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Antimalarial Activity of Extracts and Partially Purified Fractions of *Alstonia boonei* De Wild

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ABSTRACT: *Alstonia boonei* De wild is famed for several bioactive properties; this study measures its *in vivo* antimalarial potentials as well as phytochemical, nutritional and antioxidant properties. Variants of its stem bark extract were screened for phytochemical, nutritional and antioxidant properties using established procedures. *In vivo* antimalarial properties of extracts and fractions were determined against *Plasmodium berghei* NK65 infection in mice using chemo-suppressive, prophylactic, curative and mean survival time (MST) bioassays. Alkaloids, saponins, cardiac glycosides, tannins, flavonoids and phenolics were present in almost all extracts. The plant contained nitrogen free extract (NFE), crude fat, crude protein, moisture, crude ash and crude fibre in reasonable amounts. Extracts demonstrated antioxidant activity against DPPH and ferrous ion radicals. The aqueous and ethanol extracts were more active, decreased parasitaemia and increased MST relative to infected untreated control in the chemo-suppressive and prophylactic bioassays. Solvent-solvent partitioning of the hydro-ethanol extract yielded petroleum ether, dichloromethane-DCM, basified-DCM (BDCM), acidified-DCM (ADCM) and aqueous fractions. BDCM had the highest suppressive activity (70.91% suppression; MST: 16.50 ± 1.19), while the crude extract had the highest prophylactic (64.51% suppression; MST: 16.00 ± 1.22) and curative activities (69.80% suppression; MST: 16.80 ± 0.58). *Alstonia boonei* De wild possesses antimalarial properties likely associated with its phyto-nutritional composition.

Keywords: *Alstonia boonei* De wild, Antioxidant**,** Fractionation, *Plasmodium berghei*, Antimalarial

Introduction

Despite the numerous interventions by critical stakeholders in and around the health sector, malaria continues to ravage the health of several individuals and impoverish the economies of many nations, much of which are in the tropics and belong to the categorization of developing countries. Apart from favourable climatic conditions necessary for the breeding of mosquitoes, these countries are also known for their poor budgetary allocations to health, abysmal sanitary conditions and low public health awareness required to contain diseases such as malaria. For instance, while the WHO global average health spending projection per capita for low income countries is US$ 80 a person, countries especially in Africa, the epicenter of the disease, have their budgets to health hovering around 10% (WHO, 2021). The highest Nigeria has ever budgeted was 6.23% in 2012, a far cry from the 15% minimum standard she and other African Union member countries set for themselves in 2001 (dRPC, 2021). Meanwhile in 2020 alone, Nigeria accounted for over 31% of incidences of death and clinical cases of malaria worldwide (WHO, 2021), just as the African continent bore the burden of about 95% of clinical cases and 96% of mortality the same year; thus, placing them at the center of this epidemic. Of course, the situation has not changed as Nigeria continues to grapple with increasing inflation, unemployment, rising cost of healthcare, etc. (NBS, 2023). Many households have resorted to patronizing alternatives for their healthcare needs in order to complement the spiraling expensive

and inaccessible cost of orthodox care and medications. This includes the use of medicinal plants, otherwise known as herbs.

Medicinal plants are famed for their efficacies against several ailments and have gained wide acceptance around the world, where they are amongst others exploited for their medicinal and commercial gains. These plants are said to contain certain chemical molecules linked to their healing potentials, and participate in metabolic processes either as a single molecule or in synergy with other molecules to bring about the desired effects (Yuan *et al*., 2016). Some drugs currently in use were derived from medicinal plants and the prospects for getting more is high (Li-Weber, 2009). An example of these plants is *Alstonia boonei* De Wild which belongs to the family of Apocynaceae and consists of over forty (40) species, including *A. scholaris, A. congensis, A. longifolia, A. macrophylla,* etc., that are widely distributed in the continents of Africa, Asia and the Americas. In Nigeria, it is found predominantly in the lowlands and rain-forest areas of the South (Iwu, 1993: Adotey *et al*., 2012). It is a large deciduous tree measuring about 45 m in height and 1.2 m in width with a trunk that could be branchless up to 25 m in length (Adotey *et al*., 2012). In Nigeria, *Alstonia boonei* is commonly referred to as Ukhu, Uhu, Ogiegbukhu in Edo, Ukpukunu in Urhobo, Okugbe in Itsekiri, Ahun, Awun in Yoruba and Egbe, Egun, Egbu-ora in Igbo, Ndodo in Efik and Egbu in Ijaw (Aigbokhan, 2014). Its other names include patternwood, cheesewood and stoolwood (Palla, 2005; Aigbokhan, 2014). All the parts of the plant are very useful but the thick bark cut from the matured tree is the most commonly used part for therapeutic purposes (Adotey *et al*., 2012). An infusion of the fresh stem bark is used as an effective antidote against malaria, snake, rat or scorpion poison, and in treating painful micturition and rheumatic conditions (Ojewole, 1984; Olajide *et al*., 2000; Odugbemi *et al*., 2007). Fresh leaves and stem bark of *A. boonei* show good insecticidal activity against *Sesamia calamistis* making it an option as a possible crop insecticide (Oigiangbe *et al*., 2007). Also, *A. boonei* stem bark is claimed to possess analgesic, antipyretic and anti-inflammatory properties (Olajide *et al*., 2000, Osadebe, 2002).

Nonetheless, the beneficial effects of medicinal plants have not gone without criticism over their usage as they are said to be toxic and lack a proper dose regime for administration. Therefore, processing the crude form of these plants, which involves fractionation and possible isolation of active principle(s) could eliminate undesired constituents that may reduce the efficacy and quality of these plants. Also, synthesis or biotransformation of active molecules could lead to discovery of more active variants or new leads for the treatment of other ailments.

Therefore, this study seeks to evaluate the phytochemical, nutritional, antioxidant and antimalarial properties of *Alstonia boonei*, as well as partially purify its antimalarial active extract in a bioactivity-guided manner in order to narrow on activity and prepare for possible isolation of the active principle(s).

Materials and methods

Collection and preparation of plant material: The fresh stem bark samples of *Alstonia boonei* De Wild were collected from the forest area of Southwest Nigeria during the month of November. Identification and authentication were done in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, where voucher No. - UBHa210 was assigned. The samples were rid of debris and air-dried under shade, and pulverized using mortar and pestle. Thereafter, pulverized samples were stored in an air-tight plastic container at room temperature until ready for use.

Extraction of plant material: Four hundred grams each of the pulverized plant material were separately macerated in 1.5 L of distilled water, ethanol, methanol and hexane for 72 h under repeated stirring. Filtration of extracts was by Whatman filter paper No. 1 (Whatman, England), while the recovered meshes were separately macerated twice in similar solvents as previously described (Cannell, 2006). Filtrates from similar solvents were combined and dried at 45 °C *in vacuo* using a rotary evaporator (Buchi, Germany). The ensuing extracts were preserved in air-tight glass containers under refrigerated condition, until further use.

Fractionation of antimalarial active extract: The active extract of *Alstonia boonei* from preliminary antimalarial study was gotten by repeated maceration of 3.5 kg of the dried pulverized stem bark sample (as described earlier) in hydro-ethanol (30/70 v/v). Fractionation of the extract (85 g) was by acid/base solvent-solvent partitioning with graded alcohols in a separating funnel. The extract was initially defatted with petroleum ether (PE), while the aqueous residue was washed with dichloromethane (DCM). Thereafter, the resulting aqueous residue was basified using ammonium hydroxide and extracted with DCM. Lastly, the ensuing residue was acidified using acetic acid and again extracted using DCM, leaving an aqueous residue.

Estimation of nutritional content: Proximate analysis to determine the nutritional composition of the plant sample was done according to the methods of AOAC (2000). The following components were estimated; nitrogen free extract (NFE), crude fat, crude protein, moisture, crude ash and crude fibre.
Phytochemical screening: Qualitative phytochemical content of the plant extracts was determined according to the modified methods of Stahl, 1973; Sofwora, 1982; Harborne, 1998 and Evans, 2002. Each extract (5 g) was boiled for 30 min after separate mixing in 75 mL of distilled water. The filtrates were filtered hot and subsequently cooled, then used to test for the presence of tannins, saponins, phenolics, flavonoids, cardiac glycosides and alkaloids.
Estimation of antioxidant capacity: Antioxidant capacity of the extracts was determined according to the modified methods of Dinis *et al*. (1994) and Brand-Williams *et al*. (1995). This was done by testing the ability of the extracts to scavenge 2, 2–diphenyl–1– picrylhydrazyl (DPPH) radical and chelate ferrous ions.
Malaria parasites and preparation of inoculum: The chloroquine-sensitive *Plasmodium berghei* NK65 strain used in this study was obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria. Maintenance of the parasite was by a weekly intraperitoneal passage to a naive mouse. The standard inoculum was prepared from the donor mice by obtaining parasitized red blood cells-PRBCs at 30% parasitaemia via cardiac puncture under anesthesia and diluting in normal saline to give 1×10^7 PRBCs (Fidock *et al*., 2004).
Animal handling and maintenance: Experimental mice were handled according to guidelines issued by the Institute for Laboratory Animal Research (ILAR, 2011). The male Swiss albino mice weighing 20 ± 2 g and about six to eight weeks of age were used for this study. They were acquired from the Animal House of Igbinedion University, Okada, Edo State and kept under a 12 h light-dark cycle in plastic cages with wood shavings as beddings. The mice were acclimatized for 7 days and fed rat chow and water *ad libitum* for the period of experiment.
Grouping and dosing of animals: Male Swiss albino mice were randomized into three main groups of five mice each. Infection with *P. berghei* (0.1 ml) was done once intraperitoneally (*ip*), while extracts, fractions and chloroquine were orally administered once daily. Infection and treatments were according to the screening model in focus (i.e., whether suppressive, prophylactic or curative).

Group	Treatments
Group A (Negative control)	Infected and administered phosphate buffered saline
Group B (Positive control)	Infected and administered chloroquine (10 – 25 mg/kg bw)
Group C (Test Groups)	Mice were infected and each group administered extracts/fractions (800 mg/kg bw of mouse).

Antimalarial Screening Models
Suppressive test: Mice were inoculated and treated on the first day (D_0), with a two-hour interval between both procedures. Treatments (with extracts/fractions/chloroquine) continued for three days (i.e., D_0 to D_3), while on D_4, parasitaemia was ascertained under a microscope (Olympus, Japan) from thin smears made from the tail-blood of each mouse (Fidock *et al*., 2004).
Prophylactic test: In this test, mice were initially treated for four consecutive days (D_0 to D_3) and afterward infected with 10^7 PRBCs on D_4. After three days of infection (D_7), parasitaemia was evaluated under a microscope (Olympus, Japan) from thin smears made from the tail-blood of each mouse (Fidock *et al*., 2004).
Curative test (Rane's test): The ability of the extracts/fractions to clear parasite infection was determined according to the methods of Ryley and Peters (1970). Briefly, the mice were infected with 10^7 PRBCs on D_0 (the first day), while treatments commenced 72 h post-infection and lasted for four days. Parasitaemia was evaluated under a microscope (Olympus, Japan) from thin smears made from the tail-blood of each mouse.
Determination of parasitaemia: Percent parasitaemia and suppression were respectively determined by counting the number of PRBCs in random fields of microscope view under x100 objective lens-oil immersion (Fidock *et al*., 2004; Kalra *et al*., 2006.

$$\% \text{ Parasitaemia} = \frac{\text{NP}}{\text{TNR}} \text{X } 100$$

$$\% \text{ Suppression} = \frac{(\% \text{ Parasitaemia of NC} - \% \text{ Parasitaemia of TG})}{\% \text{ Parasitaemia of NC}} \text{X } 100$$

NP = Number of PRBCs; TNR = Total number of RBCs; NC = Negative Control; TG = Treated Group

Determination of Mean Survival Time (MST): The MST for each mouse was ascertained by measuring daily death and number of days of survival from infection to death of mouse.

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

Chemicals and drugs: Phosphate buffered saline, normal saline, chloroquine, ferrozine, DPPH, ferric chloride, vitamin C, glacial acetic acid, EDTA, H_2SO_4, HCl, mayers reagent, ammonia and olive oil (Silver Health Diagnostics, Nigeria). Hexane, ethylacetate, methanol and ethanol (Sigma Aldrich, Germany). Giemsa stock (Trust Chemical Lab, India), immersion oil (Scisco Research Lab, India). Items were of analytical grade and purchased from accredited suppliers.

Data analysis: GraphPad Prism 5 was used for statistical analysis and data presented as mean ± SEM. One-way analysis of variance (ANOVA) was used to compare differences between means, while comparison between differences in significance was by Turkey's HSD multiple comparison tests. Significance was set at $p < 0.05$.

Results

Phytochemical content: Table 1 shows the phytochemical content of stem bark extracts of *Alstonia boonei* De wild. From the result, all phytochemicals tested were present in the plant sample. However, the hexane extract was devoid of alkaloids, saponins and cardiac glycosides, while the methanol and aqueous extracts lacked cardiac glycosides and alkaloids, respectively.

Table 1: Phytochemical Content of Extracts of *Alstonia boonei* Stem bark

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

– not detected: + detected

Nutrient composition: The percentage nutrient composition of *Alstonia boonei* De wild stem bark is presented in Figure 1. Proximate analysis revealed a high presence of nitrogen free extract (NFE) and low composition of crude fibre and crude ash. However, crude fat, crude protein, and moisture were reasonably present.

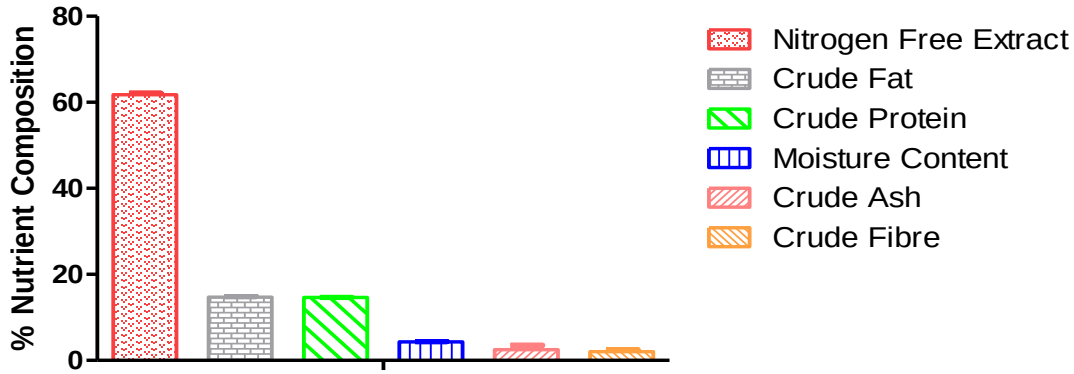


Figure 1: Nutrient composition of *Alstonia boonei* De wild Stem bark. Data represent percentage mean ± SEM of triplicate determinations.

Antioxidant capacity: The antioxidant capacity of *Alstonia boonei* stem bark against DPPH radicals and ferrous ions are shown in Figure 2. The extracts had some levels of antioxidant activity; however, it was non-significant

compared to the reference candidates (EDTA and ascorbic acid). Both methanol and hexane extracts had the most ferrous ion chelating and DPPH scavenging activities, respectively.

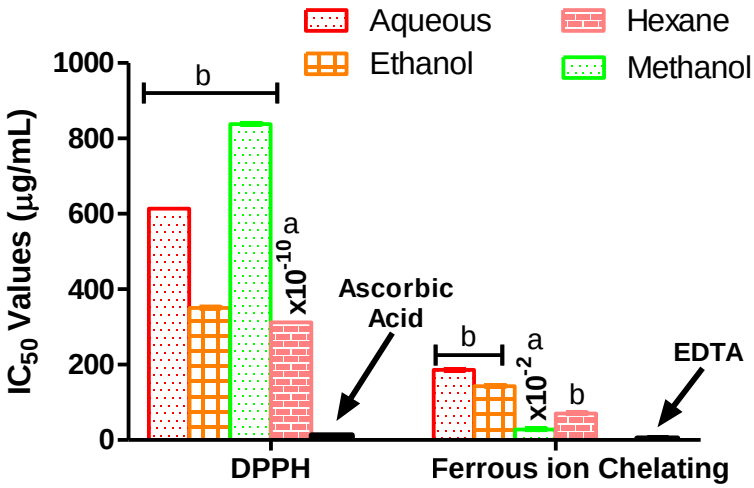
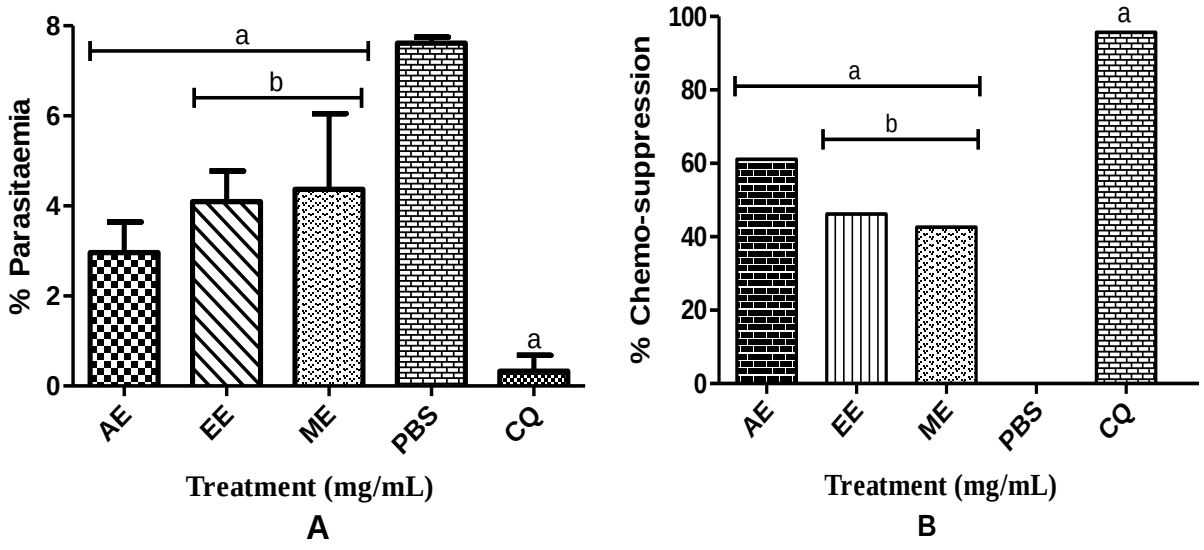


Figure 2: Antioxidant activity of extracts of *Alstonia boonei* De wild stem bark. IC_{50} values of triplicate determinations (n = 3/group). a = significant ($p < 0.05$), b = non-significant ($p > 0.05$) relative to the standard controls, ascorbic acid and EDTA.

Chemo-suppressive antimalarial activity of extracts: Figure 3 shows the suppressive activity of the crude extracts of *Alstonia boonei* De wild stem bark against *P. berghei* malaria infection in mice. The extract significantly ($p < 0.05$) reduced parasitaemia (Figures 3A and B) and increased MST (Figure 3C) ($p < 0.05$) in treated groups compared to the untreated control (PBS- pH 7.4). Suppressive activity increased in the following order: aqueous > ethanol > methanol, while chloroquine performed better than the extracts.



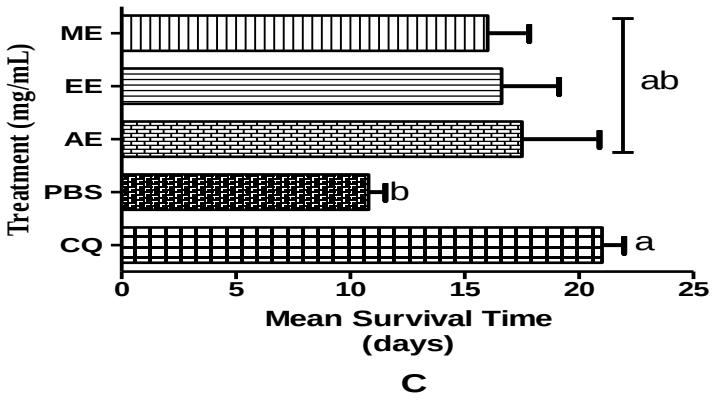


Figure 3: Four-day Chemo-suppressive Activity of *Alstonia boonei* De wild Stem bark Extracts against *P. berghei* infection in Mice. Values are presented as mean ± SEM; n = 5. AE: aqueous extract, EE: ethanol extract, ME: methanol extract, PBS: Phosphate Buffered Saline, CQ: chloroquine. a = significant ($p < 0.05$) compared to infected untreated control (Phosphate Buffered Saline- PBS- pH 7.4), b = significant ($p < 0.05$) compared to reference drug, chloroquine. $p < 0.05$.

Prophylactic antimalarial activity of extracts: Figure 4 reveals the prophylactic activity of crude stem bark extracts of *Alstonia boonei* De wild. There was significant ($p < 0.05$) prophylaxis (Figures 4A and B) against *P. berghei* infection in treated group as against untreated control (PBS- pH 7.4) with an increased MST (Figures 4C) ($p < 0.05$). Although the aqueous extract had the highest prophylactic activity, chloroquine was more effective.

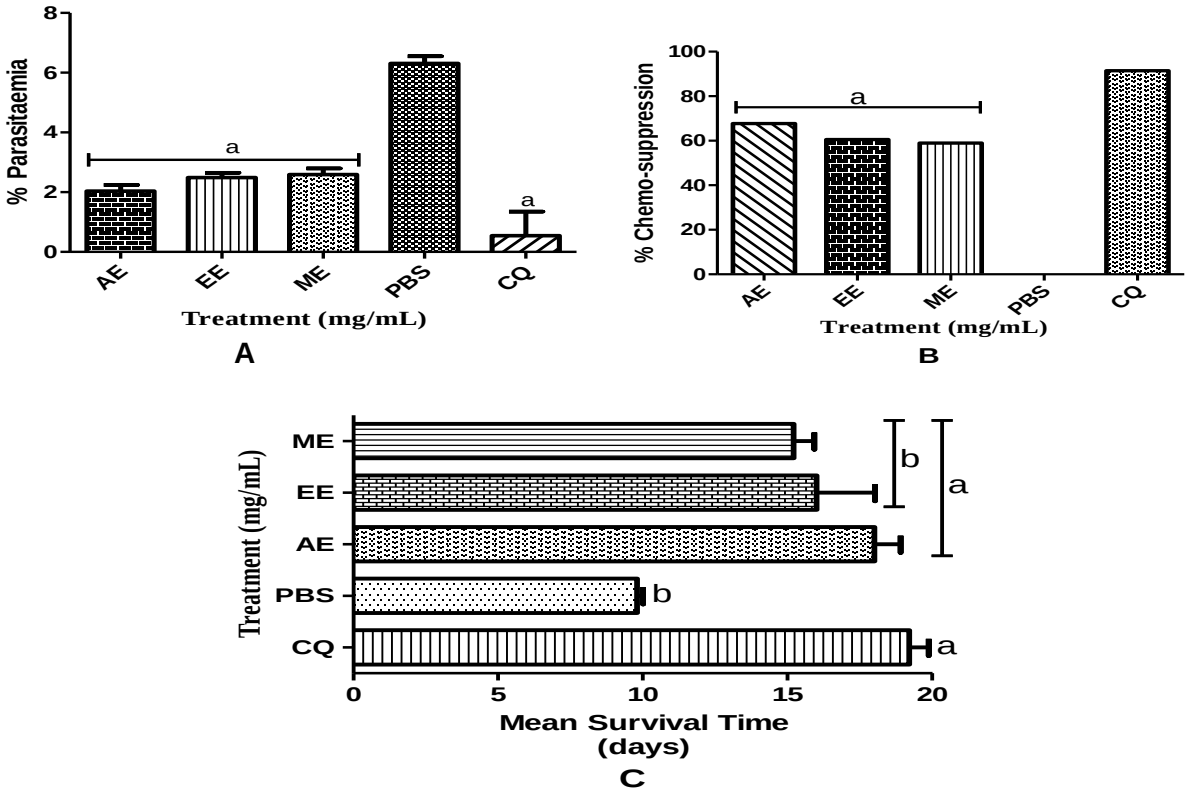


Figure 4: Prophylactic activity of *Alstonia boonei De wild* stem bark extracts against *P. berghei* infection in mice. Values are represented as mean ± SEM; n = 5. AE: aqueous extract, EE: ethanol extract, ME: methanol extract, PBS: Phosphate Buffered Saline, CQ: chloroquine. a = significant ($p < 0.05$) compared to infected untreated control (Phosphate Buffered Saline- PBS- pH 7.4), b = significant ($p < 0.05$) compared to reference drug, chloroquine. $p < 0.05$.

Percentage yield and physical characteristics of fractions: Table 2 shows the percentage yield and physical appearances of solvent fractions of crude hydro-ethanol stem bark extract of *Alstonia boonei* De wild. The aqueous residue and basified dichloromethane soluble fractions were respectively, the highest and lowest in terms of yield. Meanwhile, they varied in colour and texture.

Table 2: Percentage Yield and Physical Appearances of Fractions of Crude Hydro-Ethanol Stem bark Extract of *Alstonia boonei* De wild

Sample	Percentage Yield	Physical Appearance
Petroleum ether soluble fraction	6.47	Yellowish brown viscous liquid
Dichloromethane soluble fraction	22.71	Dark granule
Basified Dichloromethane soluble fraction	1.76	Greenish yellow solid
Acidified Dichloromethane soluble fraction	5.29	Greenish yellow solid
Aqueous soluble residue	63.77	Brownish paste

Chemo-suppressive antimalarial activity of active hydro-ethanol extracts and fractions: Figure 5 represents the chemo-suppressive activity of the crude hydro-ethanol extract and fractions of *Alstonia boonei* De wild stem bark. There was significant ($p < 0.05$) suppression of *P. berghei* growth in treated groups compared to the untreated control (PBS- pH 7.4) (Figures 5A and B). Also, MST (Figures 5C) was significantly ($p < 0.05$) increased by the extract and fractions compared to the untreated control. Although chloroquine had better activity than the test candidates, the basified DCM fraction was more active than the other fractions and the crude extract.

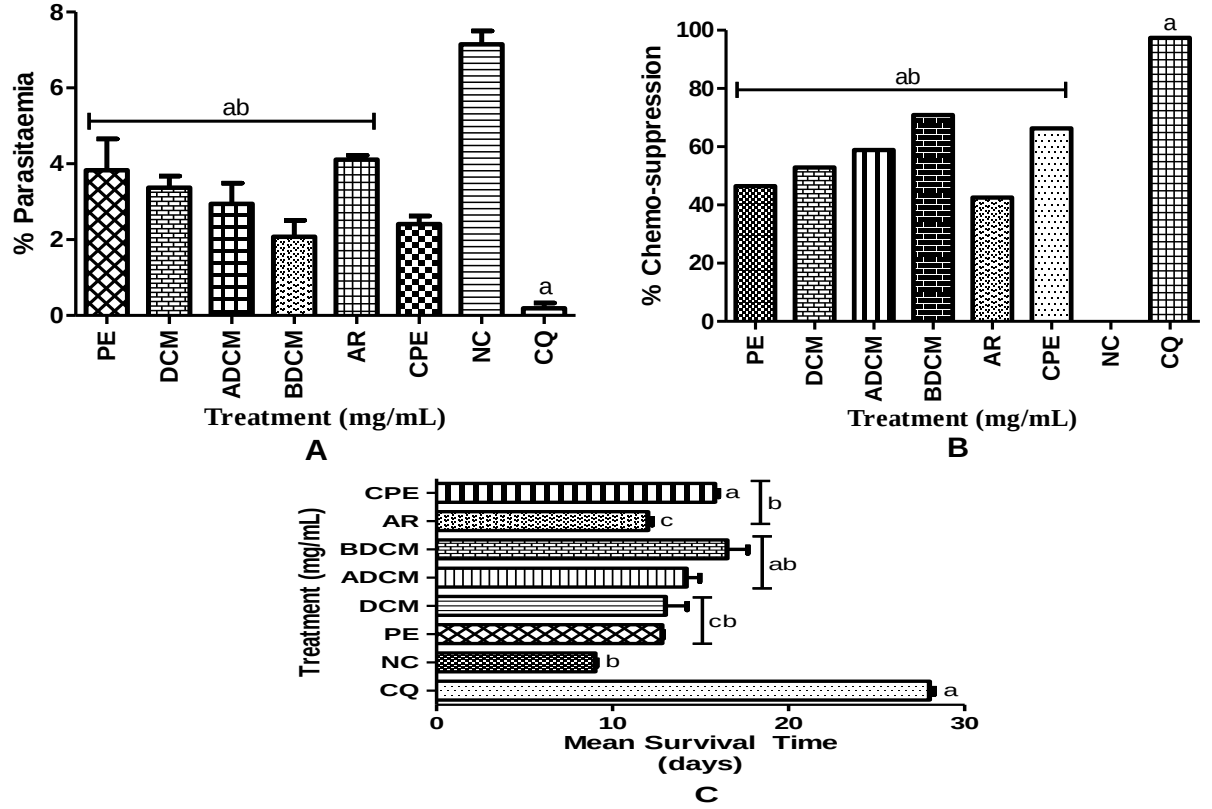


Figure 5: Comparison of four-day chemo-suppressive activity of crude extract and partially purified fractions of *Alstonia boonei* De wild stem bark against *P. berghei* infection in mice. Values are expressed as mean ± SEM; n = 5. PE: petroleum ether soluble fraction, DCM: dichloromethane soluble fraction, ADCM: acidified dichloromethane soluble fraction, BDCM: basified dichloromethane soluble fraction, AR: aqueous residue fraction, CPE: crude plant extract, MST = mean survival time. a = significant ($p < 0.05$) when compared to negative (infected untreated) control; b = significant ($p > 0.05$) when compared with chloroquine control. c = non-significant compared to negative (infected untreated) control.

Prophylactic antimalarial activity of active hydro-ethanol extracts and fractions: Figure 6 reveals the prophylactic activity of the crude hydro-ethanol extract and fractions of *Alstonia boonei* De wild stem bark against *P. berghei* infection in mice. There was significant ($p < 0.05$) prophylaxis in treated groups compared to the infected untreated control (PBS- pH 7.4) (Figures 6A and B). They also increased MST (Figures 6C), though only the BDCM fraction and crude hydro-ethanol extract were significant ($p < 0.05$) when compared with the infected untreated control. But, chloroquine still had better activity compared to the extract and fractions.

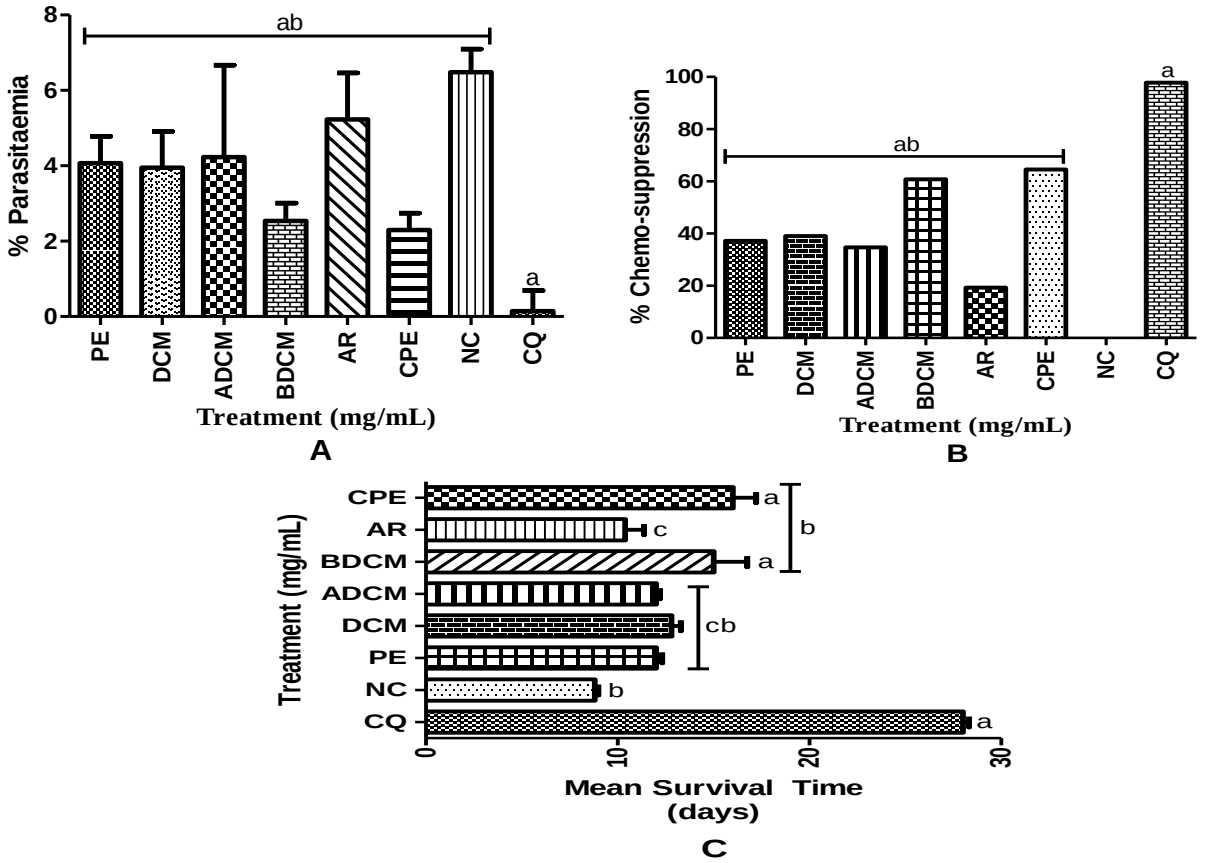


Figure 6: Comparison of prophylactic activity of crude extract and partially purified fractions of *Alstonia boonei* stem bark against *P. berghei* infection in mice. Values are expressed as mean ± SEM; n = 5. PE: petroleum ether soluble fraction, DCM: dichloromethane soluble fraction, ADCM: acidified dichloromethane soluble fraction, BDCM: basified dichloromethane soluble fraction, AR: aqueous residue fraction, CPE: crude plant extract, MST = mean survival time. a = significant ($p < 0.05$) when compared to negative (infected untreated) control; b = significant ($p > 0.05$) when compared with chloroquine control. c = non-significant compared to negative (infected untreated) control.

Curative antimalarial activity of active hydro-ethanol extracts and fractions: Figure 7 represents the curative activity (on the 9th day) of crude hydro-ethanol extract and fractions of *Alstonia boonei* De wild stem bark against *P. berghei* infection in mice. They significantly ($p < 0.05$) suppressed malaria progression compared to the infected untreated control (PBS- pH 7.4) with CPE and PE having the highest and lowest activities, respectively (Figures 7A and B). Mean survival time (Figures 7C) was significanly increased in treated mice except in the PE and AR treated groups, which were non-significant ($p > 0.05$). Chloroquine was most active, with MST greater than the 28-days.

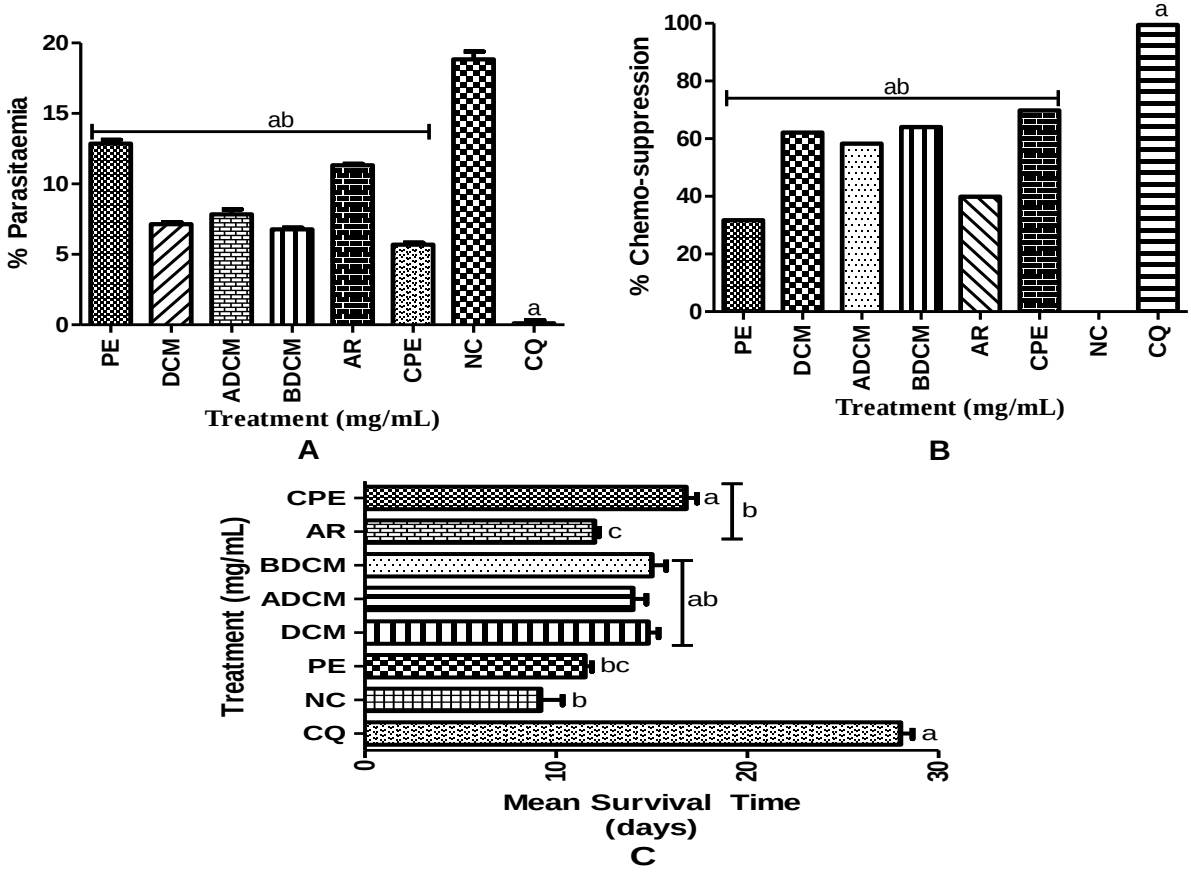


Figure 7: Comparison of antimalarial curative activity of crude extract and partially purified fraction of *Alstonia boonei* stem bark against *P. berghei* infection in Mice (9th Day). Values are expressed as mean ± SEM; n = 5. PE: petroleum ether soluble fraction, DCM: dichloromethane soluble fraction, ADCM: acidified dichloromethane soluble fraction, BDCM: basified dichloromethane soluble fraction, AR: aqueous residue fraction, CPE: crude plant extract, MST = mean survival time. a = significant ($p < 0.05$) when compared to negative (infected untreated) control; b = significant ($p > 0.05$) when compared with chloroquine control. c = non-significant compared to negative (infected untreated) control.

Discussion

With world economies slipping into recession and poverty, the average purchasing power of house-holds continues to plummet, having direct consequence on health and wellness. The complementary role of alternative medicine in ensuring wellness is unprecedented. Plants like *Alstonia boonei* De wild have been exploited for their medicinal gains and in this study, we deepen the scope by evaluating its phytochemical, nutritional, antioxidant and antimalarial properties as a validation to their usage in traditional medicine.

The bioactivities of plants have been attributed to their rich phytoconstituents which belong to several chemical families. Results from this study (Table 1) reveal the presence of these families of phytoconstituents with rich pharmacological potentials. *Alstonia boonei* was found to contain alkaloids, saponins, cardiac glycosides, tannins, flavonoids and phenolics in appreciable quantities in all extracts tested. However, cardiac glycosides and alkaloids were not detected in the hexane, methanol and aqueous extracts, while the hexane extract also lacked saponins. Thus, this phytochemical presence could make *A. boonei* a choice plant in the combat of diseases such as malaria as they participate in the defense against microorganisms (Raes *et al.*, 2015; Rathor, 2021; Yu *et al.*, 2021) and supports its use in traditional healing preparations.

Malaria patients have been observed to have low levels of essential biomolecules such as glucose, lipids and proteins (Saxena *et al*., 2016; Singh *et al*., 2017). This is not unconnected with the activities of the parasite, plasmodium, which requires these components for survival (Preuss *et al*., 2012; Saxena *et al*., 2016). Also, during such ailments, victims are said to suffer from withdrawal syndrome which complicates their feeding patterns owing to loss of appetite, thereby leading to drained energy store. However, *A. boonei* was found to contain rich food components capable of compensating for the shortages associated with malaria infection. Figure 1 indicates that the plant was rich in carbohydrate, protein and fat. It also possessed considerable amounts of moisture, ash and fibre necessary to sustain good health. Therefore, its preference for usage in healing is justified.

Similarly, the plant was observed to possess antioxidant property as it was able to scavenge DPPH radicals and chelate ferrous ions (Figure 2). Plants that are able to mop up free radicals are considered potent therapeutic leads. The actions of free radicals in the etiology of diseases have been documented (Pham-Huy *et al*., 2008; Lobo *et al*., 2010) where they have been linked to alterations in cellular processes (Percário *et al*., 2012; Tan *et al*., 2018) culminating in events such as lipid peroxidation, enzyme inhibition, DNA intercalation, etc. Therefore, resolution of the menacing activities of these radical species in disease conditions such as malaria is vital to recovery. From the study, it was discovered that methanol and hexane extracts had more ferrous ion chelating and DPPH scavenging activities, respectively, even though they were not as potent as the reference antioxidant molecules, EDTA and ascorbic acid.

The efficacy of *Alstonia boonei* against malaria infection caused by *Plasmodium berghei* NK65 strain in mice was tested using the four-day chemo-suppressive, prophylactic, curative and mean survival time-MST assays. These tests have proven to be vital in experimentally ascertaining the efficacy of drug candidates (Fidock *et al*., 2004; Kalra *et al*., 2006). From the preliminary aspect of this bioactivity guided study, the plant extracts were found to possess varying degrees of antimalarial activity. Figures 3 and 4 (suppressive and prophylactic studies, respectively) revealed the extracts to significantly ($p < 0.05$) slow down parasitaemia and increase MST ($p < 0.05$) in treated groups compared to the untreated control. The aqueous and ethanol extracts were consistent in their activity and ability to prolong survival time; hence they were more active and combined for further studies. The combined hydro-ethanol (30/70 v/v) extract of *Alstonia boonei* stem bark was further fractionated under solvent-solvent partitioning to give five soluble fractions of petroleum ether, dichloromethane-DCM, basified-DCM, acidified-DCM and aqueous residue. All fractions and the crude extract were subjected to antimalarial screening. The crude plant extract and fractions of *A. boonei* stem bark showed antimalarial activity when compared with the infected untreated control. With suppression of 3.44 folds, basified-DCM fraction had the highest activity compared to other fractions and the crude plant extract, while the aqueous residue had the lowest activity index of 1.74 fold suppression. Antimalarial activity of *A. boonei* stem bark was seen to decline accordingly: basified-DCM fraction > crude plant extract > acidified-DCM fraction > DCM fraction > petroleum ether fraction > aqueous residue fraction (Figure 5). The basified-DCM fraction was non-significantly ($p > 0.05$) higher than the crude plant extract. Here, fractionation was seen to slightly enhance activity probably by sequestering the active ingredients in more soluble fractions, thus allowing ample room for synergistic actions and possible elimination of any counteracting phytomolecules(s) that could compete with the desired effect of the active ingredient(s). Seelinger *et al*. (2012) had earlier reported that the fractionation of extract of *Pluchea odorata* enriched a spindle toxin or an indirect microfilament-targeting activity of the plant. Also, Etame *et al*. (2018) noticed enhanced activity of plant fractions over their crude extract as they found that the antibacterial activity of *Enantia chlorantha* plant was enhanced due to fractionation of its crude methanol stem back extract. These observations corroborate the findings in this study. It is believed that the activity of a plant extract or its fraction(s) would be greatly affected by several factors including methods of extraction/fractionation as well as the solvents used for these processes. In the prophylactic study, the plant fractions also demonstrated similar trend in antimalarial activity with reduced efficacy compared to results obtained in the suppressive study (Figure 6). But here, the crude plant extract, with a fold suppression of 2.82, was not significantly ($p > 0.05$) more active than the basified DCM fraction with 2.55 fold suppression. The general decline in antimalarial activity as earlier mentioned could perhaps be the fractions' inability to boost immunity of experimental mice against malaria infection well beyond the active period of their administration, perhaps due to rapid degradation of active pharmacologic components or reduction in bioavailability. Furthermore, curative potentials of the extracts and fractions of *A. boonei* stem bark on the 9th day of peak parasitaemia were also tested with results (Figure 7) confirming maximum death post-inoculation in the infected untreated mice. However, oral administration of extracts and fractions provided some significant ($p < 0.05$) amelioration as against infected untreated control; again re-enforcing the antimalarial properties of this plant. The crude extract was most active, having a slight non-significant curative effect (3.31 fold decrease in parasitaemia) over the basified-DCM fraction (2.79 fold decrease in parasitaemia), while the aqueous residue fraction had the least activity of 1.66 fold decrease in parasitaemia. This

activity also led to a significant increase in survival time for treated groups except those administered petroleum ether and aqueous residue fractions, which had a non-significant increase in MST. Meanwhile, chloroquine performed better than the test candidates in all studies conducted as compared to the infected untreated control group. While the level of parasitaemia in this group was almost undetected under microscope view, the mice survived beyond the 28 days of study. Therefore, it can be opined that the rich nutrient base of *Alstonia boonei* De wild coupled with the presence of phytoconstituents with proportionate antioxidant properties could be responsible for its pharmacologic effects as demonstrated here by its antimalarial activity.

Conclusion

The outcome of this study reinforces the use of medicinal plants like *Alstonia boonei* De wild in traditional medicine, providing complementary support to orthodox medication. It is recommended that the antimalarial molecule(s) inherent be isolated for further therapeutic gains.

Authors' contributions

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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