



Experimental and Mathematical Model for the Antimalarial Activity of Methanolic Root Extract of *Azadirachta indica* (Dongoyaro) in Mice Infected with *Plasmodium berghei* NK65

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AMO and MJO designed the study, helped in literature searches, wrote the protocol and the first draft of the manuscript. Author AMO designed the model and assisted in the statistical analysis. Author MJO designed and carry out the experimental protocols. Author AOR carry out the statistical analysis and assisted in the design of the model. All authors read and approved the final manuscript.

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Abstract

The study determines the experimental and mathematical model for the anti-plasmodial activity of methanolic root extract of *Azadirachta indica* in Swiss mice infected with *Plasmodium berghei* NK65. Phytochemical analyses, antimalarial activity of the methanolic root extract of *A. indica* was determined in mice infected with *Plasmodium berghei* NK65 using standard procedure. Liver biomarker enzymes were also determined. The model *P. berghei* induced free and *P. berghei* infected equilibrium were determined. The stability of the model equilibrium points was rigorously analyzed. The phytochemicals present in the extract include: alkaloid, flavonoid, saponin and phenolic compounds etc. The experimental study consists of five groups of five mice each per group. Group A, B, C, D and E were healthy, infected without treatment, infected mice treated with fansidar (10 mg/kg), chloroquine (10 mg/kg) and 250 mg/kg body weight of *A. indica* methanolic root extract respectively. The extract showed anti-plasmodial

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activity of 73.96%. The result was significant when compared with group B mice, though it was lower than that exhibited by fansidar (88.91%) and chloroquine (92.18%) for suppressive test. There were significant decrease ($P < 0.05$) in plasma AST and ALT levels in the treated infected mice compared to the infected untreated mice. The results of the model showed that the *P.berghei* induced free equilibrium is locally and globally asymptotically stable at threshold parameter, R_0 less than unity and unstable when R_0 is greater than unity. Numerical simulations were carried out to validate the analytic results which are in agreement with the experimental analysis of this work.

Keywords: *Azadirachta indica*; anti-plasmodial activity; mathematical model; *Plasmodium berghei* NK 65; Swiss mice.

1 Introduction

Malaria is the most devastating parasitic infection, afflicting more than 500 million people every year [1]. The mortality rate is approximated at over a million people per year and has risen in recent years, probably due to increasing resistance to antimalarial drugs [2]. Malaria is caused by parasitic protozoan of the genus *Plasmodium*, with five species known to cause the disease in humans (*Falciparum*, *Malariae*, *ovale*, *Vivax* and *Knowlesi*). Study has shown that *P. falciparum* is the leading cause of death worldwide in 2004, from a single infectious agent [3]. The disease is characterized by fever, chills, headache, nausea, tiredness and general body discomfort. In severe cases, the disease causes complications such as: convulsion, anemia and cerebral malaria. Greenwood et al. [4] study shows that the disease primarily affects poor populations in the tropical and subtropical areas, where temperature and rainfall are suitable for the development of parasites and vectors. Study has shown that Nigeria accounts for a quarter of all malaria cases in Africa [5]. Transmission in the southern part of Nigeria occurs all year round while in the north part it is more seasonal. The resistance to antimalarial drugs by *P. falciparum* poses a serious threat to malaria treatment and control. In Nigeria, a study has showed that chloroquine and sulphadoxine-pyrimethamine are no longer viable therapeutic options for the proper treatment of malaria [6]. Traditionally used medicinal plants have played important role in malaria treatment across the world [7,8]. Natural products from traditional plants can be good source of new antimalarial drugs [9]. *Azadirachta indica* commonly called Neem is one of the most useful traditional medicinal plant used in the treatment of malaria locally.

Many researchers [10-12] have studied disease transmission such as malaria disease. Mathematical models are employed to gain useful insights into understanding the behavior of the disease. Mathematical models have played greater roles in influencing decision making processes on intervention measures to prevent and control disease like malaria. In this work, we formulate and analyze a mathematical model to study the suppressive efficacies of standard drugs (fansidar and chloroquine) and *A. indica* (Dongoyaro) methanolic root extract. The Swiss male albino mice were divided into susceptible, induced infected without treatment and induced infected with treatment and asymptomatic mice respectively. Equal proportions of induced infected mice with treatment are treated with standard drugs (fansidar and chloroquine) and Dongoyaro extract respectively. Thus, our model is based on the susceptible-infective without treatment-infected with treatment-asymptomatic (SITA) in mice' population after inducing the mice with *P. berghei* NK65. The suppressive rate corresponds to how quickly parasites are reduced in the mice after treatment. The methanolic root extract of *Azadirachta indica* was screened for their antimalarial activity in *Plasmodium berghei* NK65 infected Swiss mice.

2 Materials and Methods

2.1 Collection and identification of *Azadirachta indica*

The root of *Azadirachta indica* plant was obtained from Lagos State Polytechnic Ikorodu in Lagos State, Nigeria. The plant was authenticated from the department of Botany, University of Lagos, Nigeria. Authentication number of 6967 was given.

2.2 Preparation of *Azadirachta indica* methanolic root extract

The root of *Azadirachta indica* was washed with water to remove dirt, air dried under shade in the Biochemistry laboratory, and later pulverized into coarse powder using industrial machine. Extraction was carried out by dispersing 100g of the grounded *Azadirachta indica* plant material into one litre of 70% methanol at room temperature. Shaking and maceration was done for 72 hours. The extract was filtered by passing it through cotton wool followed by Whatman No.1 filter paper and the resultant filtrate was concentrated with the help of rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was dried to complete dryness in an aerated oven at 40°C for 48 hours. The extract was later stored in a refrigerator at 4°C.

2.3 Qualitative phytochemical analysis of *Azadirachta indica*

Phytochemical analyses for phytochemical constituents were carried out on the root extract of *A. indica* using standard phytochemical procedures.

2.3.1 Determination of saponins

Froth test: 2 mL of root extract of *A. indica* solution was shaken vigorously with distilled water to form froth and was then allowed to stand for 10– 15 min. The persistent froth was considered as presence of saponin.

2.3.2 Determination of tannins

Two millilitre of the root extract of *A. indica* solution was stirred with equal volume of distilled water. Few drops of 2% FeCl₃ solution were added. The presence of tannins was indicated by the formation of a green precipitate.

2.3.3 Determination of alkaloids

Mayer's test: The root extract of *A. indica* solution was mixed with HCl and filtered. The filtrate was treated with Mayer's reagent. The formation of a yellow colour precipitate indicates the presence of alkaloids.

Wagner's test: The root extract of *A. indica* solution was mixed with HCl and filtered. The filtrate was treated with Wagner's reagent. The formation of brown or reddish precipitate indicates the presence of alkaloids.

2.3.4 Determination of flavonoids

Shinoda's test: A piece of magnesium ribbon and HCl were added to the root extract of *A. indica* solution. Purple colour confirmed the presence of flavonoids.

2.3.5 Determination of carbohydrates

Molisch's test: Two millilitre of Molisch's reagent was shaken with 3 mL of the root extract of *A. indica* solution. Then 2 mL of concentrated H₂SO₄ was added carefully down the side of the test tube. The presence of carbohydrates was indicated by a violet ring at the interphase.

2.3.6 Determination of cardiac glycosides

Liebermann's test: Two millilitre of the root extract of *A. indica* solution was mixed with 2 mL of chloroform and 2 mL of acetic acid and the solution was cooled on ice. H₂SO₄ was then added carefully. A

colour change from violet to blue to green indicates the presence of a steroidal nucleus. This indicates a glycone portion of glycoside.

2.3.7 Determination of simple phenolics

Ferric chloride (FeCl₃) test: One millilitre of the root extract of *A. indica* solution was mixed with 1–2 drops of 1% FeCl₃. Development of blue-green colouration indicative the presence of phenolic compound.

2.3.8 Tests for Protein and amino acids

Ninhydrin test: 3 ml of the *A. indica* root extract solution was heated with 3 drops of 5% Ninhydrin solution on a water bath for 10 min. The formation of blue colour indicates the presence of amino acids.

2.4 Experimental animals

All the Swiss mice used have a weight ranging from 24 to 33g. They were obtained from University of Lagos from Nigeria. They were acclimatized for one week to Laboratory condition of 23±2°C. They were kept in plastic cages and fed with commercial rat chow and supply with water *ad libitum*. The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised, [13].

2.5 Malaria parasites

The chloroquine-sensitive *Plasmodium berghei* NK65 was used to assess the antimalarial activity of methanolic root extract of *A.indica*. The parasite was obtained from National Institute for Medical Research (NIMR) Lagos, Nigeria. The parasites were maintained by continuous re-infestation into Swiss mice.

2.6 Inocula

Parasitized erythrocytes were obtained from a donor infected mouse by ocular puncturing. This was prepared by determining the percentage parasitemia and the erythrocytes count of the donor mouse and diluting them with normal saline in proportions indicated by both determinations. Each mouse was inoculated intraperitoneally with infected blood suspension containing 1×10⁷ *Plasmodium berghei* NK65 parasitized red blood cells.

2.7 Antiplasmodial activity

Suppressive test: For this study, a 4-days suppressive test described by Akuodor et al. [14] and Mbah et al. [15] were employed for this study. Twenty Swiss mice weighing between 24 to 33 gram were passaged intraperitoneally with standard inocula of *P. berghei* NK65 containing 1×10⁷ infected erythrocytes. Four hours after inoculation, the infected mice were randomly divided into four (4) groups (group B to E) of 5 mice per cage and treated for four consecutive days (D1-D4). Group A mice are not infected with *P. berghei* NK65 and received 0.2 mL of normal saline. Group B mice are infected with *P. berghei* NK65 but received no treatment. Group C and D received 10 mg/kg body weight of fansidar and 10 mg/kg body weight of Chloroquine diphosphate respectively. Group E received 250 mg/kg body weight of the methanolic root extract of *A. indica*. All doses were administered orally. On the fifth day (D5), thin films were made from the tail blood of each mouse. The films were fixed with methanol, stained with 10% Giemsa and parasite density determined microscopically by counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields [16]. The percent (%) parasitemia and percent (%) suppression of parasitemia were calculated.

1. % parasitemia = (No. of parasitized RBC / Total nob. of RBC counted) X 100

2. Percent suppression of parasitemia was calculated = (Parasitemia of negative control - parasitemia of test / Parasitemia of negative control) X 100

2.8 Determination of liver biomarkers enzymes

Plasma enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using Randox diagnostic kits.

2.9 Mathematical formulation

In this study, a mathematical model that focus on the suppressive property of standard drugs such as fansider and chloroquine; and methanolic root extract of dongoyaro (*Azadirachta indica*) is developed. The Swiss mice population under consideration is divided into Susceptible $S(t)$ rats, induced infected mice without treatment $I(t)$, induced infected mice with treatment and the asymptomatic mice. The induced infected mice with treatment were further classified into (i) infected mice treated with fansider $I_f(t)$ (ii) infected mice treated with chloroquine $I_c(t)$ and infected mice treated with *A. indica* root extract $I_D(t)$. The total mice population is given by

$$N(t) = S(t) + I(t) + I_f(t) + I_c(t) + I_D(t) + A(t) \tag{1}$$

The proportion of infected mice treated with fansider, chloroquine and *A. indica* methanolic extract are α_f , α_c and α_D respectively; $0 < \varepsilon_f, \varepsilon_c, \varepsilon_D < 1$. The efficacy of each of the treatment to suppress the *Plasmodium berghei* for fansider, chloroquine and *A. indica* are $\varepsilon_f, \varepsilon_c$ and ε_D while the rate of progression to the asymptomatic class after receiving treatment are δ_f, δ_c and δ_D for fansider, chloroquine and Dongoyaro respectively.

While developing this model, the following assumptions were made:

- i. The rats population is closed
- ii. There is no birth since all mice are male
- iii. No death occurs; whether natural or disease induced deaths. (Note that during the course of the experiment no death was recorded)
- iv. Treated mouse progress to the asymptomatic class since no mouse recovered completely in view of the number of days the treatments lasted.

Taking into account of the above considerations, the following non-linear differential equations is obtained:

$$\left. \begin{aligned} \frac{dS(t)}{dt} &= N(t) - (\alpha_1 + \alpha_f + \alpha_c + \alpha_D) \frac{\beta I(t)S(t)}{N(t)} \\ \frac{dI(t)}{dt} &= \alpha_1 \frac{\beta I(t)S(t)}{N(t)} \\ \frac{dI_f(t)}{dt} &= \alpha_f \frac{\beta I(t)S(t)}{N(t)} - \varepsilon_f \delta_f I_f(t) \\ \frac{dI_c(t)}{dt} &= \alpha_c \frac{\beta I(t)S(t)}{N(t)} - \varepsilon_c \delta_c I_c(t) \\ \frac{dI_D(t)}{dt} &= \alpha_D \frac{\beta I(t)S(t)}{N(t)} - \varepsilon_D \delta_D I_D(t) \\ \frac{dA(t)}{dt} &= \varepsilon_f \delta_f I_f(t) + \varepsilon_c \delta_c I_c(t) + \varepsilon_D \delta_D I_D(t) \end{aligned} \right\} \tag{2}$$

The variables and parameter descriptions are given in Table 1 and Table 2 respectively.

Table 1. Variable description

Variable	Description
x_1	Susceptible rats
x_2	Induced infected rats without treatment
x_3	Induced infected rats with fansider treatment
x_4	Induced infected rats with chloroquine treatment
x_5	Induced infected rats with <i>A.indica</i> extract treatment
x_6	Asymptomatic rats

Table 2. Parameter description

Parameters	Description	Value	Source
β	Induced transmission coefficient	$0 < \beta \leq 1$	Assumed
α_1	Proportion of induced infected rats without treatment	0.2	Estimated
α_f	Proportion of induced infected rats with fansider treatment	0.2	Estimated
α_c	Proportion of induced infected rats with chloroquine treatment	0.2	Estimated
α_D	Proportion of induced infected rats with Dongoyaro treatment	0.2	Estimated
δ_f	Rate of progression to asymptomatic class due to treatment with Fansider	0.89	Assumed
δ_c	Rate of progression to asymptomatic class due to treatment with chloroquine	0.92	Assumed
δ_D	Rate of progression to asymptomatic class due to treatment with Dongoyaro	0.74	Assumed
ε_f	Efficacy of fansider drug	0.89	Estimated
ε_c	Efficacy of chloroquine drug	0.92	Estimated
ε_D	Efficacy of Dongoyaro root extract	0.74	Estimated

2.10 Model analysis

In this section, attention is focused on normalizing the model system; determine the equilibrium points of the model and stability analysis using stability theories. For convenience, the system of differential equations in (2) is normalized as follows:

Let $S(t) = N(t)x_1(t)$, $I(t) = N(t)x_2(t)$, $I_f(t) = N(t)x_3(t)$, $I_c(t) = N(t)x_4(t)$, $I_D(t) = N(t)x_5(t)$, $A(t) = N(t)x_6(t)$, where $x_1(t) + x_2(t) + x_3(t) + x_4(t) + x_5(t) + x_6(t) = 1$. Thus, we obtain:

$$\left. \begin{aligned}
 \frac{dx_1(t)}{dt} &= 1 - (\alpha_1 + \alpha_f + \alpha_c + \alpha_D)\beta x_2(t)x_1(t) \\
 \frac{dx_2(t)}{dt} &= \alpha_1\beta x_2(t)x_1(t) \\
 \frac{dx_3(t)}{dt} &= \alpha_f\beta x_2(t)x_1(t) - \varepsilon_f\delta_f x_3(t) \\
 \frac{dx_4(t)}{dt} &= \alpha_c\beta x_2(t)x_1(t) - \varepsilon_c\delta_c x_4(t) \\
 \frac{dx_5(t)}{dt} &= \alpha_D\beta x_2(t)x_1(t) - \varepsilon_D\delta_D x_5(t) \\
 \frac{dA(t)}{dt} &= \varepsilon_f\delta_f x_3(t) + \varepsilon_c\delta_c x_4(t) + \varepsilon_D\delta_D x_5(t)
 \end{aligned} \right\} \tag{3}$$

The solutions $x_1(t), x_2(t), x_3(t), x_4(t), x_5(t)$ and $x_6(t)$ of system (2) with initial conditions $x_1(t) > 0, x_2(t) > 0, x_3(t) > 0, x_4(t) > 0, x_5(t) > 0$ and $x_6(t) \geq 0$ are assumed to be non-negative for all time $t > 0$, then, under this assumption, system (2) is said to be epidemiologically well-posed and meaningful.

2.11 Equilibrium points

The equilibrium point of system (2) is obtained by equating the derivatives on the left-hand-side of (2) to zero and solving the resulting algebraic equations for the dynamic variables. Thus, the equilibrium points of system (2) are:

$$E_0 = (x^0_1, x^0_2, x^0_3, x^0_4, x^0_5, x^0_6) = (1,0,0,0,0,0) \tag{4}$$

and

$$E^* = \left(\begin{array}{l} \frac{1}{\alpha_1\beta}, \frac{(\alpha_1\beta-1)}{\beta(\alpha_1+\alpha_f+\alpha_c+\alpha_D)}, \frac{\alpha_f(\alpha_1\beta-1)}{\alpha_1\beta(\alpha_1+\alpha_f+\alpha_c+\alpha_D)(1+\varepsilon_f\delta_f)}, \\ \frac{\alpha_c(\alpha_1\beta-1)}{\alpha_1\beta(\alpha_1+\alpha_f+\alpha_c+\alpha_D)(1+\varepsilon_c\delta_c)}, \frac{\alpha_D(\alpha_1\beta-1)}{\alpha_1\beta(\alpha_1+\alpha_f+\alpha_c+\alpha_D)(1+\varepsilon_D\delta_D)}, \\ \frac{(\alpha_1\beta-1)(\alpha_f(1+\varepsilon_c\delta_c)(1+\varepsilon_D\delta_D)+\alpha_c(1+\varepsilon_f\delta_f)(1+\varepsilon_D\delta_D)+\alpha_D(1+\varepsilon_c\delta_c)(1+\varepsilon_f\delta_f))}{\alpha_1\beta(\alpha_1+\alpha_f+\alpha_c+\alpha_D)(1+\varepsilon_f\delta_f)(1+\varepsilon_c\delta_c)(1+\varepsilon_D\delta_D)} \end{array} \right) \tag{5}$$

Where E_0 is referred to a *P. berghei* induced free equilibrium (PIFE). It is a state where the mice are not induced and infection free while E^* represent equilibrium in the mice population where the rats are induced and infected. This state is called *P. berghei* induced infected equilibrium (PIIE).

2.12 Stability analysis of the model equilibrium points

In the following section, focus is on the stability analysis of the model steady states by considering conditions for which local and global stability of E_0 exist. The local stability of E^* will also be considered.

2.12.1 Local asymptotic stability of Plasmodium Induced Free Equilibrium (PIFE), E_0

We discuss the Local Asymptotic Stability of E_0 by considering the following theorem:

Theorem 3.1: Consider model system (3), then the Plasmodium Induced Free Equilibrium (PIFE), E_0 is locally asymptotically stable if $R_0 < 1$ and unstable if $R_0 > 1$.

Proof:

Consider the following Jacobian matrix of system (3) determined at E_0 given as follows:

$$J(E_0) = \begin{pmatrix} -1 & -\beta A_0 & 0 & 0 & 0 & 0 \\ 0 & (\beta\alpha_1 - 1) & 0 & 0 & 0 & 0 \\ 0 & \alpha_f\beta & -(1 + A_1) & 0 & 0 & 0 \\ 0 & \alpha_c\beta & 0 & -(1 + A_2) & 0 & 0 \\ 0 & \alpha_D\beta & 0 & 0 & -(1 + A_3) & 0 \\ 0 & 0 & A_1 & A_2 & A_3 & -1 \end{pmatrix} \tag{6}$$

where $A_0 = (\alpha_1 + \alpha_f + \alpha_c + \alpha_D)$, $A_1 = \varepsilon_f\delta_f$, $A_2 = \varepsilon_c\delta_c$, $A_3 = \varepsilon_D\delta_D$. The eigenvalues of (6) are $\lambda_1 = \beta\alpha_1 - 1$, $\lambda_2 = -(1 + A_1)$, $\lambda_3 = -(1 + A_2)$, $\lambda_4 = -(1 + A_3)$, $\lambda_5 = -1$. In particular,

$$\lambda_1 = \beta\alpha_1 - 1 = (R_0 - 1) < 0, \text{ if } R_0 < 1.$$

Hence, E_0 of system (3) is locally asymptotically stable if $R_0 < 1$, which guarantee that all eigenvalues of (6) are real and negative; however, not all eigenvalues of (6) are negative if $R_0 > 1$ which makes E_0 unstable.

Remark: $R_0 = \beta\alpha_1$ is called the induced basic reproduction number and is defined as the average infection caused by inducing a mouse with plasmodium. This quantity determines if the infection persist or dies out. Suppose, E_0 is not locally asymptotically stable or the stability of E_0 does not depend on the initial size of the infected population, then it is important to look more closely at the condition for which the global stability holds. This shall be discussed in the next section.

2.12.2 Global stability of Plasmodium Induced Free Equilibrium (PIFE), E_0

Theorem 3.2: Consider system (2) that describes a male mice population, then, E_0 is of system (2) is globally asymptotically stable if $R_0 \leq 1$ otherwise E_0 is unstable.

Proof: Consider a Lyapunov function defined below:

$$L(x_2(t)) = x_2(t) \tag{7}$$

By differentiating (7) with respect to t along the solution of (3), we have

$$\begin{aligned} L'(x_2(t)) &= x_2'(t) \\ &= \beta\alpha_1 x_2(t)x_1(t) - x_2(t) \\ &= (\beta\alpha_1 x_1(t) - 1)x_2(t) \end{aligned}$$

So that at plasmodium induced free equilibrium E_0 , $x_1(t) = 1$, then

$$\begin{aligned} L'(x_2(t)) &= (\beta\alpha_1 - 1)x_2(t) \\ &= (R_0 - 1)x_2(t) \leq 0 \text{ if } R_0 \leq 1. \end{aligned}$$

Thus, E_0 is globally asymptotically stable if $R_0 \leq 1$ otherwise unstable.

2.13 Local stability of Plasmodium Induced Infected Equilibrium (PIIE), E^*

Suppose that the condition for global stability of E_0 is not satisfied, then, there is possibility of infection in the mice population. Thus, it is important to investigate the local stability of plasmodium induced infected equilibrium (PIIE), E^* . We begin this with the following Lemma:

Lemma 3.1: System (3) has a unique and positive equilibrium E^* if $R_0 > 1$.

Proof: Recall the components of E^* written as follows:

$$\left. \begin{aligned} x_1^* &= \frac{1}{R_0} \\ x_2^* &= \frac{R_0 - 1}{\beta A_0} \\ x_3^* &= \alpha_f \frac{(R_0 - 1)}{\beta A_0(1 + A_1)} \\ x_4^* &= \alpha_c \frac{(R_0 - 1)}{\beta A_0(1 + A_2)} \\ x_5^* &= \alpha_D \frac{(R_0 - 1)}{\beta A_0(1 + A_3)} \\ x_3^* &= \frac{(R_0 - 1)[\alpha_f(1 + A_2)(1 + A_3) + \alpha_c(1 + A_1)(1 + A_3) + \alpha_D(1 + A_1)(1 + A_2)]}{\beta A_0(1 + A_1)(1 + A_2)(1 + A_3)} \end{aligned} \right\} \tag{8}$$

Observe that the components of (8) will be positive and indeed give one unique endemic equilibrium if $R_0 > 1$. The result follows immediately that E^* has a positive and unique endemic equilibrium.

Theorem 3.3: Given that Lemma 3.1 holds, the plasmodium induced infected equilibrium (PIIE), E^* of system (3) is locally asymptotically stable if $R_0 > 1$ and unstable if $R_0 < 1$.

Proof: In view of the Jacobian matrix of (3) determined at E^* , we have

$$J(E^*) = \begin{pmatrix} -R_0 & -A_0 & 0 & 0 & 0 & 0 \\ \frac{(R_0-1)}{A_0} & 0 & 0 & 0 & 0 & 0 \\ \frac{\alpha_f(R_0-1)}{A_0} & \alpha_f & -(1+A_1) & 0 & 0 & 0 \\ \frac{\alpha_c(R_0-1)}{A_0} & \alpha_c & 0 & -(1+A_2) & 0 & 0 \\ \frac{\alpha_D(R_0-1)}{A_0} & \alpha_D & 0 & 0 & -(1+A_3) & 0 \\ 0 & 0 & A_1 & A_2 & A_3 & -1 \end{pmatrix} \quad (9)$$

with the following eigenvalues:

$\lambda_1 = -1$, $\lambda_2 = -1$, $\lambda_3 = -(1+A_1)$, $\lambda_4 = -(1+A_2)$, $\lambda_5 = -(1+A_3)$, $\lambda_6 = (1-R_0)$. In particular, $\lambda_6 = (1-R_0) < 0$, if $R_0 > 1$. Thus all eigenvalues of (9) are real and negative if $R_0 > 1$, which implies that the plasmodium induced infected equilibrium (PIIE), E^* is locally asymptotically stable if $R_0 > 1$ and unstable if $R_0 < 1$.

2.14 Statistical analysis

Data was expressed as mean \pm SD. Parasitemia of the different groups were assessed by unpaired student t-test using Graphpad prism InStat Demo version 5.01. Comparisons across the row was done using one way analysis of variance (ANOVA) and comparison Bonferroni's compare all pair of columns. A $P < 0.05$ was considered statistical significant.

3 Results

3.1 Phytochemical screening of *Azadirachta indica* methanolic root extract

Phytochemical screening of the methanolic root extract of *Azadirachta indica* shows the present of secondary metabolite like saponin, alkaloids, tannins, phenolic compounds, flavonoid etc (Table 3).

Table 3. The qualitative phytochemical constituents of *Azadirachta indica* methanolic root extract

Phytochemical constituent	Test performed	Inference
Alkaloids	Mayer's test	+
	Wagner's test	+
Tannins	Ferric chloride (FeCl ₃) test	+
Phenolic compounds	Ferric chloride (FeCl ₃) test	+
Saponins	Froth test	+
Flavonoids	Shinoda test	+
Protein and amino acids	Ninhydrin Test	-
Cardiac glycoside	Liebermann's test	+
Carbohydrate	Molisch's test	+

+ mean present, - absent

3.2 Evaluation of antiplasmodial activity of the methanolic root extract of *Azadirachta indica*

Azadirachta indica methanolic root extract showed a reduction of parasitemia in Swiss mice. This reduction is statistically significant ($P < 0.05$) when related to the negative control (73.96%). The result was lower than that exhibited by fansidar (88.91%) at 10 mg/kg body weight and chloroquine (92.18 %) at 10 mg/kg body weight for the suppressive test.

Table 4. Suppressive effects of methanolic root extract of *Azadirachta indica* against Swiss mice induce with *Plasmodium berghei* NK65

Group	Dose (mg/Kg)	Mean Parasitemia density (D4) for suppressive test	% Suppression for suppressive test
A	0.2mL	NIL	NIL
B	0.2mL	13.17±1.14	NIL
C	10	1.46±0.29	88.91*
D	10	1.03±0.06	92.18*
E	250	3.43±0.84	73.96*

Values are expressed in mean ± SD for five mice in each group. Unpaired student t-test was used to compare the untreated group mice and the treated groups' mice. *indicate significant difference at $P < 0.05$

3.3 Determination of liver biomarker enzymes

The plasma of Swiss mice in the suppressive test were assayed for liver biomarker enzymes activities. The result shows that AST and ALT concentration of group B mice were significantly increased ($P < 0.05$) when compared to mice in other groups.

Table 5. Determination of plasma liver biomarker enzymes of uninfected mice and infected mice treated with fansidar, chloroquine and the extract of *Azadirachta indica*

Parameters	Group A	Group B	Group C	Group D	Group E
AST (U/L)	8.22 ± 0.85 ^c	56.15 ± 4.40 ^a	21.13 ± 3.52 ^b	18.45 ± 1.80 ^b	17.85 ± 2.55 ^b
ALT (U/L)	7.16 ± 0.65 ^d	59.14 ± 5.28 ^a	28.82 ± 4.75 ^b	23.45 ± 2.24 ^{bc}	20.12 ± 3.50 ^c

The values are mean ± S.D. for five rats in each group. Comparisons across the row were done using Bonferroni's multiple comparison. Values with different alphabet superscript (a= highest, d= lowest in that order) in the same row indicate significantly different ($P < 0.05$). A $P < 0.05$ was considered statistically significant

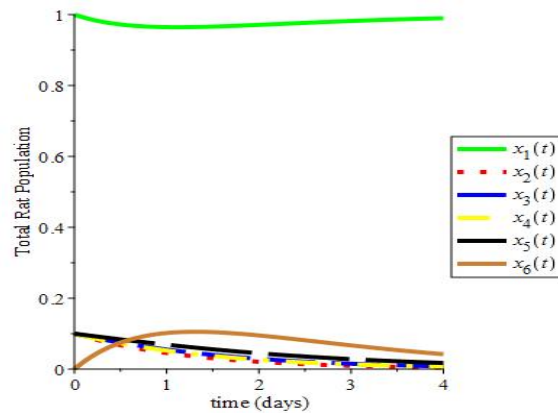


Fig. 1. The graph of the total mice population against time

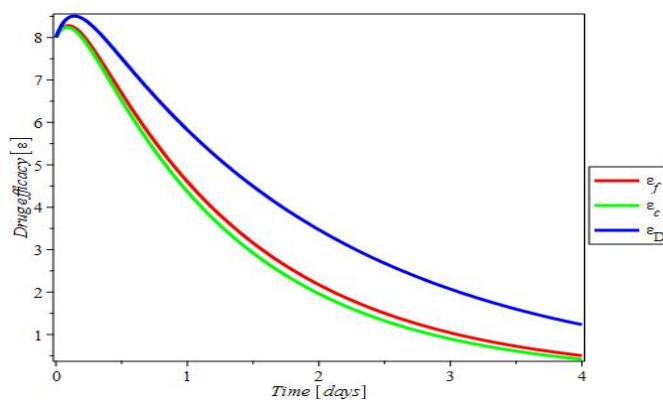


Fig. 2. Graph of drug efficacies against time

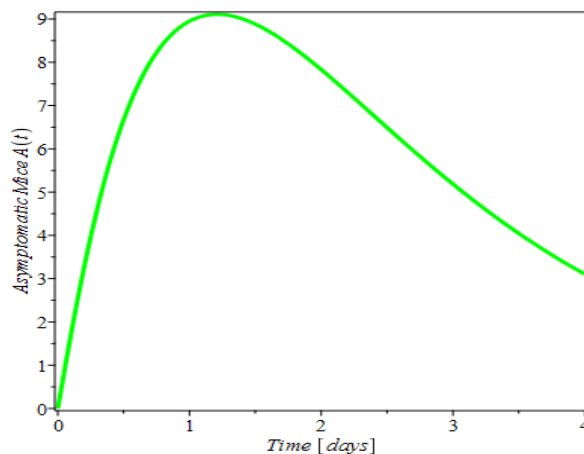


Fig. 3. Graph of the asymptomatic rats against time

4 Discussion

Phytochemicals are major constitute found in medicinal plants and are responsible for their numerous bioactivities and metabolisms. The result of the qualitative phytochemical analysis shows that the methanolic root extract of *A. indica* contain some secondary metabolites like flavonoids, alkaloids, saponins, tannin, etc. (Table 3). Manda et al. [17] study shows that *A.indica* methanolic root extract possess carbohydrate, flavonoids, alkaloids, phenolic compound, saponins, tannin. Amino acids and protein were absent. Different studies have shown that antimalarial properties of medicinal plants have been associated with a single metabolite or a combination of its secondary metabolites such as terpenoids, flavonoids, alkaloids and phenolic compounds. These metabolites have been reported in different research as having antimalarial property [18-20]. The presences of these secondary metabolites in *A. indica* root extract are responsible for the plant antimalarial activity. The GC-MS analysis of *A. indica* hexane root extract has been presented in our previous result [21]. We show that it contains seven different compounds: Hexadecanoic acid, methyl ester (7.51%), 9-Octadecenoic acid, methyl ester (70.14%), Methyl stearate (6.78%), Docosanoic acid, methyl ester (5.72%), (E)-9-Octadecenoic acid ethyl ester (4.03%), Cis-11-Eicosenoic acid, methyl ester (2.79%) and Methyl 18-Methylnonadecanoate (3.04%). The major prominent compound identified in the GC-MS result of our previous result was 9-Octadecenoic acid, methyl ester with 70.14% peak area [21]. Studies have shown that 9-Octadecenoic acid, methyl ester compound has the

following biological activities: anticarcinogenic; antioxidant activity; exists in human urine and blood where it serves as endogenous peroxisome proliferator-activated receptor ligand and dermatogenic flavor [22-24].

The *in-vivo* antiplasmodial activities of *A. indica* methanolic root extract was investigated by evaluating the suppressive activities of the plant. In this study, fansidar and chloroquine was used as the standard antimalarial drugs. One of the standard drug used in this study like Chloroquine may interrupts with heme polymerization by forming a FP-chloroquine complex. This complex reaction is responsible for the disruption of the parasite's cell membrane function and ultimately leads to auto digestion. Significant reduction ($P < 0.0001$) in percentage parasitemia of all the treated groups were found when compared with infected untreated mice (group B). The study revealed that *A. indica* methanolic root extract significantly reduces parasitemia in the animal compared to that of the standard drugs tested. The plant demonstrated antiplasmodial activity of 73.96%, for suppressive test (Table 4). The result was very significant when compared to the infected mice without treatment. The result obtained was lower than that exhibited by fansidar (88.91%) at 10 mg/kg body weight and chloroquine (92.18%) at 10 mg/kg body weight for suppressive test. *In-vivo* antiplasmodial activity study classified antiplasmodial activity as very good, good, and moderate if an extract displayed percentage parasitemia suppression equal to or greater than 50% at a dose of 100, 250 and 500 mg/kg per day respectively [25]. This study signifies that *A. indica* methanolic root extract has antiplasmodial activity. Other studies have shown the antiplasmodial activities of *A. indica* [26, 27].

Plasma activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were employed to access liver status because these enzymes can be used to test for liver disease [28]. The significant ($P < 0.0001$) elevation of ALT and AST levels were observed in the parasitized untreated mice (group B) compared to all the treated mice (Table 5). This is an indication of severe hepatic damage in group B mice compared to other infected treated mice. Study has shown that *P. berghei* NK65 infection has been reported to cause hepatomegaly and splenomegaly in mice model [29] which lead to liver damage. The ability of methanolic root extract of *A. indica* to reduce the plasma AST and ALT levels in this study, could suggest that methanolic root extract of *A. indica* ameliorate the damage caused by *P. berghei*.

The work is dedicated to validate our analytical result numerically using data obtained from the experiment. It is assumed that the mice in each group are equal which implies that $\alpha_f = \alpha_c = \alpha_D$, the inducement coefficient β of inducing the rats with the *Plasmodium berghei* is effective enough to cause the mice to become ill. Fig. 1, is the variation of the total mice population over four days. It can be seen that susceptible mice remains in good health, hence, the steady rise in the curve (solid green line). The health of induced infected and untreated mice $I(t)$ deteriorated sharply within the same period of the experiment. It can also be seen that both fansidar and chloroquine have similar impact on treated infected mice with a higher suppressive rate compared to treatment involving *A. indica* extract. Due to the suppressive power of the three treatments, it can be seen that treated mice moved to the asymptomatic class, hence, the increase observed in the number of asymptomatic mice at the end of the fourth day. The drug efficacies are considered in Fig. 2. It is clear that chloroquine drug has the highest efficacy which is closely followed by fansidar drug. The efficacy of *A. indica* root extract is lower compared to the other two standard drugs. Furthermore, as a result of the efficacies of the three drugs in suppressing the plasmodium, it is observed in Fig. 3 that the number of asymptomatic mice increased significantly before reaching a stable value.

5 Conclusion

In this work, we considered an experimental and mathematical study on the efficacies of standard drugs (fansidar and chloroquine) and *A. indica* methanolic root extract in suppressing the *P. berghei* levels in infected mice. A non-linear differential mathematical equation was developed to analyze the effect of the three drugs on infected mice population. The steady states of the model were qualitatively analyzed using stability theory. The model's local and global stability was found to be asymptotically stable if the induced reproduction number R_0 is less or equal to unity otherwise unstable. The induced infected steady state was found to be locally asymptotically stable (feasible) whenever $R_0 > 1$. The study also revealed that the

efficacy of the standard drugs are higher in suppressing the *P. berghei* in the infected rats compared to the potency of *A.indica* root extract in suppressing the *P. berghei* in infected mice. The methanolic root extract of *Azadirachta indica* A. Juss possess antiplasmodial activity in Swiss mice induced with *P. berghei* NK65 and the experimental work was supported by the mathematical model.

Disclaimer

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by Tertiary Education Trust Fund (TETFUND).

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Competing Interests

Authors have declared that no competing interests exist.

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