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# In Vivo Antimalarial Activity and Phytochemical Screening of Tree Bark Extract of Ficus Elastica

**Okereke Casmir Ogechukwu & Agbaike Precious Salt**
Department of Chemistry, Faculty of Physical science, University of Benin, Nigeria.
Email: **grand777.co@gmail.com, preciousagbaike@gmail.com**

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**Abstract**

*The phytochemical screening, antimalarial and cytotoxic activities of a medicinal plants Ficus elastica was investigated. In vivo cell-growth inhibition activities were assessed on the plant extract against Plasmodium berghei strain ANKA (PbANKA). Phytochemical screening were done according to standard methods. Alkaloids, flavonoids, phenolics, Saponins, Glycosides, terpenoids and eugenols were all present in the extract except Steroids. The prophylactic study showed an increase in the level of significance in the graded doses (100, 200 and 400 mg/kg) of Ficus elastica compared to the negative control and reference drug of the parasitemia count. However, 400 mg/kg dose proved to be more effective than the control groups in reducing the level of Plasmodium berghei. 100 mg/kg, 200 mg/kg and 400 mg/kg of the extracts (76 %, 68%, 63% and 81%) exhibited plasmodiacidal activity. Curative study of the extract against Plasmodium berghei induced malarial showed low level of significant increase ($p < 0.05$) across the graded doses (100, 200 and 400 mg/kg) of Ficus elastica compared to the negative control and reference drug with slight significant increase in the parasitemia count. Control groups of standard drug drastically reduced the level of Plasmodium berghei as recorded from the percentage inhibition with lesser effect in the graded doses of treated plant (52 %, 36%, 45% and 47%). The acute toxicity study of the animals showed no change in their normal behavior within 24hours of the Ficus elastica extract exposure, and no mortality throughout the 14days were observed.*

## 1.0. Introduction

Plants are not only meant for food and dietary supplement to living organisms but traditionally from time immemorial they have been used for treating many health problems. The exploration of plants based on their medicinal value is an ever green field in Chemistry and science in general. Investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agent [1]. Over 60% of the world human population, 80% in developing countries depends directly on plants for their medicinal purposes [2].

Malaria is a life threatening disease spread to humans by the bite of some types of mosquitoes and has brought deaths humans across different continents. Though Malaria burden is hard to estimate, the highest burden of malaria is in Africa, although many parts of Asia, particularly India and south-east Asia, also suffer from a large number of malaria cases every year. In 2021, nearly half of the world's population was at risk of malaria. That year, there was an estimated 247 million cases of malaria worldwide and the estimated number of malaria deaths stood at a staggering 619000. In

2021, the WHO Africa Region carried a disproportionately high share of the global malaria burden as the region was home to 95% of malaria cases and 96% of malaria deaths. Children under the age of 5 accounted for about 80% of all malaria deaths in the region [3] and a substantial number of children who even survive a serious attack of malaria develop physical and mental impairment [4]. In most parts of Africa, herbs are used locally in different forms to combat malaria. Nigeria is one of the countries in the world with unique wealth of medicinal and vast knowledge of use of herbal medicine for treatment of malaria and other disease conditions. This research is focused on investigating *Ficus elastica* and its anti-malaria potency.

*Ficus elastica*, from the family, *moraceae,* is a popular ornamental tree grown around the world. It is commonly known as rubber tree, rubber fig, rubber bush, rubber plant, or Indian rubber bush. It is native to southern Asia [5] but also grown in other parts of the world including Nigeria. It has become naturalized in Sri Lanka, the West Indies, and the US state of Florida [6]. It is grown as indoor potted plant in cooler climates or outdoor canopy tree in warmer tropical climates where it grows to a spectacular large spreading tree 30-40metres tall, with a stout trunk up to 2 meters in diameter. The trunk develops aerial and buttress root to anchor it in the soil and help support heavy branches. It has broad shiny leathery oval leave 10–35 centimeters long and 5–15 centimeters broad [7]. When it is mature, it unfurls and the sheath drops off the plant. Inside the new leaf, another immature leaf is waiting to develop. The plant often begins life as an epiphyte which eventually strangles and replaces the host growing independently [8].

In the Philippines decoction of the aerial rootlets is used for wounds, cuts skin eruptions, dermatitis and sores. Bark is astringent and used as styptics for wounds [9]. In folk medicine, Ficus plants are reported to have hypotensive and antidiabetic activities, also it is used to treat cough, chest conditions and also it is used as mild laxative, galactagogue, anti-rheumatic, digestive and as anti-helmintic against intestinal parasites [10]. The latex of *Ficus Elastica* contains rubber, which can be used for backache, cancer, hernia, piles, swellings, and tuberculosis of the testicles. This latex is also an irritant to the eyes and skin and can be fatal if taken internally [11]. In Panama, latex used for parasitic worms (*trichuris trichura*). In northern Cameroon, used as fertility enhancement. In West Africa, it used in the treatment of muscle and joint pain [12].

Previous studies on Ficus Elastica show that the plant extracs exhibit antioxidant, antitumor/anticancer, antimicrobial, antiprotozoal, anthelmintic, antiviral, anti-inflammatory and antiallergic properties. Reports on the anti-malarial (anti-plasmodial) activity of stem bark, root bark or leaves extracts has not been widespread. However, that of the stem bark extract has been studied [13].

This study therefore is intended to investigate the chemical constituents of the stem bark methanolic extract of *ficus elastica* with a view to authenticating the plant's anti-malarial potentials.

## 2.0. Materials and Methods

### 2.1 Collection of Plant Samples

Fresh *Ficus elastica* tree bark were collected in June, 2017 from University of Benin, Ugbowo Campus, Benin City, Nigeria. The leaves were identified and authenticated by a botanist, Dr Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, University of Benin. A voucher specimen was prepared, coded $UBH_f$ 357 and deposited in the university herbarium. Pieces of the tree bark were washed with water to remove surface pollutants after which they were air-dried and powdered with the aid of a mechanical grinder, ready for further experiment.

### 2.2 Extraction of Crude Powdered Sample

The powdered sample was extracted with 98% methanol using soxhlet extractor. The liquid extract obtained was filtered and concentrated to dryness using a rotary evaporator at reduced pressure. The concentrated extract was weighed and the percentage yield calculated based on the initial weight of

the crude powdered sample. The semi-solid brown extract obtained were weighed and yield determined. The sample was stored in an air-tight container and kept in a refrigerator at 4$^{o}$C until further experiments.

### 2.3 Phytochemical Screening

Simple chemical tests to detect the presence of secondary metabolites were done according to standard methods [14]. Approximately 5 g of the crude powdered sample was boiled with 75 mL of distilled water for 30 minutes. The solution was filtered hot and allowed to cool. The filtrate obtained was used to carry out the following tests.

#### 2.3.1 Test for Glycocides

1ml of the extract filtrate was dissolved 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of conc. $H_2SO_4$. A brown ring is required for the presence of glycosides.

#### 2.3.2 Test for steroids

2 mL of acetic anhydride was added to 0.5 g plant extract filtrate in 2 mL of dilute $H_2SO_4$. A colour change from violet to blue or green is required for the presence of steroids.

#### 2.3.3 Test for terpenoids (Salkowski test)

5 mL of plant extract filtrate was mixed in 2 mL of chloroform and 3mls of conc. $H_2SO_4$ was carefully added down the side of the inner wall of test tube to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

#### 2.3.4 Test for alkaloids

2mL of Picric acid was added to the plant extract filtrate . A yellowish precipitate test is a positive test.

#### 2.3.5 Test for Saponins

1mL of extract filtrate was diluted with 10 mL distilled water and shaken vigorously for one minute. Expected observation for a positive result: formation of a persistent frothing.

#### 2.3.6 Test for Flavonoids

2mL of extract filtrate was added to few drops of lead acetate solution. Expected observation for a positive result: formation of milky precipitate.

#### 2.3.7 Test for Phenolic compounds

2mL of plant extract filtrate was added to 5 mL of distilled water followed by 2 drops of 5% ferric chloride solution. A blank test was done by adding 2 drops of 5% ferric chloride solution to 5 ml of distilled water. Expected observation for a positive result: formation of intense coloration in the test sample.

#### 2.3.8 Test for tannins

To 2 mL of the plant extract filtrate, 10 mL of distilled water was added and boiled for 5 minutes and then filtered into halves. To about 2 drops of the filtrate, ferric ($FeCl_3$) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.

### 2.3.9 Test for Eugenols

2ml of the extract filtrate was mixed with 5ml of 5% KOH solution. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate. A pale yellow precipitate is indicative of positive test.

## 2.4 Determination of Polyphenolic and Flavonoid Content

### 2.4.1 Total Phenol

Total phenol content in the extracts was determined by the method previously described by Kim*[15]*. The extract solution (0.5 mL) of concentration 1 mg/mL was added to 4.5 mL of distilled water, and 0.5 mL of Folin-Ciocalteu's reagent (previously diluted with water 1:10, v/v) was added to the solution. After mixing, they were maintained at room temperature for 5 minutes followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of distilled water. After mixing the samples, the samples were incubated at room temperature for 90 minutes. The absorbance was measured by spectrophotometer at 765 nm. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). The standard curve was prepared using gallic acid in six different concentrations(50, 100, 150, 200, and 250 mg/L). Concentration values of extract were obtained from Gallic acid standard curve, by interpolating to the X- axis.

### 2.4.2 Total Flavonoid

Total flavonoid content was estimated using the method previously described by Figueroa *[16]*. Briefly, 0.5 mL of extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10 % aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalent per gram of extract (mg QE/g extract). The standard curve was prepared by quercetin in six different concentrations (50, 100, 150, 200, and 250 mg/L). Concentration values of extract were obtained from quercetin standard curve, by interpolating to the X- axis.

## 2.4 Anti-malarial Activity

### 2.4.1 Experimental mice

Sixty specific-pathogen-free, female and male mice (*Mus musculus*), aged 6-8 weeks old and weighted 30-35 g were purchased from the Department of Biochemistry Animal House, University of Benin, Nigeria. They were kept at 22-25°C with 12 h light/dark cycle, and given standard mouse pellet and water ad libitum. They were acclimatized for 14 days. Procedures of the animal experiments were ratified by the Ethical Committee on Animal Experimentation, Faculty of Pharmacy, University of Benin.

### 2.4.2 Rodent malaria parasite

Chloroquine-sensitive *Plasmodium berghei* strain ANKA (PbANKA) was used. They were kept alive by intraperitoneal (IP) passage in mice. Parasitemia was daily monitored by microscopic examination of Giemsa stained thin blood smear.

### 2.4.3 Treatment of the Animals

#### 2.4.3.1 Prophyllatic experiment

The mice were apportioned into Six (6) groups, five (5) animals per group for

- Group 1: 10 mg/kg Chloroquine
- Group 2: 0.2 ml Distilled water
- Group 3: Normal control
- Group 4: 100 mg/kg aqueous plant extract
- Group 5: 200 mg/kg aqueous plant extract
- Group 6: 400 mg/kg aqueous plant extract

#### 2.4.3.2 Curative experiment

The mice were apportioned into Six (6) groups, five (5) animals per group

- Group 1: 10 mg/kg Chloroquine
- Group 2: 0.2 ml Distilled water
- Group 3: Normal control
- Group 4: 100 mg/kg aqueous plant extract
- Group 5: 200 mg/kg aqueous plant extract
- Group 6: 400 mg/kg aqueous plant extract

### 2.5 Acute toxicity test

The acute toxicity test of aqueous crude extract of *F. Elastica* was carried out as previously described by Lorke, [17] though Modified. Groups of naïve ICR mice (5 mice of each) were given orally by gavage with 100, 200 and 400 mg/kg. The mice were observed for signs of toxicity which include but not limited to paw licking, salivation, stretching of the entire body, weight loss, weakness, respiratory distress and death in the first 4 h and subsequently daily for 7 days [18](Madara *et al*., 2010).

### 2.6 Antimalarial drug

Standard antimalarial drug, chloroquine diphosphate salt was used to study *in vivo* drug susceptibility of PbANKA. The drug was freshly prepared in distilled water and administered orally by gavage [19]. Drug dose, expressed in mg/kg of body weight, was adjusted at the time of administration according to the weight of each mouse. The dose of 10 mg/kg was based on the ED90 of this drug on PbANKA infected mice.

### 2.7 Statistical Analysis

Statistical analysis was done using GraphPad Prism software. The results were presented as mean + standard error of mean (SEM). Oneway ANOVA was used to compare several treatment groups. Significant differences were considered at 95% confident, $p<0.05$.

## 3.0. Results and Discussions

### 3.1.Phytochemical screening

**Table 1: Phytochemical constituents in stem bark extract of *ficus Elastica***

| Phytochemial Constituents | Result |
|---|---|
| Glycocids | + |
| Steroids | - |
| Terpenoids | + |
| Alkaloids | + |
| Saponins | + |
| Flavonoids | + |
| Phenolics | + |
| Tannins | + |
| Eugenols | + |

KEY: + ⟶ Present, - ⟶ Absent, + + ⟶ Largely present

Activities such as antioxidant, antimicrobial, anticancer, antifungal and anti-viral properties are as a result of biochemical components of various parts of plants. The phytochemical tests carried out on the stem bark extract of *F. Elastica* showed the presence of glycosides, terpenoids, alkaloids, saponins, flavonoid, phenolics, tannins, and eugenols, with no traces of steroids.However, when the phytochemical constituents of Ficus elastic extract was compared to that of the hexane extract of Datura Metel leaves, steroids were found in the hexane extract of Datura Metel leaves and tannins were absent which is present in the methanol extract of ficus elastic [20].

### 3.1.1 Total Phenolic Assay

Phytochemical screening of the methanolic extract of *F. Elastica* was conducted, and the extract showed the presence of phenolics. The total phenolic contents were determined using the Folin Ciocalteu method in terms of the Gallic acid equivalent (GAE) in mg/g of the extract. The total phenolic content was calculated using the graph shown in Figure 1, and the standard curve equation was A = 0.010c where $R^2$ = 0.947. The total phenolic contents (Gallic acid equivalents, mg/g) in the methanolic extract was calculated to be 226.60mg/g, respectively. : This calculation was carried out using the formula,

$$C = C_1 \times V/m \quad (1)$$

Where C = total phenolic content in mg/g, in GAE (Gallic acid equivalent), $C_1$ = concentration of Gallic acid established from the calibration curve in mg/ml, V = volume of extract in ml, and m = the weight of the plant extract in g

### 3.1.2 Total Flavonoids Assay

The extract *F. Elastica* also showed the presence of flavonoids. The total flavonoid content was determined using the Folin Ciocalteu method in terms of the Quercetin equivalent (QE) in mg/g of the extract. The total phenolic content was calculated with the help of the graph shown in Figure 2, and the standard curve equation was A = 0.006c where $R^2$ = 0.865. The total phenolic contents (Quercetin equivalents, mg/g) in the methanolic extract was calculated to be 127.17mg/g, respectively. The total phenolic content in the extract determined using equation 1.

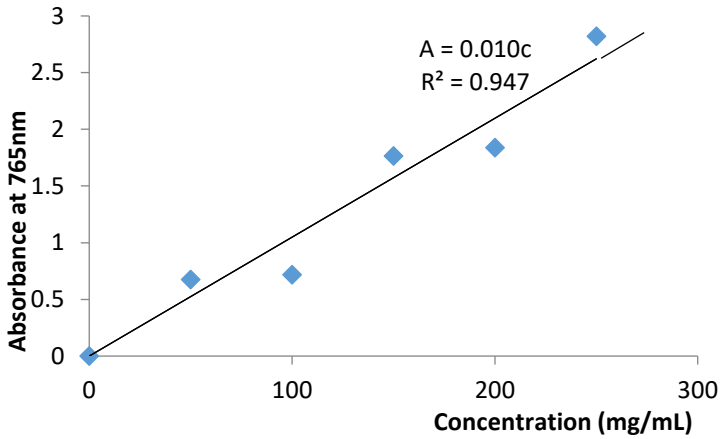


**Fig.3.1. Calibration Plot (Gallic acid standard)**

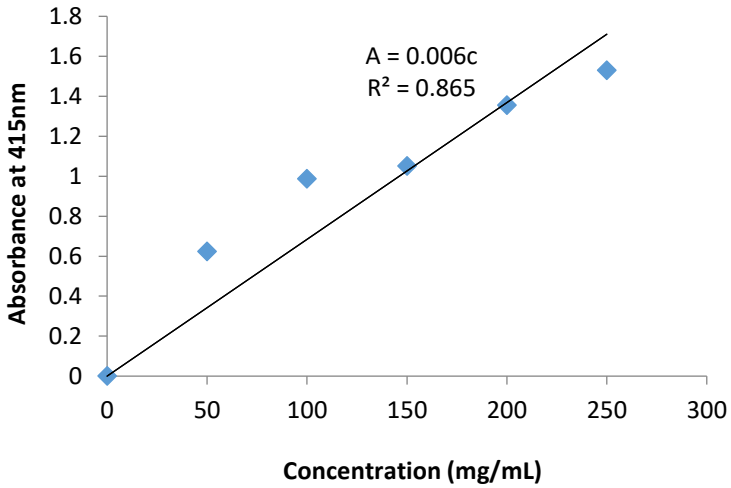


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**Fig. 2. Calibration Plot (Quercetin standard)**

**Table 2 Total phenolic and flavonoid content of the extract of *Ficus Elastica***

| Plant | Total Phenolic Content(mgGAE/g) | Total Flavonoid Content(mgQE/g) |
|---|---|---|
| *Ficus Elastica* | 226.60 | 127.17 |

**Table 3 Effect of *Ficus elastica* extracts filtrate on prophylatic treatment of *Plasmodium berghei* induced malarial.**

| Groups | Drugs | Dose (mg/kg) | Mean±SEM of Parasitemia | % inhibition | Mean±SEM of survival days | Drugs |
|---|---|---|---|---|---|---|
| **Negative control** | Distilled water | 0.2 ml | 0.00±0.00 | 0 | 7.67±0.89 | Distilled water |
| **Positive control** | Chloroquine | 10 | 0.76±0.04[a] | 76 | 20.33±0.33 | Chloroquine |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Plant extracts** | *Ficus elastica* | 100 | 0.68±0.08[a] | 68 | 20.67±1.76 | *Ficus elastica* |
| **Plant extracts** | *Ficus elastica* | 200 | 0.63±0.10[a] | 63 | 18.00±0.58 | *Ficus elastica* |
| **Plant extracts** | *Ficus elastica* | 400 | 0.71±0.29[a] | 81 | 18.67±0.88 | *Ficus elastica* |

a=p< 0.05 n=5

The prophylactic potency of *Ficus elastica* extract against *Plasmodium berghei* induced malarial showed an increase as the doses increased (100, 200 and 400 mg/kg) compared to the negative control and reference drug of the parasitemia count. As shown in Table 3, 400 mg/kg proved to be more effective and is only bested by 10 mg/kg Chloroquine. Mice that was dosed with 100 mg/kg survived for longer days when compared to mice dosed with 10 mg/kg Chloroquine as it was more sensitive to *Plasmodium berghei* and negative control (20.67±1.76, 20.33 and 7.67±0.89). A clearer picture is graphically explained on the level of decrease of the parasite level.

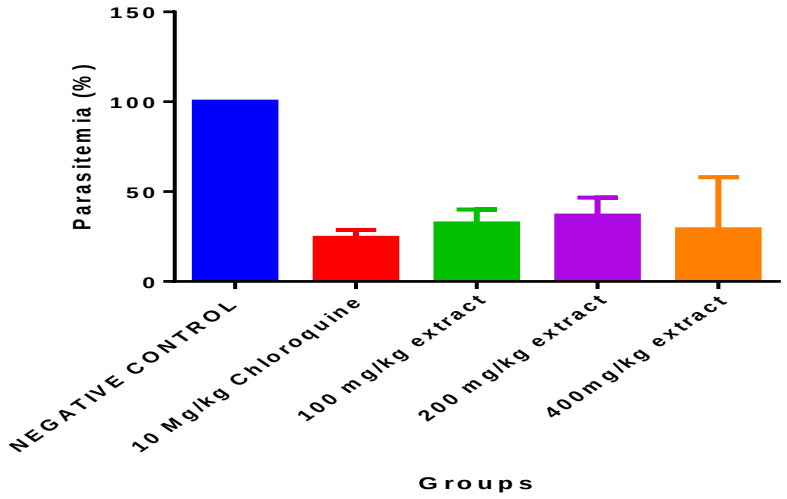


**Fig. 3 Effect of *Ficus elastica* extracts on prophylatic treatment of *Plasmodium berghei* induced malaria**

**Table 4: Effect of *Ficus elastica* extracts on curative treatment of *Plasmodium berghei* induced malaria.**

| Groups | Drugs | Dose (mg/kg) | Mean±SEM of Parasitemia | % inhibition | Mean±SEM of survival days | Groups |
|---|---|---|---|---|---|---|
| **Negative control** | Distilled water | 0.2 ml | 0.00±0.00 | 0 | 7.67±0.89 | Negative control |
| **Positive control** | Chloroquine | 10 | 0.52±0.08[a] | 52 | 17.33±1.45 | Positive control |
| **Plant extracts** | *Ficus elastica* | 100 | 0.36±0.03 | 36 | 11.67±0.88 | Plant extracts |
| **Plant extracts** | *Ficus elastica* | 200 | 0.45±0.13 | 45 | 11.67±1.76 | Plant extracts |
| **Plant extracts** | *Ficus elastica* | 400 | 0.47±0.23 | 47 | 13.33±0.88 | Plant extracts |

Curative study of *Ficus elastica* extract against *Plasmodium berghei* induced malarial showed low level of significant increase across the graded doses. Though the inhibition was quite significant when the dosage was increased from 100mg/kg to 200mg/kg, the inhibition was insignificant when the dosage was doubled to 400mg/kg. The extract exhibited less anti-malarial properties as showed in Table 4, control groups of standard drug drastically reduced the level of *Plasmodium berghei* as recorded from the percentage inhibition with lesser effect in the graded doses of treated plant (52

%, 47%, 45% and 36%) [21]. The surviving days showed lesser survival in the treated groups in comparison to the 10 mg/kg Chloroquine being sensitive to *Plasmodium berghei* and negative control (11.67±0.88, 11.67±1.76, 13.33±0.88, 17.33±1.45 and 7.67±0.89).

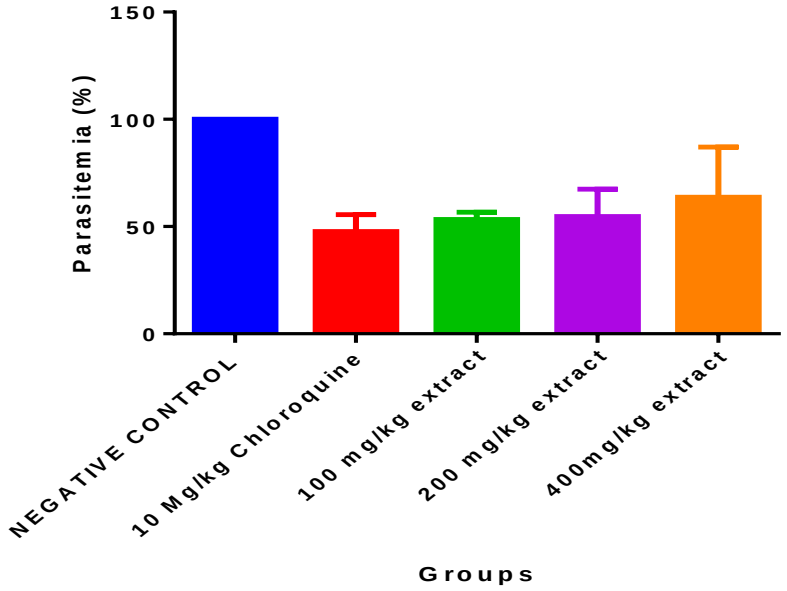


**Fig. 4: Effect of *Ficus elastica* extracts on curative treatment of *Plasmodium berghei* induced malaria**

**Table 5: Effect of *Ficus elastica* extracts on acute toxicity study.**

| Groups | **Dose (mg/kg)** | **Number of Mice** | **Mortality (%)** |
|---|---|---|---|
| *Ficus elastica* | 100 | 5 | 0 |
| *Ficus elastica* | 500 | 5 | 0 |
| *Ficus elastica* | 1000 | 5 | 0 |

The acute toxicity study of the observed animals showed no change in the normal behavior within 24hours of the *Ficus elastica* extract exposure, and no mortality was recorded during the 14days the study lasted. This showed that *Ficus elastica* extracts had no toxic effect even when the dose is increased up to 1000 mg/kg.

## 4.0. Conclusion

This study has demonstrated that oral administration of crude extracts of the stem bark of *F. elastica* presented a significant antimalarial activity in *P. berghei*–infected mice. The extract also showed a low cytotoxicity. The *in vivo* screening revealed that the extract exhibited higher prophylactic activity than curative. Thus, the plant could be considered as a promising source of organic compounds to develop new antimalarial drugs.

## 4.1. Recommendation

The isolation, purification, and structure elucidation of constituents of *F. Elastica* is recommended to support discovery of novel antiplasmodial compounds.

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