NJBMB (2022) 37(3) 194-200

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

In Vivo Antimalarial Activity of *Morinda lucida Benth.* Stem Bark in *Plasmodium berghei*-Infected Mice

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OPEN ACCESS

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ARTICLE HISTORY
Received: 27/05/2022
Reviewed: 06/06/2022
Revised: 30/08/2022
Accepted: 04/09/2022
Published: 30/09/2022

CITATION
Orumwensodia K.O. and Uadia P.O. (2022). *In vivo* Antimalarial Activity of *Morinda lucida Benth.* Stem bark in *Plasmodiumberghei*-infected Mice. *Nigerian Journal of Biochemistry and Molecular Biology*. 37(3), 194-200

ABSTRACT

Malaria eradication remains a challenge amidst the quest for new, more effective and less expensive therapeutic options. This study evaluates the secondary metabolites, antioxidant, nutritional and antimalarial potentials of *Morinda lucida* stem bark. Extracts were obtained from pulverized dry stem bark, macerated in hexane, methanol, ethanol and distilled water, separately. The secondary metabolite content of extracts and proximate analysis of dried sample were assayed using standard procedures. Antioxidant potential of the extracts was measured against DPPH and ferrous ion radicals. The antimalarial activity of the extracts was assessed using chemo-suppressive, prophylactic and mean survival time (MST) assays in male Swiss albino mice infected intraperitoneally with chloroquine-sensitive *Plasmodium berghei* NK65 strain. There was reasonable presence of flavonoids, saponins, alkaloids, phenolics, cardiac glycosides and tannins in the extracts. The extracts had antioxidant activity with aqueous extract having the highest DPPH scavenging (IC_{50}36.51 ± 0.08 µg/mL) and ferrous ion chelating (IC_{50}84.70 ± 0.17 µg/mL) activities. Percentage proximate composition was nitrogen free extract (71.22 ± 0.56%), crude protein (6.05 ± 0.02%), crude fibre (1.71 ± 0.01%), crude fat (13.02 ± 0.01%), crude ash (2.64 ± 0.04%), and moisture (5.36 ± 0.03%). The extracts demonstrated significant (p< 0.05) antimalarial activity compared to the infected untreated control. Methanol extract had the highest chemo-suppression (67.71%), prophylaxis (63.23%) and MST (16.20 ± 1.95; 15.50 ± 1.95 days, respectively) values. Therefore, *Morinda lucida* possesses rich phyto-nutritional contents possibly responsible for the observed antimalarial activity with methanol extract, derived from a near mid-polarity solvent, exerting the best antimalarial effect.

Keywords: *Morinda lucida, Antimalarial, Plasmodium berghei, Chemo-suppression, Prophylaxis, Antioxidants, Phytochemicals, Nutritional status.*

INTRODUCTION

The application of herbs otherwise known as medicinal plants in folklore medicine in places like Africa, Asia, etc., with rich plant flora predates modern history. These plants have served the purpose of cure and management of several ailments afflicting mankind. A notable example of these herbs is a member of the Rubiaceae family of plants, *Morinda lucida* Benth., which is a tropical rainforest medium-sized tree distributed along the tropical regions of the world e.g., West Africa (Sambamurty, 2005). The generic name comes from the Latin words *morus* (meaning: mulberry, i.e., judging from the appearance of the fruits) and *indica*, (meaning: of India). Other species of the genus *Morinda*are *M. citrifolia*, *M. angustifolia*, *M. ongiflora*, *M. officinalis*, etc. It thrives better on grassland, exposed hillsides, brushes, forests, often on termite mounds, sometimes in flooded planes, at elevations up to 1,300 m above sea-level (Burkhill, 1985). The evergreen shrub bearing a dense crown of slender, crooked branches and which could grow to a height of 18 m (Verdcourt, 1976; Ilodibia *et al.*, 2019), is locally referred to as Akpeko, Ikpivihilon in Edo, Ogere, Huka, Ezeogu, Njisi in Igbo, Oruwo, Owuru, Origho, Erewo in Yoruba, Ugigo in Ebira, etc. (Aigbokhan, 2014). Elsewhere in Africa, it is known as Hojologbo (Sierra Leone), Uhon (IvoryCoast) and Bompété, Wâda (Senegal) (Burkhill, 1985). However, it is commonly called Brimstone tree, Indian mulberry and Hog apple (Adeneye *et al*., 2008; Aigbokhan, 2014). All parts of the plant appear to be of medicinal value. For instance, decoctions and infusions or plasters of the root, bark and leaves (either singly or in combination) are used as fever tea, and are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis, feverish condition during childbirth and as analgesic, laxative and anti-infections (Makinde and Obi, 1985; Lawal *et al*., 2011). Other researches have shown that leaf and stem bark infusions of *M. lucida* possess hypoglycemia and antidiabetic (Daziel, 1973; Olajide *et al*., 1999), antispermatogenic (Raji, 2005), cytotoxic and genotoxic (Akinboro and Bakare, 2007), anticancer (Sowemimo *et al*., 2007) and hepatoprotective (Oduola, 2010) activities.

Despite the potentials in these plants, diseases continue to ravage significant percentage of the population of third world countries, where there exist broken infrastructure (including healthcare), low purchasing power, poor sanitation, etc. For instance, malaria has remained endemic in low income countries of Asia, parts of the Americas and sub-Saharan Africa, leading to a colossal loss in well-being, productive man-hour, and average income with its attending consequences on the economies of these nations. In 2020, the global estimate of malaria infection stood at 241 million, while mortality was put at 627,000 with Africa accounting for about 95% of clinical cases and 96% of mortality (WHO, 2021). A percentage distribution of the disease in some African countries shows Mozambique had (3.8%), Tanzania (4.1%), Democratic Republic of Congo (13.2%), while Nigeria alone accounted for 31.9% (about 8.5% increase over the previous year- 2019) (WHO, 2021). Children less than five years of age, pregnant women and the immune-compromised individuals are the most affected. Also, about 84% of mortality was in children under five years of age (WHO, 2021). This increase in disease burden has reinforced the need for the exploitation of therapeutic alternatives such as herbal remedies, believed to hold great potentials for new drug targets. Thus, this study was aimed at determining the *in vivo* antimalarial activity of *Morinda lucida* Benth stem bark in *Plasmodium berghei*-infected Swiss albino mice as well as its antioxidant, phytochemical and nutritional properties.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The fresh stem bark of *Morinda lucida* Benth obtained within November from the forest area of Southern Nigeria were identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, and voucher No. (UBHr0283) was assigned. The stem bark sample was cleaned of debris, air-dried under shade at room temperature and pulverized using mortar and pestle into a coarse form. The pulverized powdered sample was stored in an air-tight plastic container at room temperature until ready for use.

Extraction of the Plant Material

Firstly, a sensitive weighing balance (S. Mettle, Switzerland) was used to weigh about 400 g of the pulverized plant material into four portions (a portion for each solvent) and were macerated separately in 1.5 L of hexane, methanol, ethanol and distilled water for 72 h under repeated stirring. Thereafter, filtration was done with Whatman filter paper No. 1 (Whatman, England). The ensuing residues were repeatedly macerated twice under similar conditions (Cannell, 2006). The filtrates from these rounds of maceration were combined respectively and evaporated *in vacuo* to dryness using a rotary evaporator (Buchi, Germany) at 45 °C. The dried extracts were preserved in air-tight glass containers, under refrigerated condition until further use.

Phytochemical Screening

About 5 g of each extract was boiled with 75 ml of distilled water for 30 min. The solution was filtered hot and allowed to cool. The filtrates obtained were used to qualitatively assay for the presence of tannins, alkaloids, flavonoids, phenolics, saponins and cardiac glycosides according to standard methods (Stahl, 1973; Sofwora, 1982; Harborne, 1998; Evans, 2002).

Estimation of Antioxidant Capacity

The antioxidant potential of the plant extracts was determined using the modified methods of (Dinis *et al*., 1994; Brand-Williams *et al*., 1995) for 2, 2–diphenyl–1–picrylhydrazyl (DPPH) radical scavenging and ferrous ion radical chelation, respectively.

Estimation of Nutritional Content

The Nitrogen Free Extract (NFE), crude protein, crude fibre, crude fat, crude ash and water loss on drying (moisture) as an index of the plant's nutritional status were determined using the methods of AOAC (2000).

Experimental Animals

Sixty male Swiss albino mice, between six to eight weeks old, and weighing 20 ± 2 g were used for this study. They were acquired from the Animal House of Igbinedion

University, Okada, Edo State and housed in well-ventilated plastic cages ladened with softwood shavings and chips as beddings. Acclimatization was for one week, while each animal was fed rat chow and water *ad libitum* throughout the experiment. Experimental animals were handled in line with the guidelines set by the Institute for Laboratory Animal Research (ILAR, 2011).

Malaria Parasites

Chloroquine-sensitive *Plasmodium berghei* NK65 strain was used for this study. It was obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria, and was maintained via weekly intraperitoneal passage to naive mice (Fidock *et al*., 2004).

Preparation of Inoculum

A standard inoculum was prepared from an infected donor mouse at 30% parasitaemia. The parasitized red blood cells-PRBCs were obtained through cardiac puncture under anesthesia and diluted in normal saline, to give 1×10^7 PRBCs standard inoculum (Fidock *et al*., 2004).

Grouping and Dosing of Animals

Male Swiss albino mice, thirty for each experimental model, were randomized into five groups comprising five mice each.

GROUPS	TREATMENT
Group I (Negative control)	Infected and treated with phosphate buffered saline
Group II (Positive control)	Infected and treated with chloroquine (10 mg/kg bw)
Group III	Infected and treated with 800 mg/kg bw methanol extract
Group IV	Infected and treated with 800 mg/kg bw ethanol extract
Group V	Infected and treated with 800 mg/kg bw aqueous extract
Group VI (Normal control)	Uninfected and untreated

Experimental Models

Four-day Chemo-suppressive Test

The chemo-suppressive antimalarial activity of the extracts was tested using the modified method of Fidock *et al*. (2004). Inoculation and treatment of mice were done on the same day (D_0), with a two-hour interval between both procedures. Treatments were continued for the next three days (i.e., D_0 to D_3), while on D_4 (i.e., the 5th day), thin blood smears from the tail of each mouse were made on microscopic slides (Fischer Scientific, USA) and monitored for parasitaemia under the microscope (Olympus, Japan).

Prophylactic Test

The prophylactic antimalarial activity of the extracts was determined using the modified method of Fidock *et al*. (2004). The animals were first treated for four consecutive days (D_0 to D_3) with the respective extracts, and subsequently infected with the standard inoculum on D_4 (fifth day). After 72 h of infection (D_7), thin blood smears from tail-blood of each mouse were prepared on a microscopic slide and parasitaemia monitored under a microscope.

Estimation of Parasitaemia

Blood parasitaemia was determined under x100 objective lens-oil immersion by counting the number of PRBCs in random fields of microscope view. Percent parasitaemia and percent suppression were estimated accordingly to the methods of Fidock *et al*. (2004) and Kalra *et al*. (2006) for day 3 and onwards up to the 28th day.

$$\% \text{ Parasitaemia} = \frac{\text{Number of Parasitized RBCs}}{\text{Total Number of RBC}} \text{X } 100$$

$$\% \text{ Suppression} = \frac{(\% \text{ Parasitaemia of Negative Control} - \% \text{ Parasitaemia of Treated group})}{\% \text{ Parasitaemia of Negative Control}} \text{X } 100$$

Estimation of Mean Survival Time (MST)

The MST was determined by monitoring daily mortality and number of days of survival from time of infection to death of the animal. Values were recorded for each mouse in the treatment and control groups throughout the experimental period. MST was calculated accordingly;

$$\text{MST (days)} = \frac{\text{Sum of days of survival of animals in a group}}{\text{Total Number of animals in the group}}$$

Drug and Chemicals

Chloroquine, normal saline, gallic acid, quercetin, tannic acid, ferrous chloride, ferrozine, citric acid, vitamin C, DPPH, EDTA, ethanol, methanol and Hexane (Sigma Aldrich, Germany), phosphate buffered saline (Silver Health Diagnostics, Nigeria), giemsa stock (Trust Chemical Lab, India), immersion oil (Scisco Research Lab, India). All reagents were of analytical grade and procured from certified suppliers.

Data Analysis

Data were expressed as mean ± SEM and analyzed using windows SPSS version 21.0. The differences between measured means were compared using one-way ANOVA, while difference in significance was compared by Turkey's HSD multiple comparison test with significance set at p values < 0.05.

RESULTS AND DISCUSSION

Phytochemical Content of the Extracts of *Morinda lucida* Stem bark

Table 1 represents the qualitative phytochemical composition of extracts of *Morinda lucida* stem bark. Tannins, phenolic, saponins, flavonoids, alkaloids and cardiac glycosides were identified in the extracts. But, saponins were not detected in the hexane extract, alkaloids were also absent in the methanol and aqueous extracts, tannins in the hexane and aqueous extracts, while cardiac glycosides were not detected in the aqueous extract.

Table 1: Phytochemical Composition of Extracts of *Morinda lucida* Stem bar

Extracts	Phenolics	Flavonoid	Saponins	Alkaloids	Tannins	Cardiac glycoside
Hexane	+	+	–	+	–	+
Methanol	+	+	+	–	+	+
Ethanol	+	+	+	+	+	+
Aqueous	+	+	+	–	–	–

Antioxidant Capacity of Extracts of *Morinda lucida*

Figure 1 shows the antioxidant activity of *Morinda lucida* stem bark extracts (hexane, methanol, ethanol and aqueous) against DPPH and ferrous iron radicals. From the results, the aqueous extract had a relatively better antioxidant activity compared to the other extracts, though they were all non-significant in relation to the reference antioxidants (ascorbic acid and EDTA, for DPPH and iron chelation, respectively). The methanol extract was not tested.

Nutritional Content of *Morinda lucida Stem bark*

Figure 2 represents the percentage proximate composition of *Morinda lucida* stem bark. The plant stem bark was low in crude fibre and ash, but very high in nitrogen free extract (NFE). Crude fat, crude protein, and moisture was also reasonably present.

Chemo-suppressive Antimalarial Activity of *Morinda lucida* Stem bark Extracts

The chemo-suppressive antimalarial activity of the crude stem bark extracts of *Morinda lucida* are presented in Table 2. The extracts significantly ($p< 0.05$) suppressed *P. berghei* growth in treated mice compared to the mice of untreated control group (PBS- pH 7.4). Suppressive activity increased in the following order; ethanol < aqueous < methanol. Though the methanol extract had the highest activity, it was less effective than chloroquine.

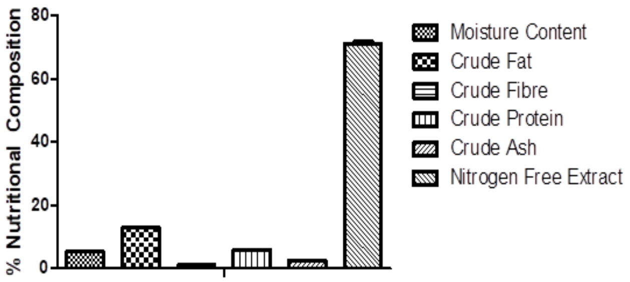

Figure 2. Nutritional Content of *Morinda lucida* stem bark.

Data represent percentage mean ± SEM of triplicate determinations.

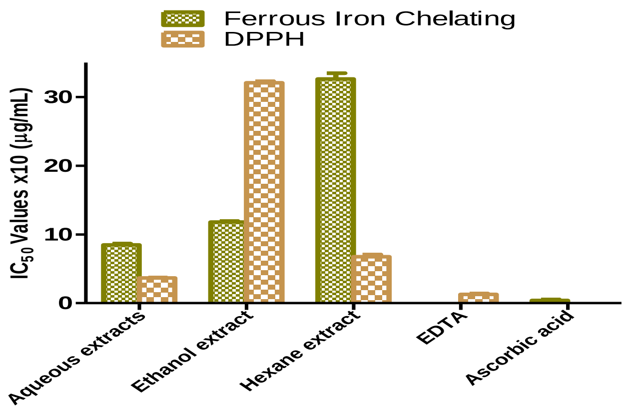

Figure 1. Ferrous ion chelating and DPPH-radical scavenging activities of the extracts of *Morinda lucida* stem bark.

IC50 value of triplicate determinations (n = 3/group).

Table 2. Four-day chemo-suppressive activity of *Morinda lucida* stem bark Extracts against *P. berghei* infection in Mice

Treatment	Dose (mg/kg)	% Parasitaemia (D_4)	% Chemo-suppresion (D_4)
Methanol extract	800	2.46 ± 0.70	67.71^{ab}
Ethanol extract	800	5.11 ± 0.18	32.94^{ab}
Aqueous extract	800	2.60 ± 0.20	65.88^{ab}
PBS (pH 7.4)	-	7.62 ± 0.13	0.00
Chloroquine	10	0.33 ± 0.35	95.67^{a}

Values are expressed as mean ± SEM; n = 5. D_4 = fifth day post-infection. Where a = significant ($p< 0.05$) compared to infected untreated control (PBS- pH 7.4), b = significant ($p< 0.05$) compared to reference drug, chloroquine. $p < 0.05$.

Prophylactic Antimalarial Activity of *Morinda lucida* Stem bark Extracts

Table 3 shows the prophylactic antimalarial activity of the crude stem bark extracts of *Morinda lucida* against *P. berghei* infected mice. The extracts had significant ($p< 0.05$) prophylactic antimalarial activity in treated mice when compared with mice of the untreated control group (PBS-pH 7.4). Prophylactic activity decreased accordingly; methanol > ethanol > aqueous. Again, although the

Table 3. Prophylactic activity of *Morinda lucida* stem bark extracts against *P. berghei* infection in mice.

Treatment	Dose (mg/kg)	% Parasitaemia (D_7)	% Chemo-suppresion (D_7)
Methanol extract	800	2.32 ± 0.35	63.23[ab]
Ethanol extract	800	3.35 ± 0.14	46.91[ab]
Aqueous extract	800	3.93 ± 0.09	37.72[ab]
PBS (pH 7.4)	-	6.31 ± 0.24	0.00
Chloroquine	10	0.54 ± 0.81	91.44[a]

Values are expressed as mean ± SEM; n = 5. D_7 = three days post-infection. Where a = significant (p< 0.05) compared to infected untreated control (PBS- pH 7.4), b = significant (p< 0.05) compared to reference drug, chloroquine. p< 0.05.

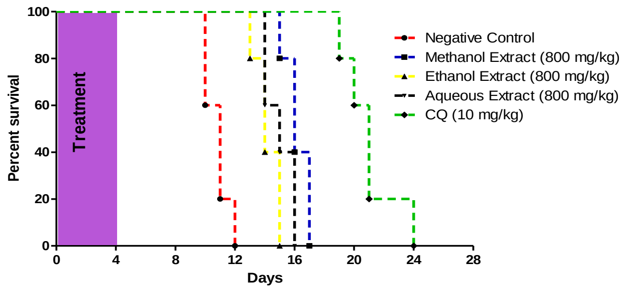

Figure 3. Chemo-suppressive activity of *Morinda lucida* leave extracts against *P. berghei*-infected mice

methanol extract performed better than the others, chloroquine, with a significant (p> 0.05) antimalarial activity was more effective.

Effect of *Morinda lucida* Extracts on Mean Survival Time (MST)

Figures 3 and 4 show the MST results of *Morinda lucida* stem bark extracts for the four-day chemo-suppressive and prophylactic tests, respectively. In the chemo-suppressive study, mice in the treated groups significantly (p< 0.05) lived longer than those in the untreated control group with mice in the methanol treated group living the longest (MST: 16.20 ± 1.95 days). But for the prophylactic study, only mice treated with methanol extract had significant MST (15.50 ± 1.95 days) compared to the untreated mice (MST: 10.80 days). Although ethanol and aqueous extracts prolonged MST, they were non-significant (p> 0.05) compared to the untreated mice. Meanwhile in both studies, chloroquine had a better effect on MST than the extracts.

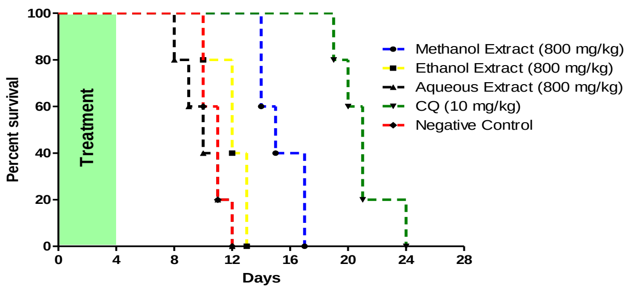

Figure 4. Prophylactic activity of *Morinda lucida* leave extracts against *P. berghei*-infected mice

Discussion

Medicinal plants have been acclaimed to possess large stores of therapeutic agents, which can serve as leads in emerging drug discoveries. These therapeutic agents widely referred to as phytochemicals (secondary metabolites) are associated with the array of bioactive properties ascribed to medicinal plants (Surh, 2003; Rathor, 2021; Yu *et al.*, 2021). Belonging to several classes of compounds e.g., alkaloids, tannins, cardiac glycosides, saponins, phenolics, flavonoids, etc., phytochemicals and their metabolic intermediates are structurally adapted to interact with cellular processes, resulting in beneficial effects against the activities of invading pathogens (Surh, 2003; Rathor, 2021; Yu *et al.*, 2021). The modulation of cellular signal transduction, immune response and enzyme activities, anti/pro-oxidation, intercalation of DNA, etc., are some proposed mechanisms of action of these secondary metabolites (Perron and Julia, 2009; Samy *et al.*, 2011; Chen and Liu, 2018). In this study, *Morinda lucida*, was screened for its secondary metabolites and was found to contain some phytochemicals (Table 1) in reasonable proportion. Also, Figure 1 showed the plant had antioxidant potentials when compared with reference antioxidant molecules such as EDTA and ascorbic acid. The aqueous, ethanol and hexane extracts of *Morinda lucida* were able to mop up radical ions like ferrous ions and DPPH radicals, though the results were non-significant (p< 0.05) compared to the reference antioxidant molecules. It was noticed that the aqueous extract was a better chelator and scavenger of ferrous ions and DPPH radicals, respectively, compared to other extracts tested. Meanwhile, the proximate analysis of the dried sample of *Morinda lucida* showed the plant was rich in carbohydrate, fat and protein (Figure 2); three essential nutrients required for synthesis of important biomolecules necessary for the sustenance of life (Ochs, 2014; Nelson and Cox, 2017).

Furthermore, it was revealed from this study that *Morinda lucida* had antimalarial activity against NK65 strain of chloroquine-sensitive *Plasmodium berghei*-induced malaria in Swiss albino mice. The efficacy of *Morinda lucida* was tested using the chemo-suppressive, prophylactic and mean survival time (MST) studies at a dose of 800 mg/kg body weight of mice. Aside conferring advantage on the plant extracts, preliminary study (unpublished results) reveals that activity was highest around a dose of 800 mg/kg bw of mice after observing a dose-dependent effect from lower doses. From the chemo-suppressive study (Table 2), the methanol, ethanol and aqueous extracts of the plant were able to significantly (p< 0.05) suppress the growth of the parasite when compared with the untreated control mice. The methanol extract exerted the highest suppressive effect, while the ethanol extract had the lowest suppression. More so, there was significant (p< 0.05) prophylactic antimalarial activity (Table 2) when compared with the infected untreated control mice. Except for the aqueous extract treated group whose effect was almost doubled, the antimalarial activity in the prophylactic study had a reduced efficacy compared to the chemo-suppressive study. This decreased efficacy of the extracts between both studies could be a reflection of the instability of the active principle(s). Metabolic degradation or biotransformation of phytomolecules could produce intermediates with shorter half-life whose potency may be diminished if not replenished with the passage of time. Therefore, a good chemo-suppressive agent may not necessarily retain its activity if subjected to prophylactic conditions. Meanwhile, it was observed that prophylactic effect of the extracts declined with decreasing polarity of extracting solvents, i.e., methanol extract < ethanol extract < aqueous extract, thus, suggesting that the prophylactic agent(s) could be very polar in nature. On mean survival time (MST) post inoculation and treatment, the extracts were able to prolong MST of treated animals beyond those of the infected untreated control (Figures 3 and 4). While survival was significant (p< 0.05) for all treated groups in the chemo-suppressive study, only the methanol extract treated group survived significant (p< 0.05) in the prophylactic study compared to the infected untreated control. Nonetheless, chloroquine performed better than the extracts in all studies conducted, exerting a significant (p< 0.05) antimalarial effect. Within local traditional healers, Lawal *et al*. (2011) reported that *Morinda lucida* was one of the four essential preparations for fever. Also, the efficacy of this plant *in vitro* against *P. falciparum* was reported by Makinde *et al*. (1985). However, Koumaglo *et al*. (1992) had earlier reported the antiplasmodial activity of two anthraquinones, viz; rubiadin 1-methyl ether and damnacanthal, isolated from the root of *Morinda lucida* with inhibitions of 98.7% and 100%, respectively, against *P. falciparum* at a dose of 30 µg/ml. These findings though observed with the root of *M. lucida* corroborate our results on the same plant and provide a scientific explanation to the use of this plant in herbal medicine against infections like malaria. It can therefore be suggested that the presence of these phytochemicals with antioxidant potential coupled with a rich nutrient composition could be responsible for the healing properties linked to *Morinda lucida* in folklore medicine.

CONCLUSION

From this study, it has been demonstrated that the stem bark of *Morinda lucida* contains rich complement of phytochemicals, nutrients and possesses antimalarial activity with the best effect exerted by the methanol extract (derived from a near mid-polarity solvent, methanol), thus, supporting its usage in herbal practice.

AUTHORS’ CONTRIBUTIONS

KOO conceptualized the study, optimized the protocol used, carried out bench work, performed statistical analysis and wrote, reviewed, and edited the manuscript. POU conceptualized, supervised the study and edited the manuscript.

FUNDING STATEMENT

This study was supported by research grants received from Tertiary Education Trust fund (TETFund) and University of Benin Research and Publication Committee (URPC), for which we are immensely grateful.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ACKNOWLEDGEMENT

The authors thank the Natural Products Research and Disease Control Laboratory (NPRDC), UNIBEN, for its facilities and Mr. Samuel for his technical assistance.

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