Alkali et al., Res J Zool 2018, 1:3
DOI: 10.4172/RJZ.1000110

Research Journal of Zoology

Research Article

A SCITECHNOL JOURNAL

Comparative Study of Antiplasdodial Activity of Aqueous Leaves Extract of *Azadirachta indica* (Juss), *Senna occidentalis* (Linn) and Standard Antimalaria on Multiplication of *Plasmodium falciparum* (Laveran)

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Received date: May 14, 2018; **Accepted date:** July 18, 2018; **Published date:** July 30, 2018

Abstract

Malaria is a major public health problem in the world, but treatment of malaria is becoming more difficult due to increasing drug resistance and high cost of antimalaria. This study investigated the antiplasmodial effects of leaves extract of *Azadirachta indica*, *Senna occidentalis* and standard antimalaria using RPMI 1640 culture media (in vitro). Parasite density was determined by counting the number of Plasmodium falciparum infected erythrocyte in 5,000 erythrocytes of the culture, thin blood smear were prepared and stained with Giemsa stain. Varying concentrations of the extracts such as 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/mL were prepared, the effect of the leaves extracts against the growth of *schizonts* were dose dependant. *A.indica* showed highest growth inhibition (96.92%) at 70m g/mL. However, the *schizonts* were found to be inhibited by the leaves extract of *S. occidetalis* at the highest concentration (100 mg/mL) with growth inhibition of 98.46%, there was no significant difference in the anti-malaria efficacy among the leave extracts and standard antimalaria drug ($p<0.05$) at 100 mg/mL. The results of phytochemical screening indicated *A.indica* and *S. occidentalis* contain Alkaloids, Flavonoids, Saponins, Saponins glycocides, Steroids and Terpenoids. The result of acute toxicity tested on rats indicates that the LD50 is greater than 3000 mg/kg body weight. The result of the study showed that *Azadirachta indica* and *Senna occidentalis* contain pharmacologically active compounds, hence they are potential antimalaria and safe to use at high dose.

Keywords: Antiplasdodial activity; *Azadirachta indica*; *Senna occidentalis*; Standard antimalaria; *Plasmodium falciparum*.

Introduction

Malaria is an ancient disease, it is an infectious disease caused by protozoan parasites from the Plasmodium genus that can be transmitted by the bite of the female Anopheles mosquito [1]. It can also be transmitted through contaminated needle or transfusion of blood, the classic symptom of malaria is fever with spikes on alternating days, headaches, malaise, fatigue, nausea, and anemia are also common. Severe forms of the disease can result in organ failure, delirium, impaired consciousness, and generalized convulsions, followed by persistent coma and death [2]. Falciparum malaria is the most deadly type. What was almost certainly malaria occur in a Chinese document from about 2700 BC, clay tablets from Mesopotamia from 2000 BC, Egyptian papyrus from 1570 BC and Hindu texts as far back as the sixth century BC [3]. Scientific studies only became possible after the discovery of the parasites themselves by Charles Louis Alphonse Laveran in 1880 and the incrimination of mosquitoes as the vectors, first for avian malaria by Ronald Ross in 1897 and then for human malaria by the Italian scientists Giovanni Battista Grassi, Amico Bignami, Giuseppe Bastianelli, Angelo Celli, Camillo Golgi and Ettore Marchiafava between 1898 and 1900 [4]. Excellent histories of this disease include those by [1,5-10]. Malaria remains the most severe and complex health challenge facing the vast majority of the countries in the sub-Saharan Africa [11]. In the World Malaria Report [12] the World Health Organization (WHO) estimated that 214 million cases of Malaria occurred worldwide in 2015 and majority of the cases (88%) occurred in the African Region, followed by the South-East Asia (10%) and Eastern Mediterranean Regions (2%) Similarly, it is estimated that in 2015 most deaths (90%) were in the WHO African Region, followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%). *Plasmodium falciparum* the most widespread etiological agent for human malaria has become increasingly resistant to standard antimalarials (e.g. chloroquine and antifolates), Artemisinin combination therapies (ACTs) are the recommended treatment for uncomplicated malaria. However, their uptake remains relatively low – in part due to availability issues, but also due to the high cost of ACTs in relation to cheaper, less effective alternatives. This is of great concern to all parties with an interest in access to medicines and the control of malaria, [2]. Medicinal plants have been the focus for the search of new antimalaria drugs in various parts of the world [13] and the present global situation indicates a recent resurgence in the severity of malaria, due to the resistance of malaria parasites to antimalaria drugs [14]. Hence, there is a need to intensify research in the development of new, cheap and effective antimalaria drugs from medicinal plants.

Azadirachta indica (Neem) is an evergreen, fastgrowing tree normally 15-20 meters in height in the mahogany family *Meliaceae*. It is one of three species in the genus *Azadirachta*, and is native to Indian subcontinent, growing in tropical and semitropical regions. It is widely available in the developing world, and known by different names such as Nim (Bengali), Tamar (Burmese) and Neem Baum (German). In Swahili, it is called Muarubaini which means the tree of the 40, as it is said to treat 40 different diseases. In northern Nigeria, it is called Dongoyaro [15]. Neem is known as reliever of sickness by traditional doctors in Sokoto [16].

Senna occidentalis (Linn), usually grows in the southern part of India which is known as Kasmard in Sanskrit, Kasondi in Hindi and Coffee Senna in English and sanga sanga in Hausa (northernwest Nigeria). The plant belongs to *Caesalpiniaceae* family. The common name is Ponnavarai in Tamil. The roots, leaves and seeds are the parts of the plant used. It is an erect herb which grows up to 2 meters and is commonly found by road sides, ditches and waste dumping sites. *Cassia occidentalis* has been widely used as traditional medicine for

the treatment of fever; Entire parts of the plant have medicinal values [17].

Materials and Methods

Collection and identification of samples

Fresh and mature leaves of *Azadirachta indica* were obtain behind new postgraduate hostel Usman Danfodio University Sokoto (permanent site). While *Senna occidentalis* fresh mature leaves were obtained behind First Bank plc, Usman Danfodio University Sokoto, (permanent site). The samples were collected separately in a clean sterile polythene bag and brought to the herbarium of the Department of Biological Science, Usman Danfodio University Sokoto, for identification and authentification. Voucher specimen (with number UDUS/ANS/0095) were prepared and deposited in the same herbarium.

Fifty grams (50 g) from each sample of *Azadirachta indica* was extracted with 1500mL of distilled water in 2000mL beaker. The soaked sample was stirred and covered with aluminum foil and keep for twenty four hours. The resultant extract was filtered using muslin cloth and each filtered was evaporated separately to dry using hot plate set at 40° C to obtain crude extract. The extract was weighted and stored in the refrigerator until use. Artemether and Lumefantrine reference standard were purchased from Sigma-Aldrich, USA.

Qualitative photochemical screening of plant extract

The leaves extract of the plant were screened for metabolites such as alkaloids, tannins, flavonoids, saponinins, balsams, anthraquinones, cardiac glycosides, glycosides, and steroids.

Preparation of Media

P. falciparum originally obtained from a positive patient from specialist hospital Sokoto, was continuously cultured based on a modified method previously described by [18]. The parasites were maintained in continuous culture on human erythrocytes (blood group O^+ obtained from the Hematology Department, Sokoto specialist hospital, in RPMI 1640 medium supplemented with 10% human AB+ serum, 25 Mm N-2-hydroxyethylpiperazine- N-2-ethanesulfonic acids (HEPES), 2g $NaHCO_3$ and 60 mg/ml gentamicin sulfates, at pH 7.2. The assay was performed in a culture flaks, the cultures were incubated at 37°C in an atmosphere of CO_2 in a candle jar for 24 hours. Parasite cultures were synchronized to the ring stage by treatment with 5% Sorbitol.

Inoculation procedure for Efficacy Test

Plant extracts 30 µL each were dropped in to different wells, each containing different concentration of 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL, and 70 mg/mL, of *A. indica* and standard antimalaria were also screened in 96 well microtitre plates. Culture media (100 µL) was parasitized with erythrocyte prasitemia at 0.5% and then inoculated in to the wells. The control well contains no treatment. The cultures were incubated at 37°C in an atmosphere of CO_2 in a candle jar for 24 hours [19]. Schizont growth inhibitions per 200 asexual parasites were counted in 25 microscopic fields. The control parasite culture was considered as 100% growth. The percentage inhibition per concentration was calculated using the formula:

Acute toxicity test

The method adopted for this study was fixed dose procedures FDP [21]. Acute toxicity of plants extracts were tested on 14 rats using 3 doses (500, 1000 and 3000 mg/kg body weight) administered orally, each dose of *A. indica* was replicated twice, control rats were kept under the same conditions without any treatments, the animals were routinely inspected for appearances or signs of toxicity such as tremors, weakness and refusal of feeds, falling off of hair, coma or even death for 48 hours. The Lethal Dose LD50 was estimated from the graph of percentage mortality converted to probit against log-dose of the extract, prosbit 5 being 50%.

Statistical analysis

Data obtained from the study were subjected to statistical analysis using statistical package for social science (SPSS) version 20.0. Analysis of variance (one way ANOVA) was carried on the data, at 95% level of significant and mean generated from this study were separated using New Duncan Multiple Range Test (DMRT).

Results

The phytochemical screening of plants material showed the presence of saponins, tannins, flavonoids, tepaenoids, cardiac glycosides, alkaloids, steroids and Saponin glycocides (Table 1).

Constituent	*A. indica*	*S. occidentalis*
Alkaloids	+	++
Saponin glycocides	+	+
Terpenoid steroids	+	+
Tannins	+	-
Saponins	+++	+++
Glycosides	-	+
Flavonoids	+	+
balsams	+	-
Cardiac glycosides	+	-
volatile oils	-	+
anthraquinones	-	-

Table 1: Qualitative phytochemical screening of *A. indica* and *S. occidentalis* leaves extracts.

Note: - Not detected; + Identified in a trace amount; ++ Identified in moderate amount; +++ Identified in high amount.

The results of the antimalaria activity of aqueous extract of *A. indica* on the schizonts growth of *P. falciparum* are presented in Table 2. The result show complete schizont growth inhibition when *P. falciparum* is treated with 70 mg/mL of *A. indica* with the mean growth inhibition of 96.92% (0.67) and the lowest inhibition was recorded at 10 mg/mL, with the mean growth inhibition of 22.72% (17.0). Similarly, complete schizont growth inhibition of 98.46% (0.33) was recorded when *P. falciparum* is treated with 100 mg/mL of *S. occidentalis* and the lowest schizont growth inhibition of 19.68% (17.67) at 10 mg/mL. Standard

antimalaria inhibited schzont growth 100% at all concentration (Table 2).

Concentrations (mg/ml)	Mean P. falciparum growth ± SE of plant extract		
	A. indica	*S. occidentalis*	Artemethe Lumefantrine
Control	22.00 ± 0.00	22.00 ± 0.00	22.00 ± 0.00
10	17.0^{c} ± 0.57	17.67^{c} ± 1.45	00.00^{a} ± 0.00
20	15.33^{c} ± 1.53	16.00^{c} ± 0.58	00.00^{a} ± 0.00
30	08.67^{b} ± 0.88	15.00^{c} ± 0.57	00.00^{a} ± 0.00
40	03.67^{b} ± 0.67	14.67^{c} ± 1.20	00.00^{a} ± 0.00
50	02.67^{b} ± 0.88	14.00^{c} ± 0.58	00.00^{a} ± 0.00
60	01.33^{a} ± 0.88	12.33^{b} ± 0.33	00.00^{a} ± 0.00
70	00.67^{a} ± 0.67	06.33^{b} ± 0.88	00.00^{a} ± 0.00
80	00.00^{a} ± 0.00	03.33^{b} ± 0.58	00.00^{a} ± 0.00
90	00.00^{a} ± 0.00	02.00^{b} ± 0.58	00.00^{a} ± 0.00
100	00.00^{a} ± 0.00	00.33^{a} ± 0.33	00.00^{a} ± 0.00

Table 2: Antimalaria activity of aqueous leaves Extract of *A. indica*, *S. occidentalis* and standard antimalaria on schizonts growth of *Plasmodium falciparum*.

Values are mean ± standard error 3 replication with different superscripts are significantly different (P<0.05).

The results of acute toxicity effects of *A. indica* and *S. occidentalis* leaves extracts on rat treated with 500 mg/kg per body weight for 48 hours are presented in Table 3.

Observation	*Azadirachta indica*	*Senna occidentalis*
Falling of hair	NIL	NIL
Behavioral patterns	NIL	NIL
Diarrhea	NIL	NIL
Loss of appetite	NIL	NIL
Tremors	NIL	NIL
Coma	NIL	NIL

Table 3: Acute toxicity studies of *Azadirachta indica* and *Senna occidentalis* leaves extracts (500 mg/kg).

The results of acute toxicity effects of *A. indica* and *S. occidentalis* leaves extracts on rat treated with 1000 mg/kg per body weight for 48 hours are presented in Table 4.

Observation	*Azadirachta indica*	*Senna occidentalis*
Falling of hair	NIL	NIL
Behavioral patterns	NIL	NIL
Diarrhea	NIL	NIL
Loss of appetite	NIL	NIL
Tremors	NIL	NIL
Coma	NIL	NIL
Dead	NIL	NIL

Table 4: Acute toxicity studies of *Azadirachta indica* and *Senna occidentalis* leaves extracts (1000 mg/kg).

The results of acute toxicity effects of *A. indica* and *S. occidentalis* leaves extracts on rat treated with 3000 mg/kg per body weight for 48 hours are presented in Table 5.

Observation	*Azadirachta indica*	*Senna occidentalis*
Falling of hair	NIL	NIL
Behavioral patterns	NIL	NIL
Diarrhea	NIL	NIL
Loss of appetite	NIL	NIL
Tremors	NIL	NIL
Coma	NIL	NIL
Dead	NIL	NIL

Table 5: Acute toxicity studies of *Azadirachta indica* and *Senna occidentalis* leaves extracts (3000 mg/kg).

Discussion

The phytochemical study of *A. indica* and *S. occidentalis* revealed the presence of tannins, saponins alkaloids, glycosides, flavonoids,

steroids, balsams, volatile oil, anthraquinones, saponin gkycocides and cardial glycocides. Qualitative phytochemical analysis of Azadiracta indica and Senna occidentalis indicated that the plants are rich sources of bioactive compounds. Similar bioactive compounds were also earlier observed on whole plant of *S. occidentalis* [22,23] reported the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycoside and balsam in *S. occidentalis*. And seed back and leaves of *Azadiracta indica* [24]. The presence of bioactive compound in *Azadiracta indica* and *Senna occidentalis* is an indication that they have medicinal potentials due to the fact that each of the bioactive compounds identified has one or more uses therapeutically [25,26]. Other studies include [27-30].

The antimalarial study revealed the activity of *A. indica* and *S. occidentalis* leaves extracts. The study revealed that A.indica is the most effective against schizonts growth of *P. falciparum* followed by *S. occidentalis*. The Artemether Lumefantrine significantly ($P<0.05$) inhibited the growth of *P.falciparum* up to 100% at the all concentration. *A. indica* leaves extract significantly inhibited the growth by 22'72% of at 10mg/ml, this differs significantly compare to Artemether Lumefantrine. At 70 mg/mL *P. falciparum* growth was inhibited significantly by 96.92%, which shows no significant difference compare to Artemether Lumefantrine drugs. This findings conforms to the report of in which similar constituents was found to exhibits antiprotozoal and antibacterial activities [31,32] who tested aqueous extracts of *Azadirachta indica* (bark), in vivo against *P. berghei* following Peter"s 4-day test and recorded about 70% parsitemia inhibition. Extracts from Nigerian neem leaves (*Azadirachta indica*) have been earlier reported to have anti-malarial activities [33,34]. But [35] demonstrated that acetone/water mixture is a more efficient solvent than water alone for the extraction of anti-malarial activity from Nigerian neem leaves. Its anti-malarial activity has been reported to be superior to chloroquine [36], gametocytocidal [35-37] against *P. falciparum* malaria parasite. The effectiveness of *A. indica* is not has been surprising as the plant shows to possess antimalarial activity [33]. This also explains the rampart use of *A. indica* by the people. *S. occidentalis* leaves extract significantly inhibited the growth by 19.68% at 10 mg/mL, this indicate there is significant difference compare to Artemether Lumefantrine. At 100 mg/mL *P. falciparum* growth was inhibited significantly by 98.46%, this indicates there is no significant difference compare to Artemether Lumefantrine. These findings are in conformity with that of [38], who worked on *Cassia occidentalis* against rat model Plasmodium and reported 60% inhibition. [39] Reported 63% inhibition of *Cassia occidentalis* leaves extracts in vitro antimalarial activity. Although less data was found about antimalarial activity of S. occidentalis. The study revealed that there is no significant difference between *A. indica* and *S. occidentalis* leaves extracts at 10 mg/mL, but they differ significantly at 70 mg/mL. There was significant difference across concentration of all plants extracts, indicating that *A. indica* and *S. occideatalis* leaves possess antimalaria potential against *P. falciparum*.

The present results show that aqueous leaves extract of Azadiracta indica and *Senna occidentalis* does not cause any apparent in vivo toxicity in an animal model. No death or signs of toxicity were observed in rats treated with extract at dose 500 mg/kg, 1000 mg/kg and 3000 mg/kg; this revealed that the LD50 is above 3000 mg/kg body weight, thus establishing its safety in use, these findings are in conformity with that of [40] who work on *A.indica* against toxicity and anti-inflammation on mice model and [41] who work on toxicity of methanol seed extract of *Cassia fistula* in Mice. Kanungo [42] reported the methanolic extract of neem stem bark demonstrated oral toxicity at LD50 of about 13g/kg on mice. The different reports in acute toxicity may be due to difference in the route of administration and the variation in the active components of the neem tree which differ from location to location.

Conclusion

Based on the present study, it can be concluded that the extracts of *Azadiracta indica* and *Senna occidentalis* possess antiplasmodial activity. The phytochemical screening revealed the presence of bioactive constituents that could be the reason for pharmacological activity. Even though they are not as effective as standard antimalaria at low concentration, both plants showed promising activity against schizons growth. Both *Azadirachta indica* and *Senna occidentalis* antimalarial activities were found to be dose dependant. Therefore, the observed antiplasmodial activity of both plant extract can be a positive attributes in the malaria control and they are safe to use at high dose.

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