

Anti-Plasmodial Activity of Methanol Extract of *Ficus sycomorus*

Abbas AY^{1*}, Ladan MJ¹, Girei AM², Achor M³

¹Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

²National Universities Commission, Abuja Nigeria

³Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

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*Corresponding author: Abbas AY

Abstract

Malaria has been reported to be one of the most debilitating illnesses of all time and is the most common parasitic disease in sub-Saharan Africa. Methanol extract of the four parts of *Ficus sycomorus* plant (leave, fruits, stem-bark and roots) were screen for *in vitro* anti-plasmodial activity using *Plasmodium falciparum*. The IC₅₀ revealed that the fruits and leave extract have an IC₅₀ > 186µg while stem-bark and roots were discovered to be the most active with IC₅₀ of 20.4µg. *In vitro* anti-plasmodial activity of organic solvent extracts (Hexane, ethylacetate and saturated butanol) of the methanol root extract revealed IC₅₀ of 40µg, 20.4µg and 20.4 µg respectively. *In vivo* anti-plasmodial activity of the saturated butanol extract of the root was investigated in albino mice. Thirty (30) mice were inoculated with the parasitized donor erythrocytes containing *Plasmodium berghei*. One week after parasite inoculation, the animals were randomly distributed into six groups of five mice each. Group 1 served as negative control (not treated), groups 2 to 5 were experimental groups and were administered 20%, 40%, 60% and 80% of the LD₅₀ (31mg, 62mg, 93mg and 124mg), while group 6 served as positive control (treated with artesunate). The mice were treated orally for seven consecutive days once daily. The results showed that the saturated butanol has anti-plasmodial activity in mice with percentage parasitemia inhibition of 50.6%, 80.8%, 100%, 100% and 100% for groups 2 to 5 and artesunate respectively. The results suggest that the plant have potential for the development of a novel anti-malarial agent.

Keywords: Malaria, Methanol, *Ficus sycomorus*, root and parasitemia.

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INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan of the *Plasmodium* family [1]. It is commonly transmitted by the bite of an infected female *Anopheles* mosquito [2]. The bite introduces the parasites from the mosquito's saliva into a person's blood. In the human body, the parasites multiply in the liver, and then erythrocytes [3, 2]. Symptoms include fever, fatigue, vomiting and headaches, in severe cases it can cause yellow skin, seizures, coma or death [4].

Malaria has proved to be one of the most debilitating illnesses of all time and is the most common parasitic disease in sub-Saharan Africa [5]. Globally, death associated with malaria is highest in Africa and most vulnerable group in the endemic areas is the people in the rural environment who often have little or no access to modern medicine [6]. It is estimated that 1 - 2 million people die yearly as a result of malaria [6]. It is the single most important cause of

ill health, death and poverty in Sub-Saharan Africa [7]. The situation is further complicated by the worldwide emergence and rapid spread of resistance to several existing anti-malarial by *Plasmodium falciparum* that threatens to increase the annual death toll by malaria [8].

The use of medicinal plants to treat diseases is as old as man he and medicinal plants have been used since ancient times to treat many illnesses [9]. Herbal medicine was the sole medical system for health care before the advent of orthodox or modern medicine [10]. Even in this present technological era, traditional medicine play a predominant role in the third world for the preservation of health of the rural majority who constitute over 70% of the total population [10]. The use of traditional remedies is common in sub-Saharan Africa, and visits to traditional healers remain a mainstay of care for many people because of preference, affordability, and limited access to hospitals and modern health practitioners [11].

Studies have shown that many medicinal plants contained antioxidant compounds and these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, antiparasitic, antifungal and antiviral activities [12]. The search for antimalarial drug from plant origin cannot be neglected since the antimalarial drugs in use today (quinine and artemisinin) were isolated from plants sources [13].

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study are of analytical grade

Ficus Sycomorus Plant

Ficus sycomorus (fruits, leaves, stem-bark and roots) was obtained from Sokoto metropolis, Sokoto State, Nigeria. The plant was identified at the Department of Biological Sciences, Usmanu Danfodiyo University, and Sokoto, Nigeria. A voucher specimen number was obtained (UDUH/ANS/0214) and specimen deposited at the herbarium.

Animals

Albino mice and rats were obtained from the Animal House of the Department of Biochemistry and Faculty of Sciences Usmanu Danfodiyo University, Sokoto, Nigeria respectively. The animals were housed in standard cages in Animal House, Department of Biochemistry. The animals were allowed access to feed and clean water *ad libitum* for seven days to acclimatize before commencement of research.

Malaria Parasites

Plasmodium falciparum

Plasmodium falciparum was obtained from Microbiology laboratory, College of Health Sciences (City Campus), Usmanu Danfodiyo University, Sokoto Nigeria.

Plasmodium berghei

Plasmodium berghei was obtained from Animal House of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria Nigeria. Blood from infected mouse was collected through the eyes, diluted in saline and 0.2 ml was intraperitoneal passaged to three mice. The mice were transported in a small cage to the Animal House of the Biochemistry Department, Usmanu Danfodiyo University, Sokoto, Nigeria

Preparation of the Plant Methanol Extracts

The four parts of the plant (fruits, leaves, stem-bark, and roots) were prepared into powdered form using pestle and mortar and then stored in plastic containers for subsequent use under room temperature. Two hundred (200) grams of each of the powdered samples (fruits, leaves, stem-bark, and roots) were soaked in 500ml of methanol for seventy two (72) hours and stirred occasionally. They were filtered with a clean muslin cloth, and the filtrates were evaporated to dryness using rotary evaporator at 45°C. The recovered methanol extracts (filtrates) were stored in plastics containers and kept under room temperature.

In vitro Anti-plasmodial Activity methanol crude extracts of the various part *Ficus Sycomorus*

Plasmodium falciparum was used for the *in vitro* anti-plasmodial activity of the methanol extracts (fruits, leaves, stem-bark and roots) 30 mg, 60 mg, 90 mg, 120 mg and 30 mg of artesunate were dissolved in 4.5ml distilled water and allow to fully dissolve for twelve [12] hours. These were used for the anti-plasmodial activity assay. Drug sensitivity assay was carried out on 96-well microtiter plates. One hundred (100µL) of the culture was placed in each well of the plate, 30µL of the different extracts and artesunate were added to the wells and the positive control, while the negative control contained no treatment (46.5µg, 93µg, 139.5µg, 186µg and 46.5µg respectively). The plate was placed in a candle jar and incubated at 37°C for 24 hours [14].

After 24 hours, blood from each well was harvested and thin film was prepared for each concentration. The slide was dried under room temperature and fixed by immersing in methanol. The slide was stained with 10% Giemsa for 20 minutes and washed with distilled water to remove excess stain. It was then dried and observed under the microscope [15]. An area of stained thin blood film where the red blood cells (RBCs) were evenly distributed was observed using X 100 objective (under oil immersion) [15]. Approximately 200 RBCs were counted and the numbers of infected RBCs amongst the 200 RBCs were also counted. The slide was moved to adjacent fields and counting was continued as mentioned earlier. Counting was continued until 5 fields were viewed and 1000 erythrocytes were counted [14]. The percentage parasitaemia was calculated by counting the number of parasitized red cells in 1000 cells in a thin blood film [15,16].

$$\text{Percentage parasitaemia (\%)} = \frac{\text{Total No. of PRBC}}{\text{Total No. RBC Viewed}} \times 100 = \frac{\text{PRBCs}}{1000} \times 100$$

Where; PRBC: Parasitized red blood cells and RBC: red blood cells.

Growth inhibition for each concentration for the extracts was determined by percentage reduction of

parasitemia in treated well compared with untreated well or negative control [16].

$$\text{Growth inhibition} = \frac{\text{PRBCs in Negative Control} - \text{PRBCs in Treated well}}{\text{PRBCs in Negative Control}} \times 100 \text{ [16].}$$

***In vitro* Anti-Plasmodial Activity of the Organic Solvent Extracts of the Roots**

The methanol root extract was fractionated with three organic solvents (Hexane, ethylacetate and saturated butanol) and *in vitro* anti-plasmodial activity for the solvent extracts was carried out following procedure and concentrations as above. Percentage parasitemia and growth inhibition were calculated following same methods as above.

In vivo* Anti-plasmodial Activity of Saturated butanol root extract of *Ficus sycomorus

Saturated butanol extract was used for the *in-vivo* anti-plasmodial activity in mice using *Plasmodium berghei*.

Inoculation of the experimental animals (Mice)

After acclimatization, thirty (30) mice were inoculated with the parasitized donor erythrocytes containing *Plasmodium berghei*. Each mouse was injected 0.2ml of the infected blood intraperitoneal using 1ml syringe [17]. One week after parasite inoculation, the animals were randomly distributed into six groups of five mice per group and were used for the experiment.

Administration of the Saturated Butanol Extract

The thirty (30) infected mice were distributed into six (6) groups (1-6). Group 1 served as negative control, groups 2 to 5 were experimental groups while group 6 served as positive control [8, 15, 18]. Animals in group 6 were treated with orthodox anti-malarial drug (artesunate) for the test period. Group 1 were treated with artesunate, groups 2 to 5 were treated with different concentrations of the saturated butanol extract as percentage of the LD₅₀; 20%, 40%, 60% and 80% of the LD₅₀ for 7 days being the usual practice for traditional medicine (31mg, 62mg, 93mg and 124mg) while Group 6 were given normal saline throughout the period of the study. The animals were treated for seven consecutive days once daily by administering the extract orally using cannula.

Estimation of Parasitemia after the Treatment

Twenty four (24) hours after the treatment, blood was collected from the tail of each mouse and smeared on to a microscope slide to make a film. A drop of the blood (3µL) was placed on one end of the slide it was smeared across with the help of another slide where a thin film was produced. The slide was

dried under room temperature and fixed by immersing in methanol. The slide was stained with 10% Giemsa for 20 minutes and washed with distilled water to remove excess stain. It was then dried and observed under the microscope [15].

An area of stained thin blood film where the red blood cells (RBCs) were evenly distributed was observed using X 100 objective (under oil immersion) [15]. Approximately 200 RBCs were counted and the numbers of infected RBCs amongst the 200 RBCs were also counted. The slide was moved to adjacent fields and counting was continued as mentioned earlier. Counting was continued until 5 fields were viewed and 1000 erythrocytes were counted [11]. The recommended procedure for estimating the percentage parasitaemia in a thin blood film is by expressing the number of infected cells as a percentage of the red blood cells. The percentage parasitaemia was calculated by counting the number of parasitised red cells in 1000 cells in a thin blood film [15].

Growth inhibition for saturated butanol extract in mice was determined by percentage reduction of parasitemia in treated mice compared with untreated mice or negative control [16]. Percentage parasitemia and growth inhibition were calculated using same methods as above.

RESULTS

In vitro* Anti-plasmodial Activity of the Methanol Extracts of the Four Parts of the *Ficus sycomorus

Table 1 showed the *in-vitro* anti-plasmodial activity assay for the four parts of the *Ficus sycomorus* (Fruits, Leaves, Stem-Bark and Roots), the negative control and the positive control for comparison. The assay revealed that methanol extracts of the fruits and leaves have little anti-plasmodial activity with leaves extract having more activity than the fruits extract. The methanol extract of the stem-bark and the roots have the highest anti-plasmodial activity, for all parasites were cleared from the least concentration of extracts. Table 1 presents The IC₅₀ values for the four parts of the plant (fruits, leaves, stem-bark and root) are presented in table 1. The concentrations used for the fruit and leaves extracts only achieved 19.8% and 39.6% growth inhibition (IC₅₀ greater than 186µg). Fifty percent (50%) growth inhibition was achieved at 20.4µg for stem-bark and roots extracts.

Table-1: *In vitro* Anti-plasmodial Activities of the Methanol Extracts of the Four Parts of the *Ficus sycomorus*

Plant Part Used	Extract	% Yield of Extract (g)	IC ₅₀ (µg)
Fruits	Methanol	7.2	> 186
Leaves	Methanol	7.5	> 186
Stem-Bark	Methanol	5.2	20.4
Roots	Methanol	4.5	20.4
Artesunate	-	-	>11.5

IC₅₀ for fruits and leaves are greater than 186µg (> 186µg) and stem-bark and roots have IC₅₀ of 20.4µg each.

Table 2 showed phytochemical components of the methanol extracts of the stem-bark and root of the *Ficus sycomorus*. The analysis revealed the presence of alkaloids, tannins, anthraquinones, saponins, balsams and steroids in both stem-bark and root extracts.

However, cardiacglycosides and saponins are only present in stem-bark while glycosides are only present in the root. Flavonoid, volatile oils are not found in both extracts.

Table-2: Qualitative and Quantitative Phytochemical Contents of *Ficus sycomorus* Root and Stem-Bark Methanol Extracts

Phytochemical Components	Plant Extracts			
	Qualitative		Quantitative Concentration (w/w)	
	Stem-bark	Roots	Stem-bark	Roots
Flavonoid	ND	ND	-	-
Tannins	+++	++	2.40	1.48
Saponins	+++	++	1.00	0.80
Glycosides	ND	++	-	-
Alkaloids	++	++	1.89	1.78
Cardiac Glycosides	+	ND	-	-
Steroids	+	+	1.51	0.88
Saponin Glycosides	+	ND	0.56	-
Balsams	++	+	-	-
Anthraquinones	++	+++	0.21	0.50
Volatile Oils	ND	ND	-	-

KEY: ND = Not detected. + = Presence in trace amount. ++ = little amount. +++ = large amount

***In vitro* Anti-plasmodial Activities of Organic Solvents Methanol roots extract (Hexane, ethylacetate and saturated butanol)**

In vitro anti-plasmodial activity assay for the three organic solvent extracts (Hexane, Ethylacetate and saturated butanol) percentage yield and IC₅₀ for the

solvent extracts are presented in table 3. Hexane extract achieved 50% growth inhibition at 40µg concentration, ethylacetate achieved growth inhibition of 50% at 20.4µg concentration and saturated butanol achieved 50% growth inhibition at 20.4µg.

Table-3: *In vitro* Anti-plasmodial Activities of Organic Solvent Methanol Roots Extracts (Hexane, ethylacetate and saturated butanol)

Plant Part Used	Extract	% Yield of the Extract (g)	IC ₅₀ (µg)
Roots	Hexane	5.5	40
Roots	Ethylacetate	4.0	20.4
Roots	Saturated butanol	3.7	20.4
Artesunate	-	-	>11.5

Percentage yield for the solvent extract are 5.5g, 4g and 3.7g for hexane, ethylacetate and saturated butanol respectively. Hexane has IC₅₀ value of 40µg. Ethylacetate has IC₅₀ value of 20.4µg concentration. Saturated butanol has IC₅₀ value of 20.4µg.

***In vivo* Anti-plasmodial Activity of the Saturated Butanol Extract of the *Ficus sycomorus* in albino Mice**

Table 4 showed the *in vivo* anti-plasmodial activity of the different concentrations of the saturated

butanol extract of the *Ficus sycomorus* used in the experimental groups, percentage parasitemia inhibition for experimental groups positive control group.

Table-4: *In vivo* Anti-plasmodial Activity of the Saturated Butanol Extract of the *Ficus sycomorus* in albino Mice

Groups	Concentration of Extract (% LD ₅₀ /mg)	% Parasitemia Inhibition
I	20% (31)	50.6%
II	40% (62)	80.8%
III	60% (93)	100%
IV	80% (124)	100%
V	Artesunate (20)	100%

The percentage parasitemia inhibition against concentration of extract in mg. 20% and 40% LD₅₀ achieved 50.6% and 80.8% and 60%, 80% and artesunate achieved 100% inhibition.

DISCUSSION

The IC₅₀ values for the *in vitro* anti-plasmodial activity of the *Ficus sycomorus* revealed that all parts of the plant have anti-plasmodial property however extracts of the fruits and leaves showed that IC₅₀ is greater (>186µg), stem-bark and roots have IC₅₀ values of 20.4µg each. The solvent extracts also showed anti-plasmodial property with the IC₅₀ values of 40µg for hexane and 20.4µg for ethylacetate and saturated butanol. The *In vivo* anti-plasmodial activity of saturated butanol extract in mice showed IC₅₀ values of 31mg being the 20% LD₅₀. The percentage parasitemia inhibition indicated that the saturated butanol in mice achieved 50% growth inhibition at 20% LD₅₀ (31mg) concentration and 100 % growth inhibition at 60 % of the LD₅₀. The phytochemical composition of the plant is important, containing alkaloid, balsams, anthraquinones, cardiac glycosides, saponins, tannins, saponin glycosides, steroids and glycosides. The high preponderance of these phytochemicals may be responsible for the antimalarial activity exhibited by the plant [15]. These results suggest that there could be scientific merit in the folkloric use of *Fycus sycomorus* in the management of microbial diseases in the northern part of Nigeria.

Some bioactive compounds have been identified to be present in the *Ficus* species (Table 2) such as tannins, saponins, flavonoids, alkaloid, steroids, anthraquinone, glycosides, and reducing sugars. Several plant constituents like, flavonoids, tannins, quinonoid, xanthene, polyphenols, and terpenoids have been reported to possess protein-binding and enzyme-inhibiting properties. Based on the foregoing, it is suggest that a likely mechanism of action of this plant may be the inhibition of key pathogenic enzymes of the malaria parasite since these bio-compounds are known to interfere with enzyme systems [19].

CONCLUSION

The qualitative phytochemical constituents of the *Ficus sycomorus* indicated the presence of secondary metabolites such as tannins, saponnins, alkaloids, saponins glycosides, steroids, cardiac glycosides and glycosides all of which are known to be pharmacologically active. The study established that the methanol extracts of the stem-bark and root exhibited the highest *in vitro* anti-plasmodial activity,

and when the roots extract was partitioned, saturated butanol extract exhibited the highest anti-plasmodial activity. The *in vitro* activity of the extract was confirmed by *in vivo* study. The results suggest that the saturated butanol extract of the plant could serve as source of novel anti-plasmodial agent(s) that can be used to develop an anti-malarial drug.

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