and Hagner's reagents, respectively. Approximately, 0.5 g of the extract was added to 10 ml of 1% aqueous hydrochloric acid (Riedel-de Haën) on a steam bath. This was filtered using Whatman Grade No. 1 filter paper and 1 ml of the filtrate treated with a few drops of each of the reagents, respectively. The formation of precipitate was taken as an indication of the presence of alkaloids.

Tannins

The presence of tannins was tested using the ferric chloride test. About 0.5 g of the extract was boiled in 10 ml of distilled water in a test tube and then filtered using Whatman Grade No. 1 filter paper. A few drops of 0.1% ferric chloride (BDH, England) solution was added and observed for green or blue-black coloration which is indicative of the presence of tannins.

Saponins

The presence of saponins was tested using the Froth test (Sofowora, 1993). To a small quantity of the powdered extract was added 95% ethanol (Riedel-de Haën), boiled and filtered using Whatman Grade No. 1 filter paper. Approximately, 2.5 ml of the filtrate was added to 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 s and allowed to stand for 1 h. Formation of persistent honeycomb froth was taken as an indication of the presence of saponins.

Flavonoids

Three tests were used to test for presence of flavonoids. First, 5 ml of dilute ammonia (Nade & Dave Ltd., England) solution was added to a portion of an aqueous filtrate of the extract. Then 1 ml of concentrated sulfuric acid (Fluka) was added. A yellow coloration that disappears on standing indicated the presence of flavonoids. Second, a few drops of 1% ammonia (Nade & Dave Ltd., England) solution were added to a portion of the aqueous filtrate. A yellow coloration indicated the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate (Riedel-de Haën) over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicated the presence of flavonoids.

Terpenes

The Liebermann-Burchard test was used to test for presence of terpenoids. Approximately, 2 ml of chloroform (Prolabo, Paris) was added to 0.5 g of the extract in a test tube and then mixed with 1 ml of acetic anhydride (Sigma-Aldrich). Then 1 ml of concentrated sulfuric acid (Fluka) was carefully added down the side of the test tube, to form a lower layer. The formation of a reddish-violet color in the chloroform (upper) layer was taken as an indication of the presence of terpenes.

Sterols

The Salkowski's test was used to test for the presence of sterols. Approximately, 2 ml of chloroform (Prolabo, Paris) was added to 0.5 g of the extract in a test tube. Then 2 ml of concentrated sulfuric acid (Fluka) was carefully added down the side of the test tube, to form a lower layer. Formation of a reddish-brown color at the interface was taken as an indication of the presence of steroidal ring.

Glycosides

The Keller-Killiani test for deoxysugars was used to test for the presence of glycosides. The extract (0.5 g) was dissolved in 5 ml of distilled water. This was added to 1 ml of glacial acetic acid (BDH, England) containing one drop of ferric chloride (Sigma-Aldrich) solution. The mixture was then carefully poured onto the surface of 1 ml sulfuric acid (Fluka) already in a test tube, to form a separate layer. Formation of a reddish-brown color at the interface of the liquids was taken as an indication of the presence of digitoxose, a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Anthraquinones

The Borntrager's test was used to test for the presence of anthraquinones. To 0.5 g of the powdered extract in a dry test tube was added 10 ml of chloroform (Prolabo, Paris) and the mixture was shaken for 5 min. and filtered using Whatman Grade No. 1 filter paper. An equal volume of ammonia (Nade & Dave Ltd., England) solution was then added to the filtrate and shaken. Appearance of a bright pink color in the upper aqueous layer was taken as an indication of the presence of free anthraquinones.

Resins

The powdered extract (0.5 g) was dissolved in acetic anhydride (Sigma-Aldrich) and 1 drop of concentrated sulfuric acid (Fluka) was added. Appearance of a purple color was taken as an indication of the presence of resins.

Acute toxicity test

The LD_{50} of the stem bark extract was tested to determine the safety of the agent using Lorke's (1983) method. Dose levels used ranged from 10–5000 mg/kg *p.o.* The acute toxicity was calculated as the geometric mean of the dose that resulted in 100 % lethality and that which caused no lethality at all. The animals were kept under the same conditions and observed for toxicity signs and mortality for 24 h.

Animals

Swiss Albino mice (22–28 g) of both sexes obtained from Animal Facility Centre (AFC), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria were used for the study. They were housed in cages with saw dust as bedding and given food and water *ad libitum*. The mice were used in accordance with NIH Guide for the care and use of laboratory Animals; NIH publication (No 83–23) revised (NIH, 1985) and NIPRD's standard operating procedures (NIPRD-SOPs).

Inocula

Parasitized erythrocytes were obtained from Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD, Abuja, Nigeria. The parasites were maintained through continuous reinfestation (*i.p*) in mice (Calvalho et al., 1991) every 4 days. The inocula consisted of *Plasmodium berghei berghei* parasitized erythrocytes. This was prepared by determining the parasitemia density/count (Table 1) of the donor mouse and diluting them with normal saline in proportions indicated by the determinations. Each mouse was inoculated intraperitoneally with 0.2 ml of infected blood containing 10^7 *P. berghei berghei* parasitized red blood cells.

Suppressive test

A 4-day suppressive test was performed using earlier infected mice following previously reported methods (David et al., 2004; Okokon et al., 2008). Thirty Swiss albino mice of either sex weighing (22–28 g) were intraperitonealy inoculated with infected erythrocytes (0.2 ml) containing 10^7 *P. berghei berghei*. The animals were divided into five groups of six mice each. They were orally administered with 100, 200, and 400 mg/kg doses of aqueous BF stem bark extract, chloroquine (Drugfield, Nigeria) 10 mg/kg and 0.2 ml of normal saline (negative control), all on the day of inoculation (D_0). Treatment then continued daily (D_1–D_4). On the fifth day (D_5), blood

was collected from the tail of each mouse, by pricking with a sterile 21 gauge needle and smeared with a spreader onto a clean microscopic slide to make a film (Saidu et al., 2000). The films were fixed with methanol (Fluka), stained with Giemsa (Sigma) for 30 min (Akuodor et al., 2010a) and parasitemia level was examined microscopically (Nikon YS2-H, Japan) by counting the parasitized red blood cells on at least 1000 red blood cells. It was observed that normal red blood cells were much more in number than the parasitized, indicating pronounced suppressive activity of the extract. The parasitized red blood cells were noted with intracellular presence of trophozoids of the parasite.

The percentage of parasitemia suppression was calculated for each dose level by comparing the parasitemia densities in infected control mice with those of treated mice.

Curative test

Evaluation of curative potentials of aqueous BF stem bark extract was carried out adopting the method described by Ryley and Peters (1970). Thirty mice were selected and intraperitonealy (*i.p*) injected with about 10^7 *P. berghei* infected erythrocytes on the first day. After 72 h, the mice were divided into five groups of six mice per cage. Group 1 received normal saline (20 ml/kg daily. Groups 2, 3, and 4 received 10 mg/kg of chloroquine (Drugfield, Nigeria) (*i.p*). Treatment continued daily (D_3-D_7). On the seventh day (D_7), blood was collected from the tail of each mouse and films made. The films were fixed with methanol (Fluka), stained with Giemsa (Sigma) for 30 min and parasitemia densities were examined microscopically by counting the parasitized red blood cells on at least 1000 red blood cells. The mice were observed for 30 days and mean survival time of each group recorded.

Statistical analysis

Results were expressed as means ± S.E.M. The data was analyzed using student's *t*-test and differences between mean were considered significant when $p < 0.05$ (Betty & Sterne, 2003).

Results

Phytochemical test

Phytochemical screening of the hot water extract of BF stem bark revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenes, sterols, and resins. All these classes of compounds are reported to show important biological activities (Kutchan, 1995; Hadacek, 2002; Ghoghari & Rajani, 2006; Panda & Kar, 2007; Banz et al., 2007). Glycosides and anthraquinones were not detected using the techniques employed (Table 2). The presence of terpenes in the aqueous stem bark extract of BF may account for the observed antiplasmodial activity of the extract because of long association of terpenoid compounds with antimalarial activity. Chinese scientists in 1971 isolated the sesquiterpene lactone, artemisinin, from *Artemisia annua* L. (Asteraceae) and showed that it was highly active against *P. falciparum* (Evans, 2005). Since then artemisinin and its derivatives have become very popular for treatment of not only chloroquine-resistant but even cerebral malaria. We recommend further studies which may include fractionation, characterization and structural elucidation of the aqueous stem back extract of BF in order to find out the actual lead compound(s) responsible for its antiplasmodial activity.

Acute toxicity test

There was no mortality observed in mice after oral administration of the hot water extract, even at doses as high as 5000 mg/kg signifying that the oral LD_{50} was greater than 5000 mg/kg. The behavioral signs of toxicity exhibited by animals are respiratory distress and abdominal constriction.

Suppressive effect

The hot water extract of BF stem barks caused a dose dependent chemosuppressive activity at different doses employed. Doses of 100, 200, and 400 mg/kg caused chemosuppression of 77, 87, and 95%, respectively. The chemosuppression exhibited by the extract was

Table 1. Curative effect of aqueous stem bark extract of BF against *P. berghei* in mice.

	Mean parasitemia count			
Drug (mg/kg)	Pre (Day 3)	Post (Day 7)	% Parasitemia inhibition	Mean survival time (Days)
Normal saline: 10 (ml/kg)	27.65 ± 0.10	35.57 ± 0.49*	0	9.00 ± 2.0
B. ferruginea: 100	22.11 ± 0.14	12.03 ± 0.25*	66.18	25.50 ± 1.2
200	24.08 ± 0.12	10.68 ± 0.12*	69.97	28.00 ± 0.9
400	25.05 ± 0.17	7.67 ± 0.22*	78.44	29.20 ± 0.5
Chloroquine: 10	23.08 ± 0.90	2.30 ± 0.33*	93.53	30.00 ± 0.0

Results are mean count ± S.E.M ($n = 6$),
*significantly different from control at $p < 0.05$.

Table 2. Result of phytochemical screening of BF aqueous stem back extract.

Metabolite tested	Alk	Tan	Sap	Flav	Terp	Sterols	Glyc	Anthra	Res
Result	+	+	+	+	+	+	–	–	+

Alk: alkaloids; Tan: tannins; Sap: saponins; Flav: flavonoids; Terp: terpenes; Glyc: glycosides; Anthra: anthranquinones; Res: Resins; +: Positive; –: negative.

significantly ($p < 0.05$) comparable to chloroquine group which had a chemosuppression of 96% (Table 3).

Curative effect

In established *P. berghei* infection in mice, the stem bark extract caused a dose dependent reduction in mean parasitemia, though lower than chloroquine treated group. However, the negative control group showed a daily increase in parasitemia. Results on Table 1 show that chloroquine 10 mg/kg gave a mean survival time of 30 days, negative control 9.0 ± 2.0 days, while 25.5 ± 1.2, 28.0 ± 0.9 and 29.2 ± 0.5 days was observed with 100, 200, and 400 mg/kg of the stem bark extract, respectively.

Discussion

Our results showed that hot water extract of BF possessed *in vivo* antiplasmodial activity that was evident from the chemosuppression it produced during the 4-day early infection study. This method of suppressive testing for antimalarial activity has become popular during scientific evaluation of potential phytomedicines for treatment of experimental malaria (Peters et al., 1993; David et al., 2004). Our investigation of the scientific reasons behind the folkloric use of BF in the treatment of malaria attack in traditional African settings can be partially satisfied with this result (Akuodor et al., 2010b). In addition, the result of chemosuppressive study can be interpreted to be that the stem bark extract of BF can suppress parasite growth to non-detectable levels in erythrocytes. It is important that scientific evaluation of traditional medicine preparations for claimed antimalarial efficacy be carried out even up to the level of finding out the degree of suppression of parasite growth in erythrocytes (Ene et al., 2008).

The aqueous stem bark extract of BF also exerted significant curative effect in established infection. Curative property of potential antimalarial agents of ethnobotanical materials should be discernible during testing for antimalarial properties. The observed antimalarial activity of the plant extract is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent. This was confirmed by the mean survival time values particularly in the group treated with 400 mg/kg of the extract. In untreated mice, the parasitemia count increased daily until the death of the animals, which was also observed in our previous studies (Akuodor et al., 2010a).

Table 3. Suppressive effect of aqueous stem bark extract of BF against *P. berghei* in mice.

Drug (mg/kg)	Parasitemia count	% inhibition
Normal Saline: 10 (ml/kg)	33.87 ± 0.70	0
B. ferruginea: 100	7.86 ± 0.17	77*
200	4.24 ± 0.27	87*
400	1.78 ± 0.16	95*
Chloroquine: 10	1.52 ± 0.24	96*

Results are mean count ± S.E.M ($n = 6$),
*significantly different from control at $p < 0.05$.

The antiplasmodial activity of stem bark extract of BF might be attributed to the presence of alkaloids, flavonoids, terpenes (Table 2), which have been variously implicated in antiplasmodial activities of many plants (Philipson & Wright, 1990; Milliken, 1997; Christensen & Kharazmi, 2001; Wright, 2005). In addition, our earlier studies have shown that hot water extract of BF stem bark possesses analgesic and antipyretic effect (Akuodor et al., 2011). Natural products possessing such activities were reported to provide relief to malaria patients (Addae-Kyeremi et al., 2001) and may specifically target some organs in their mechanism of action (Agomuo et al., 1992).

However, the active compound(s) known to give this observed activity need to be identified. Some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation (Etkin, 1997) or by inhibiting protein synthesis depending on their phytochemical constituents (Kirby et al., 1989). Therefore, some of the phytochemical groups of compounds identified in the BF extract that are known to possess haemostatic (tannins) and haemolytic (saponins) properties may also have important roles to play in its antiplasmodial activity. These compounds may be acting singly or in synergy with one another to exert antiplasmodial activity observed in the study. If further chemical characterization and structural elucidation are carried out the actual compound(s) responsible for the observed pharmacological activities may be identified.

In conclusion, the results of this study have shown that aqueous stem bark extract of *B. ferruginea* possesses antiplasmodial activity as seen in its ability to suppress chloroquine-sensitive *P. berghei* infection in the two models evaluated. We recommend that further chemical studies should be done to identify and properly characterize and develop the actual compounds responsible for the observed antiplasmodial activity. It is therefore hoped that the screening of locally used medicinal plants for antimalarial properties, can fully be investigated with a view to establishing their efficacy and to determine their potentials as sources of new antimalarial agents.

Acknowledgment

The authors are grateful to Mrs. Jemilat A. Ibrahim for her botanical assistance.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

References

Addae-Kyeremi J, Croff S, Kendrick H, Wright CW. (2001). Antiplasmodium activities of some Ghanaian plants treaditionally

used for fever/malaria treatment and of some alkaloids isolated from *Pleiocarpa mutica*. *In vivo* antimalaria activity of pleiocarpina. *J Ethnopharmacol*, 76, 99–103.

Adebayo EA, Ishola OR. (2009). Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Bridelia ferruginea*. *Afr J Biotechnol*, 8, 650–653.

Adeoye AO, Abaelu AM, Omowumi CJ, Olukoya DK. (1998). Antimicrobial activity of *Bridelia ferruginea*. Book of Abstract, symposium on drug production from natural products. Drug Research and production unit, Obafemi Awolowo University, Ile-Ife, Nigeria, 24.

Agomuo PU, Idigbe JC, Afolabi BM. (1992). Antimalarial medical plants and their impact on cell populations in various organs of mice. *Afri J Med Sci*, 21: 39–46.

Akuodor GC, Idris-Usman M, Ugwu TC, Akpan JL, Ghasi SI, Osunkwo UA. (2010a). *In vivo* schizonticidal activity of ethanol leaf extract of *Gongronema latifolium* on *Plasmodium berghei berghei* in mice. *Ibnosina J Med and Biomed Sci* 2(3): 118–124.

Akuodor GC, Idris-Uman M, Anyalewechi NA, Odo E, Ugwu TC, Akpan JL, Gwotmut MD, Osunkwo UA. (2010b). *In vivo* antimalarial activity of ethanol leaf extract of *Verbena hastate* against *Plasmodium berghei berghei* in mice. *J Herbal Med and Toxicol*, 4(2), 117–123.

Akuodor GC, Mbah CC, Anyalewechi NA, Idris-Usman M, Osunkwo UA. (2011). Pharmacological profile of aqueous extract of *Bridelia ferruginea* stem bark in relief of pain and fever. *J Med Plant Res* (in press; accepted for publication April, 2011).

Banz WJ, Iqbal MJ, Bollaert M, Chickris N, James B, Higginbotham DA, Peterson R, Murphy L. (2007). Ginseng modifies the diabetic phenotype and genes associated with diabetes in the male ZDF rat. *Phytomedicine*, 14, 681–689.

Betty K, Sterne J. (2003). *Essential Medical Statistics*. Massachusetts: Blackwell Science Ltd. 2nd ed.

Carvalho LH, Brandão MG, Santos-Filho D, Lopes JL, Krettli AU. (1991). Antimalarial activity of crude extracts from Brazilian plants studied *in vivo* in *Plasmodium berghei*-infected mice and *in vitro* against *Plasmodium falciparum* in culture. *Braz J Med Biol Res*, 24, 1113–1123.

Christensen SB, Kharazmi A. (2001). Antimalarial natural products: Isolation, characterization and biological properties. In: Tringali C, ed. *Bioactive Compounds from Natural Sources*. London: Taylor & Francis, 379–432.

Cimanga K, De Bruyne T, Apers S, Pieters L, Totté J, Kambu K, Tona L, Bakana P, van Ufford LQ, Beukelman C, Labadie R, Vlietinck AJ. (1999). Complement-inhibiting constituents of *Bridelia ferruginea* stem bark. *Planta Med*, 65, 213–217.

David AF, Philip JR, Simon RC, Reto B, Solomon N. (2004). Antimalarial drug discovery: Efficacy models for compound screening. *Nat Rev*, 3, 509–520.

De Bruyne T, Cimanga K, Pieters L, Claeys M, Domnusse R, Vlientinck AJ. (1997). Galloctechim (4-0-7) epigallocatechin: A new biflavonoid isolated from *B. ferruginea*. *Nat Prod Let*, 11, 47–52.

Ekanem OJ. (1988). *Plasmodium falciparum* infection not responding to chloroquine in Nigeria. *Trans Roy Soc Trop Med Hyg*, 97, 141–142.

Ene AC, Ameh DA, Kwanashie HO, Agomuo PU, Atawodi SE. (2008). Preliminary *in vivo* antimalarial screening of petroleum, chloroform and methanol extracts of fifteen plants grown in Nigeria. *J Pharmacol Toxicol*, 3(4), 254–260.

Etkin NL. (1997). Antimalarial plants used by Hausa in northern Nigeria. *Trop Doct*, 27 Suppl 1, 12–16.

Evans WC. (2005). *Trease and Evans Pharmacognosy*. New Delhi, India: Reed Elsevier India (pvt) Ltd. 15th ed. 174, 224–304, 411–413, 535.

Ghoghari AM, Rajani M. (2006). Densitometric determination of hecogenin from *Agave americana* leaf using HPTLC. *Chromatographia*, 64, 113–116.

Hadacek F. (2002). Secondary metabolites as plant traits: Current assessment and future perspectives. *Crit Rev Plant Sci*, 21, 273–322.

Iwu MM. (1980). Antidiabetic properties of *Bridelia ferruginea* leaves. *Planta Med*, 39, 247–251.

Iwu MM. (1983). The hypoglycemic property of *Bridelia ferruginea*. *Fitoterapia*, 54, 243–248.

Jose RA, Kayode J. (2009). The effect of *Bridelia ferruginea* bark extracts on some pathogenic microorganisms. *Ethnobot Leaf*, 13, 1042–1046.

Kirby GC, O'Neill MJ, Phillipson JD, Warhurst DC. (1989). *In vitro* studies on the mode of action of quassinoids with activity against chloroquine-resistant *Plasmodium falciparum*. *Biochem Pharmacol*, 38, 4367–4374.

Kolawole OM, Olayemi AB. (2003). Studies on the efficacy of *Bridelia ferruginea* Benth. extract for water purification. *Niger J Pure Appl Sci*, 18, 1387–1394.

Kutchan TM. (1995). Alkaloid biosynthesis—The basis for metabolic engineering of medicinal plants. *Plant Cell*, 7, 1059–1070.

Lorke D. (1983). A new approach to practical acute toxicity testing. *Arch Toxicol*, 54, 275–287.

Milliken W. (1997). Malaria and antimalarial plants in Roraima, Brazil. *Trop Doct*, 27 Suppl 1, 20–25.

Moore DV, Lanier JE. (1961). Observations on two *Plasmodium falciparum* infections with an abnormal response to chloroquine. *Am J Trop Med Hyg*, 10, 5–9.

Ngueyem TA, Brusotti G, Caccialanza G, Finzi PV. (2009). The genus *Bridelia*: A phytochemical and ethnopharmacological review. *J Ethnopharmacol*, 124, 339–349.

National Institute of Health. (1985). NIH guide for the care and use of laboratory animal (Revised). Bethesda, MD: NIH publication. No 83-23.

Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO. (2006). Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. *Afr J Tradit Complement Altern Med*, 4, 191–198.

Okokon JE, Ettebong E, Antia BS. (2008). *In vivo* antimalarial activity of ethanolic leaf extract of *Stachytarpheta cayennensis*. *Indian J Pharmacol*, 40, 111–113.

Okore VC, Eze JI, Akah PA. (1999). Recent efforts towards effective malaria chemotherapy and control in the face of increasing resistance by *Plasmodium*. *J Phytomed Therap*, 4, 59–71.

Olajide OA, Makinde JM, Awe SO. (1999). Effects of the aqueous extract of *Bridelia ferruginea* stem bark on carrageenan-induced oedema and granuloma tissue formation in rats and mice. *J Ethnopharmacol*, 66, 113–117.

Oliver-Bever B. (1986). *Medicinal Plants in tropical West Africa*. Cambridge: Cambridge University Press, 258–260.

Onorio E. (1984). The problem of *Plasmodium falciparum* drug resistance in Africa south of the Sahara. Bull. *World Health Organisation*, 62, 55–62.

Orafidiya LO, Lamikania A, Adediji JA. (1990). Coagulation of milk as an index of astringency of the bark extract of *Bridelia ferruginea* Benth and lime juice for the formulation of a traditional gargle "Ogun Efu." *Phytotherapy Res*, 4(5), 189–194.

Panda S, Kar A. (2007). *Annona squamosa* seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice: Possible involvement of quercetin. *Phytomedicine*, 14, 799–805.

Peters W, Robinson BL, Tovey G, Rossier JC, Jefford CW. (1993). The chemotherapy of rodent malaria. L. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 3: Observations on 'Fenozan-50F', a difluorinated 3,3'-spirocyclopentane 1,2,4-trioxane. *Ann Trop Med Parasitol*, 87, 111–123.

Philipson JD, Wright CW. (1990). Antiprotozoal compounds from plant sources. *Planta Medica*, 57, 553–559.

Rashidi MA, Gustafson KR, Cardilina JH, Boyd MR. (2000). A new podophyllojoxin derivative from *Bridelia ferruginea*. *Nat Prod Lett*, 14, 285–292.

Ryley JF, Peters W. (1970). The antimalarial activity of some quinolone esters. *Ann Trop Med Parasitol*, 64, 209–222.

Saidu K, Onah J, Orisadipe A, Olusola A, Wambebe C, Gamaniel K. (2000). Antiplasmodial, analgesic, and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina senegalensis*. *J Ethnopharmacol*, 71, 275–280.

Sofowora A. (1993). Medicinal plants and traditional medicine in Africa.Ibadan: *Spectrum Books*, 150.

World Health Organisation. (2010). Malaria worldwide epidermiology. www.who.int/malaria/world_malaria_report_2010/worldmalariareport2010.pdf

Wright CW. (2005). Traditional antimalarials and the development of novel antimalarial drugs. *J Ethnopharmacol*, 100, 67–71.