

Full Length Research Paper

***In vitro* and *in vivo* antimalarial activity of *Nigella sativa* L. extracts**

**Job Oyweri¹, Awadh Mohammed¹, Rahma Udu¹, Jeremiah Gathirwa², Edwin Too²,
Protus Omondi³, Francis Kimani², Suhaila Hashim⁴ and Laila Abubakar¹**

¹Department of Pure and Applied sciences, Technical University of Mombasa, P. O. Box 90420 – 80100, Mombasa, Kenya.

²Centre for Biotechnology Research and Development, Centre for Traditional Medicine and Drug Research; Kenya Medical Research Institute, P. O. Box 54840- 0002, Nairobi, Kenya.

³Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, P. O. Box 43884-00100 Nairobi, Kenya.

⁴Department of Biochemistry and Biotechnology, School of Pure and Applied sciences, Pwani University, P. O. Box 195- 80108, Kilifi, Kenya.

Received 24 September, 2019; Accepted 31 October, 2019

The Arabs, Asians and, Traditional Health Practitioners in Mombasa county found in Kenya have been using *Nigella sativa* L. seeds to traditionally manage malaria associated symptoms that is, headache, fever, chills, loss of appetite among others. The present study investigated *in vitro* antiplasmodial, *in vivo* antimalarial activities and safety of different extracts of *N. sativa*. Five extracts obtained via aqueous extraction and sequential extraction using hexane, dichloromethane, ethyl acetate and methanol were tested against *in vitro* cultures of *Plasmodium falciparum*. The most active extracts (methanolic and ethyl acetate) were assessed for cytotoxicity and toxicity. The two active extracts were evaluated *in vivo* against *Plasmodium berghei* ANKA strain at 500, 250 and 125 mg/kg/day. On *in vitro* assay, methanolic and ethyl acetate extracts showed good activity with IC₅₀ of 80.48±12.29 and 69.81±5.24 µg/ml against W2 strain and 31.93±4.31 and 53.79±6.02 µg/ml against D6 strain, respectively. The extracts exhibited weak cytotoxicity on Vero cells and high parasitemia suppression of 75.52 and 75.30% at 500 mg/kg dose of methanolic and ethyl acetate extracts respectively. Notably, there was significant decrease (p<0.001) in activity with lower doses of the extracts. The results explain the traditional use of this plant in the Middle East and Mombasa County.

Key words: *Nigella sativa* L. seeds extracts, *Plasmodium*, antimalarial activity.

INTRODUCTION

Malaria is one of the most vital parasitic infections common in many developing countries mostly affecting

sub-Saharan Africa (Nkumama et al., 2017). Amongst the *Plasmodium* species affecting human, *Plasmodium*

*Corresponding author. E-mail: joboyweri@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

falciparum is the chief cause of morbidity and mortality (Maier et al., 2018). Approximately 219 million episodes of malaria were recorded in 2017 globally in comparison to 239 million occurrences in 2010 and 217 million cases in 2016. Notably, about 435, 000 malaria deaths were witnessed in 2017 worldwide, a decrease from 451, 000 approximated deaths in 2016, and 607, 000 in 2010 (WHO, 2018). In Kenya, malaria transmission is prevalent in the western region with the target population being children under the age of 5 years and pregnant women (Kepha et al., 2016). There has been significant reduction in morbidity and mortality due to control programs and scientific interventions (Mogeni et al., 2016). However, malaria remains an essential public health problem globally and in developing countries.

Chemotherapy plays a crucial role in malaria control (Mvango et al., 2018). Effective antimalarial drug should have therapeutic activity and no toxicity on human host (Na-Bangchang and Karbwang, 2009). However, the central drawback to treatment has been the rise of parasite resistance to most antimalarial drugs (Menard and Dondorp, 2017). Chloroquine (CQ) has been used since 1940s until resistance challenged its use (Ecker et al., 2012). However, it has remained the drug of choice for *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax*, and the uncomplicated *P. falciparum* in some parts of the world where resistance to CQ is relatively low (Torres et al., 2013). A combination therapy of sulphadoxine and pyrimethamine was later introduced (Horn and Duraisingh, 2014). Afterwards, it incurred resistance associated with *dhfr*, *dhps* and *pfmdr* genes hence ineffective for *plasmodium* management (Jelinek et al., 1998; Jacques Le Bras and Durand, 2003).

Artemisinin-based combination therapies (ACTs) remain the most effective antimalarial medicines available today (Sugiarto et al., 2018; White, 1999). Tolerance of the malaria parasite to ACTs is currently increasing in South-East Asia. Resistance associated with point mutations in the “propeller” region of a *P. falciparum* kelch protein gene on chromosome 13, also known as k13 or kelch 13 has been established (Ariey et al., 2014). There is a risk of artemisinin resistance spreading to Africa allowing resistant parasites to go global. Therefore, new products for malaria management are necessary to outcompete resistance (Ashley and Phyo, 2018).

N. sativa, also well-known as Black seed belongs to the family of *Ranunculaceae*. The plant is extensively distributed especially in Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey and Saudi Arabia. There has been rising concern to this plant since its wide discovery of therapeutic potential against many ailments (Ahmad et al., 2013; Kooti et al., 2016). Among the eminent clinical importance, *N. sativa* has been shown to possess antidiabetic, anticancer and anti-inflammatory activities (Heshmati and Namazi, 2015; Heshmati et al., 2015; Periasamy et al., 2016). It has also been demonstrated to possess antiparasitic activity in the

Middle East folk medicine (Razavi et al., 2018).

The previous study conducted using ethanol, aqueous and chloroform extracts of *N. sativa* demonstrated higher percentages of parasitemia suppression against *P. berghei* PZZ1/00 strain. Parasitemia reduction of 86.19, 76.94 and 66.92% was recorded upon use of 100, 400 and 100 µL/kg doses of ethanol, aqueous and chloroform extracts respectively (Abdulelah and Zainal-Abidin, 2007). In Nigeria, methanolic and aqueous extracts' activity of *N. sativa* against *P. berghei* (nk 65) infection in mice have been reported (Ashcroft et al., 2018). However, antiplasmodial and antimalarial potential of *N. sativa* extracts is yet to be documented in Kenya.

Mombasa County located in the Coast of Kenya, which principally was our geographical site is rich in ethnomedicinal knowledge. The region is highly inhabited by Arabs and Asians who apparently migrated from Asian countries where *N. sativa* plant originates (Silberman, 1950). This important group of society (Arabs and Asians) together with the Traditional Health Practitioners (THPs) have for a long time used *N. sativa* to traditionally treat malaria associated symptoms that is, headache, fever, chills, loss of appetite amongst others (Bahekar and Kale, 2013). Lack of affordability, limited access to pharmaceutical treatment and modern treatment amenities are the key reasons (Cunningham, 2001; Katz and Kimani, 1982). This study determined the effect of *N. sativa* seed extracts on *Plasmodium* parasite as a crucial step towards seeking effective drug for malaria. In addition, cytotoxicity and toxicity of the bioactive *N. sativa* extracts were evaluated in order to determine their selective indexes and lethal doses correspondingly. This will offer valuable information towards establishing lead compounds responsible for *N. sativa* antimalarial activity.

MATERIALS AND METHODS

Plant collection and authentication

N. sativa seed samples of Saudi Arabian origin were collected from Mwembe Tayari market in Mombasa County. The plant was positively identified by a taxonomist and indigenous Mombasa residents who use it traditionally. 2 kg of *N. sativa* seeds were packed in plastic bags (voucher specimen No; PAS0023) and transported to KEMRI Centre for Traditional Medicine and Drug Research, Nairobi for processing.

Extraction

The seeds were air dried for 72 h and ground using an electric laboratory mill (Christy and Norris Ltd., Chelmsford, England). 400 g of the ground seed material was used for aqueous extraction whereas 1 kg of the material underwent sequential extraction method with different solvents of increasing polarity; hexane, dichloromethane, ethyl acetate and methanol. Briefly, ground plant material (400 g) was extracted in 1.2 L of double distilled water in a water bath at 60°C for 1 h, cooled and filtered using Whatman No 1 filter paper (Whatman, England). The filtrate was then concentrated to dryness by lyophilization.

One kilogramme (1 kg) of the plant was extracted using 1.5 L of the solvents in an order of increasing polarity by maceration at room temperature for 48 h. The soaked plant material was first filtered using gauze followed by Whatmann's No 1 filter paper (Whatman, England). The residue was re-macerated for another 48 h and filtered. The filtrates were then pooled together and concentrated to dryness under reduced pressure using rotary evaporator. The filtered plant residue was dried and the next solvent introduced as per the order of increasing polarity. Finally, the collected crude extracts were further dried in a vacuum drier to remove any possible traces of solvents and later stored at -20°C until use.

***In vitro* anti-plasmodial studies using *P. falciparum* strains**

Two different isolates of laboratory adapted *P. falciparum* cultures were used in the study. These were; Chloroquine Sensitive Sierra Leone 1 (D6) and Chloroquine-resistant Indochina 1 (W2) strains. *In vitro* anti-plasmodial study with *P. falciparum* strains was performed according to Trager and Jensen (2005) method with slight changes. The parasites were maintained in a continuous culture in RPMI 1640 medium supplemented with 10% human serum and hematocrit of O+ red blood cells under a gas mixture of 92% nitrogen, 5% carbon dioxide and 3% oxygen.

All crude extracts of the plant were initially diluted in DMSO (Sigma Chemical Co., St Louis, MO, USA) and then dilution to a concentration of DMSO was reduced to <1% in the final working solution. Distilled water was used to dissolve CQ (positive control). Semi-automated micro-dilution technique that measures the ability of extract to inhibit the incorporation of [³H] hypoxanthine into the parasite was utilized (Desjardins et al., 1979; Le Bras and Deloron, 1983). Briefly, 200 µl of 2% (v/v) suspension of parasitized erythrocytes composed of 1-2% parasitemia was added to all wells except the last well H that was the negative control having unparasitized erythrocytes. The contents in the plate were then incubated at 37°C supplied with a gas mixture of 3% CO₂, 5% O₂, and 92% N₂ for 48 h. After 48 h, 25 µl of culture medium comprising 0.5 µCi of [³H] hypoxanthine (Amersham International, Buckinghamshire, UK) was added to each well and incubated further for 18 h. Harvesting of the plates was done onto glass fibre filters, washed using distilled water and dried. The glass fibre filters were smeared with 25 µl liquid scintillation fluid. The filters were measured for radioactivity using micro-beta plate scintillation counter (Wallac MicroBeta TriLux). Counts per minute (CPM) were obtained and then used to compute the IC₅₀ values (Sixsmith et al., 1984). Non-linear regression analysis enabled calculation of the concentration of test samples that inhibited 50% (IC₅₀) growth. This calculation was carried out using Chemosen Program 2 according to the following formula;

$$IC_{50} = \text{antilog} (\log X1 + \frac{[(\log Y50 - \log Y1) \times (\log X2 - \log X1)]}{\log Y2 - \log Y1})$$

Where, Y50 is the Counts per minute (cpm) value midway between parasitized and non-parasitized control cultures, and X1, Y1, X2, Y2 are the concentrations and cpm values respectively for the data points above and below the cpm midpoints (Sixsmith et al., 1984).

***In vivo* assay**

Host, environment and parasites

Male Swiss albino mice, 6-8 weeks old, weighing 20±2 g bred in the KEMRI animal facility Nairobi were used as subjects. Test mice were housed in experimental rooms in standard microloan type II cages, well labeled with experimental details in air-conditioned rooms at room temperature and 60-70% relative humidity. They were well nourished with commercial rodent feed and water *ad*

libitum. Five mice were used for every cage per test sample. *P. berghei* ANKA parasites were used to determine parasite reduction in mice.

Four days parasite suppression test

Four days parasite suppression test study was conducted according to Peters (1975) protocol with slight adjustments. *P. berghei* strain ANKA infected blood was obtained by heart puncture from donor mouse and mixed with 1% (w/v) heparin. The infected blood was diluted in physiological saline to approximately 10⁸ parasitized erythrocytes per ml. The test animals were infected by intraperitoneal injection with 0.2 ml (2×10⁷ parasitized erythrocytes) and randomly grouped. The experimental groups were treated orally with 0.2 ml single dose of 125, 250 and 500 mg/kg of the test sample, 2 h post infection (D0) (Gessler et al., 1995). Only, extracts active as per *in vitro* assay were used (methanolic and ethyl acetate extracts). Two control groups comprising five mice each were treated with a placebo (vehicle; 3% dimethyl sulfoxide, 10% tween 80 in PBS) and 5 mg/kg of CQ for negative and positive controls respectively. The mice were consecutively treated orally for 3 days (D1, D2, and D3) with equivalent doses. Blood films were taken on the fifth day (D4) from tail snips, fixed in methanol and stained with 10% Giemsa stain (Abdela et al., 2014). Microscopic examination of thin blood film provided parasitemia counts in 4 fields of ~100 erythrocytes per view. The difference between mean numbers of parasites per view in the negative control group and those of the test groups were calculated and expressed as percentage suppression (chemo suppression) according to the formula (Tona et al., 2001):

$$PS = \frac{A-B}{A} \times 100$$

Where; A=Mean parasitemia in the negative control group on day 4, B=Corresponding parasitemia in the test group.

The mean survival time (days) for each group was determined over a period of 30 days post infection.

Toxicity evaluation

Cytotoxicity evaluation

The MTT assay that is based on the principle of conversion of yellow tetrazolium MTT to purple formazan dye by mitochondrial dehydrogenases of living cells was used for cytotoxicity determination (Van Meerloo et al., 2011). Vero cells obtained from kidney epithelial cells of the African green monkey, stored in liquid nitrogen in KEMRI; Centre for Traditional Medicine and Drug Research were used. The Vero cells were grown in Eagle's minimum essential medium, supplemented with 5% foetal bovine serum (FBS) in 25 ml cell culture flasks. They were incubated at 37°C in 5% CO₂ incubator (Kurokawa et al., 1995). The cells were sub-cultured three times a week to achieve 80% confluence. Afterwards, the cells were seeded with 5 × 10⁴ cells per well in 96-well plates. They were then incubated at 37°C for the next 2 days. Fresh MEM (GIBCO, Grand Island, NY) containing test extracts at different concentrations was added to replace the culture medium and later incubated for another 2 days. Tripsinization of the cells for each sample was done in triplicate wells to allow detachment. A hemocytometer was used to count viable cells. Data from inhibition was then plotted as dose-response curves and CC₅₀ (concentration able to cause visible alterations in 50% of intact cells) was determined. Selectivity index (SI) was used as parameter of clinical significance of the test samples by comparing general toxins and

Table 1. *In vitro* antiplasmodial activity of five extracts.

Extract/drug	Extract yield (%)	W2	D6
		IC ₅₀ (Mean±SD) (µg/ml)	IC ₅₀ (Mean±SD) (µg/ml)
H ₂ O	7.50	>200	>200
Hexane	6.00	>200	151.25±22.96
DCM	3.00	138.25±14.31	141.34±10.23
MeOH	2.75	80.48±12.29	31.93±4.31
EtOAc	3.00	69.81±5.24	53.79±6.02
Control (CQ)		65.13±17.17 ng/ml	13.54±1.20 ng/ml

The results are expressed as mean ± SD of mean of IC₅₀s of the extracts and control (CQ).

selective inhibitory effect on *P. falciparum* calculated as (Wright and Phillipson, 1990);

$$SI = \frac{CC_{50} (\text{Vero})}{IC_{50} (\text{Plasmodium falciparum})}$$

In vivo acute toxicity

6-8 weeks old, male Swiss albino mice weighing 20 ± 2 g were used to determine acute toxicity of the active extracts (methanolic and ethyl acetate) (Lorke, 1983; Tona et al., 2001). Precisely, groups of mice (5 per group) were accustomed for 5 days to laboratory conditions. 6 groups were used to determine extracts toxicity while the other group for negative control. The mice were starved of food but provided with clean water *ad libitum* for 24 h before oral treatment. The negative control group was treated once with 0.2 ml of the vehicle while the other 6 groups were subjected to 1000, 1500 and 2000 mg/Kg of body weight per extract. Mortality within the first 24 h and subsequent behavioral changes were monitored for the next 14 days.

Ethical consideration

Permission to conduct the study was granted by *Scientific and Ethics Review Unit (SERU)*, (Study KEMRI/SERU/CBRD/191/3803). The study was performed according to KEMRI guidelines on animal care and use. Furthermore; the internationally accepted principles for laboratory animal use and care as per WHO guidelines were considered. Gauge 23 needles were used to carry out IP injection. Animal-handling was in a humane way and experimental terminations were through sacrificing the mice in a chamber of carbon (iv) oxide gas. All sacrificed mice as well as carcass in the course of the experiment were bagged for disposal and incinerated.

Statistical analysis

All *in vitro* experiments were carried out in duplicate measurements and data were analyzed by Microsoft Excel 2013 using the nonlinear regression analysis aided determination of IC₅₀. *In vivo* assay analysis was done using Windows SPSS Version 25. One-way analysis of variance (ANOVA) followed by Tukey's honest significant difference post-hoc test were employed to determine statistical significance for comparison of parasitemia suppression and survival time among groups. p value of <0.05 was considered statistically significant.

RESULTS

In vitro assay

The results from the *in vitro* antiplasmodial assay of five different extracts of *N. sativa* against the two strains of *P. falciparum* and respective extract yields are listed in Table 1. The ethyl acetate and methanolic extracts showed good activity against D6 and W2 strains. However, best activity was exhibited in methanolic extract followed by ethyl acetate extract against D6 strain. Notably, ethyl acetate extract demonstrated better activity against W2 strain when compared to methanolic extract. Water extract had IC₅₀ >200 µg/ml thus no activity against the two strains of *P. falciparum*. Hexane and DCM extracts proved inactive against D6 and W2 strains with IC₅₀ >130 µg/ml. Adopted categorization of antiplasmodial activity was as follows; high (IC₅₀ ≤ 10 µg/ml), moderate (IC₅₀ 10 -50 µg/ml), low (IC₅₀ 50 -100 µg/ml) and inactive (IC₅₀ >100 µg/ml) (Gathirwa et al., 2008).

Cytotoxic activities

The two active extracts (methanolic and ethyl acetate) were tested for cytotoxicity and exhibited relatively weak cytotoxicity against Vero cells with CC₅₀s of 164.13 and 73.66 µg/ml respectively with low selectivity index indicating mild cytotoxicity as shown in Table 2.

In vivo antimalarial activity of methanolic and ethyl acetate extracts of *N. sativa* against *P. berghei* ANKA infected Swiss albino mice.

The antimalarial activity of the two potent extracts (methanolic and ethyl acetate) against *P. berghei* ANKA infected Swiss albino mice by the 4-day suppressive method is presented in Table 3. Chemosuppression was observed in a dose dependent manner after four days of antimalarial screening of *N. sativa* extracts (125-500 mg/Kg). The mean parasitemia in the groups treated with methanolic extract ranged from 4.03±0.67 to 7.18±0.73 while that of animals treated with ethyl acetate extract

Table 2. Cytotoxicity (CC₅₀) of methanolic and ethyl acetate extracts to Vero cells.

Extract	CC ₅₀ (µg/ml)	SI ^a (W2)	SI ^a (D6)
MeOH	164.13	2.04	5.14
EtOAc	73.66	1.06	1.37

^a SI-selectivity index calculated as CC₅₀/IC₅₀.

Table 3. Mean of parasitemia, % parasitemia suppression and survival time after treatment termination.

Drug/ extract	Dose (mg/Kg)	Mean ± SD parasitemia (%)	% suppression of parasite	Mean survival time (days)
MeOH	125	7.18±0.73	56.29	10.50±0.58
	250	5.58±0.39	66.05	11.25±0.96
	500	4.03±0.67	75.52	16.25±0.96
EtOAc	125	6.75±0.31	59.02	11.00±0.82
	250	4.72±0.19	71.27	12.40±1.14
	500	4.04±0.87	75.30	15.60±1.52
CQ		1.20±0.23	92.59	28.25±1.50
Vehicle (3% dimethyl sulfoxide, 10% tween 80 in PBS)		16.45±3.08		5.00±0.82

The results are expressed as mean ± SD.

varied from 4.04±0.87 to 6.75±0.31. The mean parasitemia in the negative control group was 16.45±3.08. There was a significant percentage parasitemia difference between the test groups when compared with the untreated control group ($p < 0.001$). The extracts demonstrated highest percentage parasitemia reduction at 500 mg/Kg. However, there was a slight difference in percentage parasitemia reduction between the positive control and the two extracts. The extracts were able to prolong survival of the animals after treatment termination ($p < 0.001$) in comparison to the negative control.

Acute toxicity study

The acute toxicity study results indicated that the extract caused no mortality at a dose of 1000-2000 mg/Kg within the first 24 h as well as subsequent 14 days. There were no physical and behavioral signs of over-toxicity like; loss of appetite, inability of movement, diarrhea amongst others. This suggests that LD₅₀ of the extracts is greater than 2000 mg/Kg.

DISCUSSION

There is need to seek improved antimalarial drug substitutes in order to fight resistance against *P. falciparum*

(Mueller et al., 2000). The most dependable source of remedy is possibly medicinal plants (Gasquet et al., 1993; Wright and Phillipson, 1990). The world is a rich source of medicinal plants and more research needs to be conducted to exploit their uses. Quinolones and artemisinin derivatives, the most widely effective antimalarials were principally obtained from traditional plants (Waako et al., 2005). *N. sativa*, having been used traditionally to heal many ailments, it has been mentioned to contain antiparasitic activity although underutilized in antimalarial studies (Kooti et al., 2016). This study employed *P. falciparum in vitro* culture and *P. berghei* ANKA; *in vivo* model which takes into account the prodrug effect and involvement of the immune system in infection eradication for prediction of treatment outcomes. Many conventional antimalarial agents such as chloroquine, mefloquine and lately artemisinin derivatives have been established using rodent malaria model (Waako et al., 2005). The four day suppressive test mostly assesses the antimalarial activity of candidates on early periods of infection (Verma et al., 2011). The best antimalarial drug should be safe without adverse effects (Saito et al., 2018). As a result, cytotoxicity and toxicity studies were appropriate to identify potential application of *N. sativa* in malaria management. The results indicate that the concentrations of the extracts used to carry out the *in vivo* experiments were nontoxic with no mortality recorded during the first 24 h and subsequent 14 days at 1000-2000 mg/Kg dosage. Experimental animals survived

for the entire 4 days of the 4 day suppressive test without mortality. Usually, when the mice die in the course of the 4' Day suppressive test or before the fifth day, the death is attributed to the safety of the test drug rather than the animal parasitemia levels (Satayavivad et al., 1998). Therefore, cytotoxicity and toxicity studies of the plant were important to determine selective indexes and lethal doses correspondingly. However, a weak cytotoxicity of IC₅₀s of 164.13 and 73.66 µg/ml with low selectivity index for methanolic and ethyl acetate extracts respectively was recorded. Al-Sheddi et al. (2014) noted earlier that *N. sativa* is weakly cytotoxic at higher concentrations. Additionally, our findings agree with those of Zaoui et al. (2002) that demonstrated relatively low toxicity levels and organ stability upon use of *N. sativa* extracts. This suggests that at lower concentrations, *N. sativa* extracts can be used safely to manage malaria. Therefore, the cytotoxicity and toxicity study justifies the safety of the plant in treating malaria.

The extracts demonstrated significant ($p < 0.05$) parasitemia reduction activity in all the antimalarial evaluations. The *in vitro* study showed antiplasmodial activity of the five different extracts enabling the selection of the most effective extracts. Methanolic and ethyl acetate extracts showed the best activity with IC₅₀s of 80.48±12.29 and 69.81±5.24 against W2 strain and 31.93±4.31 and 53.79±6.02 against D6 strain respectively. These two active extracts also demonstrated good activity *in vivo*. The *in vivo* assay showed significant reduction in percentage parasitemia on the test groups compared to negative control group ($p < 0.001$). These indicate the need for more studies on the use of the two solvents for extraction of active antimalarial constituents from *N. sativa* as shown by Mzena et al. (2018) in a different antimalarial plant study. The presence of single or diverse bioactive compounds possibly present in the seed extracts might have played a greater role in the antimalarial activity portrayed by the plant. Our results concurred with the findings of Ashcroft et al. (2018), who recorded much higher parasitemia suppression (>90%) upon use of methanolic extracts of *N. sativa* seeds. However, their results upon using aqueous extracts differed significantly with our findings as they reported up to 88.18% parasitemia reduction. Nonetheless, their seeds origin was different from ours. As a result, this may influence the bioactive compounds present in the seeds thus the activity. Mahmood et al. (2003) explained that the possible constituents of *N. sativa* have the ability to obstruct production of nitric oxide (NO) in macrophages. This in turn leads to escalation of tryptophan degradation via indolamine deoxygenase stimulation in human peritoneal macrophages. Consequently, the parasite dies as it is deprived of a vital amino acid. The immunomodulatory properties of *N. sativa* seeds that have been exhibited in past work done by Haq et al. (1999), might largely contribute to host-parasite interaction as noted earlier on

by Anthony et al. (2005). Consistent with this model, Salem (2005) attributed anti-microbial effects in his study to the immunomodulatory properties of *N. sativa* seeds constituents. The abovementioned factors might have singly or together achievably supported the antimalarial effects of the seed extracts exhibited in this study. The limitation of the study is that identification of specific compounds responsible for antimalarial activity was not attained as this was beyond the scope of the current study. We therefore recommend that further analysis should be done on *N. sativa* seeds to identify the specific antimalarial compounds present.

Conclusion

The results in this study provide room for future comprehensive investigations on the plant through bioassay-guided fractionation, isolation and characterization of the bioactive compounds leading to establishment of novel antimalarial compounds fighting against drug resistant malaria. This study explains the use of *N. sativa* plant traditionally in the Middle East folk medicine and in Mombasa County.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors are sincerely thankful to the Kenya National Research Fund for sponsoring this study. We are grateful to the Technical University of Mombasa for allowing progress of the work. We thank the KEMRI, Nairobi administration particular the director of Centre for Biotechnology and Research Development (CBRD) and the director of Centre for Traditional Medicine and Drug Research (CTMDR) for provision of space in the laboratories. Lastly, we value the contribution of the Mombasa local people for the information they shared on the traditional use of the plant.

REFERENCES

- Abdela J, Engdawork E, Shibeshi W (2014). In vivo antimalarial activity of solvent fractions of the leaves of *Justicia schimperiana* hochst. Ex Nees against *Plasmodium berghei* in Mice. *Ethiopian Pharmaceutical Journal* 30(2):95-108.
- Abdulah HAA, Zainal-Abidin BAH (2007). In vivo anti-malarial tests of *Nigella sativa* (Black Seed) different extracts. *American Journal of Pharmacology and Toxicology* 2(2):46-50.
- Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Anwar F (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine* 3(5):337-352.
- Al-Sheddi ES, Farshori NN, Al-Oqail MM, Musarrat J, Al-Khedhairi AA, Siddiqui MA (2014). Cytotoxicity of *Nigella sativa* seed oil and extract

- against human lung cancer cell line. *Asian Pacific Journal of Cancer Prevention* 15(2):983-987.
- Anthony JP, Fyfe L, Smith H (2005). Plant active components—a resource for antiparasitic agents? *Trends in Parasitology* 21(10):462-468.
- Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Ma L (2014). A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 505(7481):50.
- Ashcroft OF, Salaudeen OF, Mohammed K, Spencer THI, Garba MK, Nataala SU, Iduh UM (2018). Anti-malarial effect of *Nigella sativa* seeds (black seed) extract on mice infected with *Plasmodium berghei* (nk 65). *European Journal of Pharmaceutical and Medical Research* 5(10):131-137.
- Ashley EA, Phyo AP (2018). Drugs in development for malaria. *Drugs* 78(9):861-879.
- Bahekar S, Kale R (2013). Herbal Plants Used For the Treatment of Malaria-A Literature. *Journal of Pharmacognosy and Phytochemistry* 1:6.
- Cunningham AB (2001). *Applied ethnobotany: People, wild plant use and conservation*. Earthscan, London, UK. <https://www.taylorfrancis.com/books/9781849776073>
- Desjardins RE, Canfield CJ, Haynes JD, Chulay JD (1979). Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrobial Agents and Chemotherapy* 16(6):710-718.
- Ecker A, Lehane AM, Clain J, Fidock DA (2012). PfCRT and its role in antimalarial drug resistance. *Trends in Parasitology* 28(11):504-514.
- Gasquet M, Delmas F, Timon-David P, Keita A, Guindo M, Koita N, Doumbo O (1993). Evaluation in vitro and in vivo of a traditional antimalarial, "Malarial 5". *Fitoterapia-Milano* 64:423-423.
- Gathirwa JW, Rukunga GM, Njagi ENM, Omar SA, Mwitari PG, Guantai AN, Kirira PG (2008). The in vitro anti-plasmodial and in vivo antimalarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya. *Journal of Ethnopharmacology* 115(2):223-231.
- Gessler MC, Tanner M, Chollet J, Nkunya MHH, Heinrich M (1995). Tanzanian medicinal plants used traditionally for the treatment of malaria: In vivo antimalarial and in vitro cytotoxic activities. *Phytotherapy Research* 9(7):504-508.
- Haq A, Lobo PI, Al-Tufail M, Rama NR, Al-Sedairy ST (1999). Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *International Journal of Immunopharmacology* 21(4):283-295.
- Heshmati J, Namazi N (2015). Effects of black seed (*Nigella sativa*) on metabolic parameters in diabetes mellitus: A systematic review. *Complementary Therapies in Medicine* 23(2):275-282.
- Heshmati J, Namazi N, Memarzadeh MR, Taghizadeh M, Kolahtooz F (2015). *Nigella sativa* oil affects glucose metabolism and lipid concentrations in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. *Food Research International* 70:87-93.
- Horn D, Duraisingh MT (2014). Antiparasitic chemotherapy: From genomes to mechanisms. *Annual Review of Pharmacology and Toxicology* 54:71-94.
- Jelinek T, Jelinek T, Rønn AM, Lemnge MM, Curtis J, Mhina J, Warhurst DC (1998). Polymorphisms in the dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) genes of *Plasmodium falciparum* and in vivo resistance to sulphadoxine/pyrimethamine in isolates from Tanzania. *Tropical Medicine and International Health* 3(8):605-609.
- Katz SH, Kimani VN (1982). Why patients go to traditional healers. *East African Medical Journal* 59(3):170-174.
- Kepha S, Nikolay B, Nuwaha F, Mwandawiro CS, Nankabirwa J, Ndiranza J, Allen E (2016). *Plasmodium falciparum* parasitaemia and clinical malaria among school children living in a high transmission setting in western Kenya. *Malaria Journal* 15(1):157.
- Kooti W, Hasanzadeh-Noohi Z, Sharafi-Ahvazi N, Asadi-Samani M, Ashtary-Larky D (2016). Phytochemistry, pharmacology, and therapeutic uses of black seed (*Nigella sativa*). *Chinese Journal of Natural Medicines* 14(10):732-745.
- Kurokawa M, Nagasaka K, Hirabayashi T, Uyama S, Sato H, Kageyama T, Namba T (1995). Efficacy of traditional herbal medicines in combination with acyclovir against herpes simplex virus type 1 infection in vitro and in vivo. *Antiviral Research* 27(1-2):19-37.
- Le Bras J, Durand R (2003). The mechanisms of resistance to antimalarial drugs in *Plasmodium falciparum*. *Fundamental and Clinical Pharmacology* 17(2):147-153.
- Lorke D (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology* 54(4):275-287.
- Maier AG, Matuschewski K, Zhang M, Rug M (2018). *Plasmodium falciparum*. *Trends in Parasitology*. *Plasmodium falciparum* 35(6):481-482.
- Mahmood MS, Gilani AH, Khwaja A, Rashid A, Ashfaq MK (2003). The in vitro effect of aqueous extract of *Nigella sativa* seeds on nitric oxide production. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 17(8):921-924.
- Menard D, Dondorp A (2017). Antimalarial drug resistance: A threat to malaria elimination. *Cold Spring Harbor Perspectives in Medicine* 7(7):a025619.
- Mogeni P, Williams TN, Fegan G, Nyundo C, Bauni E, Mwai K, Osier F (2016). Age, spatial, and temporal variations in hospital admissions with malaria in Kilifi County, Kenya: A 25-year longitudinal observational study. *PLoS Medicine* 13(6):e1002047.
- Mueller MS, Karhagomba IB, Hirt HM, Wemakor E (2000). The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: Agricultural, chemical and clinical aspects. *Journal of Ethnopharmacology* 73(3):487-493.
- Mvango S, Matshe WM, Balogun AO, Pilcher LA, Balogun MO (2018). Nanomedicines for malaria chemotherapy: Encapsulation vs. polymer therapeutics. *Pharmaceutical Research* 35(12):237.
- Mzena T, Swai H, Chacha M (2018). Antimalarial activity of *Cucumis metuliferus* and *Lippia kituiensis* against *Plasmodium berghei* infection in mice. *Research and Reports in Tropical Medicine* 9:81.
- Na-Bangchang K, Karbwang J (2009). Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. *Fundamental and Clinical Pharmacology* 23(4):387-409.
- Nkumama IN, O'Meara WP, Osier FH (2017). Changes in malaria epidemiology in Africa and new challenges for elimination. *Trends in Parasitology* 33(2):128-140.
- Periasamy VS, Athinarayanan J, Alshatwi AA (2016). Anticancer activity of an ultrasonic nanoemulsion formulation of *Nigella sativa* L. essential oil on human breast cancer cells. *Ultrasonics Sonochemistry* 31:449-455.
- Peters W (1975). The four-day suppressive in vivo antimalarial test. *Annals of Tropical Medicine and Parasitology* 69:155-171.
- Razavi SM, Asadpour M, Malekpour SH, Jafari A (2018). The field efficacy of *Nigella sativa* and *Berberis vulgaris* methanolic extracts against *Haemoproteus columbae*. *Avicenna Journal of Phytomedicine* 8(2):114.
- Saito M, Gilder ME, McGready R, Nosten F (2018). Antimalarial drugs for treating and preventing malaria in pregnant and lactating women. *Expert Opinion on Drug Safety* 17(11):1129-1144.
- Salem ML (2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *International Immunopharmacology* 5(13-14):1749-1770.
- Satayavivad J, Noppamas S, Aimon S, Yodhathai T (1998). Toxicological and antimalaria activity of *Eurycoma longifolia* Jack extracts in mice. *Thai Journal of Phytopharmacy* 5:14-27.
- Silberman L (1950). The social survey of the old town of Mombasa. *Journal of African Administration* 2:15-21.
- Sixsmith DG, Watkins WM, Chulay JD, Spencer HC (1984). In vitro antimalarial activity of tetrahydrofolate dehydrogenase inhibitors. *The American Journal of Tropical Medicine and Hygiene* 33(5):772-776.
- Sugiarto SR, Moore BR, Makani J, Davis TM (2018). Artemisinin therapy for malaria in Hemoglobinopathies: A systematic review. *Clinical Infectious Diseases* 66(5):799-804.
- Tona L, Mesia K, Ngimbi NP, Chirwami B, Okond'Ahoka Cimanga K, Totte J (2001). In-vivo antimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus niruri*. *Annals of Tropical Medicine and Parasitology* 95(1):47-57.
- Torres REM, Banegas EI, Mendoza M, Diaz C, Bucheli STM, Fontecha GA, Zambrano JON (2013). Efficacy of chloroquine for the treatment

- of uncomplicated *Plasmodium falciparum* malaria in Honduras. *The American Journal of Tropical Medicine and Hygiene* 88(5):850-854.
- Trager W, Jensen JB (2005). Human malaria parasites in continuous culture. *Journal of Parasitology* 91(3):484-486.
- Van Meerloo J, Kaspers GJ, Cloos J (2011). Cell sensitivity assays: The MTT assay. In *Cancer cell culture*. Springer. pp. 237-245. Doi: 10.1007/978-1-61779-080-5_20.
- Verma G, Dua VK, Agarwal DD, Atul PK (2011). Anti-malarial activity of *Holarrhena antidysenterica* and *Viola canescens*, plants traditionally used against malaria in the Garhwal region of north-west Himalaya. *Malaria Journal* 10(1):20.
- Waako PJ, Gumede B, Smith P, Folb PI (2005). The in vitro and in vivo antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch. Et Thonn. *Journal of Ethnopharmacology* 99(1):137-143.
- White N (1999). Antimalarial drug resistance and combination chemotherapy. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 354(1384):739-749.
- World Malaria Report (2018). Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.
- Wright CW, Phillipson JD (1990). Natural products and the development of selective antiprotozoal drugs. *Phytotherapy Research* 4(4):127-139.
- Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M (2002). Acute and chronic toxicity of *Nigella sativa* fixed oil. *Phytomedicine* 9(1):69-74.