IAA Journal of Scientific Research 10(2):89-101, 2023 ISSN: 2736-7319

Antimalarial Activity and Levels of Inorganic ions of the Aqueous Leaf Extract of *Maesa lanceolata* Forssk

Jacqueline Njeri Muchiri[1,2], Martin Amanya[1,], Wilberforce Mfitundinda[2], Maniga Nyabayo Josephat[3,4] and John Odda[2,5]

[1] Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda.
[2]School of Pharmacy, Kampala International University Western Campus, Bushenyi, Uganda
[3] Department of Biological Sciences, Kisii University, Kenya.
[4]Department of Microbiology and Immunology, Kampala International University Western Campus, Bushenyi, Uganda
[5]Department of Pharmacology and Therapeutics, Makerere University, Kampala, Uganda.
Correspondence: Jacqueline Njeri Muchiri, Department of Pharmacology, Kampala International University, P.O.BOX 71 Bushenyi, Uganda , Tel +256779752951, +256706106193
Email: jmnjeri600@gmail.com

ABSTRACT

Drug resistance, inaccessibility, unaffordability and side effects of conventional medicines used for malaria treatment require that new drugs be developed. Plants like *Maesa lanceolata* are a potential source of new drugs. *Maesa lanceolata* which is one of the most commonly used in the traditional management of malaria in Uganda was evaluated for curative antimalarial activity using Reley and Peters method and levels of copper and zinc ions by using the atomic spectroscopy method. *Maesa lanceolata* aqueous leaf extract showed no antimalarial activity on *Plasmodium berghei* infected mice with all the three tested doses exhibiting less than 50% parasitemia suppression. Copper ions in the dried leaf of *Maesa lanceolata* were 9.33 parts per million. Level of zinc were 8.53 parts per million. The amount of copper consumed by patients surpasses the recommended daily allowance which warrants further research on posology of this plant using a greater sample size as well as further investigations on possible effects of excess copper consumption. The lack of antimalarial activity in mice calls for more research on other factors such as possible fever and pain reducing effect and appetite increase that could be contributing to the relief experienced by malaria patients being managed using this herb.
Keywords; antimalarial, Maesa lanceolata, Plasmodium berghei, aqueous leaf extract

INTRODUCTION

Malaria is an acute febrile illness caused by infection with *Plasmodium* parasites and is transmitted from one person to another by infected female anopheles mosquito. There are five *Plasmodium* species namely: *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*. *Plasmodium falciparum* is the most virulent and most common parasite in Uganda. Malaria still is one of the most fatal communicable diseases globally with an estimated 241 million cases and 627,000 deaths in 2020 which is an increase from 227 million cases and 558,000 deaths reported in 2019[1]. This could be attributed to the disruption of malaria services by the covid-19 pandemic [1]. The greatest burden is in Sub-Saharan Africa where in 2020, the region accounted for 96% of all malaria cases and deaths, with children under 5 years contributing more than 77% of all deaths globally[1]. In Uganda, Malaria remains one of the most significant diseases, responsible for high morbidity, mortality and negative socio-economic

effects. In 2020, Uganda had the highest proportion of malaria infections in East and Southern Africa standing at 23.7% and 95% of the country experiences stable perennial malaria transmission [2] Uganda hospital records suggest that 30 to 50 percent of outpatient visits, 15 to 20 percent of admissions, and 9 to 14 percent of inpatient deaths are due to malaria [3]. Uganda ranks third (5%) in the total number of malaria infections out of the twenty six countries that account for 96 percent of *P. falciparum* infections in sub-Saharan Africa following the Democratic Republic of the Congo (12%) and Nigeria (27%) [1]. Currently, with no available effective vaccine against malaria, the continued malaria parasite's ever evolving resistance to conventional anti-malarial medications, their unaffordability for majority of developing countries, and the counterfeit drugs on the market, herbal preparations and extracts remain a valid and cost-effective alternative for malaria treatment.

About 80% of the population in the developing countries depend on herbal medicine as their primary source of healthcare due to poverty and limited access to modern medicine and this is because the plants from which these herbal preparations and extracts are obtained are available and perceived to be effective, cheap, affordable and sustainable[4].

Maesa lanceolata FORSSK is among many plants that have been documented to be used in folk medicine in Uganda and other tropical countries for the treatment of various ailments,[5]. A fresh leaf extract of *Maesa lanceolata Forssk* has also been used in Western Uganda in the traditional management of malaria related symptoms [6].

Phytochemical studies of various plant parts including the leaves, roots and stem barks of *Maesa lanceolata* have shown the presence of many phytochemicals such as phenols, terpenoids, anthraquinones, flavonoids, saponins and alkaloids with medicinal properties resulting from water and dichloromethane extracts [7]. The extracts and compounds isolated from *M. lanceolata* have shown a wide spectrum of pharmacological activities including antibacterial activity, [7], anthelminthic and antidermatophytic properties [8].

The chloroform extract of *M lanceolata* leaves showed good antiplasmodial activity *In vitro* [6]. However, it is known that some compounds may show *In vitro* activity and lack *In vivo* activity as a result of immunological and pharmacokinetic factors [9].

A variety of herbal plants have been analyzed for the presence and quantity of inorganic ions which would have found their way in the plant constituent from soil during cultivation. Some of these ions are known to play immune-modulatory and dietary roles. For instance, a study which analyzed the dried samples and extracts of *Phyllanthus amarus (PA)* and *Phyllanthus fraternus* (PF) using Atomic Absorption Spectrophotometry (AAS) found that the ions of Aluminium (Al), Magnesium (Mg), Iron (Fe), Manganese (Mn), Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn), Chromium (Cr), and Nickel (Ni) were present [10]. *Phyllanthus amarus and Phyllanthus fraternus* [10] were also found to contain appreciable amounts of trace metallic ions though they were all below the Food and agricultural Organization (FAO) and World Health Organization (WHO) maximum permissible limits in vegetables.

The deficiency of several metallic ions in the human body has been reported to increase its susceptibility to several infectious diseases. Zinc and copper are important for normal immune response of the body because their presence contributes to the immune response of the host when sick [11]. It is shown by astudy conducted in Southern Sudan, that zinc supplementation reduced the number of clinic visits due to malaria [12].

Another study conducted in 2013 by [13] in Burkina Faso assessed the potential benefits of combining zinc and a large dose of vitamin A and demonstrated a significant decrease in the incidence of malaria in the supplemented group when compared with the control (placebo) group and a delay in subsequent malaria attacks/relapses [13].

The mineral copper plays an important role in the development and maintenance of the immune system function as evidenced by cases of copper deficiency that leads to pro-inflammatory effects in neutrophils and causing the number of neutrophils in peripheral blood to be significantly reduced [14]. Additionally, interleukin 2 is predominantly reduced in copper deficiency and this is the likely mechanism by which T cell proliferation is curtailed. Copper deficiency is generally associated with suppressed cell mediated as well as humoral immunity [15]. Therefore malaria patients will have boosted immunities and abilities to fight off malaria infections when taking herbal medicines rich in Zn and Cu given the fact that these ions have been reported to have immunomodulation capabilities. Thus, determining the quantities of these metallic ions in *M. lanceolata* extracts is pertinent to confirming its perceived efficacy and affirming its claimed effectiveness against malaria parasites.

Materials and methods

Plant collection and identification

The fresh leaves of *M. lanceolata* were collected from Rukararwe Botanical Gardens- Bushenyi. They were harvested by plucking them from their stems at the stage when the plant was blossoming as it is done traditionally and were carried in perforated paper boxes to maintain freshness. The leaves were transported to Mbarara University Pharmacology Laboratory. Identification of the plant part was done by a botanist at the department of Biology, Faculty of Science at Mbarara University of Science and Technology and a voucher specimen number 'Jacqueline Njeri 001'was issued.

Extraction

The collected leaves were dried in shade until constant weight was obtained. The dried leaves were pulverized using a ceramic mortar and a pestle. Subsequently extraction was done by cold maceration of 500 grams in 2.5 liters of water for eight hours followed by sieving. Evaporation was done using a rotary everporator at 70^0c and the product was concentrated in a hot air oven at 40^0c [16]. The concentrated extract was then stored in amber bottles and was always used within 24 hours of reconstitution. The percentage yield of the extract upon concentration was calculated as follows; -

$$\text{Percentage yield (\%tage yield)} = \frac{\text{Weight of concentrated extract}}{\text{Weight of plant powder}} x\ 100$$

Test animals:

The study utilized 20 albino mice both male and female weighing 18 – 22 grams acquired from the animal house at Mbarara University of science and technology. The mice were allocated to five groups: two control groups (positive receiving chloroquine 5mg/kg and negative receiving 2ml distilled water) and three treatment groups (receiving extract 250mg/kg, 500mg/kg & 1000mg/kg) [16].

Preparation of inoculum

Chloroquine (CQ) sensitive *Plasmodium berghei* (strain ANKA) was kindly acquired from BEI resources USA. The parasites were maintained by continuous blood passage in mice. A standard inoculum of 1 x 10^7 parasitized erythrocytes was prepared by dilution of blood from a donor mouse (with parasitaemia levels reaching at least 30%) with distilled water and administered intraperitoneally to each test mouse.

The percentage of the parasitemia of the donor mouse was calculated as follows:

Percentage parasitemia =P.RBC/T.RBC× 100

Where P.RBC = parasitized red blood cells, T.RBC= total red blood cells. The total number of red blood cells counted on different fields were at least 1000 cells[17].

Determination of antimalarial activity

The antimalarial activity of the plant extract was evaluated using the method described by Ryley and Peters also known as the curative test or established infection test [18]. Seventy two hours after infecting the test mice with parasitized erythrocytes via intraperitoneal injection and confirming parasitemia levels above 30%, a four day treatment course followed where animals in the three treatment groups received single daily doses of the test substance (250, 500, 1000mg/kg) while two other groups served as positive control receiving a daily dose of chloroquine 5mg/kg and negative control group receiving distilled water 2mls [17]. On each day of the administration, blood was collected from the tail of each mouse by nipping and a blood smear was made onto a microscope slide to create a thin film. The blood smears collected from day 0 up to day 3 were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 minutes and parasitaemia was examined microscopically. Screening was then done at low magnification of 20X objective lens using 100X oil immersion lens [17]. Parasitized RBCs were counted against 1000 RBC. Average parasitemia of each slide was determined using the equation below:

$$Percentage\ parasitemia = \frac{\text{Number of parasitized RBC}}{Total\ number\ of\ RBC\ counted}\ x\ 100\% \quad [16]$$

The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in negative controls with those of treated mice using the formula below:

$$\text{Average percentage suppression} = 100\left\{\frac{A-B}{A}\right\}$$

Where;

A – The average percentage parasitaemia in the negative control group

B – The average percentage parasitaemia in the test group

Data storage and handling

The data collected were stored on a password protected computer hard disk with backups on a password protected flash disk and on Goggle cloud-based server that is also password protected as well.

Levels of zinc and copper

The second phase of this study which involved the determination of inorganic ion levels was done at Makerere University chemistry Laboratory which is equipped with an atomic spectroscopy machine.

Determination of the quantities of inorganic ions (Zinc and Copper) in the plant extract

The method that was used was the atomic spectroscopy machine Agilent-model 240FS (German). The study was conducted for two weeks in the chemistry Laboratory of Makerere University. The metallic ions were determined using nitrous oxide - acetylene flame at a wavelength of 324.8nm and atomization temperature of 2300^{0}C for copper, and at a wavelength of 213.9nm and atomization temperature of 2000^{0}C for zinc. The slit width, ignition temperature and nitrogen flow rate were 0.5nm, 800^{0}C and 3L/min, respectively. These were the standard atomic absorption conditions for copper and zinc respectively. The standards were run on the spectrometer from 0.2 to 2.0ppm for each of the metallic ion separately and the calibration curve for each metallic ion was obtained prior to running the samples for the determination of metallic ions in the extract [18].

Preparation of the calibration standards for determining linearity and calibration of the spectrometer

Calibrators for copper analysis

A stock solution of 1000 mg/l (1000ppm) was diluted to obtain concentrations of 0.2 mg/ml, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml by diluting 2 µl, 5 µl, 10 µl and 20 µl of stock respectively with deionized water, to 10 ml and these were used for the calibration of the machine.

Calibrator solutions for analysis of zinc

A stock solution of 500mg/l (500ppm) was diluted to obtain concentrations of 0.2 mg/ml, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml by diluting 4 µl, 10 µl, 20 µl, and 40 µl respectively with deionized water to 10 ml.

Preparation of the sample for inorganic ion analysis

Maesa lanceolata extract of 0.5g was weighed in four replicates and placed in digestion flasks containing 3ml of concentrated sulphuric acid, 1ml of hydrogen peroxide and 1ml of lithium sulphate with a little amount of selenium powder added to each flask [19]. Flasks were then sealed so that they were airtight and placed in a block digester where digestion occurred at 350^{0}C [20]. After digestion, the flasks were cooled in air and the clear colorless solutions contained in the digestion flasks were obtained and then introduced into 50ml volumetric flasks. The digestion flasks were rinsed twice with 15ml of deionized water and the rinses were collected in the volumetric flasks and then diluted to 50ml to give a concentration of 10mg/ml.

Preparation of the Blank solution

A mixture of 3ml of concentrated sulphuric acid, 1ml of hydrogen peroxide (30%), 1ml of lithium sulphate and a little amount of selenium powder was put in a digestion flask and the digestion was carried out in the same manner as for the test solution above.

Calibrating the machine and analysis of the sample

Calibration for copper analysis

Calibration for zinc analysis

The calibrator solutions with concentrations as for copper standardized together with the Blank solution were aspirated into the machine using the zinc cathode lamp at a wavelength of 213.9nm, atomization temperature of 2000^{0}C and using a nitrous oxide - acetylene flame. The obtained results of absorbance were treated as those for copper above.

Analysis of the sample

A sample digest of 1ml was pipetted and diluted to 10 ml with deionized water to give a concentration of 1mg/ml. This was then sprayed with the copper standard in four replicates in the Atomic Absorption Spectrometer with a copper lamp whose absorption wavelength was 324.8nm and a slit width of 0.5nm. This particular wavelength was chosen because 324.8nm has the best sensitivity and gives the most intense emission line while decreasing the uncertainty in the measured absorbance [21]. The results of absorbance were then recorded in the result's sheer. The same procedure conducted for the analysis of copper was repeated for the analysis of zinc (zinc lamp with absorption wavelength of 213.9nm and slit width of 0.5nm) and results of absorbance were then recorded accordingly.

Determining the amounts of Zinc and copper in the sample

Microsoft excel was used to generate the graph of absorbance against concentration. Using the calibration curve of absorbance of working standards

against concentration for each metallic ion, the coefficient of determination (R^2), the coefficient of correlation (R) and the regression line equation were obtained. Also using the regression equation, the concentrations of each metallic ion was calculated. The concentrations of copper and zinc in *M. lanceolata* extract were determined using the regression line equations obtained from the determination of the linearity of the standard series of solutions of both metallic ions [21].

Determination of the accuracy and precision of analysis method

The determination of accuracy of the analysis method was done by the standard addition method [21]. The method involved weighing 0.5gm of the powdered extract in four replicates and adding 0.47µL of the 1000mg/L solution of copper, which was 10% of the amount of copper found in the sample, to each sample and digesting the samples. The clear solutions obtained were then diluted to 50ml using deionized water. 1ml of each solution was obtained and then diluted to 10ml with deionized water and finally assayed separately in the machine to obtain the absorbencies. Using the regression equation, the concentrations of copper were obtained, and from this, the percentage recovery was obtained.

For zinc, 0.5gm was weighed to make 0.15 µL of the 500mg/ml solution of zinc, which was 10% of the amount of zinc found in the powder. This powder was treated in a similar manner to that of copper above. Percentage recovery was then obtained. Precision of the analysis method was done by calculating the relative standard deviation (RSD) of the percentage of recovery for both copper and zinc. Accuracy and precision of results were determined from the percentage recovery and the percentage RSD respectively [21].

Ethical and biosafety considerations

Ethical clearance was granted by the Research Ethics Committee (REC) of MUST reference number MUREC1/7. The number of animals used was limited to only those absolutely required to carry out the study as per the principles of 3R's (Replacement, Reduction, and Refinement). The animals were housed under a 12-hour light – dark cycle with free access to water and these mice were fed on recommended commercial food pellets. The mice were inbred and did not require acclimatization. Animals were handled in accordance with internationally accepted standard guidelines for Care and Use of Laboratory animals [22].

Data analysis

The mean and standard deviation (SD) of the mean were used to express and present the results after using SPSS Version 20.0 for antimalarial data analysis while microsoft excel was used for analysis of the inorganic ions' profile.

RESULTS

Characteristics of study subjects (Laboratory mice)

The albino mice (males and females) used for antimalarial activity in this study were sourced from the Animal house in the Department of pharmacology, Faculty of m Medicine, Mbarara University of Science and Technology. Their mean age and mean weights were 4 weeks and 20 grams at the beginning of the study respectively [22].

Anti-malarial activity

Findings regarding the *In vivo* antimalarial tests are summarized in table 1 below. The *Plasmodium berghei* mice model was used since it is supposed to be the first recommended step to screen most of the *In vivo* antimalarial activities of new molecules and new therapeutics [22]; [16]. The water extract of the leaves of *M. lanceolata* FORSSK showed 39% suppression of parasitemia at the dose of 250mg/kg, 33% suppression of parasitemia at 500mg/kg and 6% suppression of parasitemia at the dose of 1000mg/kg as summarized in Table 2 below:

Table 1: Activity of aqueous extract of the shoot of *M. lanceolata* FORSSK against *P. berghei* in mice.

Extract	Dose (mg/Kg/day)	Ant malaria activity	
		% Parasitemia ±SD	**% Suppression**
Code	NC	18.0±0.06671[a]	**00.00%**
Group 1(GP 1)	250	11.0±0.5117[b]	**39.00%**
Group II (GP II)	500	12.0±0.00[c]	**33.00%**
Group III (GP III)	1000	17.0±0.01225[d]	**06.00%**
Code	PC	5.0±0.01785	**72.00%**

Values are mean ± SD, n = 4 except in 500 mg/Kg dose group where 1 mouse died on day 2 of the treatment process; NC: Negative control (0.2mL N/S); PC is positive control (CQ 5mg/kg); a, b, c.d) = values in the same column followed by the same letter do not differ significantly. Mean survival time (days) was expressed as the average number of days ± standard deviation that the mice remained alive from the day they were inoculated by the parasite as shown on the graph below.

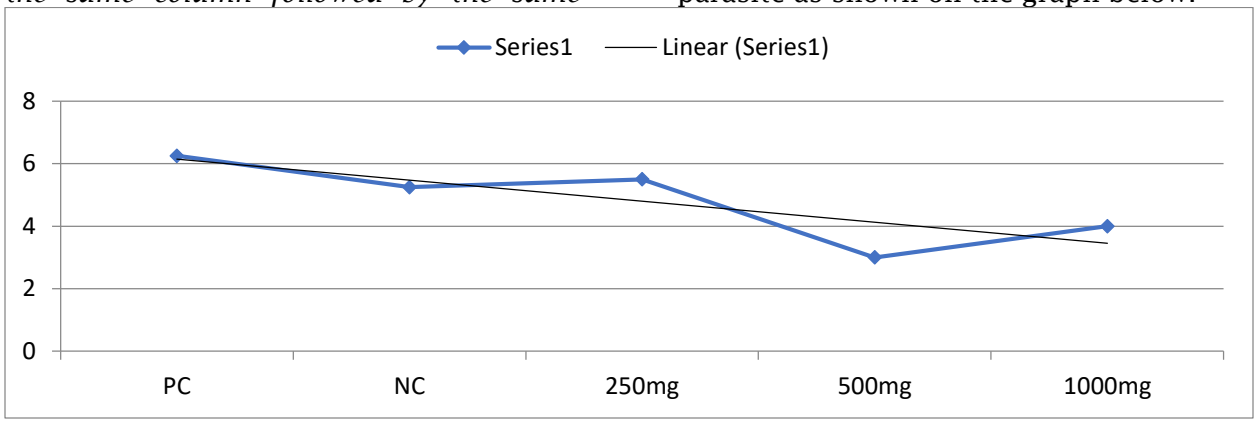

Figure 1: A linear graph showing the survival time for mice.

Levels of inorganic Zinc and Copper

Levels of inorganic Zinc and Copper in the dried leaf powder of *Maesa lanceolata* FORSSK were analyzed using the Atomic absorption spectrometer system (AAS): Agilent-model 240FS (Germany). The plant sample was analyzed in four replicates at a wavelength of 324.7nm for copper and 213.9nm for zinc from which mean detector responses were determined and the findings are in the results section.

Results of copper and zinc levels

The sample was analyzed in four replicates at a wavelength of 324.7nm for copper and 213.9nm for zinc from which mean detector responses were determined and the findings are shown in tables 2 and 3.

Table 2: A table showing mean detector response copper (ppm)

Samples	Concentration	conc- (mean Blk)	% Concentration.
Blank 1	0.022	0.00	
Blank 2	0.023	0.00	
Replicate 1	0.114	0.0915	9.15
Replicate 2	0.114	0.0915	9.15
Replicate 3	0.119	0.0965	9.65
Replicate 4	0.116	0.0935	9.35
Mean			**9.33**

By conversion to conventional units, there are 9.33mg of copper per liter of solution of the sample.

Table 3: A table showing mean detector response zinc (ppm)

Samples	Concentration	conc- (mean Blk)	% Concentration
Blank 1	0.0762	0.00	
Blank 2	0.078	0.00	
Replicate 1	0.1482	0.0711	7.11
Replicate 2	0.1413	0.0642	6.42
Replicate 3	0.1792	0.1021	10.21
Replicate 4	0.1809	0.1038	10.38
Mean			**8.53**

By conversion to conventional units, there are 8.53mg of zinc per liter of solution of the sample.

DISCUSSION

In this study the *In vivo* model was used to assess antimalarial activity of *Maesa lanceolata* because *In vitro* antiplasmodial studies were previously done by Katuura et al 2007. However, from a pharmacological point of view, *In vitro* studies alone cannot be used to predict a patient's clinical response during the drug development process. This is because *In vitro* studies may fail to detect the activity of chemicals which behave like prodrugs-these are chemicals that need to undergo enzymatic transformations for their pharmacological activity to be realized [16]. Other factors may be pharmacokinetic and immunological factors which are absent in the *In vitro* testing system [9]. It is on the basis of this that the *Plasmodium berghei* mice-model was used in the current study. The *Plasmodium berghei* mice-model uses both male and female mice weighing between 18 and 22 grams. The mice are divided into five groups; positive control group (choloroquine), negative control group (placebo) and three treatment groups which receive the test substance at 250, 500 and 1000mg per kg body weight. According to this model, when the % inhibition is higher than 50% at a dose of 1000mg/kg/day, the activity is considered moderate; otherwise it is considered inactive. This dose is made use of to detect if an active product is present in only small amounts in the extract. When the percentage inhibition is equal to or higher than 50%, at a dose of 500mg/kg/day, the activity is also considered moderate; and when the % inhibition is equal to or higher than 50%, at a dose of 250mg/kg/day, the activity is considered good[16]. Based on this analysis, it was interesting to note that the activity decreased with increase in dose, where the lowest dose of 250mg/kg achieved the highest percentage suppression of 39% even though it did not reach the WHO cutoff value of 50% parasitemia suppression. The medium dose which was 500mg/kg showed 33% parassiemia suppression and the highest dose of 1000mg/kg showed 6%

parasitemia suppression (table 1). Whereas the acceptable cutoff of parasitemia suppression was not achieved, by this dose, it might be possible that as the dilution is increased the activity seems to be increasing. With increased dilution there could be increased number of ions which could increase the activity. Probably it could be of interest to investigate this in other studies beyond the scope of this work. Nevertheless, according to the cutoff criteria and within the scope of the model used in this study, it can be stated that, among the treatment groups, the group receiving 250mg/kg showed the highest percentage parasitemia suppression of (39%) which is regarded as negligible parasitemia clearance and the percentage suppression did not increase with an increase in dose, given that the group receiving 1000mg/kg of the extract showed a mere 6% parasitemia suppression (table 1). Moreover, there was no dose dependent relationship observed in this study and there was no overall antimalarial activity of the aqueous leaf extract of *M. lanceolata* in mice infected with *Plasmodium berghei*. This situation is not unique to *M. lanceolata* alone as other studies have found inactivity of various medicinal plants despite their traditional use as anti-malarials for instance, most of the *Commiphora africana* extracts used by the Maasai were found to be inactive against malaria parasites [23]. *Maesa lanceolata In vitro* activity against *Plasmodium falciparum* was previously studied by Katuura *et al* in which study they found the chloroform leaf extract to have good antiparasitic activity (IC_{50} = 1.6 µg ml $^{-1}$) [6].The overall lack of activity in all the investigated doses ranging from 250mg/kg to 1000mg/kg of the aqueous leaf extract of *M. lanceolata* could be due to variation in the active constituents possibly related to seasonal or geographical setting. Further explanation may have stemmed from the extraction process since some of the phyto chemicals extracted within the aqueous extraction could have masking activity of other phyto constituents which may result in reduction of the activity of the extract [23]. In this case it would be of interest if some *In vivo* investigations using the model are done on chloroform leaf extract. Furthermore, the specific traditional preparation techniques often employ mixtures of plants [6], and it is possible that the concoction with phyto chemicals from the other botanicals have synergistic effect with those from *M. lanceolata.* However, this requires further investigation. During the cause of the study the animals were allowed food and water ad libitum, there is a possibility that the presence of food in the GIT interfered with drug absorption causing the volume of distribution to be too low to clear the parasites. First pass metabolism might also have been another contributing factor. Furthermore, *Maesa lanceolat*a is used traditionally to improve appetite [24,25,26,27,28,29], most patients when suffering from malaria have reduced appetite which coupled with fever contributes to the patient being lethargic and feeling more sick. Improved appetite may reverse this and make the patient feel stronger hence better; furthermore eating a balanced diet will improve a patient's immune system making it easier for the body to fight a disease [25]. Many patients and herbalists may be using *Maesa lanceolata* FORSSk in the management of malaria fever and reaping benefits from it even when it is not directly efficacious against the infective organism *Plasmodium falciparum*. It is therefore paramount that more research is carried out to check for another possible activity of the plant like antipyretic, analgesic and appetite inducing effects [26]. Mean survival time was highest in the group treated with chloroquine at 6.25±1.9 days followed by the group dosed with 250mg/kg of the extract with 5.5±2.1 days while the lowest survival time was observed in the group treated with 500mg/kg at 3±0.7 days (figure 1). Similar to the observation made in the percentage parasitemia suppression, the survival time of the mice decreased with increase in the extract dose except for the group receiving 500mg/kg which showed the least survival time average and this was due to

the death of one mouse on day two of treatment which might not have resulted from the parasite itself. In our view this could have been due to a technical error during administration. Analysis of copper and zinc were mandated in this study as they have been documented to play a key immune-modulatory role which is important in survival of patients with malaria. On the other hand, metals like copper can have detrimental effects like Wilson's disease when taken in excess. Copper ions in the dried leaf extract of *Maesa lanceolata* were found in the concentration of 9.33mg/L (table 2). According to the National Academy of Science 2019, the recommended daily intake of copper is 0.4 – 1.0 mg per day for children and 1.5- 3.0 mg per day for adults. According to one traditional healer who lives and treats malaria patients at her home in Bwegeragye in Ishaka, a patient suspected to be suffering from malaria is advised to consume 500mls twice a day, which is after breakfast and after dinner of a prepared solution of *Maesa lanceolata* leaves for about a week. This means that a patient consumes in total, one liter of solution per day and hence approximately 9mg of copper daily at the minimum during treatment. The same patient could be getting additional copper from other consumable sources like food and other drinks that he may take in the cause of the day. The amount of copper consumed by a patient on treatment with *Maesa lanceolata* FORSSK, is above 9mg daily which is way above the upper limit of recommended daily intake of the ion which is 3 mg per day. However, this information may not be generalized because the information of dosage was obtained from an herbalist from one locality. Further studies on posology from many practitioners in the study area are necessary to gather enough information on doses used. The mineral Copper plays an important role in the development and maintenance of the immune system function as evidenced by cases of copper deficiency where the number of neutrophils in human peripheral blood is significantly reduced together with their ability to generate superoxide anions and kill ingested microorganisms [15]. This would go on to mean that the copper ions may enhance the immune system function helping the patient to fight off the infection and recover from malaria even though there is no direct activity against the malaria parasites elicited by the extract. However, excess consumption of copper ions may have detrimental effects like causation of Wilson's disease. It is possible that these results may not be generalized as it is well known that most traditional medicines are not standardized. The posology of *Maesa lanceolata* may differ in terms of dosage, frequency and duration from one traditional healer to another.

It may also differ depending on the ailment being managed, for instance the dose used for malaria management may be different from that used to treat abdominal discomfort not to mention the variation in preparation method, parts used and possible combinations entailed in the use of herbal products including *Maesa lanceolata*. Usually most traditional healers do not follow up their patients and they do not document their practice; it is therefore difficult to find out if there is a prevalence of Wilson´s disease due to the use of *Maesa lanceolate* in Western Uganda. More research should deliberately be done to find out how posological practice of *Maesa lanceolata* differs and possibly follow users up to find out if there is any detrimental effect associated with the high levels of copper in the plant.

Zinc ions in the dried leaf extract of *Maesa lanceolata* were found in the concentration of 8.53mg/L (table 3). According to the National Academy of Science 2019, the recommended daily intake of zinc is 3– 5 mg per day for children and 8-11 mg per day for adults. When a patient consumes one liter of the prepared solution of *Maesa lanceolata* he would therefore be consuming approximately 8 mg of zinc per day which is well within the range of recommended daily intake of the ion. This would mean that the consumer's immunity could be enhanced because zinc is necessary for

the normal development and function of cells mediating innate immunity that is neutrophils and natural killer cells. Zinc deficiency affects the production of macrophages, phagocytosis, intracellular killing, and cytokines. In addition to enhancing T-cell and B-cell growth and function, Zinc has also been shown to possess antioxidant and anti-inflammatory properties [11] that would help patients combat infection and recover from malaria even though there is no direct action against malaria parasites induced by the extract.*Maesa lanceolata* FORSSK is widely used to manage a variety of ailments including abdominal discomfort and loss of appetite, malaria and fever, and sexually transmitted diseases [24, 25,26,27,28,29].

CONCLUSION

In conclusion, this study has shown that whereas the aqueous extract of *M. lanceolata* lacked antimalarial activity *In vivo*, it might still play a role in the management of malaria and other lethargic patients because it may improve their appetite therefore making them feel stronger and recover faster as well as giving their immunity a better fighting chance.

RECOMMENDATIONS

Possible effects of copper in people taking *M. lanceolata* should be determined. It is recommended that further *In vivo* antimalarial studies using different models which controls for food and other pharmacokinetic effects be carried out. Antimalarial activity of Maesa lanceolarta chloroform extract should be done. The plant extract should be evaluated for antipyretic activity which might justify its use in malaria patients. A study to find out posology of *M. lanceolata* among Western Uganda practitioners should be carried out.

Data Availability

Data in tables and figures used to support the findings of this study are included within the article. Raw materials used in this study can be produced from the authors in request.

Conflicts of Interest

All authors declare that there are no conflicts of interest existing in regard to this study and publication of the findings

Authors' Contributions

This work was conducted in collaboration between all authors. All authors have made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; took part in drafting the article and revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

Acknowledgements

The effort of Dr. Ajayi Clement on the help provided during the infection procedures in the mice is hereby acknowledged. We are grateful to **BEI resources** USA for the provision of *Plasmodium berghei* parasites ANKA

REFERENCES

1. WHO. *World Malaria Report 2021*.; 2021.
2. Knowledge sharing for severe malaria. *Sev Malar Obs*. Published online 2020.
3. NMCP. Guidelines for preparedness and response for malaria epidemics. *Natl Malar Control Progr*. Published online 2014.
4. Vijayan N, Swapna TS, Seghal Kiran G, Abdulhameed S. Medicinal plants of India: Implications in modern medicine. In: *Bioresources and Bioprocess in Biotechnology*. ; 2017. doi:10.1007/978-981-10-3573-9_18
5. Okello D. Exploring Antimalarial Herbal Plants across Communities in Uganda Based on Electronic Data. 2019;2019.
6. Katuura E, Waako P, Tabuti JRS,

Bukenya-ziraba R, Ogwal-okeng J. Antiplasmodial activity of extracts of selected medicinal plants used by local communities in western Uganda for treatment of malaria. 2007;45:94-98.

7. Timothy C, Lizzy M, Richard K, Angela M, Christine B. Antimicrobial Activity and Safety of Maesa lanceolata for the Treatment and Management of Selected Bacterial Pathogens. 2018;8(3):1-8. doi:10.9734/JAMB/2018/39450
8. Kipkore W, Wanjohi B, Rono H, Kigen G. A study of the medicinal plants used by the Marakwet Community in Kenya. *J Ethnobiol Ethnomed*. 2014;10(1). doi:10.1186/1746-4269-10-24
9. Waako PJ, Gumede B, Smith P, Folb PI. The in vitro and in vivo antimalarial activity of Cardiospermum halicacabum L. and Momordica foetida Schumch. Et Thonn. *J Ethnopharmacol*. Published online 2005. doi:10.1016/j.jep.2005.02.017
10. Etornam Adukpo G, Kwaku Adotey J, Gyingiri Achel D, et al. Trace and heavy metals analysis of Phyllanthus amarus and Phyllanthus fraternus in Ghana. *Int J Biol Chem Sci*. 2012;6(1):438-445. doi:10.4314/ijbcs.v6i1.39
11. Prasad AS. Zinc in human health: Effect of zinc on immune cells. *Mol Med*. Published online 2008. doi:10.2119/2008-00033.Prasad
12. Saad AA, Doka YA, Osman SM, Magzoub M, Ali NI, Adam I. Zinc, copper and C-reactive protein in children with severe Plasmodium falciparum malaria in an area of unstable malaria transmission in eastern Sudan. *J Trop Pediatr*. 2013;59(2):150-153. doi:10.1093/tropej/fms056
13. Owusu-Agyei S, Newton S, Mahama E, et al. Impact of vitamin A with zinc supplementation on malaria morbidity in Ghana. *Nutr J*. 2013;12(1):1-9. doi:10.1186/1475-2891-12-131
14. Dinicolantonio JJ, Mangan D, O'Keefe JH. Copper deficiency may be a leading cause of ischaemic heart disease. *Open Hear*. 2018;5(2):1-8. doi:10.1136/openhrt-2018-000784
15. Shizgal HM. Nutrition and immune function. *Surg Annu*. 1981;13(2):15-29. doi:10.30726/esij/v7.i2.2020.72005
16. Odda J, Waako P, Obua C, Kabasa JD. In Vivo Antiplasmodial Activity and Safety of the Aqueous Ethanolic Shoot Extracts of Phyllanthus amarus Schum . and Thonn. *Int J Pharm Pharm Res*. 2018;12(3):265-280.
17. Ahmed O, Olusola S, Olusoji A, Mojeed A. Activities of aqueous extracts of Mangifera Indica on Parasitaemia level and blood profile of Plasmodium Berghei - infected Albino Mice. 2016;4(4).
18. Ryley JF, Peters W. The antimalarial activity of some quinolone esters. *Ann Trop Med Parasitol*. Published online 1970. doi:10.1080/00034983.1970.11686683
19. Knight DJ, Peters W. The antimalarial activity of N-benzyloxydihydrotriazines. *Ann Trop Med Parasitol*. 1980;74(4):393-404. doi:10.1080/00034983.1980.11687360
20. Okalebo. *LAB METHODS OF SOIL AND PLANT ANALYSIS.Pdf*.; 2002.
21. Nerdy N, Manurung K. Spectrophotometric method for antioxidant activity test and total phenolic determination of red dragon fruit leaves and white dragon fruit leaves. *Rasayan J Chem*. Published online 2018. doi:10.31788/RJC.2018.1134018
22. Gosh M. *Fundamentals of Experimental Pharmacology*.; 2015.
23. Kweyamba PA, Zofou D, Efange N, Assob JCN, Kitau J. In vitro and in vivo studies on anti - malarial activity of Commiphora africana and Dichrostachys cinerea used by the Maasai in Arusha region ,

Tanzania. *Malar J*. Published online 2019:1-6. doi:10.1186/s12936-019-2752-8

24. Kiringe JW. A Survey of Traditional Health Remedies Used by the Maasai of Southern Kaijiado District, Kenya. *Ethnobot Res Appl*. Published online 2006. doi:10.17348/era.4.0.61-74
25. Rodgers,K (2023).Antimalarial Drug Utilization and Adherence to Treatment Guideline in a Secondary Hospital in Sheema District Western Uganda.NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES 3 (2), 100-106.
26. Omara C (2023).Antibacterial Activity of the Ethanolic Bark Extract of Syzygium cumini on Escherichia coli Recovered from Surface Waters Used in Ishaka Municipality. NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES 3 (2), 144-151.
27. Ugwu, O. P.C., Nwodo, O. F.C., Joshua, P. E., Odo, C. E., Bawa, A., Ossai, E. C. and Adonu C. C. (2013). Anti-malaria and Hematological Analyses of Ethanol Extract of Moringa oleifera Leaf on Malaria Infected Mice. International Journal of Pharmacy and Biological Sciences,3(1): 360-371.
28. Ugwu OPC, OFC Nwodo, PE Joshua, CE Odo, EC Ossai, B Aburbakar (2013). Ameliorative effects of ethanol leaf extract of Moringa oleifera on the liver and kidney markers of malaria infected mice. International Journal of Life Sciences Biotechnology and Pharma Research, 2(2): 43-52.
29. Nwaka AC, MC Ikechi-Agba, PC Ugwu Okechukwu, IO Igwenyi, KN Agbafor, OU Orji, AL Ezugwu (2015). The effects of ethanol extracts of Jatropha curcas on some hematological parameters of chloroform intoxicated rats. American-Eurasian Journal of Scientific Research,10(1): 45-49.

Jacqueline Njeri Muchiri, Martin Amanya, Wilberforce Mfitundinda, Maniga Nyabayo Josephat and John Odda (2023). IAA Journal of Scientific Research 10(2):89-101.