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Research Article

Effects of Methanol Leaves Extracts of *Azadirachta indica* (Juss.), *Senna occidentalis* (Linn) and *Senna siamea* (Lam) on the *In vitro* Growth of *Plasmodium falciparum* (Laveran) Trophozoites

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ABSTRACT

Malaria is a major parasitic disease in the world, especially in Africa and antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. This study investigated the antiplasmodial effects of methanol leaf extract of *Azadirachta indica, Senna occidentalis*, *Senna siamea,* and standard antimalarial drugs. Plant samples were collected and screened for qualitative phytochemicals. *Plasmodium falciparum* was obtained from symptomatic patients, confirmed using RDTs, and cultured in RPMI 1640 media supplement with 10% human AB^+ serum. Varying concentrations of the extracts, such as 100, 200, and up to 1000 µg/ml were prepared. The results of phytochemical screening indicated that *A. indica*, *S. occidentalis,* and *S. siamea* contain Alkaloids, Flavonoids, Saponins, Saponins glycosides, Steroids, and Terpenoids. Methanol plant extracts showed that Combination therapy exhibited the highest growth inhibition, achieving 98.48% effectiveness at 400 µg/ml. The acute toxicity studies in rats showed no significant adverse effects at doses up to 3000 mg/kg. These findings highlight the pharmacological potential and safety of these plant extracts, supporting further investigations for malaria treatment.

Keywords: *Azadirachta indica;* Methanol; Phytochemical; *Plasmodium falciparum; Senna occidentalis*; *Senna siamea;* Trophozoites

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INTRODUCTION

Malaria is a contagious illness caused by protozoan parasites from the *Plasmodium* genus, which are transmitted through the bite of a female *Anopheles* mosquito (Sato, 2021). Common symptoms include fever, headaches, malaise, fatigue, nausea, and anemia. In severe cases, the disease can lead to organ failure, delirium, loss of consciousness, seizures, and eventually a prolonged coma or death (Bleakley, 2007). *Plasmodium falciparum* is the most lethal strain, responsible for high rates of illness and death (Bleakley, 2007).

Malaria remains the most serious and complex health issue affecting most countries in sub-Saharan Africa (Uneke, 2009). Globally, approximately 3.4 billion people are at risk of contracting the disease. According to the World Health Organization, there were an estimated 249 million malaria cases worldwide in 2022, with around 608,000 deaths, slightly lower than the 610,000 recorded in 2021. That year, the region accounted for 94% of malaria cases (233 million) and 95% of malaria-related deaths (580,000). Additionally, children under the age of five made up about 80% of all malaria fatalities in the region (WHO, 2023). The *World Malaria Report 2021* indicated that malaria cases rose from 227 million in 2019 to 241 million in 2020. The estimated number of malaria-related deaths in 2020 was 627,000, marking an increase of 69,000 deaths from

the previous year, with around two-thirds of these additional deaths (47,000) attributed to disruptions caused by the COVID-19 pandemic (WHO, 2021). A total of 29 countries accounted for 96% of malaria cases worldwide, with six nations—Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%), Angola (3.4%), and Burkina Faso (3.4%)—contributing to approximately 55% of global cases (WHO, 2021). By 2023, malaria cases were estimated to have risen to 263 million, with an incidence rate of 60.4 cases per 1,000 people at risk. This represents an increase of 11 million cases from the previous year and a rise in incidence from 58.6 cases per 1,000 in 2022 (WHO, 2024)

Plasmodium falciparum, the most prevalent cause of human malaria, has developed increasing resistance to commonly used antimalarial drugs such as chloroquine and antifolates. Artemisinin-based combination therapies (ACTs) are the preferred treatment for uncomplicated malaria. However, their adoption remains relatively low, partly due to supply challenges and the high cost of ACTs compared to more affordable but less effective alternatives. This poses a significant concern for stakeholders focused on improving access to medications and malaria control efforts (WHO, 2021). Cases of ACT-resistant malaria have been reported in Cross River State, Nigeria (Oboh *et al.,* 2022). Zhao *et al.* (2021) found that 17% of 167 *Plasmodium falciparum* isolates from Nigeria, analyzed in Henan, China, exhibited a high prevalence of resistance-associated mutations in *Pfdhfr* and *Pfdhps*. Additionally, the issue of substandard and counterfeit antimalarial drugs remains a significant challenge (Nayyar *et al.,* 2012). Therefore, there is an urgent need to develop new, affordable, and effective antimalarial treatments derived from medicinal plants. In Africa and other countries where malaria is endemic, traditional medicinal plants are frequently used to treat or cure malaria (Willcox and Bodeker, 2004). It is a fact that conventional antimalarials such as chloroquine, quinine, and artemisinin derivatives originated from plants (Habibi *et al.,* 2022).

Azadirachta indica (Neem) is a fast-growing evergreen tree that typically reaches a height of 15–20 meters and belongs to the mahogany family (*Meliaceae*) (Sarkar *et al.,* 2021). It is one of three species in the *Azadirachta* genus and is native to the Indian subcontinent, thriving in tropical and subtropical regions (Alzohairy, 2016). Neem is widely available in developing countries and is known by various names, including *Nim* in Bengali, *Tamar* in Burmese, and *Neem Baum* in German. In Swahili, it is called *Muarubaini*, meaning "the tree of 40," as it is believed to cure 40 different ailments. In northern Nigeria, it is referred to as *Dongoyaro* (Awofeso, 2011). And traditional healers in Sokoto regard it as a remedy for various illnesses (Alebiosu *et al.,* 2012).

Senna occidentalis is a plant commonly found in southern India, where it is known as *Kasmard* in Sanskrit, *Kasondi* in Hindi, and *Coffee Senna* in English. In north-western Nigeria, the Hausa people refer to it as *Sanga Sanga or Rai Daure*. It belongs to the *Caesalpiniaceae* family and is commonly called *Ponnavarai* in Tamil (Awofeso, 2011). The plant, which can grow up to 2 meters tall, is an erect herb typically found along roadsides, near ditches, and in waste disposal areas. Its roots, leaves, and seeds are the primary parts used for medicinal purposes (Awofeso, 2011). *Cassia occidentalis* has been widely utilized in traditional medicine for various treatments. In treatment of fever; entire parts of the plant have medicinal values (Mohammed *et al.,* 2012).

Senna siamea also known as kassod tree, Malga or marke **(**Hausa), ewe kasia (Yuroba) is an angiosperme native of Southeast Asia (Kamagaté *et al.,* 2014). Firstly classified in Caesalpiniacae family, then in those of Leguminoseae, *S. siamea* is now classified among the Fabaceae ((Kamagaté *et al.,* 2014). *S. siamea* is better known by the tropical populations for it various medicinal value (Shivjeet *et al.,* 2013). It is also known for its various common uses in cattle rearing, agriculture environment and furniture (Rojas-Sandoval and Acevedo-Rodríguez, 2022).

MATERIALS AND METHODS

Ethical Consideration

The study was approved by Barau Dikko Teaching Hospital Kaduna with ethical letter reference (BDTH/2022/118/VOL./1).

Collection, Identification and Authentication of Plant Samples

Fresh and mature leaves of *Azadirachta indica*, *Senna occidentalis* and *Senna siamea* were obtained at Jiddari polo Maiduguri, Borno State. The samples were collected separately in a clean sterile polythene bag and brought to the herbarium of the Department of Biological Sciences, Nigerian Defence Academy Kaduna, for identification and authentication with Voucher specimen number issued NDA/BIOH/2023/50 (*Azadirachta indica*), NDA/BIOH/2023/51 (*Senna siamea) and* NDA/BIOH/2023/52 (*Senna occidentalis*). All that material was deposited in the same herbarium.

The powdered samples (50 g) were extracted with methanol solvent (500 ml) by using a Soxhlet extractor for 72 h. After complete extraction, the methanol solvent was evaporated using rotary evaporator (Yamato, model-RE 801 Japan) under

reduced pressure to obtain methanol crude extract. The methanol crude extract from each sample was suspended in water (60 ml). All crude extracts were filtered separately through Whatman filter paper to remove particles. The particle free crude extract were evaporated completely using rotary evaporator under reduced pressure to obtain dry crude extracts. The residue left in the separator funnel was re-extracted twice following the same procedure and filtered (Patil and Gaikwad, 2010).

Qualitative Phytochemical Screening of Leaf Extracts of *A. indica*, *S. occidentalis*, and *S. siamea*

The leaf extracts of these plants were analyzed for various metabolites, including alkaloids, tannins, flavonoids, saponins, terpenoids, steroids, anthraquinones, glycosides, cardiac glycosides, saponin glycosides, volatile oils, and balsams.

Alkaloid Test (Dragendorff's Test)

Approximately 0.2 g of each plant sample was mixed with 3 mL of hexane in a test tube, shaken, and filtered. Then, 5 mL of 2% HCl was added to the mixture and heated before filtering. A few drops of picric acid were added to the filtrate. The formation of a yellow precipitate indicated the presence of alkaloids (Wadood *et al.,* 2013).

Tannin Test (Ferric Chloride Test)

Two millilitres (2ml) of each extract were mixed with 2 mL of water, followed by the addition of 1–2 drops of diluted ferric chloride solution. The appearance of a dark green or blue-green color signified the presence of tannins (Wadood *et al.,* 2013).

Flavonoid Test (H_2SO_4 Test)

About 0.5 g of each extract was dissolved in 10 mL of distilled water in a test tube. Then, 5 mL of dilute ammonia solution was added to a portion of the filtrate, followed by 1 mL of concentrated H_2SO_4. The development of a yellow color indicated the presence of flavonoids (Wadood *et al.,* 2013).

Saponin Test (Frothing Test)

A small volume of distilled water was added to 1 mg of each extract in a test tube, and the solution was shaken vigorously. A stable froth that persisted for 20 minutes confirmed the presence of saponins (Sabri *et al.,* 2012).

Terpenoid and Steroid Test (Liebermann Burchard's Test)

Ten milliliters of each extract were evaporated, and the residue was dissolved in 0.5 mL of hot acetic anhydride. Next, 0.5 mL of chloroform was added, followed by treatment with Liebermann-Burchard's reagent. A blue-green coloration at the interface confirmed the presence of steroids (Wadood *et al.,* 2013).

Anthraquinone Test (Borntrager's Test)

Two milliliters of 10% hydrochloric acid were added to each extract in a test tube and boiled for two minutes. Equal volumes of chloroform were added and vortexed twice. The chloroform layer was extracted, and an equal amount of ammonia was added. A pink-colored layer indicated the presence of anthraquinones (Efe *et al.,* 2016).

Glycoside Test (Fehling's Test)

About 2.5 mL of 50% sulfuric acid was added to 5 mL of each extract in a test tube, then heated in boiling water for 15 minutes. After cooling, the solution was neutralized with 10% NaOH, followed by the addition of 15 mL of Fehling's reagent. The mixture was boiled, and a brick-red precipitate confirmed the presence of glycosides (Ayoola *et al.,* 2008).

Cardiac Glycoside Test (Keller-Kiliani's Test)

Two milliliters of 0.5% ferric chloride solution were added to 1 mL of each extract in a test tube and allowed to stand for one minute. Then, 1 mL of 10% H_2SO_4 was carefully poured along the inner wall of the test tube. A reddish-brown ring at the interface indicated the presence of cardiac glycosides (Gul *et al.,* 2017).

Saponin Glycoside Test (Fehling's Test)

About 2.5 mL of Fehling's reagent was mixed with 2.5 mL of each extract in a test tube. The formation of a bluish-green precipitate confirmed the presence of saponin glycosides (Gul *et al.,* 2017).

Volatile Oil Test (HCl Test)

Each plant extract was mixed with 90% HCl. The appearance of a white precipitate confirmed the presence of volatile oils (Efe *et al.,* 2016).

Balsam Test (Ferric Chloride Test)

Approximately 2.5 mL of each extract was combined with an equal volume of 90% ethanol. Two drops of alcoholic ferric chloride solution were added. A dark green color indicated the presence of balsams (Gul *et al.,* 2017)

Preparation of Culture Media

Before preparing the culture media, all glassware was thoroughly washed with detergent, rinsed with tap water followed by distilled water, air-dried, and then sterilized in a hot oven at 100°C for one hour. The inoculation room and chamber were sterilized by boiling a 4% formalin solution for 30 minutes. Additionally, the inoculation chamber and table surfaces were disinfected using cotton wool soaked in 70% alcohol before use.

Plasmodium falciparum was obtained from a symptomatic patient at Barau Dikko Teaching Hospital, Kaduna, and its presence was confirmed using an RDT malaria diagnostic kit. The parasite was continuously cultured following a modified protocol based on the methods of Trager and Jensen, as adopted by Jadhav *et al.* (2014), Achi *et al*. (2018), Alkali *et al.* (2018), and Abdulrazaq *et al.* (2020). The parasites were maintained in continuous culture using human erythrocytes

(blood group O+), sourced from the Hematology Department at Barau Dikko Teaching Hospital, Kaduna. The culture medium consisted of RPMI 1640 supplemented with 10% human AB+ serum, 25 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES), 2 g $NaHCO_3$, and 60 mg/mL gentamicin sulfate, adjusted to pH 7.2. The assay was conducted in a culture flask, with incubation at 37°C in a CO_2-enriched atmosphere using a candle jar for 24 hours. Synchronization of the parasite culture to the ring stage was achieved using 5% sorbitol treatment (Srinivas & Puri, 2002).

Preparation of Plant Extracts for Sensitivity Test

Stock solution was prepared using 0.05g of the dried plant extract dissolve in 10ml of distilled water and was shaked until desolved completly, 40 ml of distlled water was added to make a stock solution of 1 mg/ml. From the stock solution, working concentration were prepared from stock solution using dilution formula as follows:

$$C_1V_1 = C_2V_2$$

Where C_1 = present concentration, C_2 = required concentration, V_1 = volume to use, V_2 = required volume (Mary *et al.,* 2005).

Different Serial concentrations of the plant extracts of *A. indica, S.occidentalis*, *S. siamea* aqueous extract individual and combined therapy and standard antimalaria were prepared to test for the antimalaria activity, the serial concentrations were: 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000µg/ml. The above concentrations were prepared by taking 0.2, 0.4, 0.6, 0.8, l, 1.2, 1.4, 1.6, 1.8 and 2ml of the stock solution and dissolved in test tubes containing sterilized distilled water to make 2ml of the solution.

Inoculation and Sensitivity Test

Plant extracts 50µl each was dropped in to different wells using micro pipette, each containing different concentration of 100, 200, 300, 400, 500, 600, 700, 800, 900 and absolute stock of *A. indica, S.occidentalis* and *S. siamea* was also screened. The 96 well microtitre plates are labeled at rows (1-12) and at Column (A-H), each Column contains different treatment while each row contains different concentration of the treatments. Culture media (100µl) was parasitized with erythrocyte parasitemia at 10% 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 (Moll *et al.,* 2008). Three (3) 96 well microtitre plates labelled 1, 2 and 3 were used for the inoculation, in each microtitre plates, row 1-10 reprsented the concentration from 100 µl - 1000µl, column A represented *A. indica*, B represented *S. occidentalis,* D represented *S. siames*, E represented combimation theraphy, G represented standard antimalarial and H control wells with no treatment, The 3 well represented 3 replication.

Acute toxicity test

Adults Wister rats were randomly selected and purchased at the department of applied Biology, College of Engineering, Kaduna Polytechnic. The method adopted for this study was fixed dose procedures FDP (Erhirhie *et al.,* 2018). Acute toxicity of aqueous, methanol and hexane crude plants extracts was tested on 60 rats using 3 doses (500, 1000 and 3000 mg/kg body weight) administered orally, there were 9 treatments and each treatment requires six animals. The six animals were divided into three groups (three replication) of two animals in each group. Each group of animals were administered different doses of *A. indica*, *S.occidentalis* and *Senna siamea.* Control rats were kept under the same conditions without any treatments, the animals were inspected for appearances or signs of toxicity such as tremors, weakness, and refusal of feeds, falling off of hair, coma or even death after 24 hours. Biochemical parameters (liver function test) was performed on RAYTO CHEMTY 120 Japan, LFT of the rats were observed. The rats were fasted overnight with free access to water and samples were administered orally. After 24 h, Blood samples were collected in lithium heparin coated tubes. Serum was separated by centrifugation of whole blood at 3000 r/min (Hettich, EBA 20) for 10 minutes and was used to determine concentrations of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), and total protein (Odiegwu *et al.,* 2021).

Data Analysis

Data obtained from the study were subjected to statistical analysis using statistical package for social science (SPSS version 25.0.) Analysis of Variance (ANOVA) was carried on the data to determin the significant defference between groups, at 95% level of significant and mean generated from this study were separated using List Significant Difference (LSD).

RESULTS

The phytochemical analysis of the plant materials revealed the presence of various bioactive compounds, including saponins, tannins, flavonoids, terpenoids, cardiac glycosides, alkaloids, steroids, and saponin glycosides, as summarized in Table 1.

Table 1: Qualitative Phytochemical Components of Methanol Leaf Extracts of *Azadirachta indica, Senna occidentalis* and *Senna siamea*

Phytochemical Components	A. *indica*	*S. occidentalis*	*S siamea*
Alkaloids	+	+	+
Saponin glycocides	+	+	+
Steroids	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Cardial Glycosides	+	+	+
Flavonoid	+	+	+
Balsams			
Volatile Oils	+	+	+
Saponins	+	+	+
Anthraquinones	+	+	+

Key: - Absent. + Present

The results of the antimalarial activity of methanol extracts of *A. indica S. occidental, S. siamea* individual and combined therapy and standard antimalaria on the growth of *P.falciparum* trophozoites were presented in Table 3.The result showed complete trophozoites growth inhibition when *P. falciparum* trophozoites were treated with 1000 µg/ml of *A. indica* with the mean trophozoites growth of 0.00 and the lowest inhibition was recorded at 100 µg/ml, with the mean growth of18.33, Similarly, complete trophozoites growth inhibition of 0.00 was recorded when *P. falciparum* trophozoites were treated with 1000 µg/ml of *S. occidentalis* and the lowest trophozoites growth of 18.67when *P. falciparum* trophozoites were treated with 100 µg/ml. *siamea* showed complete trophozoites growth inhibition when *P. falciparum* trophozoites were treated with 600 µg/ml and the lowest trophozoites growth of16.67when *P. falciparum* trophozoites were treated with 100 µg/ml. Combined Therapy of all three plant methanol crude extract showed complete trophozoites growth inhibition when *P. falciparum* trophozoites were treated with 500 µg/ml and the lowest trophozoites growth of 9.00 when *P. falciparum* trophozoites were treated with 100 µg/ml. Standard antimalaria inhibited trophozoites growth 100% when *P. falciparum* trophozoites were treated with 500 µg/.

The results of the Percentage Inhibition of methanol extract of *A. indica S. occidental, S. siamea* individual and combined therapy and standard antimalaria on the growth of *P. falciparum* trophozoites were presented in Table 3. The result showed complete trophozoites growth inhibition when *P. falciparum* trophozoites were treated with 1000 µg/ml of *A. indica* with trophozoites growth Inhibition 100 % the lowest inhibition was recorded at 100 µg/ml, with growth Inhibition of 15.41%, complete trophozoites growth inhibition of 100 % was recorded when *P. falciparum* trophozoites were treated with 1000 µg/ml of *S. occidentalis* and the lowest *trophozoite* growth inhibition of 13.84% when *P.falciparum* trophozoites were treated with 100 µg/ml. Similarly, *S. siamea* showed complete trophozoites growth inhibition of 100% when *P. falciparum* trophozoite were treated with 600 µg/ml and the lowest trophozoites growth inhibition of 23.07% when *P. falciparum* trophozoite were treated with 100 µg/ml. Combined Therapy of all three plant methanol crude extract showed complete trophozoites growth inhibition when *P. falciparum* trophozoites were treated with 500 µg/ml and the lowest trophozoites growth inhibition of 58.47% when *P. falciparum* trophozoites were treated with 100 µg/ml. Standard antimalaria inhibited trophozoites growth 100% when *P. falciparum* trophozoites were treated with 500 µg/ml. and 46.13% trophozoites growth inhibition at 100 µg/m.

Table 2: Antimalaria Activity of Methanol Leaf Extracts of *A. indica, S. occidentalis, S. siamea* Individual and Combined Therapy and Standard Antimalaria on Trophozoites Growth

Concentrations of plant extracts (µg/ml)	*Azadirachta indica* Mean growth ±SD	*Senna occidentalis* Mean growth ±SD	*Senna siamea* Mean growth ±SD	Combine therapy Mean growth ±SD	Artemethe Lumefantrine Mean growth ±SD
control	21.67±1.15	21.67±1.15	21.67±1.15	21.67±1.15	21.67±1.15
100	18.33±1.53^{c}	18.67±0.58^{c}	16.67±1.53c	9.00±2.00^{a}	11.67±0.5^{b}
200	14.67±0.08^{b}	16.00±1.00^{b}	14.67±1.53^{b}	7.00±0.00^{a}	8.00±1.00^{a}
300	12.67±0.58^{e}	11.00±1.00^{d}	7.67±0.58^{c}	3.00±1.00^{a}	5.33±0.56^{b}
400	10.33±1.53^{c}	10.00±2.00^{c}	3.67±1.15^{b}	0.33±0.57^{a}	1.00±1.00^{a}
500	9.33±2.08^{c}	6.00±1.00^{b}	1.67±1.15^{a}	0.00±0.00^{a}	0.00±0.00^{a}
600	6.67±0.57^{c}	4.33±0.57^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
700	3.33±0.57^{b}	3.00±1.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
800	2.00±1.00^{b}	1.33±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
900	0.67±0.57^{a}	0.67±0.57^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
1000	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

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

Table 3: Percentage Inhibition of Methanol Leaf Extracts of *A. indica, S. occidentalis, S. siamea* Individual and Combined Therapy and Standard Antimalaria on Trophozoites Growth.

Concentrations of plant extracts (µg/ml)	*Azadirachta indica* Inhibition (%)	*Senna occidentalis* Inhibition (%)	*Senna siamea* Inhibition (%)	Combine therapy Inhibition (%)	Artemethe Lumefantrine Inhibition (%)
control	0.00	0.00	0.00	0.00	0.00
100	15.41	13.84	23.07	58.47	46.13
200	32.30	26.17	32.30	67.70	63.08
300	41.53	49.34	64.61	86.16	75.43
400	52.33	50.85	86.16	98.48	95.39
500	56.92	72.31	92.29	100	100
600	69.22	80.01	100	100	100
700	84.63	86.16	100	100	100
800	90.77	93.86	100	100	100
900	96.91	96.91	100	100	100
1000	100	100	100	100	100

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

The results of acute toxicity (LFT) of *A. indica S. occidental, S. siamea* of methanol leaf extracts at 500 mg/kg body weight were presented in Table 4. The result showed *S. siamea* with highest of 171.83U/L ALP, 145.00U/L AST. Control group with highest ALT with 42.33U/L, *A. indica* with highest of total protein of 5.31 g/dL and *S. occidentalis* with ALB of 3.70g/dL. *S. occidentalis* showed lowest ALP of 166.66 U/L and ALT of 31.50 U/L, control with lowest AST of 112.50 U/L, *S. siamea* with total protein of 34.33 g/dL and *A. indica* with lowest ALB of 3.00 g/dL.

The results of acute toxicity (LFT) of *A. indica*, S. occidental, S. siamea of methanol leaf extracts at 1000 mg/kg body weight were presented in Table 5. The result showed control group with highest ALP of 169.16 U/L and AST of 112.50 U/L, *S.occidentalis* with highest ALT of 46.00 U/L and total protein of 5.76 g/dL and control group with highest ALB of 3.70 g/dL. *S. occidentalis* showed lowest ALP of 108.83 U/L and AST of 81.00 U/L and with lowest ALT of 39.00 U/L and *A. indica* with total protein of 5.41 g/dL and lowest ALB of 3.40 g/dL.

The results of acute toxicity (LFT) of *A. indica S. occidental, S. siamea* of methanol leaf extracts at 3000 mg/kg body weight were presented in Table 6. The result showed *S. siamea* with highest ALP of 246.33 U/L, *A. indica* with highest AST of 155.50 U/Land ALT of 62.50 U/L, *S. siamea* with highest total protein of 5.90 g/dL and control with highest ALB of 3.70 g/gL. *S. occidentalis* showed lowest ALP of 156.50 U/L, *S. siamea* with lowest AST of 140.00 U/L, lowest ALB of 3.35g/dL, and control with lowest ALT of 42.33 U/L, total protein of 5.06g/dL.

Table 4: Biochemical Acute Toxicity Study of Methanol Leaf Extracts of *A. indica, S. occidentalis* and *S. siamea* orally adminitered to Rats at (500 mg/kg) Body Weight

Biochemical	control	*A. indica*	*S.occidentalis*	*Senna siamea*
ALP (U/L)	169.16±83.88^{a}	173.33±13.69 ^{a}	166.66±60.85 ^{a}	171.83±87.50 ^{a}
AST (U/L)	112.50±55.74^{a}	142.66±10.98 ^{a}	140.00±19.57 ^{a}	145.00±40.14 ^{a}
ALT (U/L)	42.33±19.33^{a}	36.66±7.34 ^{a}	31.50±15.35 ^{a}	35.66±17.35 ^{a}
TPROT(g/dL)	5.06±0.82^{a}	5.31±0.19^{a}	5.16±0.63^{a}	5.00±0.94^{a}
ALB (g/dL)	3.70±0.20^{a}	3.00±0.14^{a}	3.78±0.43^{a}	3.78±0.54^{a}

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

Table 5: Biochemical Acute Toxicity Study of Methanol Leaf Extracts of *A. indica, S. occidentalis* and *S. siamea* rally adminitered to Rats at (1000 mg/kg) Body Weight

Biochemical	control	*A. indica*	*S.occidentalis*	*Senna siamea*
ALP (U/L)	169.16±83.88^{b}	126.33±10.23^{ab}	108.83±11.75^{a}	145.00±22.87^{ab}
AST (U/L)	112.50±55.74^{a}	95.16±17.05 ^{a}	81.00±22.91^{a}	90.00±16.78^{a}
ALT (U/L)	42.33±19.33^{a}	42.16±41.11^{a}	46.00±7.16^{a}	39.00±49.33^{a}
TPROT(g/dL)	5.06±0.82^{a}	5.41±0.32^{ab}	5.76±0.52^{b}	5.71±0.30^{b}
ALB (g/dL)	3.70±0.20^{b}	3.40±0.10^{a}	3.50±0.54^{ab}	3.50±0.06^{a}

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

Table 6: Biochemical Acute Toxicity Study of Methanol Leaf Extracts of *A. indica, S. occidentalis* and *S. siamea* orally adminitered to Rats at (3000 mg/kg) Body Weight

Biochemical	control	*A. indica*	*S.occidentalis*	*Senna siamea*
ALP (U/L)	169.16±83.88^{a}	186.16±49.43^{a}	156.50±16.19^{a}	246.33±133.52^{a}
AST (U/L)	112.50±55.74^{a}	155.50±10.44^{b}	144.33±8.62^{ab}	140.00±6.16^{ab}
ALT (U/L)	42.33±19.33^{a}	62.50±7.56^{b}	49.00±4.70^{a}	48.33±5.68^{a}
TPROT(g/dL)	5.06±0.82^{a}	5.73±1.63^{c}	5.40±0.74^{b}	5.90±1.17^{c}
ALB (g/dL)	3.70±0.20^{b}	3.46±0.63^{a}	3.36±0.23^{a}	3.35±0.41^{a}

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

DISCUSSION

This study has revealed that methanol leaf extracts of *A. indica, S.occidentalis* and *S.siamea* possesed phytochemical components such as steroids, terpenoids, cardial glycocides, tannins, saponins alkaloids, flavonoids, balsams, anthraquinones, and saponin. This could be attributed to the availability of the extractable component of different polarities. The findings indicated that the plants are rich sources of phytochemical components. Similar components were also earlier observed on whole plant of *S. occidentalis* (Egharevba *et al.,* 2010). Egharevba *et al.* (2013), 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* (Niyi, 2011; Padal, *et al.,* 2013; Susmitha *et al.,* 2013; Abdulrazaq *et al.,* 2020). The most important of these plants phytochemicals Constituents are alkaloids, tannins, flavonoids, anthraquinonnes, and phenolic compounds (Okwu, 2001). Alkaloids, anthraquinonnes, cardiac glycosides, flavonoids, phlobatannin, polyphenols, saponins, steroids, tannins and terpenoids were groups of phytochemicals observed in *S.siamea* (Kendeson *et al.,* 2018). Other study include Ronan *et al*., 2009; Sheeba *et al*., 2009 and Odeja *et al.,* 2015) that reported presence of similar phytochemical components in *A. indica,* S.occidentalis *and S.siamea*. This report is in line with report by Tamasi *et al.,* (2012) who reported presence of terpenoid in *S. occidentalis* and Oyun and Oyetayo (2020) in *A. indica*

The antimalarial study of *A. indica. S.occidentalis and S.siamea* methanol leaves *extracts.* The study revealed that the combination therapy is the most effective against trophozoites growth of *P.falciparum,* with no significant difference with standard antimalarial even at higher concentration, followed by *S. siamea*. The standard antimalaria significantly (P<0.05) inhibited the growth of trophozoites up to 95.39% at 400µg/ml. *A. indica* leaves extract significantly inhibited *trophozoite* growth by 15.41% of at 100 µg/ml, which showed significant difference compared to standard antimalaria drugs but showed no significant difference with *S.occidentali* with 13.84% growth inhibition and *S. siamea* with 23.07% growth inhibition. At 900 µg/ml mg/mL *trophozoite* growth were inhibited significantly by 96.91%.this differs significantly compared to standard antimalarial drug and combination therapy. This could be attributed to the Synergistic effects of combination of phytochemicals of *A. indica, S. occidentalis* and *S. siamea*.

This result is in conformity the findings of Some Zuleta-Castro *et al.,* (2021) that reported extract fractions showed impressive activity against *P. falciparum* with IC_{50} values of 13.66 and 9.80 µg ml^{-1} which was higher than that of the crude extract in an in-vitro assey agains *P. falciparum*. Yusuf *et al.,* (2011) reported antiplasmodium activity of neem leave extract with 3.86ug/ml growth inhibition 50 after 32 hours, using varying concentration on clone *Plasmodium falciparum*. After working on rats infected with *Plasmodium berghei,* but this result did not confirm with the findings of Farahna *et al*., (2010), that concluded Neem extract was not protective against malaria symptoms and signs in this mouse model infected with *P. berghei*.

The difference may be attributed to the location of plant sample collection, period or species of *Plasmodium* used for the experiment. *S.siamea* leaves extract significantly inhibited the growth by 96.91% at 900 µg/ml, this indicate there is significant difference compared to standard antimalaria. While *S.siamea* at 500 µg/ml inhibited trophozoites growth significantly by 92.29%, this indicates there is significant difference between the two plants, and when compared to standard antimalaria drugs. Combination therapy inhibited trophozoites growth significantly by 98.48% at 400 µg/ml this indicates that there is significant difference compared to standard antimalaria drugs. These findings are in conformity with that of Mogaka *et al.,* (2023) who reported that the methanol extracts of *S. occidentalis* show antiplasmodial activity with a calculated IC_{50} of 1.76 µg/mL against 3D7 strain. The methanolic extract was also tested in vivo (*P. berghei*), showing an antiplasmodial effect of 200 mg/kg and effective dose (DE_{50}) of 34.13 mg/kg. Another report of in vitro antiplasmodial activity of seven ethanol extracts by Tona *et al.,* (2004) revealed a high antiplasmodial activity (IC_{50}<3 µg/ml) was observed for *Cassia occidentalis* leaves. Other findings includes doses (Ajaiyeoba *et al.,* (2008); Javeres *et al.,* (2019); Daskum, (2020) and Yakub *et al.,* 2022) who reported similar antiplasmodial avtivity from the same plants.

The present study demonstrated that the methanol leaf extracts of *Azadirachta indica*, *Senna occidentalis*, and *Senna siamea* do not cause significant in vivo toxicity in an animal model. No mortality or visible signs of toxicity were observed in rats treated with extract doses of 500 mg/kg, 1000 mg/kg, and 3000 mg/kg. Biochemical parameters, including ALP, AST, ALT, total protein (TPROT), and albumin (ALB), showed no significant differences compared to the control group at 500 mg/kg and 1000 mg/kg. However, *A. indica* showed a significant difference in AST levels compared to the control group (155.50 U/L). Additionally, methanol extracts of all three plants showed significant differences in TPROT levels (*A. indica*: 5.73 g/dL, *S. occidentalis*: 5.40 g/dL, *S. siamea*: 5.90 g/dL), but these values remained within the normal range of 5.0–7.6 g/dL (UCLA, 2013). The absence of significant toxic effects in the extracts of *Azadirachta indica*, *Senna occidentalis*, and *Senna siamea* can be attributed to several factors, including their phytochemical composition, traditional use, and metabolic properties and the ability of the liver and kidneys to process and excrete these compounds.

These findings are consistent with previous studies. Dorababu *et al.,* (2016) reported that the LD_{50} of aqueous extracts of *A. indica* is above 2.5 g/kg, while Kingsley *et al.,* (2012) and Ghatule *et al.,*

(2012) found that the LD_{50} of ethanolic extracts exceeded 5 g/kg in animal models. Similarly, Mirtes *et al.,* (2012) observed no mortality or signs of toxicity in rats treated with *S. occidentalis* at 5 g/kg over 14 days of observation. Other studies by Martin and Annick (2023), Alkali *et al.,* (2018), and Nabukenya *et al.,* (2014) also confirmed the safety of aqueous and ethanol extracts, reporting LD_{50} values above 5000 mg/kg. Additionally, Yakubu *et al.,* (2022) reported that the LD_{50} of methanol leaf extracts of *S. siamea* in rats exceeded 5000 mg/kg, with another study by Vattem *et al.,* (2012) estimating an LD_{50} of 9600 mg/kg. Furthermore, Ntandou *et al.,* (2015) observed no mortality in albino Wistar rats up to 3000 mg/kg following oral administration of aqueous and ethanol stem bark extracts of *Cassia siamea*. Agbodjogbe *et al.,* (2017) further confirmed that *A. indica*, *S. occidentalis*, and *S. siamea* are safe for use.

Overall, the present study supports the safety of these medicinal plant extracts, as evidenced by their high LD_{50} values and the absence of significant toxicity in animal models. These findings reinforce the potential of *A. indica*, *S. occidentalis*, and *S. siamea* as promising candidates for further pharmacological and clinical evaluations in malaria treatment.

CONCLUSION

This study confirms that methanol leaf extracts of *Azadirachta indica*, *Senna occidentalis*, and *Senna siamea* contain bioactive phytochemicals with potential pharmacological benefits. The extracts significantly inhibited *Plasmodium falciparum* trophozoite growth, with combination therapy showing the highest efficacy, comparable to standard antimalarial drugs. Among individual extracts, *S. siamea* exhibited the strongest antiplasmodial activity. All plant extracts showed dose dependent.

Toxicity tests revealed no significant adverse effects in animal models, supporting the safety of these plant extracts with LD_{50} values of above 3000 mg/kg. These findings suggest that *A. indica*, *S. occidentalis*, and *S. siamea* could serve as promising candidates for new antimalarial drug development. Further research should focus on isolating active compounds and conducting clinical trials to validate their efficacy and safety in humans

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

Abdulrazaq A., B. Abdulkadi, N. T. Isyaku, M. Yahaya, Badru O. A., L. Adamu and Alkali K. (2020). In Vitro Antimalarial Activity of Extracts of Some Indigenous Plant Species in Kebbi State. *UMYU Journal of Microbiology Research,* 5 (2): 1- 10.

Achi N. K., C. Onyeabo, D. A. Nnate, C. A. Ekeleme-Egedigwe, I. K. Kalu, I. C. Chibundu and G. C. Wokoma (2018). Therapeutic effects of Azadirachta indica A.Juss. leaves in malaria induced male Wistar rats. *Journal of Pharmacy & Pharmacognosy Research,* 6 (3), 191-204

Agbodjogbe K. W. D., A. G. Houngbeme, M. Haïdara, R. Sanogo, A Laleye and H. P. Dansou (2017). In-Vitro and In-Vivo Toxicological Evaluation of Aqueous Extracts From beninese plants: Senna siamea leaves and Sarcopcephalus733 latifolus stem roots. I*nt J Pharmacogn*. 2017;4(2):60–70.

Ajaiyeoba E.O., Ashidi J.S., Okpako L.C., Houghton P.J., Wright C.W. (2008). Antiplasmodial compounds from *Cassia siamea* stem bark extract. *Phytotherapy Research*. 22:254–255.

Alebiosu CO, Ugwah OM, Ugwah Oguejiofor CJ, Njan AA, (2012) Ethno botanical studies of medicinal plants used in the management of Peptic ulcer disease in Sokoto State, North Western Nigeria. Int Res J Pharm Pharmacol 2:225-230.

Alkali K, Abdullahi K, Mshelia HE, Yahaya MA, Abubakar N and Tarbutu AG (2018) 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). *Research Journal of Zoology* 1:3.

Alzohairy, M.A. (2016). Therapeutics Role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine*. pp. 1-11 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4791507/pdf/ECAM2016-7382506.pdf

Awofeso N (2011) Neem tree extract and malaria control: Prospects and challenges. *Spatula* DD 1:167-174.

Ayoola GA.,HA Coker, SA Adesegun, AA Adepoju-Bello, K Obaweya, EC Ezennia and TO Atangbayila (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, 7 (3): 1019-1024.

Bleakley, H. (2007). "*Malaria in the Americas: A Retrospective Analysis of Childhood Exposure*," University of Chicago. **773:**834-2192.

Daskum, A. M., Chessed, G., Qadeer, M. A.2 and Ling, L. Y. (2020). Phytochemical screening, Gas Chromatography Mass Spectroscopy (GC-MS) and *in vitro* antiplasmodial analysis of *Senna siamea* leaves as antimalarial, Yobe State, Nigeria. *Nigerian Journal of Parasitology,* 41(1): 60-67.

Dorababu, M.; Joshi, M.C.; Bhawani, G.; Kumar, M.M.; Chaturvedi, A.; Goel, R.K. (2016). Effect of aqueous extract of neem (Azadirachta indica) leaves on offensive and diffensive gastric mucosal factors in rats. *Indian Journal of Physiology* and *Pharmacology*. 50, 241–249.

Efe M. Omwirhiren, S A. James and A O. Abass (2016). The Phytochemical Properties and Antimicrobial Potentials of Aqueous and Methanolic seed Extract of Cola nitida (Vent.) and Cola acuminata (Beauvoir) grown in South West, Nigeria. *Saudi Journal of Medical and Pharmaceutical Sciences,* 2 (12): 354-363

Egharevba, H.O., Odigwe A. C., Abdullahi M.S., Okwute, S.K., kogun, J.I., (2013). Phytochemical Analysis and Broad Spectrum Antimicrobial Activity of *Cassia Occidentalis* L. (whole plant); *New York Science Journal*; 3(10), 74-81

Egharevba, H.O., Odigwe, O.C., Abdullahi, M.S., Okwute, S.K., and Okogun, J.I., (2010) Phytochemical Analysis and Broad Spectrum Antimicrobial Activity of *Cassia Occidentalis* L. (whole plant); *New York Science Journal*; 3(10)

Erhirhie E. O., C. P. Ihekwereme, E. E. Ilodigwe (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology.*11 (1): 5–12

Farahna M, Bedri S, Khalid S, Idris M, Pillai CR, Khalil EA. (2010). Anti-plasmodial effects of Azadirachta indica inexperimental cerebral malaria: Apoptosis of cerebellar Purkinje cells of mice as a marker. *North American Journal of Medical Sciences;* 2:518-525.

Ghatule, R.R.; Shalini, G.; Gautam, M.K.; Singh, A.; Joshi, V.K.; Goel, R.K. (2012). Effect of Azadirachta indica leaves extract on acetic acid-induced colitis in rats: Role of antioxidants, free radicals and myeloperoxidase. *Asian Pacific Journal* of *Tropical Disease*. 2, S651–S657.

Gul R.**,** S U Jan, S Faridullah, S Sherani, and N Jahan (2017). "Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan", *The Scientific World Journal*, 2017: 7 pages, https://doi.org/10.1155/2017/587364

Habibi P., Y Shi, M F Grossi-de-Sa and I Khan (2022). Plants as Sources of Natural and Recombinant Antimalaria Agents, review. *Molecular Biotechnology*. 64:1177–1197

Jadhav, P., Lal H., Kshirsagar N., (2014) Pharmacodynamic evaluation for antiplasmodial activity of *Holarrhena antidysentrica* (Kutaja) and *Azadirachta indica* (Neemb) in *Plasmodium berghei* infected mice model; *Asian Pacific Journal of Tropical Medicine* doi:10.1016/S1995-7645(13)60089-9

Javeres M N. L., S M. Nurulain, O G. Hamadama, H J. Bello and A Muazu (2019). In vivo Anti-Plasmodium Activity and Toxicity of Afzelia bipindensis and Senna Siamea Extracts: A Murine Model. *The Open Medicinal Chemistry Journal*, 13: 50-57.

Kamagaté M., C Koffi, N M Kouamé, A Akoubet, N A Roland Yao and H M Die-Kakou (2014). Ethnobotany, phytochemistry, pharmacology and toxicology profiles of Cassia siamea Lam. *The Journal of Phytopharmacology,* 3(1): 57-76

Kendeson A. C, Abdulkadir A. G, Danja A B, Abdu Z, Alfazazi A (2018). Phytochemical Screening, Proximate and Elemental Analyses of *Senna siamea* (Lam.) leaves from Kashere metropolis of Gombe State, Nigeria. *International Research Journal of Chemistry and Chemical Sciences*, 5(1): 082-085,

Kingsley, O.-A.; Oseni, L.A.; Quasie, O.; Antwi, S.; Mavis, T. (2012). A comparative evaluation of in vivo antiplasmodial activity of aqueous leaf exracts of Carica papaya, Azadirachta indica, Magnifera indica and the combination thereof using Plasmodium infected BALB/c mice. *International Journal of Applied Biology* and *Pharmaceutical Technology*. 3, 372–378.

Martin S. Y and T. Annick (2023). *The Biology, Physiology, and Control of Termite: Termiticidal Activity of Senna occidentalis and Tithonia divercifolia Extract.* Intechopen. Pp. 1-16. DOI: http://dx.doi.org/10.5772/intechopen.1002365

Mary, A.H., Robert, L., Kimball, J. and Peterson, C. (2005). Mathematics Exercises in Biotechnology: *National Science Foundation*. Pp 1- 152

Mirtes G.B. S., Ticiana P. A., Carlos F.B. V., Pablo A. F., Bruno A. A., Igor M.A. C., João H. C., Almir G. W., Simone S.L. L, (2012). Acute and subacute toxicity of Cassia occidentalis L. stem and leaf in Wistar rats, *Journal of Ethnopharmacology,* 136, (2): 341-346,

Mogaka, S.; Molu, H.; Kagasi, E.; Ogila, K.; Waihenya, R.; Onditi, F.; Ozwara, H. (2023)**.** Senna occidentalis (L.) Link Root Extract Inhibits Plasmodium Growth in Vitro and in Mice. *BMC Complementary Medicine and Therapies*. 23, 71

Mohammed M, Aboki MA, Saidu HM, Victo O, Tawakalitu A,. (2012) Phytochemical and some antmicrobial activity of cassia occidentalis L. (Caesalpiniaceae). Int J Sci Tech 2:133-154.

Nabukenya I, Rubaire-Akiiki C, Mugizi D, Kateregga J, Olila D and J. Höglund. (2014) Sub-acute Toxicity of Aqueous Extracts of Tephrosia vogelii, Vernonia amygdalina and Senna occidentalis in Rats. Nat Prod Chem Res 2: 143.

Nayyar, G.M., J.G. Breman, P.N. Newton, and J. Herrington. (2012). "Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa." *Lancet Infectious Diseases* 12 (6):488–496.

Niyi, A., (2011) Neem tree extract *Azadiracta indica* and malaria control Prospects and challenges: *spatula DD.* 1(3):167-174

Ntandou, G. N., Bassoueka, D. J., Banzouzi J. T. , E. Ossibi, E. A. W., Itou, E. R. D. G., Makambila, M. C., Ramos, S., Benoit-Vical, F., Abena, A. A., & Ouamba, J. M. (2015). Assessment of Cassia siamea stem bark extracts toxicity and stability in time of aqueous extract analgesic activity. *African Journal of Pharmacy and Pharmacology*, 9(41), 988-994.

Oboh, M.A., Faal, F., Adeniji O., S. Correa, A. U. Amawu, E. Ogban, E. Heinz, G. Hughes, M. M. Meremikwu and A Amambua-Ngwa (2022). Multiple Plasmodium falciparum drug resistance polymorphisms identified in a pregnant woman with severe malaria and a concomitant spontaneous abortion in Cross River, *Nigeria, West Africa.* *Malaria Journal* 21, 160 https://doi.org/10.1186/s12936-022-04176-9

Odeja O., G Obi, C Ogwuche, E E Elemike and Y Oderinlo (2015). Phytochemical Screening, Antioxidant and Antimicrobial activities of Senna occidentalis (L.) leaves Extract. *Clinical Phytoscience,* 1:6

Odiegwu C.N.C., Chianella I., Azubike N.C., Odiegwu U.O and Ogbuowelu O.S (2021). Liver function tests values in Albino Wistar rats administered with isolated Nigeria Achatina achatina snail lectin. *GSC Biological and Pharmaceutical Sciences,* 15(02), 092–102

Okwu, D.E. (2001) Evaluation of the Chemical Composition of Indigenous Spices and Flavoring Agents. Global Journal of Pure and Applied Sciences, 7, 455-459

Oyun J F and V O Oyetayo (2020). Assessment of the Preservative Efficacy of Azadirachta indica A. Juss. And Psidium guajava L. Leaf Extracts on Capsicum annuum L. (Bell Pepper). *South Asian Journal of Research in Microbiology,* 7(2): 19-33.

Padal, S. B., B. Sandhya sri, P. Chandrasekhar and Y.Vijayakumar (2013). Folklore treatment of skin diseases by the tribes of G. Madugula Mandalam, Visakhapatnam District, Andhra Pradesh, India. *Journal of Environmental Science, Toxicology and Food Technology*, 4, (1) 26-29

Patil, U.H. and Gaikwad, D.K. (2010). Phytochemical evaluation and bactericidal potential of *Terminalia arjuna* stem bark. *International Journal of Pharmaceutical Sciences and Research*. 2(3): 614-619.

Rojas-Sandoval J and P. Acevedo-Rodríguez (2022). Senna siamea (yellow cassia), *CABI International, cabicompendium.*11462, https://doi.org/10.1079/cabicompendium.11462

Ronan K. R., M F. Canoy and K. J. Burke (2009). Child Maltreatment: Prevalence, Risk, Solutions, Obstacles. *Australian Psychologist*. 44 (3) Pp. 195-21

Sabri F Z., Belarbi M, Sabri S, Alsayadi Muneer M.S (2012). Phytochemical Screening and identification of some compounds from Mallow. *Journal of Natural Product and Plant Resources*, 2 (4):512-516

Sarkar S, Singh RP, and Bhattacharya G. (2021). Exploring the role of Azadirachta indica (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. *Biotech;* 11(4):178. Doi.

Sato S. (2021). Plasmodium-a brief introduction to the parasites causing human malaria and their basic biology. *Journal of Physiological Anthropology* (1):1.

Sheeba C J., R. P. Andrade, D. Duprez and I. Palmeirim (2009). Comprehensive analysis of fibroblast growth factor receptor expression patterns during chick forelimb development. International Journal of Developmental Biology. 54: 1517-1526

Shivjeet S., Sandeep K.S., Ashutosh Y. (2013). Review on Cassia species: Pharmacological, Traditional and Medicinal Aspects in Various Countries. American Journal of Phytomedicine and Clinical Therapeutics; 1: 291-312.

Srinivas S D and Puri S K (2002). Time Course of in Vitro Maturation of Intra-erythrocytic Malaria Parasite: a Comparison between Plasmodium falciparum and Plasmodium Knowles. Memórias do Instituto Oswaldo Cruz, Rio de Janeiro, Vol. 97(6): 901-903

Susmitha S., K.K. Vidyamol, P. Ranganayaki and R. Vijayaragava (2013). Phytochemical Extraction and Antimicrobial Properties of Azadirachta indica (Neem). Global Journal of Pharmacology 7 (3): 316-320,

Tasiam E, Primaharinastiti R and Ekasari W (2020). *In Vitro* Antimalarial Activity and Toxicity Studies of Johar (*Cassia Siamea*) Leaves From Three Different Locations. *African Journal* of *Infectious Disease*. 14(2):23-29.

Tona L., R.K. Cimanga, K. Mesia, C.T. Musuamba, T.De Bruyne, S. Apers, N. Hernans, S. Van Miert, L. Pieters, J. Totté, A.J. Vlietinck, (2004). In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo, *Journal of Ethnopharmacology*, 93 (1): 27-32.

Uneke, C. J. (2009). Impact of home management of *Plasmodium falciparum* malaria on Childhood malaria control in sub-Saharan Africa. *Tropical Biomedicine;* 26: 182–199

Vattem, D.A., V. Maitin and C.R. Richardson, 2012. Evaluation of antibacterial and toxicological effects of a novel sodium silicate complex. Res. J. Microbiol., 7: 191-198.

Wadood, A., Ghufran, M., Jamal, S.B. and Naeem, J. (2013). Phytochemical analysis of medicinal plants occurring in local area of mardan. *Analytical Biochemistry*, 4(2): 1-3

WHO (2021). Global technical strategy for malaria 2016–2030, 2021 update. Geneva: World Health Organization; (https://apps.who.int/iris/handle/10665/342995.

WHO (2023). World malaria report 2023 [EN/AR/RU/ZH] Geneva, World Health Organization, Pp 1-283

WHO (2024). World malaria report 202, Addressing inequity in the global malaria response**.** Geneva, World Health Organization, Pp. 1-283

Willcox M. L and G. Bodeker, (2004). Traditional herbal medicines for malaria, British Medical Journal 13; 329(7475): 1156–1159

Yakub A U., M A Garba, A M Umar, U I Adam, S Yahay, Ankita Mathur (2022). In-Vitro Antiplasmodial Activity of Aqueous and Ethanol Stem and Leaf Extracts of Senna occidentalis (Coffee Senna). *Journal of Indian Research,* 10 (2). 61-77.

Yusuf H., Suraiyatu and Mariyatu (2011). Antiplasmodium Activity of Neem Leave Extract (*Azadirachta Indica,)* On *Plasmodium Falciparum* In Vitro. *Proceeding of Annual International Conference Syiah Kaula University, Banda Aceh,* Indonesia. Pp. 29-30.

Zhao D, Zhang H, Ji P, Li S, Yang C, Liu Y, Qian D, Deng Y, Wang H, Lu D, Zhou R, Zhao Y. (2021). Surveillance of Antimalarial Drug-Resistance Genes in Imported Plasmodium falciparum Isolates From Nigeria in Henan, China, 2012-2019. *Front Cell Infect Microbiol*. 11:644576. doi: 10.3389/fcimb.2021.644576. PMID: 33968801; PMCID: PMC8102827

Zuleta-Castro, C., Rios, D., Hoyos, R., and Rozco-Sanchez, F. (2021). "In Vitro Antiplasmodial Activity of an Azadirachta indica Cell Culture Extract" *Proceedings of the National Academy of Sciences, India, Section B: biological sciences* 91 (1): 81-88.