

**IN VITRO ANTIPLASMODIAL ACTIVITY
AND CYTOTOXICITY OF LEAF EXTRACTS
FROM *JATROPHA TANJORENSIS***

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Summary

We evaluated the *in vitro* antiplasmodial activity and cytotoxicity of extracts from *Jatropha tanjorensis* leaves. The plant leaves were successively extracted into three (3) extract forms including aqueous extract, ethanolic extract and hydro-ethanolic (50:50 v/v) extracts. For quality control, high performance thin layer chromatography (HPTLC) was performed on extracts. Antiplasmodial activity was assessed

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in vitro by using a 3D7 chloroquine sensitive clone of NF-54 isolate of *Plasmodium falciparum*. Parasite growth inhibition was estimated based on the 48 hours microassay technique with the extracts dissolved in either DMSO or water. Chloroquine served as the positive control and the negative control contained only parasitized red blood cells in medium. Cytotoxicity of extracts against non-cancerous *vero* cell lines (C-1008 kidney fibroblasts from African green monkey) was determined with doxorubicin serving as the standard cytotoxic drug. The spectra from the chemical finger-print of the extracts showed several peaks which were more in the ethanolic extract than in the other extracts. The ethanolic extract also had the highest antiplasmodial activity (IC₅₀ 10.86 ± 1.52 ug/ml), cytotoxicity (IC₅₀ 86.8 ± 4.8 ug/ml) and the lowest selectivity index (8.0) when compared with the other extracts. The results justify local claims on the use of *Jatropha tanjorensis* leaves in the treatment of malaria infection.

Keywords: Antiplasmodial, malaria infection, cytotoxicity, vero cell lines, Jatropha tanjorensis, Plasmodium falciparum, chemical finger-print, doxorubicin.

INTRODUCTION

Malaria is one of the most serious pathogenic diseases in endemic areas of the world, particularly in Africa, Asia, and Latin America. In Africa alone, it is estimated that over 350 million people are infected by malaria parasite, commonly *Plasmodium falciparum* (one of the four species of malaria parasites infecting humans), with a reported 90 million clinical cases and 2 million deaths annually (1, 2, 3). The

problem is further compounded by the upsurge in the resistance strain of the parasite. Thus, the search for novel and more effective antimalarial compounds especially from medicinal plants extracts is of utmost importance (4).

Traditional methods of malaria treatment have remained a promising source of new antimalarial compounds. In Africa, the use of indigenous plants plays an important role in the treatment of malaria. Medicinal plants may provide good source for the detection of novel antiplasmodial compounds (3). The therapeutic properties ascribed to most medicinal plants have been linked to the phytochemical compounds contained in them. Phytochemicals such as alkaloids, triterpenes, flavonoids, etc have been reported in many papers as contributing to the antimalarial property of most plants (5, 6, 7, 8, 9).

Jatropha tanjorensis (Euphorbiaceae family), is a perennial herb, a hybrid species which shows intermediacy in phenotypic characters between *Jatropha curcas* and *Jatropha gossypifolia* (10, 11). The common names include: catholic vegetables, 'Hospital too far', *Iyana ipaja* (Yoruba). The plant leaves were initially popularly consumed in Nigeria as soups and as a tonic with the claim that it increases blood volume. The leaves are also employed traditionally in the treatment of anaemia (as a haematinic agent), diabetes and cardiovascular diseases (12). However, the plant's popularity was doused by unproven claims that the whitish latex emanating from the leaf stem and stalk may be toxic to man.

This study therefore reports the *in vitro* cytotoxicity of extracts from *Jatropha tanjorensis* leaves in non-cancerous vero cell lines. Also, the *in vitro* antiplasmodial activity of extracts of the plant was tested in chloroquine sensitive *Plasmodium falciparum* 3D7 (NF-54) clone.

Materials and Methods

Collection of Plant Materials

J. tanjorensis leaves were collected during the rainy season between April and June from private farms at different locations in Benin City, Nigeria. The leaves were authenticated by a Botanist, and voucher specimens of the plant leaves (with voucher number: JT/104/07) was deposited in the herbarium of the University of Benin, Benin City, Nigeria. The plant leaves were selected based on claims, by traditional healers, of their efficacy in the treatment of anaemia and malaria fever in the southern region of Nigeria.

Chemicals

All chemicals used were of analytical grade (from E-Merck India Ltd). Chloroquine standard and doxorubicin were purchased from Sigma Chemicals Ltd. (Bangalore, Karnataka India).

Preparation and Chemical Finger-Print of the Extracts

The plant extracts including the alcoholic, aqueous, and hydro-ethanolic (50:50 v/v) extracts were prepared from air-dried leaves of the plant according to previously reported standard procedure (13). The extracts were subjected to HPTLC Chemical Finger-Printing for quality assurance. The HPTLC system consisted of TLC plates 10 × 10 cm silica gel 60F₂₅₄ (Merck). 100mg of plant extract was dissolved in 1.0ml of methanol by means of ultra-sonication for 30 minutes, and centrifuged at 8000rpm for 10 minutes. The supernatant obtained was used for the HPTLC chemical profile with chloroform: methanol (9:1, v/v) serving as the

mobile phase. Sample application plates were developed for one hour in a Camag 10 × 10 twin trough glass solvent developing chamber initially pre-saturated with the mobile phase.

The plates were allowed to air-dry, and then scanned using a Camag TLC Scanner model 3 (with slit size 10 × 0.40mm and wavelength of 254nm) equipped with Wincats software and absorption-reflection scan mode. The calibration curve of peak area vs. concentration was prepared.

Determination of in vitro Antimalarial activity of plant Extracts

Plasmodium falciparum (clone NF-54) were maintained at 5% hematocrit (human type O-positive red blood cells) in complete RPMI 1640 medium (RPMI 1640 medium supplemented with 25 mM HEPES, 370 μM hypoxanthine, 40 μg ml⁻¹ gentamycin, 0.25 μg ml⁻¹ Fungizone and 0.5% [wt/vol] AlbuMax II) in 60mm petri dish by the candle jar method described previously (14). The culture was routinely monitored through Geimsa staining of the thin smears.

Parasite growth and drug susceptibility assay

Antiplasmodial activity was determined *in vitro* by parasite growth inhibition assay with some minor modifications. Chloroquine sensitive *Plasmodium falciparum* 3D7 (NF-54 clone) were maintained at 5% haematocrit (human type o-positive red blood cells) in complete RPMI 1640 medium by the candle jar method (14). The culture was routinely monitored using Giemsa staining of thin smears. Standard drug, chloroquine or extracts (at different concentrations of 1, 5, 10, 50, 100, 500, and 1000ug/ml) were prepared in either water (chloroquine; Sigma) or DMSO (Artemisinin; Aldrich and test extracts) and then diluted to achieve the required concentrations.

The synchronized culture with parasitaemia of 1.5% and 3% haematocrit were incubated in 96-well microtitre plate predisposed with multiple concentrations of compounds/extracts for 48 hrs at 37°C in candle jar. Blood smears from each well were fixed in methanol, Geimsa stained and the number of infected RBCs per 5000 cells was counted. The antimalarial activity of the test extract was expressed as 50% inhibitory concentration (IC₅₀) determined from dose-response curve by non-linear regression analysis (curve-fit) using Graph Pad Prism (version 4) software. All experiments were performed in triplicates and the results were expressed as percentage of growth inhibition. Crude extracts with IC₅₀ values > 50 µg/ml were considered to be inactive (6).

Statistical Analysis

Data were analyzed using Graph Pad Prism (version 4) software and results were expressed as means ± standard deviation (SD) (15).

Results and Discussion

The chemical profile of extracts of *Jatropha tanjorensis* leaves showed several peaks in the spectra which suggest the presence of some phytochemical compounds (figures 1 to 3). Clear peaks were observed more in the ethanolic extract with eleven peaks (between 22.8 - 427.8 AU), followed by hydro-ethanolic extract with eight peaks (33.1 – 234.4 AU) and the lowest peaks shown by the aqueous extract - six peaks of between (19.5 – 211.2 AU).

In vitro antiplasmodial study revealed that the ethanolic extract of *Jatropha tanjorensis* was most active, when compared with the other extracts, against the chloroquine sensitive 3D7 strain of *Plasmodium falciparum* with 50%

inhibitory concentration of parasite growth (IC_{50}) observed at $10.86 \pm 1.52 \mu\text{g/ml}$ when compared with that of the aqueous ($44.0 \pm 2.40 \mu\text{g/ml}$) and hydro-ethanolic ($48.0 \pm 1.34 \mu\text{g/ml}$) extracts (figures 4 to 6 and table 1). From literature, an extract is regarded as highly active if $IC_{50} < 10 \mu\text{g/ml}$, moderately active if IC_{50} is between $10\mu\text{g/ml}$ and $50\mu\text{g/ml}$ and inactive if $IC_{50} > 50\mu\text{g/ml}$ (8). Based on this classification, the three extracts forms were found to be moderately active against *Plasmodium falciparum* when compared with the standard antimalarial drug chloroquine ($IC_{50} < 0.087 \pm 0.0003 \text{ug/ml}$).

The antiplasmodial property of the plant extracts, particularly the ethanolic extracts may be attributed to presence of some phytochemicals which might have relatively conferred some protective / antioxidative effect against oxidative stress induced by the malaria parasite. Moreover, previous papers have shown that the plant leaves contain alkaloids, saponins, anthraquinones, tannins, and flavonoids (11, 16), one or more of which may be responsible for its antimalarial activity. For instance, phytochemicals such as phenolic compounds (e.g. flavonoids) have been suggested to be responsible for the antioxidative activities of edible and non-edible plant products by offering protection against harmful reactive species (17, 16). The HPTLC chemical finger-print profile of the plant extracts further attest to the presence of these phytochemical compounds. This is evidenced by the several yet to be identified absorption peaks.

In addition, other constituents in the plant leaves may possess therapeutic effect other than antiparasitic effect, such as antipyretic, antioxidant, anti-inflammatory, analgesic, immunomodulatory (5, 8) and cytotoxic properties (8, 18). All the extracts showed little or no cytotoxic activity against normal vero cells with the exception of the moderate

cytotoxic activity shown by ethanolic extract ($IC_{50 \text{ vero}} = 86.8 \pm 4.8 \mu\text{g/ml}$) which was still less toxic than the standard cytotoxic drug doxorubicin ($IC_{50 \text{ vero}} = 1.8 \pm 0.42 \mu\text{g/ml}$). The selectivity index (S.I.) was however lower for ethanolic extract group (8.0) than the other extracts. This is suggestive of lower selectivity of the ethanolic extract when compared with the other extracts (see table 1).

The relatively high antiplasmodial and low cytotoxic activity of the crude ethanolic extract of *Jatropha tanjorensis* leaf justifies local claims on the efficacy of the plant leaves in tradomedicinal practices. Investigations to identify its active principles may be the focus of future study. Nevertheless, further work is necessary to ascertain the plant's toxicity *in vivo* and optimum dosage so as to provide effective and low cost intervention during malaria infection. This study therefore report for the first time the *in vitro* antimalarial activity and chemical finger – print of ethanolic extract of *J. tanjorensis* leaves.

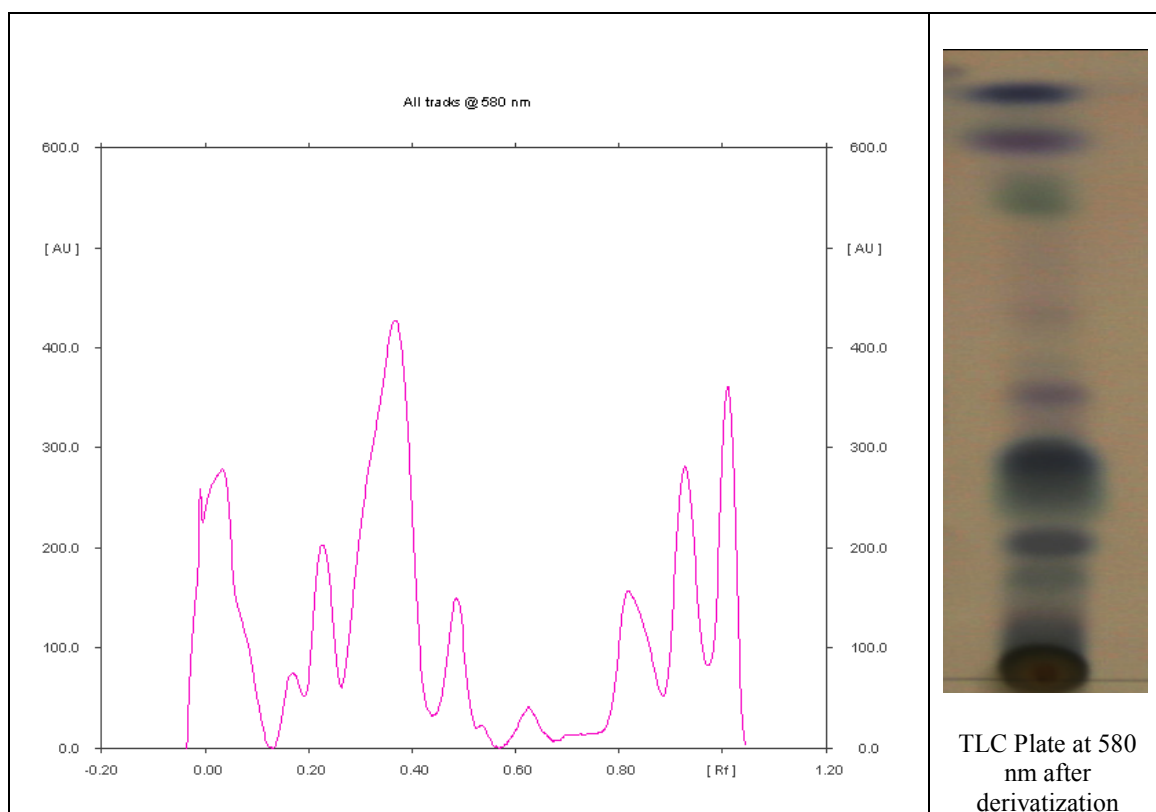


Figure 1: Chemical Fingerprint of Ethanolic Extract of *Jatropha tanjorensis* Leaves

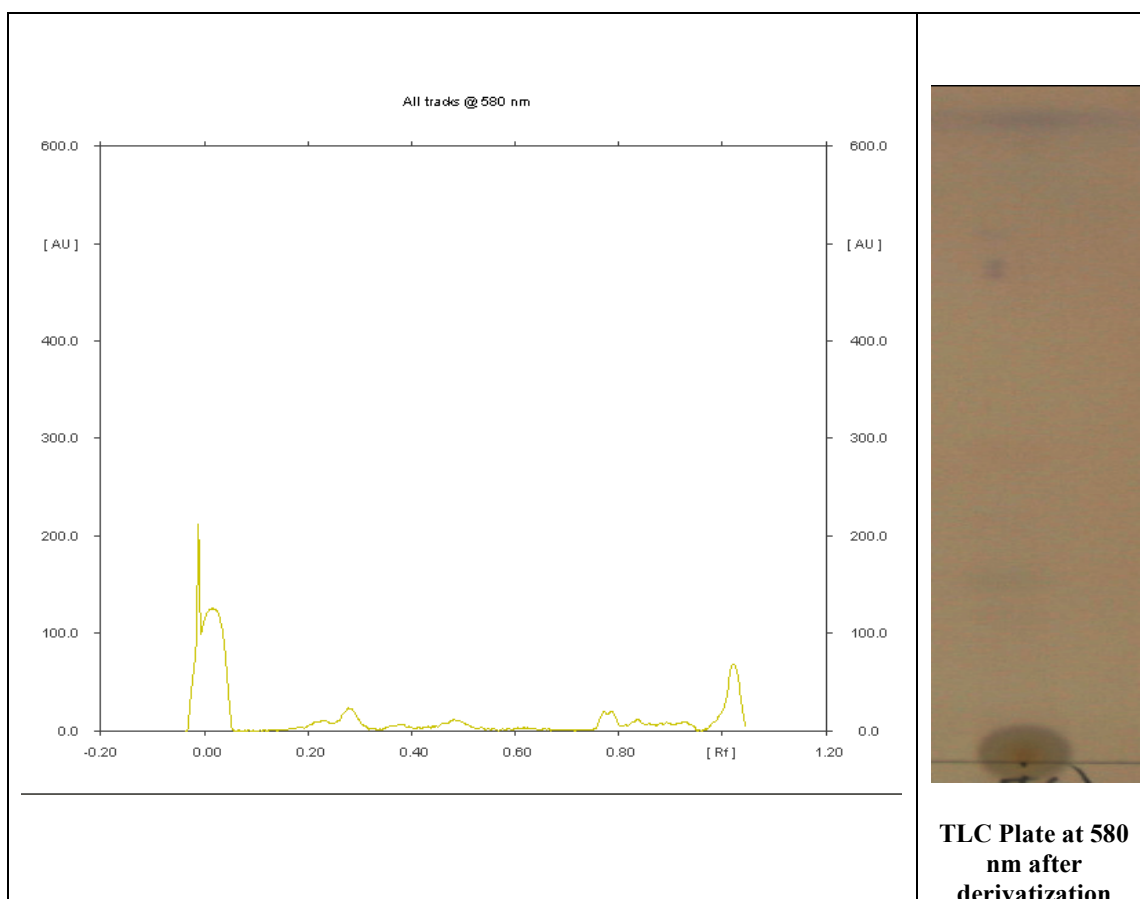


Figure 2: Chemical Fingerprint of Aqueous Extract of *Jatropha tanjorensis* Leaves

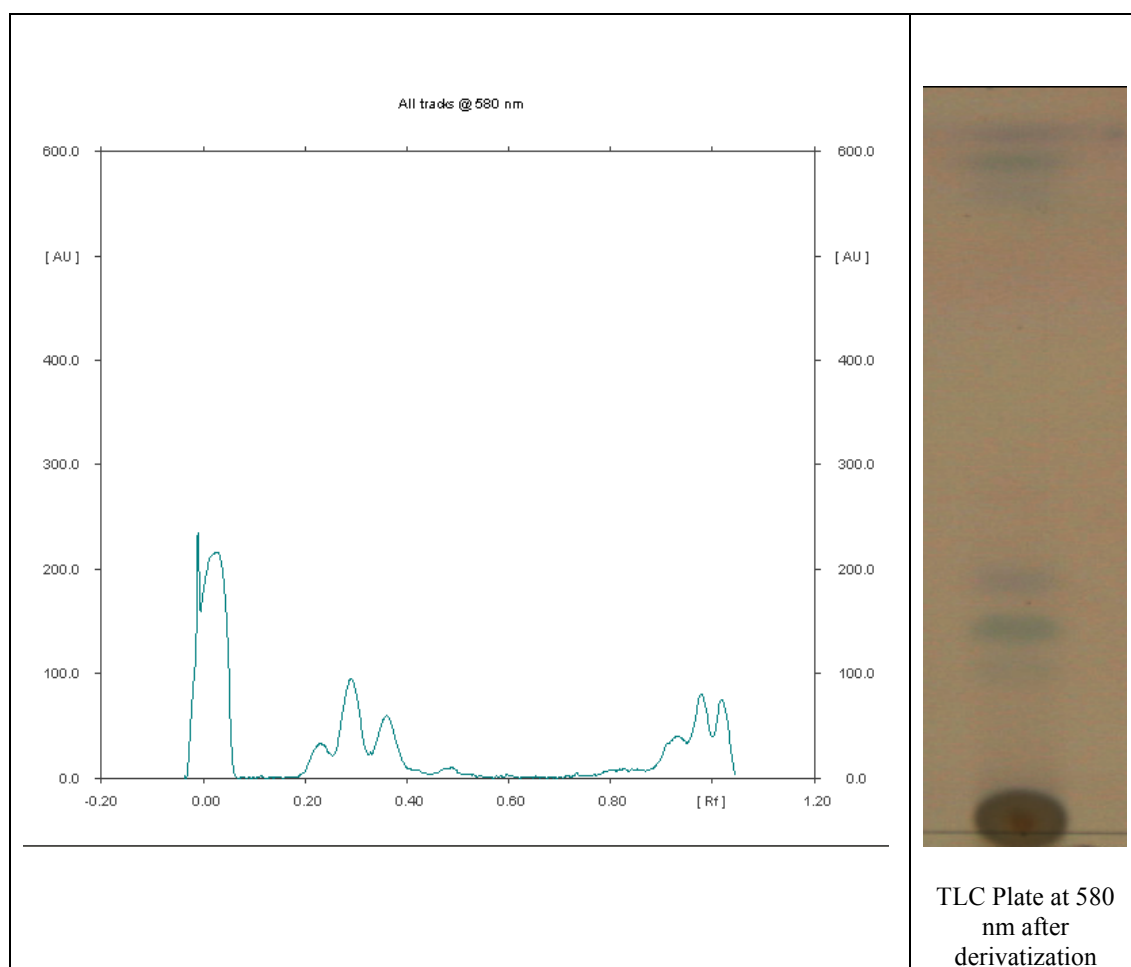


Figure 3: Chemical Fingerprint of Hydro-ethanolic (50:50) Extract of *Jatropha tanjorensis* Leaves

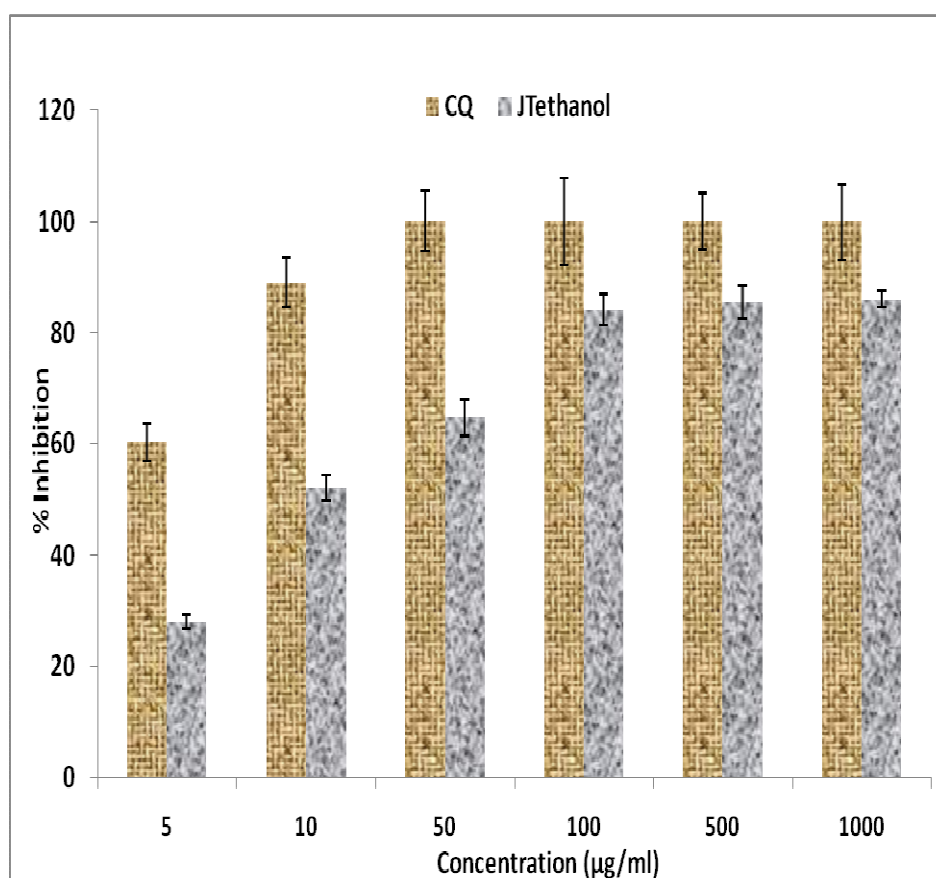


Figure 4 showing effect of different concentrations of ethanolic extract of *Jatropha tanjorensis* and chloroquine on *in vitro* growth of *P. falciparum*

Data are Mean \pm S.D. from two independent experiments performed in triplicates. JT_{ethanolic} (*J. tanjorensis* ethanolic extract); IC₅₀ chloroquine 0.090 µg/ml.

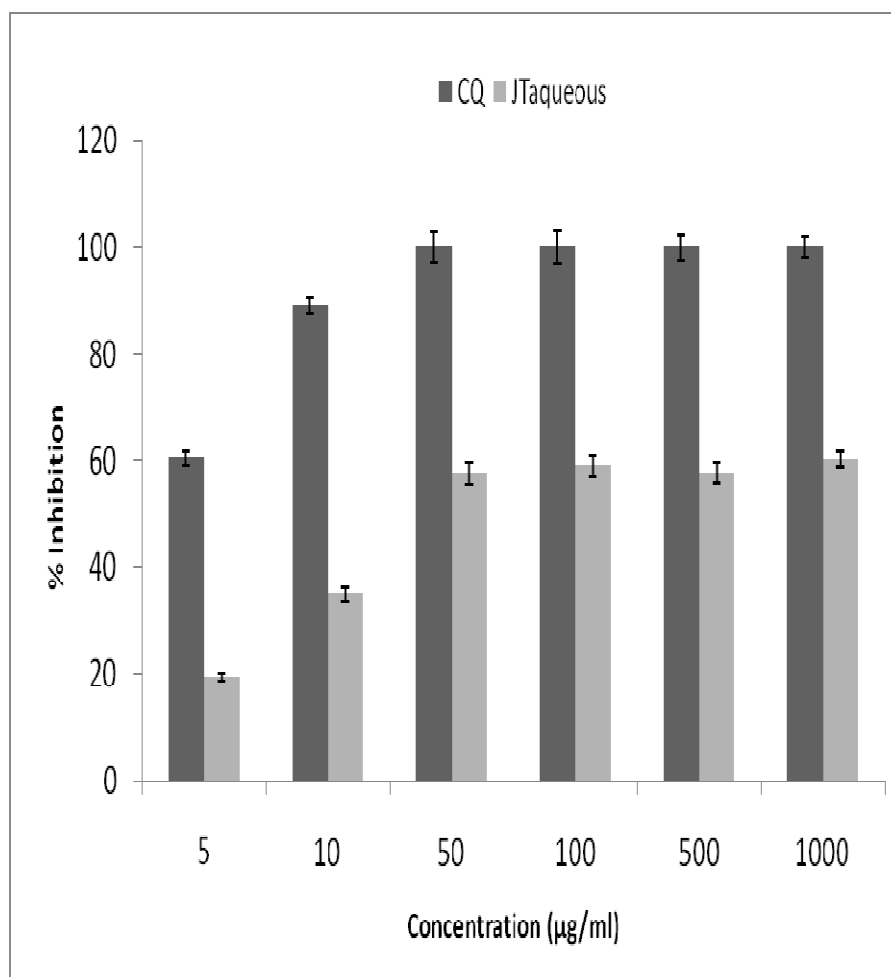


Figure 5: Effect of different concentrations of aqueous extract of *Jatropha tanjorensis* and chloroquine on *in vitro* growth of *P. falciparum*

Data are Mean \pm S.D. from two independent experiments performed in triplicates. JT_{aqueous} (*J. tanjorensis* aqueous extract); IC₅₀ chloroquine 0.090 µg/ml.

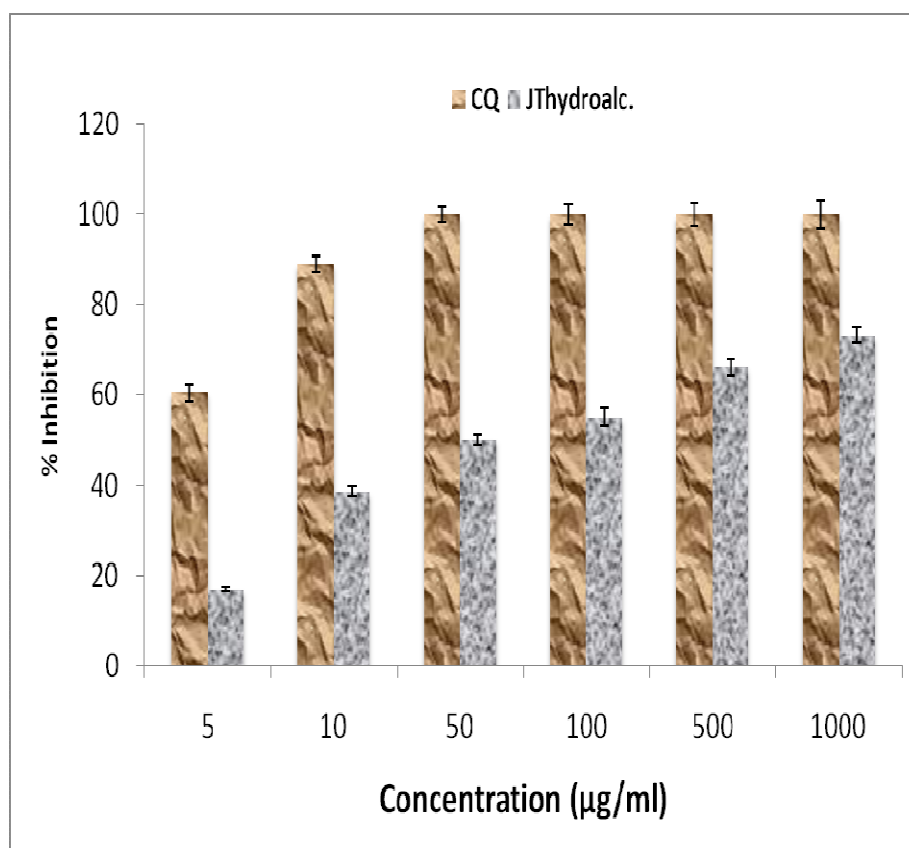


Figure 6: Effect of different concentrations of hydro-ethanolic extract of *Jatropha tanjorensis* and chloroquine on *in vitro* growth of *P. falciparum*

Data are Mean \pm S.D. from two independent experiments performed in triplicates. JT_{hydro-ethanol} (*J. tanjorensis* hydro-ethanolic extract); IC₅₀ chloroquine 0.090 µg/ml.

Table 1 showing *in vitro* Antiplasmodial Activity, Cytotoxicity and Selectivity Index of various Leaf Extracts of *Jatropha tanjorensis*

Extracts	IC ₅₀ (µg/ml) Plasmodium	IC ₅₀ (µg/ml) Vero ^a Cells	Selectivity Index (SI) ^b
JT _{ethanol}	10.86 ± 1.52	86.8 ± 4.8	8
JT _{aqueous}	44 ± 2.4	NC ^c	NC ^c
JT _{hydro-ethanol}	48 ± 1.34	547 ± 9.4	11.4

^a Vero cell lines (C-1008) from African green monkey kidney fibroblast. ^b Selectivity Index = IC₅₀ Vero Cells / IC₅₀ Plasmodium

^c Not cytotoxic at the highest test concentration of 1000 µg/ml. Data are Mean ± S.D. from two independent experiments performed in triplicates. JT_{ethanol} (*J. tanjorensis* ethanolic extract), JT_{aqueous} (*J. tanjorensis* aqueous extract); JT_{hydro-ethanol} (*J. tanjorensis* hydro-ethanolic extract); IC₅₀ chloroquine (standard reference drug) = 0.090 µg/ml; Doxorubicin hydrochloride (standard cytotoxic compound) = 1.8 ± 0.42 µg/ml.

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