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Original Research Article

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTI-PLASMODIAL ACTIVITY OF *JATROPHA TANJORENSIS* LEAF EXTRACTS AGAINST *PLASMODIUM FALCIPARUM*

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ABSTRACT

The challenge of antimalarial drug resistance necessitates scientific research to validate herbal preparations as cost-effective and accessible alternative therapies with equal or superior efficacy to synthetic drugs. This study evaluates the phytochemical constituents and anti-plasmodial activity of *Jatropha tanjorensis* leaf extracts against *Plasmodium falciparum*. Extracts were obtained through crude and sequential maceration methods, and their phytochemical constituents were analysed qualitatively and quantitatively using standard techniques. The *P. falciparum*-infected blood samples were cultured *in vitro*, and the susceptibility of the fresh isolates to artemether oil-based injection was evaluated. The isolates with the highest IC_{50} values for artemether were further assessed for *in vitro* susceptibility to the crude extracts of *Jatropha tanjorensis* leaves. The results indicated the presence of cardiac glycosides, tannins, phenols, saponins, flavonoids, alkaloids, steroids, terpenoids, proteins, and amino acids in the crude leaf extracts of *J. tanjorensis*. Among the extracts, ethyl acetate extract exhibited the highest total phytochemical content, particularly in tannins, phenols, saponins, flavonoids, and alkaloids. The *P. falciparum* isolates showed susceptibility to artemether, with IC_{50} values ranging from 0.24 to 0.77 µmol/L. The ethyl acetate extract also demonstrated significant anti-plasmodial activity against the isolates, with IC_{50} values of 4.77 µg/ml and 4.98 µg/ml at the early and late trophozoite stages of the *P. falciparum* intraerythrocytic cycle respectively. These findings highlight the potential of ethyl acetate extract of *J. tanjorensis* leaf as a natural antimalarial agent, necessitating additional research into its therapeutic potential.

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INTRODUCTION

Malaria is still a major public health issue, especially in tropical and subtropical areas like Africa, Southeast Asia, and the Americas, where it causes high mortality and morbidity [1]. *Plasmodium* protozoan parasites are the cause of the disease, with *Plasmodium falciparum* being the most lethal species, mostly to blame for mortality from malaria, particularly in sub-Saharan Africa [2]. Despite advancements in treatment, drug-resistant forms of *P. falciparum* emerging and proliferating have posed a substantial challenge to malaria control efforts [1]. Resistance to previously effective drugs such as chloroquine and sulfadoxine-pyrimethamine, and reduced

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efficacy of artemisinin-based combination therapies (ACTs) has spurred the need for alternative therapies [3].

Historically, plant-based compounds have played a critical role in developing antimalarial treatments. Notable examples include quinine, derived from the bark of *Cinchona* species, and artemisinin, extracted from *Artemisia annua*, which have been crucial in combating malaria [4]. These successes have underscored the potential of medicinal plants in the search for novel antimalarial agents. Among these plants is *Jatropha tanjorensis*, a member of the Euphorbiaceae family, traditionally used for its medicinal properties in some parts of Nigeria. It is locally consumed for its purported blood-boosting effects and has been reported to possess a range of therapeutic properties, including antimicrobial, antidiabetic, and antimalarial activities [5,6,7,8, 9].

Preliminary studies have demonstrated the anti-plasmodial activity of *Jatropha tanjorensis* leaf extracts, particularly against *P. falciparum* [9]. This suggests that the plant could serve as a valuable source of bioactive substances for developing novel antimalarial drugs. With the growing problem of drug-resistant malaria, exploring the phytochemical properties of *Jatropha tanjorensis* leaf and its potential anti-plasmodial effects is both timely and critical. This study aims to investigate the phytochemical composition and in vitro anti-plasmodial activity of *Jatropha tanjorensis* leaf extracts against *Plasmodium falciparum*, to search for effective plant-based treatments for malaria.

MATERIALS AND METHODS

Ethical Consideration

The management of Ahmadu Bello University Medical Centre, Samaru Main Campus, Zaria, permitted the collection of Malaria parasite-positive blood samples with approval number UHS/ADM/S-8.

Collection and authentication of Plant material

The fresh leaves of *Jatropha tanjorensis* (commonly known as Catholic vegetable) were harvested in February 2023 from private gardens located within Zaria Local Government Area, Kaduna State (11°04'N, 7°42'E). A sample was authenticated at Ahmadu Bello University's Herbarium Unit in the Department of Botany. The plant was assigned the voucher number V/N-ABU01911, and a voucher specimen was placed in the Herbarium.

Preparation of Plant Material

The fresh leaves of *Jatropha tanjorensis* were prepared according to the procedure outlined by Aiwonegbe *et al.* [7]. After being thoroughly cleaned with running tap water, the leaves were allowed to air dry in a shaded area at room temperature (20 to 23°C) for three weeks, and then ground into a fine powder. The powdered leaves were kept for further analysis in an airtight glass jar.

Plant Material Extraction

The *Jatropha tanjorensis* leaf powder was extracted using the maceration technique, as outlined by Chibuogwu *et al.* [10]. Two types of extraction were conducted: crude extraction and sequential extraction.

Crude Extraction: 100 g of dried leaf powder was placed in a 500 mL conical flask and mixed with 400 mL of 70% methanol. The mixture was vortexed periodically for seventy-two hours at room temperature. The combination was first filtered through a muslin towel and then Whatman No. 1 filter paper after 72 h. The solvent was evaporated in a water bath at 45°C to concentrate the filtrate. Before being used again, the resultant crude extract was kept at 4°C in a sterile, sealed glass container. After measuring the extract's yield, the yield was calculated in percentage using Equation 1:

$$\%\ \text{yield of crude extract} = \frac{\text{weight of the crude extract}}{\textit{weight of dry sample}}\ \boldsymbol{x\ 100} \quad \text{(Equation 1)}$$

Sequential Extraction: n-hexane, ethyl acetate, and methanol were the solvents of increasing polarity used in the sequential extraction process. The first step involved soaking 500 g of dried leaf powder in n-hexane for 72 h at room temperature while stirring occasionally. Following filtration through Whatman No. 1 filter paper and a muslin cloth, the n-hexane extract was concentrated in a water bath at 45 °C and kept in an airtight container at 4 °C. The n-hexane extract's yield was determined using Equation 2:

$$\%\ \text{yield of n-hexane extract} = \frac{\text{weight of n}-\text{hexane extract}}{\textit{weight of dry sample}}\ \boldsymbol{x\ 100} \quad \text{(Equation 2)}$$

The residue (marc) from the n-hexane extraction was soaked in ethyl acetate for 72 hours, following the same procedure. The resulting extract was concentrated and stored similarly. The percentage yield of the ethyl acetate extract was calculated based on the weight of the residue from the n-hexane extraction in Equation 3:

$$\%\ \text{yield of ethyl acetate extract} = \frac{\text{weight of ethyl acetate extract}}{\textit{weight of n}-\textit{hexane residue}}\ \boldsymbol{x\ 100} \quad \textbf{(Equation 3)}$$

Finally, the residue from the ethyl acetate extraction was soaked in methanol and processed using the same method. The yield was calculated using the weight of the residue from the ethyl acetate extraction in Equation 4:

$$\%\ \text{yield of methanol extract} = \frac{\text{weight ofmethanol extract}}{\textit{weight of ethyl acetate residue}}\ \boldsymbol{x\ 100} \quad \textbf{(Equation 4)}$$

Phytochemical Screening of *Jatropha tanjorensis* Extracts

Screening for phytochemicals qualitatively

The qualitative phytochemical screening of Alkaloids, carbohydrates, glycosides, saponins, flavonoids, steroids,

tannins, phenols, and anthraquinones was carried out using standard methods [11].

Detection of Alkaloids: A few drops of dilute H_2SO_4 were added to the extract, followed by Mayer's, Wagner's, Hager's, and Dragendorff's reagents. The formation of white or cream colour (Mayer's reagent), brownish black (Wagner's reagent), yellow crystalline (Hager's reagent), and orange or orange-red (Dragendorff's reagent) precipitates indicated the presence of alkaloids.

Detection of Carbohydrates (*Molisch test*): Molisch reagent and concentrated H_2SO_4 were added to the extract, and the appearance of a violet ring at the interface confirmed the presence of carbohydrates.

Detection of Cardiac Glycosides (*Keller-Kiliani Test*): The formation of a brown ring upon adding glacial acetic acid, $FeCl_3$, and H_2SO_4 indicated the presence of glycosides.

Detection of Saponins (*Frothing test*): The formation of persistent foam after shaking the extract with distilled water and olive oil confirmed the presence of saponins.

Detection of Phenols (*Lead acetate test*): A yellow precipitate formed upon adding lead acetate indicated the presence of phenols.

Detection of Tannins (*Bromine Water test*): The addition of $FeCl_3$ resulted in a blue-black colouration, indicating tannins.

Detection of Flavonoids (*Alkaline Reagent Test*): A yellow colouration that turned colourless upon adding of acid confirmed flavonoids.

Detection of Proteins and Amino Acids: Concentrated nitric acid was added to the extracts in little drops. When a yellow tint developed, it meant that proteins were present.

Detection of steroids (*Salkowski's Test)*: The formation of a red precipitate after adding chloroform and H_2SO_4 indicated the presence of steroids.

Detection of Triterpenes (*Liebermann Burchard Test)*: A small amount of extract was mixed with 2.0 ml of acetic acid and 2 ml of chloroform. Concentrated H_2SO_4 was added after the liquid had cooled. The steroidal component of glycosides, aglycone, was identified by its green hue.

Detection of Anthraquinones (*Bontrager's Test*): A red colouration in the ammoniacal layer after shaking with dichloromethane confirmed anthraquinones.

Quantitative Phytochemical Screening

The concentration of flavonoids, phenols, saponins, alkaloids, tannins, triterpenes, and steroids in the plant sample was determined using the method of Aiwonegbe *et al.* [7].

Total Flavonoids Content (TFC): Determined by an aluminum chloride colourimetric assay using quercetin as the standard.

Total Phenols Content (TPC): Measured using the Folin-Ciocalteu method with gallic acid as the standard.

Total Saponins Content (TSC): Quantified using a vanillin-sulfuric acid reaction, with diosgenin as the standard.

Total Alkaloids Content (TAC): Measured by the bromocresol green spectrophotometric method, with atropine as the standard.

Total Tannins Content (TTC): Determined using the Folin-Ciocalteu method with tannic acid as the standard.

Total Triterpenes Content (TTPC): Quantified using vanillin-glacial acetic acid, with ursolic acid as the standard.

Total Steroids Content (TSTC): Measured using cholesterol as the standard.

All experiments were conducted in triplicate, and results were expressed as mg equivalents per gram of extract for each phytochemical.

In vitro* Anti-Plasmodial Activity of *Jatropha tanjorensis* against *Plasmodium falciparum

Culture and Maintenance of *Plasmodium falciparum*

Microscopy

Microscopic identification of *P. falciparum* by thick and thin blood smear method was carried out following WHO guidelines [12]. Following Giemsa's staining, both smears were screened for *P. falciparum*-infected red blood cells under a microscope with an oil immersion high-power objective (×100) and parasitaemia was determined by calculating the average number of infected erythrocytes relative to the total number of erythrocytes across 10 microscopic fields. Blood samples with detected parasitaemia were cultured for further analysis.

Preparation of Culture Medium

The method described by Jensen and Trager [13] was adopted to prepare the Complete medium from the Incomplete medium. Exactly 500ml of prepared sterile incomplete culture medium, Rose Park Memorial Institute (RPMI 1640) supplemented with L-Glutamine was prepared based on the manufacturer's instruction. Gentamicin was added to the solution to prevent microbial contamination (625µl of 40mg/ml). Then it was dispensed aseptically in sterile glass bottles (100ml each) and stored at 2^{o}C as an incomplete medium. A complete medium was prepared from 100ml of incomplete medium by supplementing the medium with sterile 4ml of 5% NaHCO3 and 10ml of O+ human serum with pH 7.4.

Preparation of Erythrocytes

The method by Jensen and Trager [13] was used to prepare uninfected and infected erythrocytes for *Plasmodium falciparum* culture. Human blood in acid dextrose citrate was stored at 4°C and used to prepare a 5% haematocrit solution. For uninfected erythrocytes, blood was centrifuged to remove the plasma and the buffy coat, washed, and resuspended in RPMI and human serum. Infected erythrocytes were prepared similarly using blood samples with parasitaemia. The uninfected and infected erythrocyte suspensions were combined, diluted with a complete medium, and dispensed into sterile cell culture flasks for *P. falciparum* cultivation.

Culture Procedure

The method described by Trager [14] was adopted for the cultivation of *Plasmodium falciparum*. Bottles containing 1.5ml suspension of erythrocytes were placed in a candle jar. Approximately 3% CO2 and 17 % O2 were set in the candle jar when the candle was lit and covered with the stopcock open. The stopcock was closed when the candle flame went out and then the jar was incubated at 37°C for 48hrs. Parasitaemia level after incubation was maintained for the study.

Culture Maintenance

The method described by Jensen and Trager [13] was adopted to maintain *Plasmodium falciparum* cultures. Fresh medium was added daily by aspirating the old medium and gently adding fresh complete culture medium. The cultures were kept in a candle jar and incubated at 37° C for 48hrs.

Artemether Susceptibility Assay

The anti-plasmodial activity of Artemether against *Plasmodium falciparum* was evaluated following the method of Frosch *et al.* [15]. A stock solution of Artemether (80 mg/ml) was prepared in 70% ethanol and distilled deionized water and varying concentrations were obtained through serial dilution. Each concentration was added to a 96-well plate, mixed with complete medium, and *P. falciparum*-infected erythrocytes. The plates were incubated for 30 h at 37°C. Following incubation, the smears were prepared from the treated wells, air-dried, and stained with 5% Giemsa for screening under a microscope. Parasitaemia in treated wells was computed by comparing them to control wells, and the inhibition was determined in percentage. IC_{50} values were determined using a non-linear regression curve on GraphPad Prism, and isolates with the highest IC_{50} values were selected for further analysis based on the peak plasma concentration of Artemether (1.81 µmol/L).

***Jatropha tanjorensis* Leaf Extracts Susceptibility Assay**

The anti-plasmodial activity of *Jatropha tanjorensis* leaf extract against *Plasmodium falciparum* was evaluated following the method of Jensen and Trager [13]. The standard drugs used as positive controls were Artemether-Lumefantrine (53.5µmol/L, 20 mg/120 mg, PPC=2.1442ug/ml) and Quinine (55.18µmol/L, 83mg/ml, PPC=2.2115ug/ml). Stock solutions of these drugs and the *Jatropha tanjorensis* leaf extracts were prepared in 5% Dimethyl sulphuroxide (DMSO). The working concentrations were obtained through serial dilution. For the preparation of the inoculum, the ring stage of *Plasmodium falciparum* was synchronized by mixing late schizonts with fresh erythrocytes, incubating under culture conditions, and treating with 5% sorbitol to kill later stages and isolate young rings. This synchronized inoculum was used for anti-plasmodial assays at both the early and late trophozoite stages of the erythrocytic cycle. The assays involved incubating the prepared mixtures in a candle jar at 37°C for 30 h, after which the prepared smears were examined under an oil immersion objective lens. The percentage inhibition of parasitaemia was calculated, and IC_{50} values were determined using GraphPad Prism Version 9.3.1. The statistical differences between the IC_{50} values of the extracts and positive controls were analysed using two-way ANOVA.

RESULTS

Phytochemical Constituents of Extracts of *Jatropha tanjorensis* Leaf

Qualitative Phytochemical Constituents of Extracts of *Jatropha tanjorensis* Leaf

The results of qualitative phytochemical screening conducted on the extracts of *Jatropha tanjorensis* leaf are presented in Table 1 showing the presence of Carbohydrates, Cardiac Glycosides, Tannins, Phenols, Saponins, Flavonoids, Alkaloids, Steroids, Terpenoids, Proteins and Amino Acids in the C-MeOH extract, S-Ethyl A extract and S-MeOH extract. Cardiac Glycosides, Steroids, Terpenoids, Proteins, and Amino Acids were present in the S-nHex extract. Anthraquinones were not detected in all the extracts.

Quantitative Phytochemical Contents in Extracts of *Jatropha tanjorensis* Leaf

The results of quantitative phytochemical contents in extracts of *Jatropha tanjorensis* leaf are expressed as milligrams of different phytochemical equivalents per gram of extract as presented in Table 2. The results show that the S-Ethyl A extract had the highest quantity of Flavonoids (25.676±2.182 Mg QE/g of extract), Phenols (24.433±0.095 Mg GAE/g of extract), Saponins (20.172±0.126 Mg DE/g of extract), Alkaloids (15.770±0.058 Mg AE/g of extract) and Tannins (28.522±0.126 Mg TAE/g of extract). The quantity of Flavonoids was higher in S-MeOH extract (20.137±0.133 Mg QE/g of extract) than in C-MeOH extract (11.833±0.068 Mg QE/g of extract). However, S-MeOH extract had the lowest phytochemical contents in terms of Alkaloids (10.061±0.069 Mg AE/g of extract), Tannins (14.600±0.404 Mg TAE/g of extract), Saponins (8.687±0.126 Mg DE/g of extract) and Phenols (10.617±0.317 Mg GAE/g of extract). Flavonoids, Phenols, Saponins, Alkaloids, and Tannins were not detected in the S-nHex extract but it had the highest quantity of steroids while the S-MeOH extract had the highest quantity of triterpenes among other extracts.

In vitro Anti-Plasmodial Activity of *Jatropha tanjorensis* Leaf Extracts against *Plasmodium falciparum*

Plasmodium falciparum Culture and Maintenance

Plasmodium falciparum was confirmed in ten (10) blood samples by microscopy as presented in Figure 1. The percentage of parasitemia before and after culture shows that MPS-3 had the highest percentage of parasitemia at 4.3% while MPS-9 didn't indicate any parasitemia after culture. However, all other samples had an increase in percentage parasitemia at different levels.

Susceptibility Pattern of *Plasmodium falciparum* Isolates to Artemether

The result showing the susceptibility pattern of *Plasmodium falciparum* isolates to artemether oil-based intramuscular injection is presented in Figure 2 based on the peak plasma concentration (PPC) of artemether by WHO given as 1.81 µmol/L, the results show that the concentrations of artemether that inhibited 50% of the parasites (IC_{50}) from all the isolates were below the PPC of artemether and thus, all the isolates were considered sensitive to artemether. However, the IC_{50} value of artemether against the isolates were at different concentrations where Isolate *Pf*-4 had the highest IC_{50} value of 0.77 µmol/L followed by isolate *Pf*-8 with IC_{50} value of 0.50 µmol/L. These two isolates were selected for further studies.

Anti-Plasmodial Activity of *J. tanjorensis* Leaf Extracts against *Plasmodium falciparum* Isolates at Intraerythrocytic Stages

Figure 3 shows the activity of the extracts and positive control against the early trophozoite stage of *Pf-4* and *Pf-8* where IC_{50} values are significantly different at (p <0.05). From the results, Sequential Ethyl acetate (S-Ethyl A) extract among other extracts had the lowest IC_{50} values of 5.83 µg/mL and 4.77 µg/mL against *Pf-4* and *Pf-8* respectively. This was followed by the Crude Methanol (C-MeOH) extract which had IC_{50} values of 6.66 µg/mL and 5.10 µg/mL against *Pf-4* and *Pf-8* respectively while the Sequential Methanol (S-MeOH) extract had IC_{50} values of 11.39 µg/mL and 8.54 µg/mL against *Pf-4* and *Pf-8* respectively. The Sequential n-Hexane (S-nHex) extract had the highest IC_{50} values of 16.71 µg/mL and 10.72µg/mL against the isolates. However, the positive controls had lower IC_{50} values compared to the extracts. The differences were statistically significant (p <0.05).

Figure 4 shows the activity of the extracts and positive control against the late trophozoite stage of *Pf-4* and *Pf-8* where IC_{50} values are significantly different at (p <0.05). From the results, Sequential Ethyl acetate (S-Ethyl A) extract among other extracts had the lowest IC_{50} values of 5.52 µg/mL and 4.98 µg/mL against *Pf-4* and *Pf-8* respectively. This was followed by the Crude Methanol (C-MeOH) extract which had IC_{50} values of 6.56 µg/mL and 7.45 µg/mL against *Pf-4* and *Pf-8* respectively while the Sequential Methanol (S-MeOH) extract had IC_{50} values of 7.41 µg/mL and 10.85 µg/mL against *Pf-4* and *Pf-8* respectively. The Sequential n-Hexane (S-nHex) extract had the highest IC_{50} values of 13.56 µg/mL and 13.60µg/mL against the isolates. The positive controls also had lower IC_{50} values compared to the extracts. The differences were statistically significant (p <0.05).

DISCUSSION

The results of this study reveal that secondary metabolites such as cardiac glycosides, tannins, phenols, saponins, flavonoids, alkaloids, steroids, terpenoids, proteins, and amino acids were detected in the crude leaf extracts of *Jatropha tanjorensis*. These results are consistent with the findings of previous studies by Babayemi *et al.,* [16] Aiwonegbe *et al.,* [7] and Ezendiokwere *et al.,* [6] which also identified similar phytochemical constituents in the crude leaf extracts of *J. tanjorensis*. The presence of these secondary metabolites in the various extracts of *J. tanjorensis* leaves indicates their potential anti-plasmodial activity.

The sequential ethyl acetate leaf extract of *Jatropha tanjorensis* exhibited the highest total flavonoid content, measured at 25.676±2.182 mg QE/g of extract. This finding contrasts with the results reported by Aja *et al.* [8] and Aiwonegbe *et al.* [7], who documented total flavonoid contents of 15.91±1.60 mg QE/g and 145.922 ± 0.674 QE/g, respectively, in the methanol leaf extract of *J. tanjorensis*. The total flavonoid content of this study's sequential methanol leaf extract was 20.137±0.133 mg QE/g of extract. The observed discrepancies in total phytochemical content can be attributed to variations in the solvent's polarity and the extraction methods applied. Ri *et al.* [17] reported that ethyl acetate extract had the highest phenol and flavonoid content in their study on *Aurea helianthus* extracts, which supports the findings of this study. Flavonoids, a group of polyphenolic compounds prevalent in leafy vegetables, are noted for their antimicrobial, antioxidant, and anti-inflammatory activities, significantly reducing the risk of various diseases [18,19].

In this study, *Plasmodium falciparum* detected in human blood samples was successfully cultured *in vitro* for 48 h. Figure 1 shows an increase in parasitemia in malaria parasite-positive blood samples, ranging from 0.5% to 4.3%, except in sample MPS-9, which recorded 0% after culture despite an initial 0.5% parasitemia. This anomaly could be attributed to the possibility that the sample was collected from a patient who had already taken some antimalarial treatment before the hospital visit, rendering the parasite vulnerable before culture. Basco [20] reported that the growth rates and adaptability of new isolates of *P. falciparum* to *in vitro* culture vary depending on initial parasitemia, serum nutritional characteristics, recent drug treatment exposure, and the degree of red cell dilution. Jensen and Trager [13]; and Smith *et al.* [21] also noted that the unknown intrinsic properties of the donor's erythrocytes' that encourage parasite growth may have resulted in these variances.

The *in vitro* sensitivity pattern of *P. falciparum* isolates to artemether revealed that the concentrations inhibiting 50% of the parasites (IC_{50}) were below the Peak Plasma

Table 1: Qualitative Phytochemical Constituents of Extracts of *Jatropha tanjorensis* Leaf

Phytochemical Constituents	C-MeOH	S-nHex	S-Ethyl A	S-MeOH
Alkaloids	+	-	+	+
Carbohydrates	+	-	+	+
Saponins	+	-	+	+
Cardiac glycosides	+	+	+	+
Phenols	+	-	+	+
Tannins	+	-	+	+
Flavonoids	+	-	+	+
Steroids	+	+	+	+
Triterpenes	+	+	+	+
Proteins/Amino acids	+	+	+	+
Anthraquinones	-	-	-	-

Present (+); Absent (-); C-MeOH = Crude Methanol extract; S-nHex = Sequential n-Hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract.

Table 2: Quantitative Phytochemical Contents in Extracts of *Jatropha tanjorensis* Leaf

Phytochemical Contents	C-MeOH	S-nHex	S-Ethyl A	S-MeOH
TFC (Mg QE/g)	11.833±0.068^{a}	ND	25.676±2.182^{c}	20.137±0.133^{b}
TAC (Mg AE/g)	13.927±0.473^{b}	ND	15.770±0.058^{c}	10.061±0.069^{a}
TTC (Mg TAE/g)	18.378±0.280^{b}	ND	28.522±0.126^{c}	14.600±0.404^{a}
TSC (Mg DE/g)	15.636±0.182^{b}	ND	20.172±0.126^{c}	8.687±0.126^{a}
TPC (Mg GAE/g)	17.633±0.113^{b}	ND	24.433±0.095^{c}	10.617±0.317^{a}
TTPC (Mg UAE/g)	24.17±0.007^{b}	18.230±0.014^{a}	23.67±0.014^{c}	26.80±0.014^{d}
TSTC (Mg CE/g)	17.61±0.029^{a}	39.58±0.011^{d}	28.38±0.060^{c}	19.97±0.011^{b}

Values are expressed as Mean ± SD (n= triplicates). Values with different superscripts across rows are significantly different ($p<0.05$). ND = Not detected; TFC = Total Flavonoids Content; QE = Quercetin Equivalent; TPC = Total Phenols Content; TTPC = Total Triterpenes Content; TSTC = Total Steroids Content; GAE = Gallic Acid Equivalent; TSC = Total Saponins Content; DE = Diosgenin equivalent; TAC = Total Alkaloids Content; AE = Atropine Equivalent; TTC = Total Tannins Content; TAE = Tannic Acid Equivalent; UAE = Ursolic Acid Equivalent; CE = Cholesterol Equivalent; C-MeOH = Crude Methanol extract; S-nHex = Sequential n-Hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract.

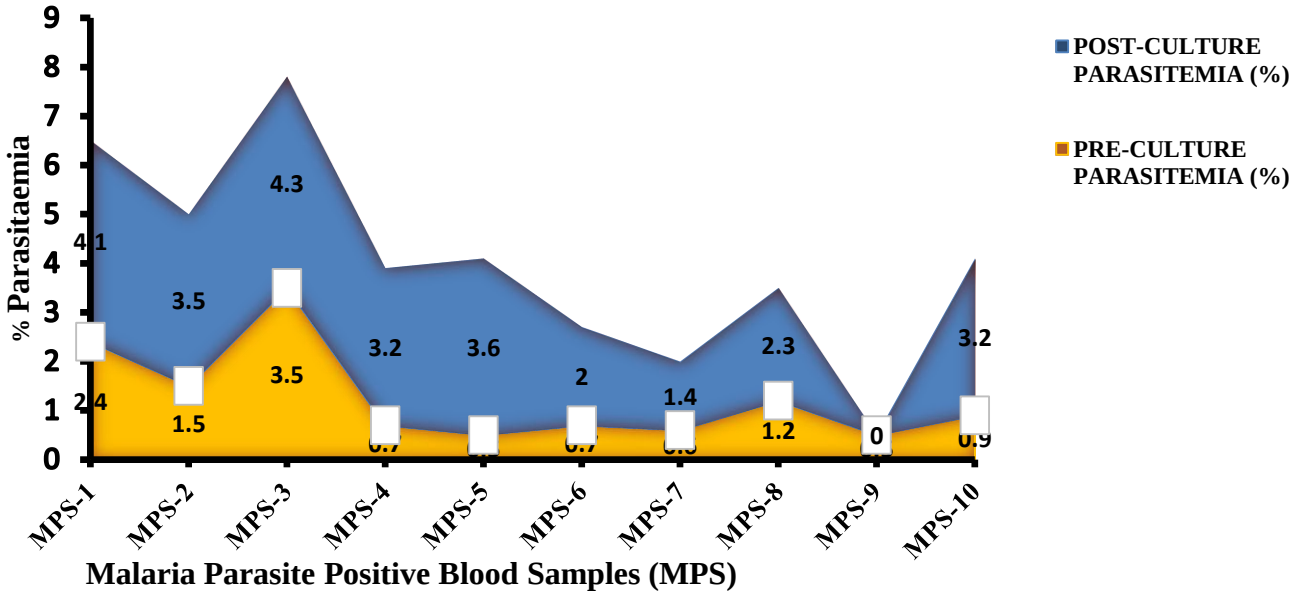

Figure 1: Growth Rates of *Plasmodium falciparum* in *In vitro* Culture

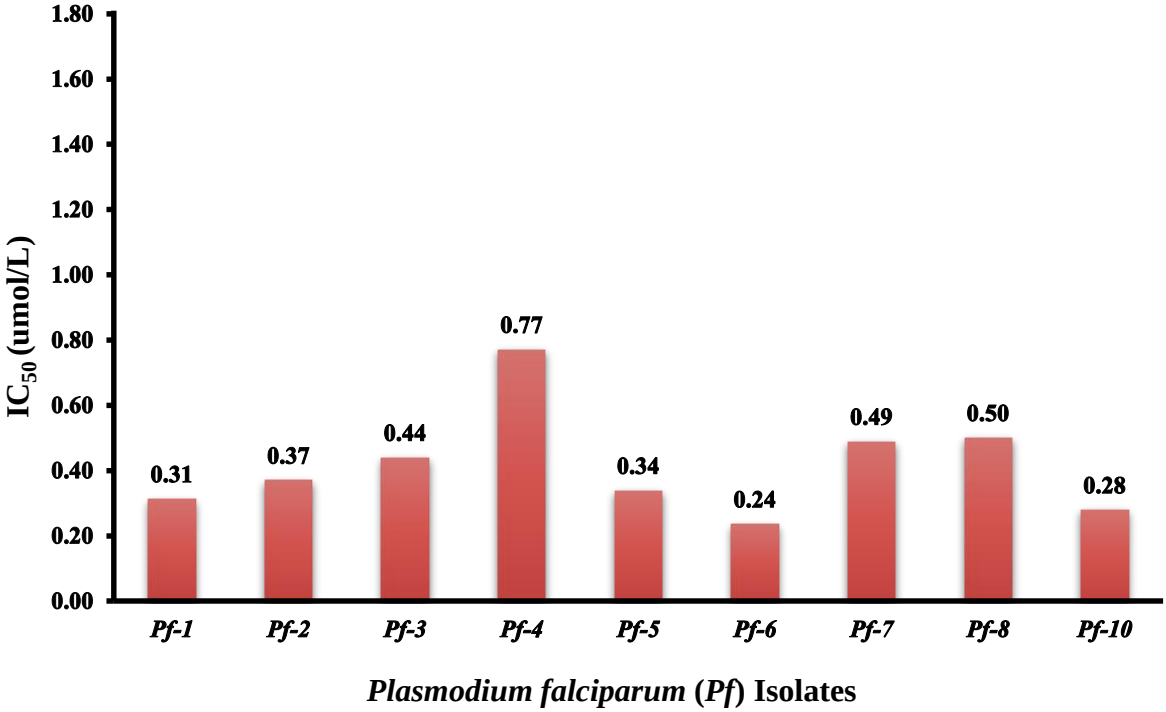

Figure 2: Susceptibility Pattern of *Plasmodium falciparum* Isolates to Artemether

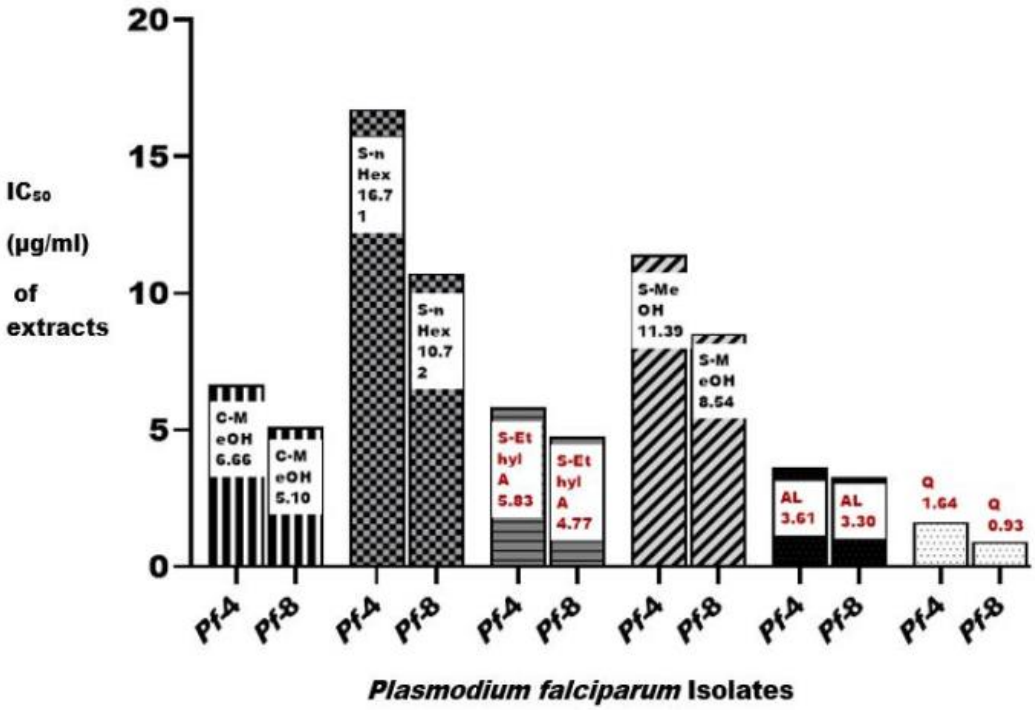

Figure 3: Anti-Plasmodial Activity of Extracts of *Jatropha tanjorensis* Leaf Against *Plasmodium falciparum* Isolates at the Early Trophozoite Stage

Key: n= 12; Two-way ANOVA; IC_{50} values are significantly different at $p<0.05$. C-MeOH = Crude Methanol extract; S-nHex = Sequential n-Hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract; A/L=Artemether Lumefantrine; Q=Quinine; *Pf*-4=*Plasmodium falciparum* Isolate 4; *Pf*-8= *Plasmodium falciparum* Isolate 8.

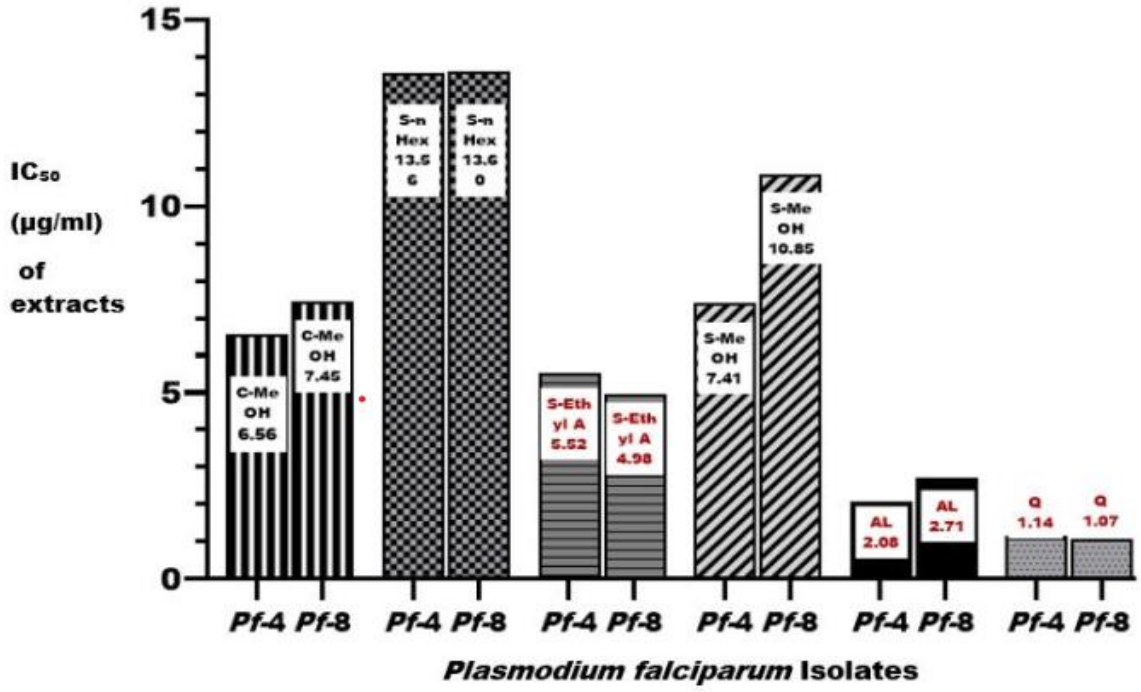

Figure 4: Anti-Plasmodial Activity of Extracts of *Jatropha tanjorensis* Leaf Against *Plasmodium falciparum* Isolates at the Late Trophozoite Stage

Key: n= 12; Two-way ANOVA; IC_{50} values are significantly different at $p<0.05$. C-MeOH = Crude Methanol extract; S-nHex = Sequential n-Hexane extract; S-Ethyl A = Sequential Ethyl acetate extract; S-MeOH = Sequential Methanol extract; A/L=Artemether Lumefantrine; Q=Quinine; *Pf*-4=*Plasmodium falciparum* Isolate 4; *Pf*-8= *Plasmodium falciparum* Isolate 8**.**

Concentration (PPC) of 1.81 µmol/L as established by WHO [22]. The IC_{50} values in this study ranged from 0.24 to 0.77 µmol/L, indicating the sensitivity of the isolates to artemether. However, the highest IC_{50} values recorded by isolates Pf-4 and Pf-8 were 0.77 and 0.50 µmol/L, respectively, suggesting a potential future resistance to artemether. Recent studies on the anti-plasmodial activity of artemether and artesunate against *P. falciparum* have shown that artemether is almost as active as artesunate even though it is absorbed via intramuscular injections more slowly as an oil-based injection compared to the rapid absorption of water-soluble artesunate [23]. Esu *et al.* [24] also reported that intramuscularly administered artemether is as effective as quinine in preventing children's mortality due to severe malaria. The complete sensitivity of all the isolates to artemether in this study confirms the continued efficacy of artemether oil-based intramuscular injections in malaria treatment.

The *in vitro* anti-plasmodial activity of *J. tanjorensis* leaf extracts against *P. falciparum* isolates at both the early and late trophozoite stages in this study validates the results of Omoregie and Sisodia [9], who reported the anti-plasmodial activity of ethanol and hydro-ethanol leaf extracts of *J. tanjorensis* against *P. falciparum* with an IC_{50} value of 10.86 ± 1.52 µg/ml. This indicates the potential of ethyl acetate extract as a new source of antimalarial treatment. The anti-plasmodial activity of artemether-lumefantrine (A/L) against *P. falciparum* isolates at the early and late trophozoite stages indicated reduced sensitivity of the isolates, based on the WHO's Peak Plasma Concentration (PPC) of A/L, which is 2.1442 µg/mL (53.3 µmol/L) [22]. In contrast, quinine exhibited complete sensitivity against *P. falciparum* isolates at the early and late trophozoite stages, with IC_{50} values from 0.93 to 1.64 µg/ml, aligning with its PPC of 2.2115 µg/mL (55.18 µmol/L) [22]. The result on reduced sensitivity to artemether-lumefantrine aligns with reports of declining efficacy of this drug in parts of sub-Saharan Africa. For instance, three instances of artemether-lumefantrine-resistant parasites were recorded in Nigeria after 3 days of therapy [25], and a similar trend was reported in Portugal [26]. In western Myanmar, more than 10% of patients remained parasite-positive on day 3 following treatment with artemether-lumefantrine [27]. In Mali and Burkina Faso, a randomized controlled trial found higher rates of recurrent *P. falciparum* parasitemia in patients treated with artemether-lumefantrine compared to other regimens [28]. Adamu *et al.* [29] identified *P. falciparum* strains in Northern Nigeria that were less sensitive to lumefantrine. The result in this study as supported in previous studies [25, 26, 27, 28, 29] suggests a potential threat to the efficacy of artemether-lumefantrine in West Africa. The findings also reveal the antimalarial potential of ethyl acetate extract of *Jatropha tanjorensis* leaf with indications of significant phytochemical constituents responsible for the antimalarial effects as supported in previous works [30, 9].

CONCLUSION

In conclusion, secondary metabolites such as cardiac glycosides, tannins, phenols, saponins, flavonoids, alkaloids, steroids, terpenoids, proteins, and amino acids were identified in the crude leaf extracts of *Jatropha tanjorensis*. Among the extracts, ethyl acetate extract exhibited the highest total phytochemical contents, particularly in tannins, phenols, saponins, flavonoids, and alkaloids. *Plasmodium falciparum* isolated from human blood samples demonstrated susceptibility to artemether, with IC_{50} values ranging between 0.24 and 0.77 µmol/L. Isolates Pf-4 and Pf-8, which had the highest IC_{50} values of 0.77 and 0.50 µmol/L respectively, were further evaluated for susceptibility to the crude extracts of *J. tanjorensis* leaves. The ethyl acetate extract showed significant antiplasmodial activity against isolates Pf-4 and Pf-8, with IC_{50} values of 4.77 µg/ml and 4.98 µg/ml at the early and late trophozoite stages of the intraerythrocytic cycle. Despite the potential loss of synchronicity in *Plasmodium falciparum* intraerythrocytic stages in the culture system, further studies on the antiplasmodial activity of *J. tanjorensis* leaf extracts against all the intraerythrocytic stages are recommended to understand the mechanism of its antiplasmodial activity.

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AUTHORS' CONTRIBUTION

Jumare, F.M.: project management, resources, conceptualization, data gathering, formal analysis, illustration, composition, and writing. H. I. Inabo: supervision, methodology, validation, visual aids, and editing. M.H.I. Doko: supervision, validation, data curation, visual aids, writing reviews, editing, and writing. Adeshina, G.O: supervision, verification, editing, and review.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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