

## Full Length Research Paper

# The antimalaria effect of *Momordica charantia* L. and *Mirabilis jalapa* leaf extracts using animal model

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*Momordica charantia* L. (Cucurbitaceae) and *Mirabilis jalapa* L. (Nyctaginaceae) are medicinal plants used extensively in almost all folklore remedies around the world to treat malaria. This experiment investigated the effects of *M. charantia* L. and *M. jalapa* L. on malaria in a 4-day suppressive test. Animals received 50, 100, or 200 mg/kg of methanolic extracts orally. *M. charantia* and *M. jalapa* methanolic extracts had intrinsic antimalarial properties that were dose-dependent. The result showed that *M. charantia* was effective in suppressing malaria at the highest dose tested (200 mg/kg) while *M. jalapa* gave the highest chemosuppression of parasitemia at the lowest tested dose of 50 mg/kg body weight of mice. The result also showed that the standard reference drug, Chloroquine, had its highest chemosuppression of parasitemia (100%) at 20 mg/kg when administered orally. This research affirms the uses of these plants for the treatment of malaria.

**Key words:** Antimalarial, *Plasmodium berghei*, *M. charantia*, *M. jalapa*.

## INTRODUCTION

Malaria is still one of the most significant diseases in the world. The World Health Organization (WHO) reported that half of the world's population is prone to malaria in which 1-2 million deaths occur annually (WHO, 2012; Vogel, 2010).

*Plasmodium falciparum*, among the protozoan species of the genus *Plasmodium*, causes most of the severe cases of this ailment (Nogueira and Lopes, 2011). Many drugs have been used to treat malaria e.g. quinine, chloroquine, mefloquine, artemisinin amongst others, but

the parasite has developed resistance against a lot of these treatment regimens (White, 2004).

In the search for new therapeutic substances, a great number of researchers have resorted to plant sources (Chin et al., 2006; Fabricant and Farnsworth, 2001; Addae-Mensah et al., 2011). This is due to the fact that many of these plants are used in African traditional medicine (ATM) (Ginsburg and Deharo, 2011) and drugs from natural products have been summoned for use as origin of development of new antimalarials (Guantai and

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Chibale, 2011; Cruz et al., 2013; Wells, 2011; Anthony et al., 2012) so as to eradicate drug resistance problems (Taylor, 2002).

*Momordica charantia* (Family: Cucurbitaceae, Plate 1) is widely called bitter melon, bitter gourd, Balsam pear, Karela and Pare. It develops naturally in tropical areas of the Amazon, East Africa, Asia, India, South America, and the Caribbean. The plant is perennial, herbaceous and tendril climber which grows to six meters or longer. It has a lengthen fruit that looks like a warty gourd or cucumber. The unripe fruit is either white or green in colour and has a bitter taste that becomes more noticeable as the fruit ripens. The Latin name *Momordica* means "to bite" (describing the uneven edges of the leaf, as if they had been bitten) (Bakare et al., 2010). The leaves are simple, alternate (4-12 cm across) and palmately veined having ununited 3-7 deep lobes. *M. charantia* is a strong nutrient-based herb which consists of a composite collection of phytochemicals such as bioactive compounds, vitamins, minerals and antioxidants that contribute to its extraordinary ability in treating a whole lot of sicknesses. Bitter melon has separate yellow male and female flowers. The leaves and fruits of *M. charantia* are rich in vitamin A, vitamin B, vitamin C, vitamin E, iron, calcium, phosphorus and beta carotene. They are also rich in dietary fibers. The values of the calories present in leaf, fruit and seed were 213.26, 241.66 and 176.61 Kcal/100 g respectively (Snee et al., 2011). The activity of bitter melon has been ascribed to the level of antioxidant in it (Kirtikar and Basu, 2001).

A steroid saponin called charantin and a polypeptide named gurmardin, which is similar to insulin in composition were isolated from the fruits and leaves of *M. charantia*. These bioactive constituents were reported to be responsible for its hypoglycemic activity (Raman and Lau, 1996). *M. charantia* has some interesting biological and pharmacological activities. Previous investigations have shown that aqueous extracts of the leaf and fruit of *M. charantia* exhibited high antioxidant activity (Kubola and Siriamornpun, 2008). The role of free radicals and active oxygen in treating chronic diseases including cancer, aging and atherosclerosis has been recognized (Mathew and Abraham, 2006). Therefore, much attention has been focused on the use of antioxidants in protecting against the threat of damage of free radicals. It is a wonderful herbal medicine for human health. *M. charantia* is used in folkloric medicine to treat diabetes, HIV, coughs, skin diseases, sterility in women, parasiticide, antipyretic and as purgative among others.

*M. jalapa* (Plate 2) belongs to the family Nyctaginaceae which is popularly known as beauty of the night, four o'clock, or marvel of Peru. It is an herbaceous climber growing up to 2 m high. It has opposite leaves, very big impressive flowers, curvaceous, obovoid fruits and conspicuous tuberous roots. *Mirabilis* in Latin means 'wonderful' and Jalapa is a popular name in Central and North America. The precise source of *M. jalapa* is

unknown, but it is believed to originate from parts of tropical America (Yi-Fen et al., 2002). It is approximately 0.9 m high. It is mostly grown among the species of *Mirabilis* and has diverse of colours. Each flower is spattered with different colours and the designs are known as sectors (whole sections of flower), flakes (stripes of varying length), and spots. A flower can be plain yellow, pink or white, or mixture of sectors, flakes and spots (Miko, 2008). *M. jalapa* is also known for its colour-changing attribute.

For instance, yellow variety flower changes to dark pink colour gradually as it matures, so also is white flowers which change to light violet. The flowers normally unfold from late afternoon onwards, which led to its name 'the four o'clock plant'. The flowers produce long lasting sweet-smell all through the night, and fold up in the morning. The flower of *M. jalapa* developed from the pigmented modification of the calyx and not from the petals. It produces flowers from July to October, and the seeds ripen from August to October. The fruits with single seed are spherical, wrinkled and black when matured (Wang Yi-Fen et al., 2002). Several components such as  $\beta$ -sitosterol, stigmasterol, ursolic acid, oleanolic acid, brassicasterol, and *Mirabilis* antiviral protein, rotenoids (mirabijalone A-D, boeravinones C and F) were isolated from the aerial parts and roots of *M. jalapa* (Siddiqui et al., 1990, Siddiqui et al., 1994; Yi-Fen et al., 2002). Aoki et al. (2008) and Oskay et al. (2007) reported that *M. jalapa* had numerous biological activities such as antispasmodic, antibacterial, antiviral, antifungal and protein synthesis inhibition. *M. jalapa* is used in herbal medicine for the treatment of diarrhea, dysentery, conjunctivitis, edema, inflammation, swellings, muscular pain and malarial (Daniel, 2006).

In this work, we evaluated the antimalarial activities of *M. charantia* and *M. jalapa* using animal model with the view to justify the ethnomedicinal claim of the indigenous usage of the species in the cure of malaria.

## MATERIALS AND METHODS

### Plant specimens

The plant specimens used for this study were fresh leaves of *M. charantia* and *M. jalapa*. They were collected at the botanical garden of Adekunle Ajasin University, Akungba Akoko and authenticated in Forestry Research Institute of Nigeria (FRIN). The voucher sample was deposited at FHI (Forest Herbarium, Ibadan) with herbarium numbers FHI 110131 and 110132 respectively. *M. charantia* (179 g) and 160 g of *M. jalapa* leaves were air-dried, powdered and macerated with 70% methanol for five days. The filtrates were concentrated to dryness *in vacuo* and weighed (89.88 and 91.95 g respectively). Dilutions of dried extracts were prepared to give appropriate concentrations used for the assay.

### Preliminary phytochemical analysis

Phytochemical screening of the plants was carried out using standard procedures to test for alkaloids, saponin, cardiac



**Plate 1.** *Momordica charantia* L.



**Plate 2.** *Mirabilis jalapa* L.

glycosides, sterioids, flavonoids and tannins (Sofowora, 1993; Trease and Evans, 1986).

#### **Alkaloids**

1 g of powdered sample was stirred in 10 ml of 10% (v/v) HCL on a steam bath followed by filtration. The filtrate (1 ml) was mixed with a few drops of Meyer's reagent. To another 1 ml of the filter was added few drops of Wagner's reagent and a few drops of Drangendorff reagent was added to another 1 ml of the filtrate. The mixtures were observed for turbidity or formation of precipitate.

#### **Saponins**

1 g of powdered sample was boiled with 10 ml of distilled water for 10 min. The sample was filtered while hot, cooled and the following tests were performed:

1. Frothing test: 2.5 ml of the filtrate was diluted to 10ml with distilled water and shaken vigorously for 20 min. The formation of persistent foams was taken as evidence for the presence of saponins.
2. Emulsifying property: 2 drops of olive oil were added to 2.5 ml of



the filtrate and shaken vigorously for 30 min. Observation was made for the formation of stable emulsion.

### Tannins

1 g of powdered sample was boiled in 10ml of distilled water, filtered whilst hot and cooled. The filtrate was adjusted to 10 ml with distilled water. Then a few drops of 1% ferric chloride reagent were added to 1 ml of the filtrate. The mixture was observed for the formation of blue, blue black, green-black colouration or precipitate.

### Flavonoids

1 g of powdered sample was boiled with 10 ml of ethanol.

1. To 5 ml of the extract was added 2 drops of ferric chloride. A dusty green colour was considered positive.
2. To 5 ml of the extract, a small quantity of dilute NaOH was added and drops of Conc. HCL were run down the side of the tube. A reddish colouration indicated the presence of flavonoids.

### Cardiac glycosides

1 g of the sample was extracted with 10ml of 80% ethanol for five minutes on a water bath. The extract was filtered and diluted with equal volume of distilled water. A few drops of lead acetate solution were added, shook and filtered after standing for a few minutes. The filtrate was then extracted with aliquots of chloroform; the extract was divided into two portions in evaporating dish and evaporated to dryness on a steam bath.

### Keller killiani test

One portion from above was dissolved in 2 ml of glacial acetic acid containing one drop of  $\text{FeCl}_3$  solution in a clean test tube. 2 ml of concentration sulphuric acid was then poured down the side of the tube so as to form a layer below the acetic acid. The formation of a purple or reddish-brown or brown ring at the interface and a green colour in the acetic layer was taken for positive result (Sofowora, 1993).

### Kedde test

The second portion was mixed with 1ml of 2% 3, 5-dinitrobenzoic acid in ethanol. The solution was made alkaline with 5% NaOH after mixing. The formation of a transient purple, which turned brown on standing, was considered positive.

### Steroids

1 g methanolic extract was dissolved in 1 ml acetic anhydride and then 1 ml of dichloromethane. The solution was transferred into a dry test tube and by the means of pipette 2 ml of concentrated sulphuric acid was added at the bottom of the test tube. At the contact zone of the two liquids, a brownish-red ring was formed; the supernatant layer became greenish denoting presence of sterols and triterpenes.

### Experimental animals

Adult Swiss albino mice weighing between 20 and 40 g of both

sexes were used for the estimation of antimalarial properties of the plant extracts. They were obtained from the animal house, Department of Physiology, University of Ibadan. All experimental protocols were in accordance with internationally accepted principles for laboratory animal use and care as found in the US guidelines (Makinde et al., 1988). The animals were caged under standard conditions and fed with a stock diet and water *ad libitum*.

### Parasites

The antimalarial activities of the methanol extract of *M. charantia* and *M. jalapa* leaves were evaluated with chloroquine-susceptible strain of *Plasmodium berghei* (NK 65). Parasite was acquired from the Malaria Research Laboratories, Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan.

### Assessment of early malarial infection (4-day suppressive test)

The antimalarial activities of *M. charantia* and *M. jalapa* leaves were investigated using a 4-day suppressive test in *P. berghei*-infected mouse model (Peters and Robinson, 1992; Tona et al., 2001).

Twenty adult Swiss albino mice were grouped into five of four each and the mice that donated the parasite were infected with 200  $\mu\text{l}$  of *P. berghei* inoculum. The blood of each donor mouse that had been infested with parasite was collected from the tail vein and thinned with 0.9% sodium chloride. Normal saline suspension of  $1 \times 10^7$  parasitized erythrocytes (0.2 ml) was introduced into the mice by intra-peritoneal (i. p.) injection (Day 0). Four hours later, the first three groups were treated with 50, 100 and 200 mg/kg/day doses of the extracts for four successive days, while the fourth and fifth groups were treated with 20 mg/kg/day of chloroquine diphosphate (positive control) and 5 ml of normal saline (negative control) respectively for four sequential days. On the fourth day, thin blood films were prepared from blood collected from the tails of all mice. The films were air-dried, fixed in methanol for 30 s, and stained with 10% giemsa for 20 min on the previously washed slides. Parasitaemia of each mouse was counted under microscope and the percentage of suppression of parasitaemia for each dose was calculated:

$$\% \text{ Suppression} = \frac{\text{Parasitaemia of negative control} - \text{Parasitaemia of test drug}}{\text{Parasitaemia of negative control}} \times 100$$

The reduction in the percentage of parasite denotes the antimalarial activities of the extracts. (Philipson and Wright, 1991). Results were represented as mean values. Comparison of difference in quantitative variables between more than two and two groups was performed using Analysis of Variance (ANOVA) tests (SPSS version 16.0, SPSS Inc., CO, USA). Statistical significance level was set at  $P < 0.05$  for all tests.

## RESULTS AND DISCUSSION

The percentage yields of the crude extracts were 57.50% w/w for *M. jalapa* leaves and 50.21% w/w for *M. charantia* leaves. Results of the phytochemical screening (Table 1) showed the presence of some bioactive components in the leaves. They contain alkaloids, saponins, tannins, flavonoids, cardiac glycosides and steroids. It was noted that results of the screening showed abundant presence

**Table 1.** Qualitative phytochemical analysis of *M. omordica charantia* and *M. jalapa* leaves.

Test	Observation	<i>M. charantia</i>	<i>M. jalapa</i>
<b>Alkaloids</b>			
Dragendorff's reagents	Reddish brown precipitate	+++	+++
Mayer's reagents	Pale cream precipitate	+++	+++
Wagner's reagents	Reddish brown precipitate	+++	+++
<b>Saponins</b>			
Frothing Test	Persistent frothing	++	+++
Emulsification	Oily layer emulsified	++	+++
TANNINS	Greyish green colouration	+++	+++
<b>Flavonoids</b>			
Ferric Chloride solution	Dusted green colour	+++	+++
Reaction with Sodium Hydroxide	Reddish-brown colouration	+++	+++
<b>Cardiac-glycosides</b>			
Keller-Kiliani test	Brown ring formed at interphase	++	++
Kedde test	Brown purple precipitate	++	++
<b>Steroids</b>			
Acetic anhydride and chloroform	Solution turns brown	++	++
Concentrated sulphuric acid	Brown-red ring formed at interphase and supernatant layer turns green	++	++

+++ . Highly positive; ++, positive.

**Table 2.** Antimalarial activity of methanolic leaves extracts of *M. charantia* and *M. jalapa* in animal model.

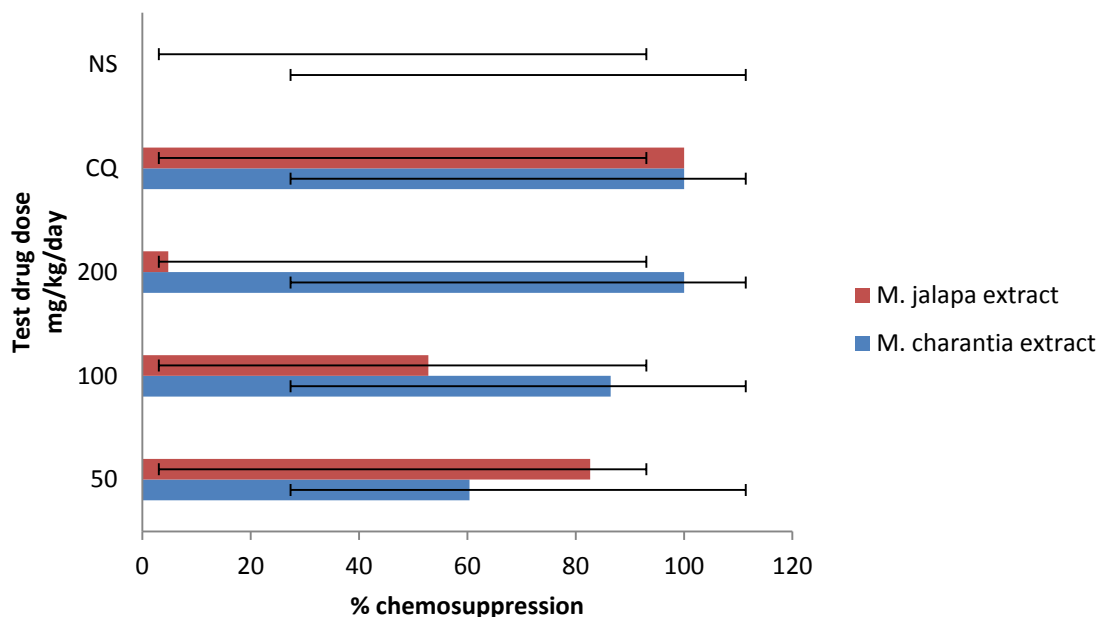
Test drug dose (mg/kg body weight/day)	<i>M. charantia</i> methanolic extract		<i>M. jalapa</i> methanolic extract	
	Parasitaemia (%)	Chemosuppression (%)	Parasitaemia (%)	Chemosuppression (%)
50	2.40 <sup>a</sup> ±3.09	60.39	1.05 <sup>a</sup> ±1.84	82.67
100	0.82 <sup>a</sup> ±1.45	86.46	2.86 <sup>a</sup> ±2.90	52.80
200	0.00 <sup>a</sup> ±0.00	100	5.77 <sup>b</sup> ±1.89	4.78
Chloroquine	0.00 <sup>a</sup> ±0.00	100	0.00 <sup>a</sup> ±0.00	100
Normal saline	6.06 <sup>b</sup> ±0.20	-	6.06±0.20	-

Value is represented as mean ± SD, n=4. Different alphabets in the same column denote significantly different values ( $p < 0.05$ ) as separated by analysis of variance.

of alkaloids, saponins, tannins and flavonoids in *M. jalapa* leaves and alkaloids, tannins, flavonoids and steroids in *M. charantia* leaves.

Phytochemicals such as terpenoids (e.g. Artemisinin) are involved in the antiprotozoal and antiplasmodial potential of diverse plants (Francois et al., 1996; Ghoshal et al., 1996; Asase et al., 2010; Tasdemir et al., 2006). Flavonoids exhibit substantial antiparasitic potentials against different strains of malaria, trypanosome and leishmania (Waako et al., 2007). There were records that alkaloids derived from plants have a lot of contributions to the development of anti-malarial drugs (Schwikkard and Van Heerden, 2002; Bero et al., 2009). *M. charantia* and *M. jalapa* plants are popularly used in herbal medicine to

cure malaria. The results of this experiment revealed antimalarial activities (Table 2) for the *M. charantia* and *M. jalapa* in the 4-day suppressive antimalarial test in mice infected with *Plasmodium berghei* (NK 65). The results showed high antimalarial potency when compared with the results of the standard reference drug (chloroquine) which gave 100% at the dose of 20 mg/kg. The bioassay carried out on the plants gave varying results. The mean percentage parasitaemia of *M. charantia* was 2.40 ± 3.09, 0.82 ± 1.45 and 0.00 ± 0.00% at 50, 100 and 200 mg/kg respectively, while positive and negative controls gave 0.00 ± 0.00 and 6.06 ± 0.20% respectively; the mean percentage parasitaemia of *M. jalapa* was, 1.05 ± 1.84, 2.86 ± 2.90 and 5.77 ± 1.89% for



**Figure 1.** Antimalarial activity bar chart showing % chemosuppression of *Momordica charantia* and *Mirabilis jalapa* methanolic leaves extract.

50, 100 and 200 mg/kg respectively, positive (chloroquine) and negative (normal saline) controls also gave  $0.00 \pm 0.00$  and  $6.06 \pm 0.20\%$ , respectively. Figure 1 showed the mean percentage chemosuppression for *M. charantia* and *M. jalapa* leaves. The values for *M. charantia* were 60.39, 86.46 and 100% at 50, 100 and 200 mg/kg respectively, while positive and negative controls gave 100 and 0%, respectively. The mean percentage chemosuppression of *M. jalapa* were 82.67, 52.80 and 4.78% for 50, 100 and 200 mg/kg respectively, positive and negative controls gave 100 and 0% respectively. *M. charantia* had its lowest mean percentage parasitaemia at the dose of 200 mg/kg ( $0.00 \pm 0.00\%$ ) while *M. jalapa* had its lowest mean percentage parasitaemia at the dose of 50 mg/kg ( $1.05 \pm 1.84\%$ ). *M. charantia* leaves extract induced the highest chemosuppression of parasitaemia (100%) at dose of 200 mg/kg compared to chloroquine (20 mg/kg), positive control group which had a chemosuppression of 100% while *M. jalapa* leaves had its highest activity of 82.67% at 50 mg/kg. The standard drug, chloroquine however gave 100% chemosuppression at 20 mg/kg. *M. charantia* leaves (100%) showed a significantly ( $p < 0.05$ ) higher percentage chemosuppression of parasitaemia than *M. jalapa* leaves (82.67%). The values were observed to increase as extract concentration increased in *M. charantia* leaves and decreased as extract concentration increased in *M. jalapa* leaves that is, the activity is dose dependent.

The activities might be attributed to the presence of one of the phytochemicals identified to be present; or even a combined action of more than one of the metabolites.

However, the active compound(s) known to give this observed activity need to be identified. In this regard, efforts are presently directed towards biologically guided fractionation of these plants so as to isolate, identify and characterize the active metabolite(s) and also investigate on its cytotoxic activity.

## Conclusion

This current research revealed the potency of *Momordica charantia* and *Mirabilis jalapa* methanolic leaves extracts against malarial *in vivo*. Therefore, the use of these plants traditionally for malarial is justified.

## Conflict of interests

The authors have not declared any conflict of interests.

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