Therapeutic Potentials of Combined Leaf Extract of Morinda lucida and Ficus exasperata on Plasmodium berghei Infected Mice

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Abstract

Background Control and eradication of malaria depend on effective anti-malarial medications. There is increasing concern over the development of drug resistance by the *Plasmodium* species which has led to an unending search for potent therapeutic agents for malaria case management.

Methods This study investigated the therapeutic potentials of combined leaf extracts of *Morinda lucida* and *Ficus exasperata* on mice infected with *Plasmodium berghei* and the effect on the histology of the liver. This was aimed at assessing the possible synergistic efficacy of these two already-proven antimalarial plants. One hundred and eighty (180) mice were divided into 12 groups of 15 mice each. The extracts were orally administered at single dose per day for 3 days. Blood samples were collected from the mice at Days 0 and 4 of treatment for parasitological examination. Liver sections were collected and used for histopathology. Data obtained were analysed in R Version 4.4.1.

Results Parasitological examination of blood samples for suppressive potentials of the extracts showed that 1500 mg/kg of *F. exasperata* extract and the standard drug had significant suppressive effect ($p < 0.05$) on parasitaemia. The curative test indicated that parasitaemia was significantly ($p < 0.05$) lower in all the combined extracts treated groups and 1500 mg/kg single extracts of the two plants. Contrary to the expectation, 1500 mg/kg of single extracts of these plants had better antiplasmodial potency than the mixture. There were diffuse degeneration of the hepatocytes following infection with *P. berghei* and was not reversed by the combined extracts of *M. lucida* and *F. exasperata* rather by 1500 mg/kg of the single plant extracts.

Conclusion This study showed that *M. lucida* and *F. exasperata* possess therapeutic potentials for malarial case management. It also revealed that single extracts of these plants had better antimalarial activities than their combinations. It is thus inferred that these plants contain antagonistic compounds that are affecting the potency of their combination. However, *M. lucida* and *F. exasperata* have proven to be potent antimalarials which further justify their use in traditional medicine, hence recommended for continuous use for malaria treatment especially as single extracts.

Background

Malaria is the most prevalent parasitic disease and major cause of ill health and death in the tropics (WHO, 2019). It plays a significant role in the current increase in the number of mortalities in the tropics. It is a disease caused by *Plasmodium* species. The different species of this haemoparasite that cause malaria infection include *Plasmodium falciparium*, *P. vivax*, *P. ovale* and *P. malariae*. Transmission of these parasites is by infected female *Anopheles* mosquito during a blood meal (WHO, 2018; Oladeji et al., 2022). There is an alarming increase in the population density of this malaria vector in the endemic regions which can be associated with climate change such as rainfall patterns, temperature and environmental factors, including lack of drainage systems and indiscriminate dumping of wastes. The risk of infection with malaria varies from place to place depending on the local mosquito fauna, season and level of environmental sanitation. Many countries of the world are at risk of malaria infection. About

247 million people were reportedly infected with the disease in 2021 in 85 countries while the mortality rate stood at 619,000 lives (WHO, 2022).

Control and eradication of malaria depend on effective anti-malarial medications. The World Health Organization currently approved artemisinin-based combination treatments (ACTs) as the therapy for malaria. The medication combines a partner medication with derivatives of artemisinin, such as artemether, dihydroartemisinin, and artesunate (WHO, 2013). Different other drugs have been earlier used for the treatment of malaria such as chloroquine, quinoline, however, there is increasing concern over the development of drug resistance by the *Plasmodium* species (Dondorp et al., 2010; WHO, 2022). This has led to an unending search for a more potent therapeutic agent for the treatment of malaria, especially in endemic areas. Research efforts are tilting towards medicinal plants because they are perceived to be effective, safe, cheap and readily available in the surroundings (Dondorp et al., 2010).

The application of plant extracts for medicinal purposes dates back to the ancient times, quite before the advent of chemical therapeutic agents (Idowu et al., 2014). About 80% of the world population use plant concoctions as medicines to treat various diseases. Medicinal plants have been used particularly for prevention and management of malaria in different parts of the world (Dike et al., 2012). Stem bark extract (Quinine) from Cinchona tree was used for the treatment of malaria as early as 1632 (Idowu et al., 2014). It has been argued that if not for the effectiveness of plant extracts used in the treatment of malaria and fever, malaria would have wiped off the African continent (Elujoba, 2005). Currently, medicinal plants still serve as the bases for traditional medicine as well as modern pharmaceuticals used for the treatment of different diseases (Kamba and Hassan, 2010).

There are clear indications that medicinal plants are the oldest, most widely distributed and most acceptable form of medication. Despite the increasing use of synthetic drugs, most medicines are still derived directly from plant sources (Memvanga et al., 2015). The elimination and eradication of malaria would be far-fetched if treatments only rely on the use of synthetic drugs (Bello et al., 2009). This is as a result of the multi-drug resistance which is the most significant challenge in the control of malaria over the years. These parasites resistance to chloroquine led to the recommendation by the World Health Organization that Artemisinin combination therapy which offers more improved efficacy be used as a replacement (WHO, 2003). However, the use of artemisinin combination therapy may be limited as a result of reports of development of resistance by the parasites (Kambale et al., 2022). This has caused renewed research for solutions of which the assessment of medicinal plants for malaria treatment occupies a prime position. The production of artemisinin from the plant "*Artemisia annua*" has also motivated researches in the field of malaria control to revisit the medicinal plants regularly applied for the management of malaria in traditional medicine (Koumaglo et al., 1992).

There is a gradual shift in the Primary Healthcare System of many African countries as a result of the use of plant-based products for disease management (Kambale et al., 2022). This is however, in agreement with the "Traditional Medicine Strategy" of the World Health Organization (WHO, 2013; Memvanga et al., 2015).

Many tropical medicinal plants have been confirmed for their potentials to yield antimalaria medicine (Memvanga et al., 2015). Notable among these plants are *Morinda lucida* and *Ficus exasperata*, either alone or in combination with other plants (Ebiloma et al., 2011., Lawal et al., 2012). These plants are composed of many different biological properties which are yet to be explored and documented. The presence of phenols, flavonoids, triterpenoids acids, anthraquinones, tannins and alkaloids informed the idea for the application of these plants extracts for malaria treatment (Koumaglo et al., 1992; Cimanga et al., 2006).

Morinda lucida and *Ficus exasperata* have been proven to have antiplasmodial actions against drug resistant *P. berghei* in mice (Bello et al., 2009; Ebiloma et al., 2011., Lawal et al., 2012). *M. lucida* has been revealed to possess anthraquinones, methylanthraquinones, tannins and heterosides which are antimalarial agents (Idowu *et al.*, 2010). The aqueous leaf extract of *M. lucida* has been reported to have chemosuppression properties with 85.05% in *P. berghei* infected Mice (Ebiloma et al., 2011; Idowu et al., 2014). The leaves of *M. lucida* (“Isi mkpi” in my dialect) are traditionally used for the treatment of malaria and fever in various areas of south eastern Nigeria. The leaves are boiled and the aqueous extracts are taken orally when still warm. In some cases, the sick also use the water that contains the extract for bathing as it is believed that the bioactive molecules penetrate through the skin.

Ficus exasperata has enjoyed similar reputation in traditional medicine in many African countries (Uzama et al., 2018). It is widely distributed in different vegetations in West Africa (Kar, 2007). Various parts of *F. exasperata* are traditionally applied for treatment of ailments. The leaves are particularly applied for malaria case management in many West African countries including Nigeria (Buniyamin et al., 2007; Titanji et al., 2008). The root is used for the treatment of gonorrhea, asthma, tuberculosis, cough and as antihelmintic (Uzama et al., 2018). The bark of the stem is applied for treatment of leprosy, wounds and stomach discomfort. They are also used in traditional medicine for alleviation of body pains, diuresis, haemorrhoids and venereal diseases (Ahmed et al., 2012).

Studies have shown that majority of the active compounds of these plants are not toxic to human cells, while some are very toxic and damaging to cells and organs of the body (Phillipson, 2001; Wilcox and Boderke, 2001; Kitua and Malebo, 2004). There is therefore need to investigate the therapeutic and toxicity properties of the bioactive compounds of these medicinal plants. This is because, there is a progressive increase in their use as they are readily available, cheap, and as well have prospective efficacy when compared to synthetic drugs.

Materials and methods

Collection and identification of plants

The leaves of *Morinda lucida* were collected from nearby bushes in Nsukka while those of *Ficus exasperata* were gotten from University of Nigeria, Nsukka, Enugu State. The plants were identified by a

plant taxonomist in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The voucher specimens were kept in the herbarium of the Department.

Preparation of methanolic leaf extracts and phytochemical screening

The powdered leaf extracts were prepared based on the procedure used by Siqueira et al., (2011). The fresh leaves of *M. lucida* and *F. exasperata* were washed with water and allowed to air- dry separately at room temperature for two weeks.

The phytochemistry of the methanolic extracts of the leaves of *M. lucida* and *F. exasperata* was conducted using standard methods to identify the compositions of the extracts of the two plants. Methods described by Harbone (1973), Sofowara (2008) were used.

Procurement and preparation of antimalarial drug

Artemisinin Combination Therapy (Artemether and Lumefantrine) tablets were purchased from a certified pharmacy store in Nsukka, Enugu State, Nigeria. This was administered by oral intubation at a dose of 20 mg/kg body weight of mice and used as standard control to compare and assess the therapeutic effect of combined leaf extracts of *Morinda lucida* and *Ficus exasperata* on mice infected with *Plasmodium berghei*.

Experimental design

This study adopted a completely randomized block design. At the beginning of the experiment, 180 mice were divided into 12 groups of 15 mice each. Nine (9) mice from each group were used for curative test while 6 were used for suppressive test. These treatment groups were A_1, A_2, A_3 (received 500, 1000 and 1500 mg/kg of *M. lucida* extract respectively); groups B_1, B_2, B_3 (received 500, 1000 and 1500 mg/kg of *F. exasperata* extract respectively); groups C_1, C_2, C_3 (received 1500 mg/kg of the combined extracts in the ratios; 1:1 of *M. lucida* and *F. exasperata*, 2:1 of *M. lucida* and *F. exasperata*, and 1:2 of *M. lucida* and *F. exasperata* extracts respectively); group D (received standard drug); group E (negative control) and group F (normal control). Each group was in triplicate. Mice in each experimental group were marked for identification. The dosing treatments were orally administered on the mice daily for 3 days. The treatment was initiated after 5 days (for curative test) and after 4 hours (for suppressive test) of inoculation of the parasite and the dosing was maintained at single dose per day. Food and water were provided *ad libitum* while observing 12 hours light and dark cycle. Blood samples were collected from the mice at Day 0 and 4 of treatment for parasitological examination while liver tissues were collected for histopathology.

Aquisition and acclimatization of mice

Disease-free mice (*Mus musculus*) were obtained from the Genetics and Animal Breeding unit of the Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State. The mice were housed in a hygienic experimental animal house in iron cages. Water and Chikun finisher meal pellets were given to the mice as needed. Before the experiment started, they were given two weeks to acclimate. The National Research Council's guidelines on experimental animal use (NRC, 2011) were followed in handling the mice. Much-needed attention was given to the mice's wellbeing in terms of reducing their discomfort and suffering (Passantino, 2008).

Acquisition of Plasmodium berghei and inoculation to the mice

Plasmodium berghei-infected mouse was purchased from the National Institute for Medical Research (NIMR), Lagos. Blood was drawn from the mouse that was already infected with *P. berghei* (Nk 65). The mouse was anaesthetized using chloroform. Sterile needles were used to draw infected blood from the mouse through ocular puncture. The blood and normal saline were diluted in a 1:10 ratio, respectively. Five days before treatment, each experimental mouse was intraperitoneally infected with parasitized red blood cells in a volume of 0.2 ml (David et al., 2004).

Administration of the plant extracts on the mice

Constituted extracts of each of the plant extracts and their combinations were administered to the mice 5 days after inoculation (curative test) and 4 hours after inoculation (suppressive test) with *Plasmodium berghei*. The extracts were administered orally using a feeding cannula.

Collection and examination of blood samples

Blood was drawn from each mouse in the different groups five (5) days following the parasite inoculation using ocular puncture. To ascertain the baseline parasitaemia, thin and thick blood smears were prepared and examined under the microscope.

Histopathological studies

Following 3 days administration of the plant extracts and determination of parasitaemia, the mice were sacrificed and the liver harvested for histopathology. The samples were transported to the histopathology unit of Department of Veterinary Anatomy, University of Nigeria, Nsukka for histopathological preparations using standard procedures in histopathology (Paget and Thompson, 1979).

Histopathological techniques

Liver samples of 3–4 mm thick were fixed in 10% formalin for 24 hours. This enabled the preservation of the tissue for subsequent treatments. Water contained in the tissue was removed using graded alcohol

concentrations, starting from 70% for 60 minutes through 90% for 45 minutes to absolute alcohol for 45 minutes. The alcohol was later removed from the dehydrated tissues using xylene. These tissues were placed in three phases of xylene each for 60 minutes. This allowed impregnation to take place. This was followed by infiltration of the tissue with molten paraffin wax which made the tissue suitable for microtomy.

The tissues were then ready for sectioning using the microtome. The cut paraffin embedded tissues were placed in the thermostatically controlled water at a temperature of about 4–60 C below the melting point of paraffin wax. The section was then flouted flat. In order to pick up a portion from a slide, the slide was vertically submerged to a fourth of its length, smeared with adhesive, while the section is being brought into contact with the slide (Joshua et al., 2022). The part was flattened on the slide when it was raised vertically out of the water. After that, it was allowed to air dry on the staining rack to blot it.

The slide was then stained with haematoxylin. This was followed by treating the section in scot's water for 5 minutes in order to deepen the blue colour of the nuclei. It was then washed with tap water and counter stained in 1% aqueous eosin for 2 minutes and washed. It was dehydrated in graded alcohol in ascending order of 70%, 80%, 90% and absolute alcohol for 10 minutes each.

The section was finally cleared and mounted with cover slip using Canadan blalsum (Dibutylphthalute Polystyrene Xylene) and was viewed under the microscope.

Data analysis

Data was analyzed in R version 4.4.1 (R Core Team, 2024), preliminary data exploration to assess for outliers, normality of distribution, and equality of variance were done using graphs and descriptive statistics. Q-Q plots, scatter plots, histogram and density plots were used for graphical examination while Shapiro-Wilk and Levene's tests were used for normality and equality of variance tests respectively. Parasitaemia in the suppressive test for single and mixtures of the extracts was not normally distributed, hence it was compared across the treatment groups by Kruskal-Wallis H test followed by Dunn's post-hoc test. In the curative treatment with combination of the extracts, parasitaemia was normally distributed; and was thus compared amongst the groups by two-way analysis of variance (ANOVA) accompanied by Tukey HSD post-hoc test. Generalized linear models (GLMs) were fitted to estimate the main and interaction effects of treatment and duration. This was accompanied by Tukey HSD post-hoc test where necessary. Results were presented as mean ± standard deviation (SD); and level of significance set at $p \leq 0.05$.

Results

Table 1
Suppressive effect of single leaf extracts of *F. exasperata* and *M. lucida*

Treatment	Conc. (mg/kg)	Mean ± SD	Median (IQR)	Min. – Max.	CV (%)
ML	500	7.75 ± 3.10	7.0 (3.25)^{ab}	5–12	39.9
	1000	3.75 ± 1.50	4.0 (2.25)^{ab}	2–5	40.0
	1500	4.33 ± 4.04	5.0 (4.0)^{ab}	0–8	93.3
FE	500	10.75 ± 6.06	10.5 (7.25)^{ab}	4–18	56.3
	1000	8.00 ± 7.00	5.0 (6.5)^{ab}	3–16	87.5
	1500	0.00 ± 0.00	0 (0)^{a}	0–0	-
Control	Standard	0.00 ± 0.00	0 (0)^{a}	0–0	-
	Negative	12.75 ± 4.99	12.5 (7.75)^{b}	8–18	39.2

Median (IQR) values with different alphabet superscripts along a column were significantly different between treatments ($p \leq 0.05$). ML – *Morinda lucida*; FE – *Ficus exasperata*; IQR – interquartile range; CV – coefficient of variation; SD – standard deviation.

Table 2
Curative effect of single leaf extracts of *F. exasperata* and *M. lucida*

Treatment	Conc. (mg/kg)	Initial		Final	
		Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
ML	500	25.25 ± 6.70	24.5 (6.25)^{a}	22.25 ± 9.88	22.0 (14.25)^{a}
	1000	15.75 ± 6.08	15.5 (7.25)^{a}	13.33 ± 11.02	8.0 (10.0)^{a}
	1500	19.00 ± 2.65	20.0 (2.50)^{a}	17.67 ± 2.08	17.0 (2.0)^{a}
FE	500	19.75 ± 11.15	19. 5 (15.75)^{a}	27.25 ± 12.04	23.5 (13.25)^{a}
	1000	17.25 ± 6.80	17.5 (10.25)^{a}	13.50 ± 4.93	13.5 (6.5)^{a}
	1500	16.33 ± 4.73	18.0 (4.50)^{a}	7.33 ± 3.06	8.0 (3.0)^{a}
Control	Standard	16.50 ± 4.43	16.0 (5.50)^{a}	8.00 ± 3.61	9.0 (3.5)^{a}
	Negative	24.00 ± 4.58	25 (4.50)^{a}	38.33 ± 5.51	38.0 (5.5)^{a}

Median (IQR) values with different alphabet superscripts along a column were significantly different between treatments ($p \leq 0.05$). ML – *Morinda lucida*; FE – *Ficus exasperata*; IQR – interquartile range; SD

– standard deviation.

Table 3
Curative effect of different combinations of *F. exasperata* and *M. lucida* methanol leaf extract.

Treatment	Conc. (mg/kg)	Initial		Final	
		Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
ML	1500	19.00 ± 2.65[a]	20.0 (2.5)	17.67 ± 2.08[ab]	17.0 (2.0)
FE	1500	16.33 ± 4.73[a]	18.0 (4.5)	7.33 ± 3.06[a]	8.0 (3.0)
1:1 ML-FE	750:750	19.33 ± 0.58[a]	19.0 (0.5)	22.00 ± 2.65[b]	23.0 (2.5)
2:1 ML-FE	500:1000	20.67 ± 2.08[a]	20.0 (2.0)	17.00 ± 1.00[ab]	17.0 (1.0)
1:2 ML-FE	1000:500	23.33 ± 8.74[a]	21.0 (8.5)	25.33 ± 2.52[b]	25.0 (2.5)
Control	Standard	16.00 ± 5.29[a]	14.0 (5.0)	8.00 ± 2.61[a]	9.0 (3.5)
	Negative	24.00 ± 4.58[a]	25.0 (4.5)	38.33 ± 5.51[c*]	38 (5.5)

Mean ± SD values with different alphabet superscripts along a column were significantly different between treatments ($p \leq 0.05$). *Significantly different from the initial parasitaemia ($p < 0.05$). ML – *Morinda lucida*; FE – *Ficus exasperata*; SD – standard deviation; IQR – interquartile range.

Histopathology of Liver of the Experimental Mice

Plate 1

Photomicrograph of liver sections from the experimental groups A1(*infected with malaria parasite + treatment with 500 mg/kg Morinda lucida) and A2 (infected with malaria parasite + treatment with 1000 mg/kg Morinda lucida) showing moderate degeneration of hepatocytes (arrows) while A3 (infected with malaria parasite + treatment with 1500 mg/kg Morinda lucida) shows normal hepatocytes (white arrows) and portal tracts (PT) and F (uninfected control) shows normal hepatocytes (white arrows) and portal tracts (PT).* H and E stain × 400.

Plate 2

Photomicrograph of liver sections from the experimental groups B1 (*infected with malaria parasite + treatment with 500 mg/kg Ficus exasperata) and B2 (infected with malaria parasite + treatment with 1000 mg/kg Ficus exasperata) showing moderate degeneration of hepatocytes (black arrows) while B3 (infected with malaria parasite + treatment with 1500 mg/kg Ficus exasperata) shows normal hepatocytes (white arrows) and portal tracts (PT) and F (uninfected control) shows normal hepatocytes (white arrows) and portal tracts (PT).* H and E stain × 400.

Plate 3

Photomicrograph of liver sections from the experimental groups C (*infected with malaria parasite + treatment with combination of 1500mg/kg Morinda lucida and Ficus exasperata) and E (infected untreated group) showing diffuse degeneration of hepatocytes (arrows) while D (infected with malaria parasite + treatment with 20mg/kg ACT) and F (uninfected control) shows normal hepatocytes (white arrows) and portal tracts (PT).* H and E stain × 400.

Discussion

The potency of *M. lucida* and *F. exasperata* in inhibiting the establishment of *Plasmodium* parasite in the liver and red blood cells was investigated. The extracts were administered four hours after infection with the parasite and continued for three consecutive days. The result showed that there were varying levels of suppression of the parasite based on the plant extract and concentration of the extract.

Mean parasitaemia were observed to be lower in the treated mice compared to the infected-untreated negative control. The result revealed that *P. berghei* parasitaemia was completely suppressed by the standard control (ACT) and the 1500 mg/kg of *F. exasperata* after administration to the infected mice. Thus, differences in level of parasitaemia were only significantly ($p < 0.05$) low in standard control and the 1500 mg/kg *F. exasperata* compared to the negative control. It was also observed that the single 1500 mg/kg of *M. lucida* and *F. exasperata* suppressed *P. berghei* parasitaemia better than the different combinations of *M. lucida* and *F. exasperata* used. The reason for this lower performance of the combined extracts is not clear, however, it is suspected that there may be antagonistic compounds possessed by these two plants that were mixed.

This result showed that *M. lucida* and *F. exasperata* have high schizontidal properties which are dose dependent, hence can serve prophylactic purposes at higher doses. This result is in consonance with the work of Oladeji et al. (2022) who reported a difference in percentage parasitaemia in chloroquine and *M. lucida* treated groups. The findings of this present study however disagree with a section of their result as they reported that the effect of the extract on level of parasitaemia was not dose dependent. It also agrees with the report of Okon et al. (2014) who reported high antimalarial potency of *F. exasperata* in suppressing *P. berghei*. The chemosuppressive effect of these plant extracts is also in agreement with previously reported works on this subject (Bello et al., 2009; Ebiloma et al., 2011; Idowu et al., 2014; Ugwu et al., 2020; Adepiti et al., 2021).

The leaves of *M. lucida* and *F. exasperata* have been shown to possess antimalarial properties (Obih et al., 1985; Tona et al., 1999). Generally, natural products of plant sources have been the focal point as sources of safer and more efficacious phytochemicals for malaria control (Dike et al., 2012). This is as a result of the understanding that medicinal plants have been and have remained the mainstay for the prevention and treatment of malaria in the world over (Koumaglo et al., 1992).

Assessment of the curative potentials of *M. lucida* and *F. exasperata* as well as their various combinations showed that treatments with 500, 1000 and 1500 mg/kg of *M. lucida* and *F. exasperata* extract reduced the parasite load, especially at the 1000 and 1500 mg/kg of extract concentrations. The variation in parasitaemia between the initial parasitaemia and final parasitaemia was however not significant ($p > 0.05$) between any two of the treatment groups despite mean and median parasitaemia being much less in the two higher concentrations of *F. exasperata*. The antiplasmodial activity of the 1500 mg/kg of *F. exasperata* was also observed to be same with the standard drug as the average parasitaemia of the two groups were comparable. This shows that *F. exasperata* has a better performance and antiplasmodial activity than *M. lucida*. It was also observed that the efficacy of these plant extracts for malaria treatment was concentration dependent as increase in concentration increased their antiplasmodial effects. It is thus inferred that higher concentrations of these plant extracts more than the concentrations used for this study would achieve greater parasitaemia clearance effect. This supports the reports of Idowu et al. (2014) and Oladeji et al. (2022) who revealed that antimalarial activity in the extract-treated groups may be a result of the terpenoids, alkaloids and phenolic contents. It is also in line with the work of Bello et al. (2009) who confirmed the antimalaria activity of *M. lucida* and further reported that the antiplasmodial activity of these plants reside mainly in the N-Hexane and chloroform fractions. The result of this study is in agreement with other previous antimalaria studies involving *M. lucida* and *F. exasperata* with only differences in the average final percentage parasitaemia level which can be attributed to variations in concentration and geographical location of the plants (Obih et al., 1985; Tona et al., 1999; Buniyamin et al., 2007; Titanji et al., 2008; Ebiloma et al., 2011; Adeleye et al., 2018; Oshiobugie et al., 2019; Ugwu et al., 2020; Afolabi and Abejide, 2020; Doma et al., 2023).

Studies on synergistic effects of combination of extracts of two different plants used for the treatment of malaria have revealed greater efficacies more than the single use of those extracts (Liu et al., 1992; White, 1999; Ishih et al., 2003; Fivelman et al., 2004; Edwards and Biagini, 2006; Guantia and Chibale, 2011; Rasoanaivo et al., 2011;). The result of the administration of the different combinations of *M. lucida* and *F. exasperata* in this work showed that the final parasitaemia decreased significantly ($P < 0.05$) at the end of three days treatment with the extracts. On the other hand, contrary to the expectation and reports of earlier published works on combinations of plant extracts for malaria treatment (Bello et al., 2009; Idowu et al., 2014; Memvanga et al., 2015; Afolabi and Abejide, 2020; Kambale et al., 2022,), this study showed that single extracts of *M. lucida* and *F. exasperata* had better antiplasmodia potency than the mixture. It was however suspected that these two plant extracts may probably contain antagonistic compounds. The result of the combination of extracts of *M. lucida* and *F. exasperata* is a confirmation of claims of traditional medicine practitioners in Osun State, Nigeria who stated that the leaves of *F. exasperata* should not be used with any antimalarial plant as it will impair their antimalarial activities (Adepiti et al., 2021). The poor antimalarial activity of the combination may also be a result of resistance of the species and strain of *Plasmodium* used for this study against the mixture. Moreso, the physiological state of the animal may have played some inhibitory roles against the therapeutical effect

of the extracts. This study thus, provided a baseline data for further studies on the combination of *M. lucida* and *F. exasperata* for malaria treatment.

The highest potency was observed in 1500 mg/kg of *F. exasperata* which had the same curative effect as the standard drug. This is a confirmation of the rationale for its reliable use for treatment of malaria in African traditional medicine. *F. exasperata* has been reported to be effective, cheap and readily available in the surroundings, safe and without side effects which have motivated its wide use (Phillipson, 2001; Wilcox and Boderke, 2001; Kitua and Malebo, 2004; Alaribe, 2008; Joseph and Raj, 2010; Graz *et al*., 2011; Ahmed et al., 2012; Dike et al., 2012; Okon et al., 2014; Memvanga et al., 2015).

The results of histopathology of the liver showed that infection with the parasite caused diffuse degeneration of the hepatocytes. This is likely a result of the multiplication of the schizonts (schizogony) in the liver cells during the exoerythrocytic stage of the *Plasmodium* lifecycle. The administration of single extracts of *M. lucida* and *F. exasperata* restored the integrity of the liver cells. The activity of the extracts in the liver was found to be dose-dependent, because, hepatocytes were restored to their normal state only in the 1500 mg/kg of *M. lucida* and *F. exasperata* compared to the 500 mg/kg and 1000 mg/kg of the extracts. This supports the hepatoprotective properties of these plant extracts and attestation of their long history of human use (Adepiti et al., 2021). The result of the combination of the two plant extracts showed that the combined extract failed to reverse the liver destruction caused by the malaria infection. This still supports the poor performance of these combined leaf extracts on *P. berghei* infected mice

The results obtained for the histopathology of the liver indicated that *M. lucida* and *F. exasperata* leaf extracts have no toxic effect on the liver. This agrees with previous reports on the toxicity of these plant extracts (Oduola et al., 2010; Arise et al., 2013; Adeleye et al., 2018; Joppa et al., 2019). It, however, disagrees with the report of Ahmed et al. (2012) who revealed several damages on the liver and kidney following administration of *F. exasperata*. It also disagrees with the reports of some other authors (Sowemino *et al*., 2007; Bafor and Igbinuwem, 2009; Ikpeme et al., 2010). This is likely because of prolonged use of the extracts at higher doses. It has already been revealed that pathological and histological changes in liver and kidney may be noticed over prolonged administration of these extracts (Umeh *et al*., 2014). Hence, the extracts are safe for short term oral administration.

Conclusion

The outcome of this study showed that *M. lucida* and *F. exasperata* possess therapeutic potentials for malaria case management. It also revealed that single extracts of these plants had better antimalarial activities compared to the combinations. This is also true for their histopathological activities. It is thus inferred that these plants contain antagonistic compounds that are affecting the potency of their combinations. Hence, further studies on fractionation, isolation and purification of the phytochemical constituents of these plants are advocated. However, *M. lucida* and *F. exasperata* have proven to be

potent antimalarials which further justify their use in traditional medicine, hence recommended for continuous use for malaria treatment especially as single extracts.

Declarations

Author contributions

The conception, design of study, literature materials and work draft were carried out by Ijem A. Nnachi and Ikem C. Okoye. Data analysis was done by Felix A. Andong and Daniel E. Echude. Hope C. Ezinwa conducted a critical revision of the manuscript. The research was conducted in collaboration among all authors. All authors endorsed the final version of the manuscript and accepted responsibility for every aspects of the work.

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Availability of data and materials

All data and materials are contained within the manuscript

Ethical approval

Approval for use of the animals was granted by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The Approval Reference Number is: FVM-UNN-IACUC-2024-06/156.

Consent to participate

Not applicable

Consent for publication

We declare that all authors have read and approved the manuscript for submission to The Journal of Basic and Applied Zoology. We hereby affirm that the manuscript is original and has not been previously, nor is it currently under consideration for publication elsewhere.

Competing interests

The authors declare no competing interests.

References

1. Adeleye, O. O., Ayeni, O. J. and Ajamu, M. A. (2018). Traditional and medicinal uses of *Morinda lucida*. *Journal of Medicinal Plants Studies*, 6(2): 249-254.

2. Adepiti, A. O., Agbaje, K. O. and Afolabi, M. O. (2021). Knowledge of traditional medical practitioners in Osun State, Nigeria about herbal antimalarials: a survey and in vivo evaluation of selected plants in mice. *West African Journal of Pharmacy,* 32(2): 18-29.
3. Afolabi, O. J and Abejide, A. E. (2020). Antiplasmodial activities of *Morinda lucida* (Benth) and *Alstonia boonei* (De wild) in mice infected with Plasmodium berghei. *Bulletin of the National Research Centre*, 44 (85).
4. Ahmed, F., Ahmed, M. K., Abedin, M. Z. and Karim, A. A (2012). Traditional uses and pharmacological potentials of *Ficus exasperata* Vahl. *Systematic Reviews in Pharmacy*, 3 (1): 15-23.
5. Arise, R. O., Malomo, S. O. and Bolarinwa, O. A. (2013). Comparative antimalarial and safety attributes of methanolic extracts of leaves of *Tithonia diversifolia* and *Morinda lucida* in animal models. *American Journal of Drug Discovery and Development*, 3: 140-148.
6. Bafor, E. E. and Igbinuwem, O. (2009). Acute toxicity studies of the leaf extract of *Ficus exasperata* on haematological parameters, body weight and body temperature. *Journal of Ethnopharmacology*, 123: 302-307.
7. Bello, I. S., Oduola, T., Adeosun, O. G., Omisore, N. O., Raheem, G. O. and Ademosun, A. A. (2009). Evaluation of antimalarial activity of various fractions of *Morinda lucida* leaf extract and Alstonia boonei stem bark. *Global Journal of Pharmacology*, 3 (3): 163-165.
8. Buniyamin, A. A., Eric, K. I. Q. and Fabian, C. A. (2007). Pharmacognosy and hypotensive evaluation of *Ficus exaperata* Vahl (Moraceae) leave. *Acta Polomae Pharmaceutical Drug Research*, 64 (6): 543-546.
9. Cimanga, R. K., Tona, G. L., Mesia, G. K., Kambu, O. K., Bakana, D. P. and Kalenda, P. T. D. (2006). Bioassay-guided isolation of antimalaria triterpenoid acids from leaves of *Morinda lucida*. *Pharmaceutical Biology*, 44(9): 677-681.
10. David, A. F., Philip, J. R., Simon, I. C., Reto, B. and Solomon, N. (2004). Antimalarial drug discovery efficacy models for compound screening. *Nature Review*, 3: 509-520.
11. Dike, I. P., Obeembe, O. O. and Adebiyi, F. E. (2012). Ethnobotanical survey for potential antimalarial plants in South western Nigeria. *Journal of Ethno-pharmacology*, 144 (3): 618-626.
12. Doma, A. I., Maitama, A. Y., Ameera, A. R., Abubaka, A. R., Muhammad, Y. A. and Yaro, A. H. (2023). In Vivo antiplasmodial activity and short term safety assessment of methanol leaf extract of *Ficus asperifolia* (Moraceae). *FUW Trends in Science and Technology Journal*, 8(3): 405-414.
13. Dondorp, A. M., Yeung, S., White, L., Nguon, C., Day, N. P. J., Socheat, D. and von Seidlein, L. (2010). Artemisinin resistance: current status and scenarios for containment. *Nature Reviews Microbiology,* 8: 272-280. DOI: 10.1038/nrmicro2385.
14. Ebiloma, G. U., Omale, J. and Aminu, R. O. (2011). Suppressive, curative and prophylactic potentials of Morinda lucida (Benth) against erythrocytic stage of mice infected with *Plasmodium berghei* NK-65. *British Journal of Applied Science and Technology*, 1 (3): 131-140.
15. Edwards, G. and Biagini, G. A. (2006). Resisting resistance: dealing with the irrepressible problem of malaria. *British Journal of Clinical Pharmacology*, 61: 690-693.

16. Elujoba, T. (2005). Book review "Traditional medicinal plants and malaria. *African Journal of Traditional Complementary Alternative Medicine*, 2 (2): 206-220.
17. Fivelman, Q. L., Adagu, I. S. and Warhurst, D. C. (2004). Modified fixed-ratio isobologram method for studying in vitro interactions between atovaquone and proguanil or dihydroartemisinin against drug-resistant strains of *Plasmodium falciparum*. *Antimicrobial Agents Chemotherapy*, 48:4097-4102. DOI: 10.1128/AAC.48.11.4097- 4102.2004.
18. Guantia, E. and Chibale, K. (2011). How can natural products serve a viable source of lead compounds for the development of new/novel antimalarials. *Malaria Journal*, 10: S2.
19. Harborne, J. B. (1973). *Phytochemical Methods: A Guild to Modern Techniques of PlantAnalysis*. Chapman and Hall, London, Pp388.
20. Idowu, O. A., Babalola, A. S., Adenubi, O. T. and Olukunle, J. O. (2014). Antiplasmodial activities of combined extracts of *Morinda morindiodes, M. lucida* and *Vernonia amygdalina* in *Plasmodium berghei* infected mice. *The Zoologist*, 12: 40-45.
21. Ikpeme, E. V., Udensi, O., Ekaluo, U. B. and Efleneokwu, N. (2010). Biological response of male wistar rats to crude extract of *Ficus exasperata* (Vahl). *International Journal of Current Research*, 7:9-13.
22. Ishih, A., Suzuki, T., Watanabe, M., Miyase, T. and Terada, M. (2003). Combination effects of chloroquine with febrifugine and isofebrifugine mixture against a blood-induce infection with chloroquine-resistant *Plasmodium berghei* NK65 in ICR mice. *Phytotherapical Research*, 17: 1234-1236.
23. Joppa, K. M., Metowogo, K., Missebukpo, A., Agbere, S., Vivor, A. and Aklikokou, K. (2019). Preventive and curative effect of *Morinda lucida* extract on the anaemia and its toxicological evaluation. *Research Journal of Medicinal Plants*, 13(3): 89-95.
24. Joshua, B. I., Nanchak, L. R., Ayo, A. C., Jummai, B. D., Emmanuel, L. L., Suleiman, I., Alesa, U. M., Dominic, U. A., Pwajok, C. T. P., Gunya, D. Y. and Elisha, I. L. (2022). Gross and histopathological lesions associated with *Echerichia coli* infection in chickens examined at ECWA Veterinary Clinic, Bukuru, Plateau State, Nigeria. *Acta Scientific Microbiology*, 5 (3): 74-81.
25. Kamba, A. S. and Hassan, L. G. (2010). Phytochemical screening and antimalaria activities of *Euphorbia balasamifera* leaves, stem and root against some pathogenic microorganisms. *African Journal of Pharmaceutical Sciences and Pharmacy*, 1:57-64.
26. Kambale, E. K., Mukubwa, G. K., Mwabonkolo, M. M., Musuyu, D. M., Nkanga, C. I. and Memvanaga, P. B. (2022). *In vivo* antimalaria activity of self-nanoemulsifying drug delivery systems containing ethanolic extract of *Morinda lucida* in combination with other Congolese plants extracts. *Brazilian Journal of Pharmaceutical Sciences*, DOI: 10.1590/s2175-97902022e20074.
27. Kar, A. (2007). *Pharmacognosy and Pharmacobiotechnology*. 2nd edition. New age international LTD. New Delhi. 600Pp.
28. Kitua, A. Y. and Malebo, H. M. (2012). Malaria control in Africa and the role of traditional medicine In: *Traditional Medicinal Plants and Malaria*. Willcox M. L., and Rasoanaivo, P. (eds), Boca Raton: CRC Press, 3-18.

29. Koumaglo, K. M., Gbeassor, O., Nikabu, C., Souza, de and Werner, W. (1992). Effects of three compounds extracted from *Morinda lucida* on *Plasmodium falciparum*. *Planta Medicine*, 58: 533-534.
30. Lawal, H. O., Etatuvie, S. O. and Fawehinmi, A. (2012). Ethnomedicinal and Pharmacological properties of *Morinda lucida*. *Journal of Natural Products*, 5: 93-99.
31. Liu, K. S., Yang, S. L., Roberts, M., Elford, B. and Phillipson, J. (1992). Antimalarial activity of *Artemisia annua* flavonoids from whole plants and cell cultures. *Plant Cell Reports*, 11: 637-640. DOI: 10.1007/BF00236389.
32. Memvanga, P. B., Tona, G. L., Mesia, G. K., Lusakibanza, M. M. and Cimanga, R. K. (2015). Antimalaria activity of medicinal plants from the Democratic Republic of Congo: a review. *Journal of Ethnopharmacology*, 169: 76-98.
33. NRC (2011). *Guide for the Care and Use of Laboratory Animals*. 8th Edition. National Research Council of the National Academics, National Academic Press, Washington, D.C.
34. Obih, P. O., Makinde, J. M. and Laoye, J. V. (1985). Investigation of various extracts of *Morinda lucida* for antimalarial actions on *Plasmodium berghei* in mice. *African Journal of Medical Science*, 14: 45-49.
35. Oduola, T., Bello, I., Adeosun, G., Ademosun, A., Raheem, G. and Avwioro, G. (2010). Hepatotoxicity and nephrotoxicity evaluation in wistar albino rats exposed to *Morinda lucida* leaf extracts. *New American Journal of Medical Research*, 2(5): 230 – 233.
36. Okon, O. E., Gboeloh, L. B. and Udo, S. E. (2014). Antimalarial effect of combined extracts of the leaf of *F. exasperata* and stem bark of *Anthocleista vogelii* on mice experimentally infected with *P. berghei* (NK 65). *Research Journal of Medicinal Plants*, 8(3): 99-111.
37. Oladeji, O. S., Oluyori, A. P. and Dada, A. O. (2022). Antiplasmodial activity of *Morinda lucida* Benth. leaf and bark extracts against *P. berghei* infected mice. *Saudi Journal of Biological Sciences,* 29 (4): 2475-2482.
38. Oshiobugie, M. J., Damazio, O. A., Ajetunmobi, A. O., Babalola, A. O., Adekunle, O. M., Busari, N. O. and Musa, A. A. (2019). Phytochemical analysis and antiplasmodial (curative) activities of methanolic leaf extract of *Morinda lucida* in male swiss mice infected with *Plasmodium berghei* (NK 65). *International Journal of Tropical Diseases and Health*, 37(1):1-13.
39. Paget, E. G. and Thompson, R. (1979). "*Standard Operating Procedures" In: Pathology*, MTP Press, Lancaster. Pp. 134 -139.
40. Passantino, A. (2008). Application of the 3Rs principles for animal used for experiments at the beginning of the 21st century. *Annual Review of Biomedical Sciences*, 10: 27-32.
41. Phillipson, J. D. (2001). Phytochemistry and Medicinal Plants. *Phytochemistry*, 56: 237-243.
42. R Core Team (2024). _R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

43. Rasoanaivo, P., Wright, C. W. and Willcox, M. L (2011). Whole plant extracts versus single compounds for the treatment of malaria: Synergy and positive interactions. *Malaria Journal*, 10 (S4).
44. Siqueira, S., Falcao-Silva, V. D. S., Agra, M. D. F., Dariva, C., Siqueira-Junior, J. P. D and Fonseca, M. J. V. (2011). Biological activities of *Slanum paludosum* Moric. Extracts obtained by maceration and supercritical fluid extraction. *The Journal of Supercritical Fluids*, 58 (3): 391-397.
45. Sofowora, E. A. (2008). *Medicinal Plants and Traditional Medicine in Africa*. 3rd Edition, John Wiley and Sons Limited, New York.
46. Sowemimo, A. A., Fakoya, F. A., Awopetu, I., Omobuwajo, O. R. and Adesanya, S. A. (2007). Toxicity and mutagenic activity of some selected Nigerian plants. *Journal of Ethnopharmacology*, 113: 427-432.
47. Titanji, V. P., Zofou, D. and Ngemenya, M. N. (2008). The antimalaria potential of medicinal plants used for the treatment of malaria in Cameroon folk medicine. *African Journal of Traditional and Complementary Alternative Medicine*, 5: 302-321.
48. Tona, L., Ngimba, N. P., Tsakala, M., Mesia, K., Cimanga, K. and Apers, S. (1999). Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasha, Congo. *Journal of Ethnopharmacology*, 68 (1-3): 193-203.
49. Ugwu, C., Ugorji, L., Diovu, E., Nwaije, C., Amadi, B., Ezegbe, A. and Ugwu, N. (2020). Formation and evaluation of *Morinda lucida* based-phytosome complexes for malaria treatment. *Pharmacologyonline*; 3: 345-357.
50. Uzama, D., Abdullahi, S., Okeniyi, S. O. and Adeniyi, M. M. (2018). Antimicrobial activities and phytochemical properties of *Ficus exasperata roots extracts*. *Journal of Chemical Society of Nigeria*, 43 (2): 198-204.
51. White, N. (1999). Antimalarial drug resistance and combination chemotherapy. *Philosophical Transactions of Royal Society of Biological Science*, 354: 739-749. DOI: 10.1098/rstb.1999.0424.
52. WHO (2013). Traditional Medicine Strategy 2014-2023, WHO Press. Geneva, Switzerland.
53. WHO (2018). World malaria report, World Health Organization, Geneva. https://apps.who.int/iris/bitstream/handle/10665/275867/9789241565653-end.pdf?ua=1. Accessed 15 June, 2023.
54. WHO (2019). World Malaria Report, WHO Press. Geneva, Switzerland.
55. WHO (2022). World Malaria Report, World Health Organization, Geneva. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report- 2022. Accessed on 4th June, 2023.
56. Willcox, M. L. and Bodeker, G. (2001). Traditional herbal medicines for malaria. *British Medical Journal*, 329: 1156-1159.

Plates

Plates 1-3 are available in the Supplementary Files section.

Figures

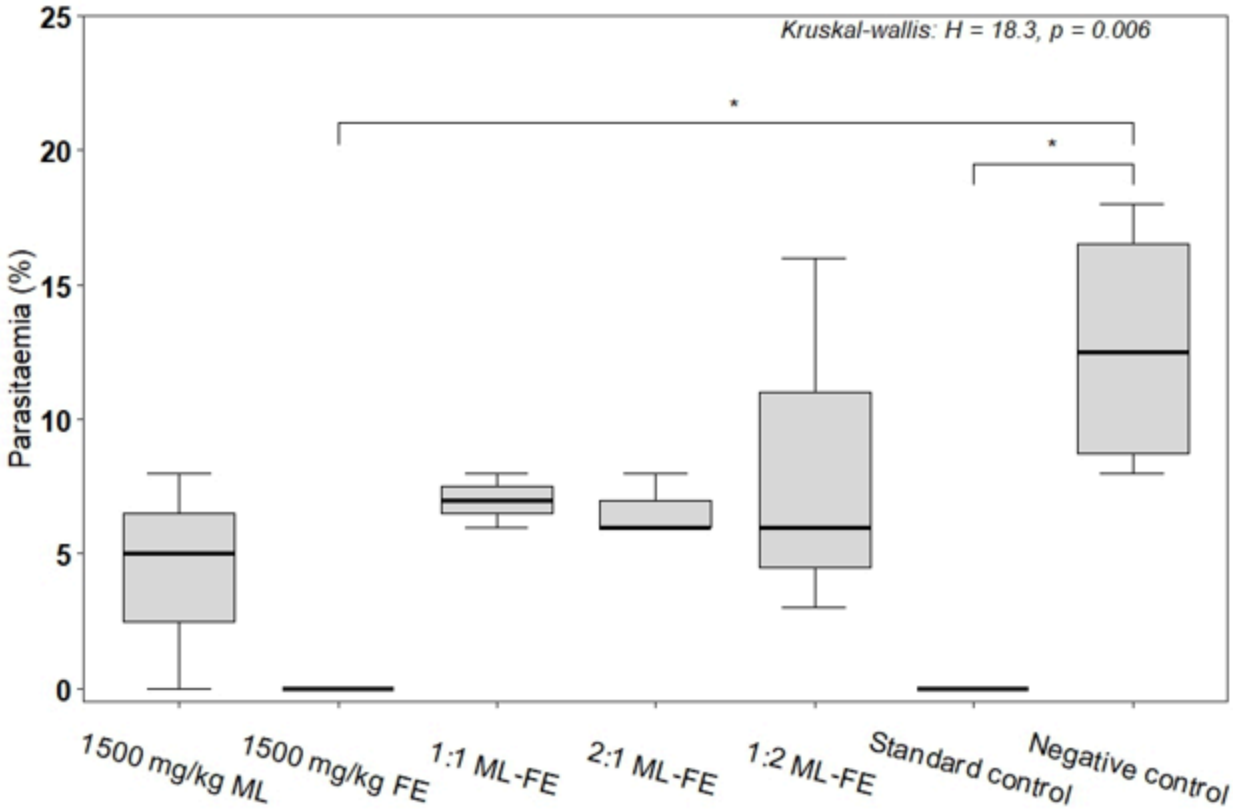

Figure 1

Suppressive effect of combinations of different concentrations of *F. exasperata*and *M. lucida* methanol leaf extract. *Significantly different at $p \leq 0.05$.

Supplementary Files

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