Exertion of Malaria Prophylaxis by Bioactive Constituents of a Medicinal Apocynaceae Plant

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Abstract

One of the strategies employed in malaria control and possible elimination efforts is the prevention of establishment of infection of the malaria parasite using prophylactic antimalarial drugs. This strategy has been negatively affected with the development of resistance of the malaria parasite to the currently used antimalarial drugs. This development has increased the urgent need for new antimalarial compounds with prophylactic properties against malaria. This study was thus carried out to evaluate the malaria prophylactic potential of bioactive compounds from extracts of *Alstonia boonei*, a medicinal plant used locally for malaria treatment in Nigeria and other African countries. The bioactive compounds were identified by Gas Chromatography – Mass Spectrometer analysis technique and then subjected to antimalarial tests to determine their prophylactic activity in mice experimentally infected with *Plasmodium berghei*. Results from the tests showed that bioactive compounds possess significant dose – dependent prophylactic property at $p < 0.05$ at the doses used. From these findings it was concluded that the plant is a potential source of prophylactic antimalarial molecules and should be further researched on for antimalarial drug development.

Keywords: Exertion, malaria, prophylaxis, bioactive constituents, drug development

1.0 Introduction

Malaria is a fatal insect-borne tropical disease caused by the *Plasmodium* parasite and is transmitted by the bite of the female Anopheles mosquito. Malaria continues to pose public health challenges globally and is a major cause of mortality, especially in areas where it is endemic (WHO, 2019). Malaria remains a major global public health problem following the increasing reports of incidences of resistant to the majority of antimalarial drugs (WHO, 2012). This development of resistance to commonly used antimalarial drugs is a threat to the efforts so far made to prevent and control malaria. In order to effectively prevent and control malaria, there is the need for new antimalarial molecules which are available in plants such as *Alstonia boonei* (Arise and Lawal, 2012; Asuzu and Anaga, 1991).

Alstonia boonei belongs to the family Apocynaceae and is an herbal medicinal plant of West African origin (Odugbemi *et al.*, 2007; Ogbuehi and Ebong, 2015; Ojewele, 1984; Osadebe, 2002). Products from nature, such as those from plants, play important roles as leads for the discovery and development of new drugs **(**Kumaratilake *et al., 1992;* Madhiri and Vijayalakshini, 2018; Muhseen *et al.*, 2021*).*

Malaria prophylaxis is aimed at the prevention of being infected by the Plasmodium parasite even when exposed to it by the bite of the female anopheles mosquito that transmits it (WHO, 2019). The development of new and novel prophylactic antimalarial molecules with novel modes of action will play key roles in malaria prevention and control **(**Madhiri and Vijayalakshini, 2018; WHO, 2019; Muhseen *et al.*, 2021*)***.**

2.0 Materials and methods

2.1 Preparation of Plant materials

The leaves, stem bark and roots of *Alstonia boonei* were separately cut into small pieces, washed and air dried for two weeks under room temperature. The dry samples were then ground into powder with a mechanical blender (Afolabi and Abejide, 2020).

2.2 Extraction methods

2.2.1 Aqueous extraction

A measurement of 500 g each of the ground fine powder obtained from the leaves, stem bak and roots was percolated in 1600 mL of water for 72h after which it was filtered. This was followed by evaporating the filtrate collected to dryness using a temperature-regulated water bath pre-set at 40^{o}C to yield the extract concentrate which was stored in the refrigerator at 4^{o}C before use (Amole and Ilori, 2010).

2.2.2 Ethanolic extraction

Another 500 g each of the ground plant material (leaves, stem bark and roots) powder was measured and dispersed in 2.5 L of ethanol. The mixture was shaken with a mechanical shaking machine for 72 h after which it was vacuum filtered. The resultant extract was then concentrated using a rotary evaporator at a temperature not exceeding 400^{o}C. The concentrate was then heated over a temperature-regulated water bath pre-set at 40^{o}C to obtain a solvent free extract. The extract thus obtained was stored in the refrigerator at 4^{o}C until use (Amole and Ilori, 2010).

2.3 Gas Chromatography-Mass Spectrometer (GC-MS) analysis of the bioactive compounds

The bioactive compounds present in the ethanolic and aqueous leaf, stem bark and root extracts of *Alstonia booei* were determined and identified by Gas Chromatography and Mass Spectrometer methods using (Angilent 6890 series) equipment following the procedures described by Eswarainh *et al,* 2019. The identification of the compounds was done based on retention time and integral area of peaks. The similarity of compounds matched with listeings based on NIST and Mass Hunter library searches (Eswarainh *et al,* 2019).

2.4 Experimental animals

The animal tests were carried out according to the National Institute of health (NIH) guide for the care and use of laboratory animals, NIH publication (volume 25, number 28), revised 1996. Inbred albino mice of both sexes weighing between 20 and 22g were used for this study. The animals were obtained from the animal house of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. They were acclimatized for seven days and fed mice feed and tap water *ad libitum* (Afolabi and Abejide, 2020).

2.5 Parasite strain for the study

Mefloquine (a prophylactic antimalarial drug) - sensitive *Plasmodium berghei* NK65 strain was used for this study. It was obtained from the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria and maintained in mice by serial passage (Afolabi and Abejide, 2020).

2.6 Malaria prophylactic tests

Antimalarial tests were carried out on the bioactive compounds to evaluate their malaria prophylactic effects against infections of *P. berghei* in mice using procedures described by ref with some modifications. On the first day a set of mice of both sexes were randomly selected and administered orally with 100, 200 and 400mg/kg body weights of the bioactive compounds separately using corn meal as the vehicle. Another set of mice, the negative control, were given 5 $mLkg^{-1}$ distilled water while another set of mice, the positive control, were given $5mgkg^{-1}$ of mefloquine. Treatment was conducted for three consecutive days. On the fourth day the mice were infected with 10^7 *P. berghei* and after 72 hours, blood was taken from the tail of each mouse and examined microscopically to determine the level of parasitaemia suppression and recorded accordingly.

2.7 Data analysis

The data obtained was analyzed using Statistical Package for the Social Science (SPSS) version 20. The mean values of the parameters studied were compared using one-way Analysis of Variance (ANOVA) at 95% confidence interval, and separated using Turkey-b post hoc comparison. Probability values of $p < 0.05$ were considered significant. Results were expressed as mean +/- standard error of mean (SEM).

3.0 Results

Table 1. Prophylactic activity of 1,3,5-Triazine, 2-methylamino-4,6-bis(nonafluoro-tert-butyl) a compound from aqueous leaf extract of *A. boonei* and mefloquine in mice infected with *Plasmodium berghei*

Treatments	Suppression (%)
Distilled water $5mlkg^{-1}$	0.00 ± 0.00^{a}
Compound $100mgkg^{-1}$	25.93 ± 0.58^{b}
Compound $200mgkg^{-1}$	51.22 ± 0.64^{c}
Compound $400mgkg^{-1}$	59.99 ± 0.58^{d}
Mefloquine $5mgkg^{-1}$	86.76 ± 0.64^{e}

[1]All values expressed as mean ± standard error (±SE).

[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison. The antimalarial tests showed that all the doses (100, 200 and 400mg/kg body weight) used produced significant ($p < 0.05$) dose-dependent prophylactic activity against the parasite infection in the treated mice.

Table 2. Prophylactic activity of androsta--2,4,16-triene-3,6,17-triol, tri-TMS a compound from aqueous stem bark extract of *A. boonei* and mefloquine in mice infected with *Plasmodium berghei*

Treatments	Suppression (%)
Distilled water $5mlkg^{-1}$	0.00 ± 0.00^{a}
Compound $100mgkg^{-1}$	46.12 ± 0.58^{b}
Compound $200mgkg^{-1}$	55.65 ± 2.83^{c}
Compound $400mgkg^{-1}$	65.94 ± 1.15^{d}

Mefloquine 5mgkg^{-1}	90.48 ± 1.21^{e}

[1]All values expressed as mean ± standard error (±SE).

[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison. The antimalarial tests showed that all the doses (100, 200 and 400mg/kg body weight) used produced significant ($p < 0.05$) dose-dependent prophylactic activity against the parasite infection in the treated mice.

Table 3. Prophylactic activity of_1-Oxo-forskolin a compound from aqueous root extract of *A. boonei* and mefloquine in mice infected with *Plasmodium berghei*

Treatments	Suppression (%)
Distilled water 5mlkg^{-1}	0.00 ± 0.00^{a}
E Compound xtract 100mgkg^{-1}	52.71 ± 0.64^{b}
Compound 200mgkg^{-1}	69.88 ± 1.15^{c}
Compound 400mgkg^{-1}	71.66 ± 1.62^{c}
Mefloquine 5mgkg^{-1}	93.44 ± 1.41^{d}

[1]All values expressed as mean ± standard error (±SE).

[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison. The antimalarial tests showed that all the doses (100, 200 and 400mg/kg body weight) used produced significant ($p < 0.05$) dose-dependent prophylactic activity against the parasite infection in the treated mice.

Table 4. Prophylactic activity of Aldosterone, N-methoxy-tri-TMS a compound from ethanolic ethanolic leaf extract of *A. boonei* and mefloquine in mice infected with *Plasmodium berghei*

Treatments	Suppression (%)
Distilled water 5mlkg^{-1}	0.00 ± 0.00^{a}
Compound 100mgkg^{-1}	50.22 ± 3.00^{b}
Compound 200mgkg^{-1}	63.35 ± 1.21^{c}
Compound 400mgkg^{-1}	71.55 ± 1.79^{d}
Mefloquine 5mgkg^{-1}	94.68 ± 0.52^{e}

[1]All values expressed as mean ± standard error (±SE).

[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison. The antimalarial tests showed that all the doses (100, 200 and 400mg/kg body weight) used produced significant ($p < 0.05$) dose-dependent prophylactic activity against the parasite infection in the treated mice.

Table 5. Prophylactic activity of Hecogenin acetate a compound from ethanolic stem bark extract of *A. boonei* and mefloquine in mice infected with *Plasmodium berghei*

Treatments	Suppression (%)
Distilled water 5mlkg^{-1}	0.00 ± 0.00^{a}
Compound 100mgkg^{-1}	38.79 ± 1.21^{b}
Compound 200mgkg^{-1}	50.77 ± 1.62^{c}
Compound 400mgkg^{-1}	64.22 ± 1.28^{d}

Mefloquine 5mgkg^{-1}	93.04 ± 1.73^{e}

[1]All values expressed as mean ± standard error (±SE).

[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison. The antimalarial tests showed that all the doses (100, 200 and 400mg/kg body weight) used produced significant ($p < 0.05$) dose-dependent prophylactic activity against the parasite infection in the treated mice.

Table 6. Prophylactic activity of βeta-Dithiodilactic acid a compound from ethanolic root extract of *A. boonei* and mefloquine in mice infected with *Plasmodium berghei*

Treatments	Suppression (%)
Distilled water 5mlkg^{-1}	0.00 ± 0.00^{a}
Compound 100mgkg^{-1}	30.11 ± 1.83^{b}
Compound 200mgkg^{-1}	49.60 ± 0.46^{c}
Compound 400mgkg^{-1}	56.98 ± 1.73^{d}
Mefloquine 5mgkg^{-1}	94.15 ± 1.21^{e}

[1]All values expressed as mean ± standard error (±SE).

[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison. The antimalarial tests showed that all the doses (100, 200 and 400mg/kg body weight) used produced significant ($p < 0.05$) dose-dependent prophylactic activity against the parasite infection in the treated mice.

4.0 Discussion

Analysis of the bioactive compounds from the extracts showed that they belong to different classes of compounds including phenols, alkaloids, carboxylic acids, terpenes, acridones and lactones. Similar to the findings from this study, several bioactive compounds have been found in *Alstonia boonei* such as phenols, alkaloids (Adotey *et al.*, 2012; Babatunde, 2017), carboxylic acids (Balogun et *al.*, 2016; Batista *et al.*, 2009; Imam *et al.*, 2017), terpenes (Marini-Bettolo *et al.*,1983; Olanlokun *et al.*,2021; Rohloff, 2015; Ruikar, *et al.*, 2010) tannins, saponins (Uzor, 2020; Ajayi *et al.*, 2019; Otuokere *et al.*, 2016; Saleh *et al.*, 2019; Saxena *et al.*, 2003), glycosides (Taiye and Pass 2014; Tarkang *et al.*, 2014), acridones and lactones (Uraku, 2015 and Vasanth *et al.*, 1990). Studies by Wilairatana and Looareesuwan 1994, Winter *et al.*, 2006 and Yamuna *et al.*, 2017 also revealed several other bioactive compounds present in *Alstonia* plants including phenols, esters, and diterpenoids. Other studies on bioactive compounds of the plant also revealed similar compounds as in this study comprising carboxylic acids (Wong *et al.*, 2021), diterpenoids (Saleh, 2019), acridones (Winter, 2006), diosenin (Omonirri *et al.*, 2021) and lactones (Chea *et al.*, 2006).

Antimalarial tests were carried out on the bioactive compounds from the extracts in order to determine their antimalarial prophylaxis activity. The antimalarial tests showed that all the doses (100, 200 and 400mg/kg body weight) used produced significant ($p < 0.05$) dose-dependent prophylactic effect against *P. berghei* parasite infection in the treated mice as follows:

Aqueous leaf bioactive compound prophylactic effect of 25.93 ± 0.58, 51.22 ± 0.64, 59.99 ± 0.58 for the 100, 200 and 400 mg/kg body weights respectively; aqueous stem bark bioactive compound prophylactic effect of 46.12 ± 0.58, 55.65 ± 2.83, 65.94 ± 1.15 for the 100, 200 and 400 mg/kg body weights respectively; aqueous root bioactive compound prophylactic effect of 52.71 ± 0.64,

69.88 ± 1.15, 71.66 ± 1.62 for the 100, 200 and 400 mg/kg body weights respectively; ethanolic leaf bioactive compound prophylactic effect of 50.22 ± 3.00, 63.35 ± 1.21, 71.55 ± 1.79 for the 100, 200 and 400 mg/kg body weights respectively;

ethanolic stem bark bioactive compound prophylactic effect of 38.79 ± 1.21, 50.77 ± 1.62, 64.22 ± 1.28 for the 100, 200 and 400 mg/kg body weights respectively and ethanolic root bioactive compound prophylactic effect of 30.11 ± 1.83, 49.60 ± 0.46, 56.98 ± 1.73 for the 100, 200 and 400 mg/kg body weights respectively, with all values expressed as mean ± standard error (±SE).

The prophylactic activity of the bioactive compounds tested, namely, 1,3,5-Triazine, 2-methylamino-4,6-bis(nonafluoro-tert-butyl) a methoxymethylbutyl class compound from aqueous leaf extract of *A. boonei* indicated that the minimal prophylactic effect was found in the lowest administered dose of 100mg/kg body weight while the optimal prophylactic effect was in the highest administered dose of 400mg/kg body weight as shown in Table 1. In a similar study on methoxymethylbutyl class compound, Wong *et al.,* 2021 evaluated chemical constituents from the crude extract of *Dendrocalamus asper* using chromatographic methods for their antimalarial potential and of all the chemicals tested, dimethyl-15,16-dibutoxytriconta-11,13,17,19-tetraenedioate, methoxyl-4-hydroxybenzoate and 1-methoxy-4-(methoxymethyl)benzoate showed promising antimalarial activity with IC_{50} between 0.8-2.2 ug/Ml.

In the prophylactic activity of androsta--2,4,16-triene-3,6,17-triol, tri-TMS a lactone class compound from aqueous stem bark extract of *A. boonei* (Table 2), it showed that the minimal prophylactic effect was found in the lowest administered dose of 100mg/kg body weight while the optimal prophylactic effect was in the highest administered dose of 400mg/kg body weight as shown in Table 2. Other similar studies have reported the antimalarial effects of lactones from plants. Chea *et al.,* 2006 in their study found out that lactones possess antimalarial activity. They tested three lactones and compounds 1 8 alpha-tigloyloxy-hirsutinolide-13-*O*-acetate, 7 8 alpha-(4-hydroxymethacryloyloxy)-hirsutinolide-13-*O*-acetate and 8-alpha-(4-hydroxytigloyloxy)-hirsutinolide-13-*O*-ac exhibited significant antimalarial activity with IC_{50} 3.9, 3.7 and 3.5um respectively.

The result from the prophylactic test of 1-Oxo-forskolin a diterpenoid compound from aqueous root extract of *A. boonei* revealed that the lowest prophylactic effect was in the lowest administered dose of 100mg/kg body weight while the highest prophylactic effect was in the highest administered dose of 400mg/kg body weight as recorded in Table 3. Saleh 2019, carried out a study on the therapeutic potential of the Labdone Diterpenoid Forskolin and also recorded a significant antimalarial activity by the compound which agrees to this finding.

The prophylactic activity of Aldosterone, N-methoxy-tri-TMS an acridone class compound from ethanolic leaf extract of *A. boonei* showed that the lowest prophylactic effect was found in the lowest administered dose of 100mg/kg body weight while the highest prophylactic effect was in the highest administered dose of 400mg/kg body weight as shown in Table 4. The antimalarial potential of acridone compounds have been reported in other studies. Winter 2006 reported significant antimalarial activity of acridone compounds.

In the prophylactic activity of Hecogenin acetate a diosgenin compound from ethanolic stem bark extract of *A. boonei*, it was revealed that the lowest prophylactic effect was found in the lowest administered dose of 100mg/kg body weight while the highest prophylactic effect was in

the highest administered dose of 400mg/kg body weight. Omonirri *et al.,* 2021 studied the antimalarial activity of diosgenin compound and reported that the compound exerted significant antimalarial activity on the tested murine model which is akin to the result of this study.

The result from the prophylactic test of βeta-Dithiodilactic acid a compound from ethanolic root extract of *A. boonei* revealed that the lowest prophylactic effect was in the lowest administered dose of 100mg/kg body weight while the highest prophylactic effect was in the highest administered dose of 400mg/kg body weight as recorded in Table 6. This result revealed the antimalarial prophylaxis potential of this compound.

5.0 Conclusion and Recommendations

The antimalarial tests carried out in this study revealed that the bioactive compounds exerted significant dose – dependent prophylaxis against malaria parasite infection on *Plasmodium berghei* treated mice. Thus these bioactive compounds should be further studied and positioned for novel antimalarial drug development.