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Antimalarial Efficacy of Ethanolic Leaf Extract of *Vernonia amygdalina* against *Plasmodium berghei* in Infected Swiss Albino Mice

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ABSTRACT

There is a considerable increase in mortality caused by malaria due to the rapid spread of drug-resistant strains of Plasmodium falciparum and Plasmodium berghei. The parasite has develop resistant to orthodox drugs over the years, thus need for herbal remedy. This study is sought to investigate the antimalarial efficacy of ethanolic leaf extract of Vernonia amygdalina against Plasmodium berghei in infected Swiss albino mice. Fresh plants of V. amygdalina were harvested from the Institute of Agricultural Research and Training, Ibadan. The leaves were dried, milled into powder and extracted using soxhlet apparatus and ethanol as the solvent. Thirty Swiss albino mice obtained from the Federal University of Agriculture, Abeokuta, were *acclimatized for seven (7) days and divided into six groups. Each mouse in groups 2 to 6 was inoculated intraperitoneally with infected blood suspension containing about 1x10^7 Plasmodium berghei parasitized red blood cells on day zero while those in group 1 were not infected and this served as the normal control group. Animals in group 2 were administered 0.2 ml normal saline, those in group 3 were administered Chloroquine diphosphate at 5 mg/kg body weight, those in groups 4, 5 and 6 were administered 100, 200 and 400 mg/kg of the ethanolic leaf extract of V. amygdalina respectively. All treatments were orally done once per day for five consecutive days from when parasites were first seen in the infected animal blood. Parasitemia Count and PCV were done using standard methods. V. amygdalina extract exhibited antimalarial properties especially at 200 and 400mg/kg and the results were not different from that of chloroquine. The result of this present study confirmed that ethanolic leaf extract of V. amygdalina which displayed good activities against P. berghei are suitable for their traditional use in the treatment of malaria fever.*

Keywords: *Vernonia amygdalina*, Antimalarial efficacy, *Plasmodium berghei*, Ethanolic leaf extract, Swiss albino mice

1. INTRODUCTION

Malaria is still considered a major public health problem in the 106 countries where the risk of contracting the infection with one or more of the *Plasmodium species* exists one of the serious health problems in most of the tropical countries [1]. Malaria is endemic throughout most of the tropics. Ninety-five countries and territories have ongoing transmission. Of the approximately 3.2 billion people living in malarious countries, 1.2 billion are at high risk; the World Health Organization (WHO) states that there were 214 million (range 149 to 303 million) cases of symptomatic malaria in 2015 [2]. It is the leading

cause of morbidity and mortality in sub-Saharan Africa, especially in young children and pregnant women [3], with an estimated 429 000 malaria deaths (range 235 000–639 000) worldwide in 2015 [4]. The attack of malaria during pregnancy usually results in severe anemia and impairment of fetal nutrition which contribute to the low birth weight, premature delivery, mental retardation and 60% miscarriages. In 2015, there were an estimated 438 000 malaria deaths worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) [2].

Malaria is caused by protozoan of genus *Plasmodium* transmitted to the vertebrate by female Anopheles mosquitoes. In the vertebrate host, the sexual blood forms of the parasite are the life cycle stage that is responsible for the morbidity and mortality of plasmodial infections [5]. *P. falciparum* is the most virulent parasite, and is responsible for the majority of malaria related morbidity and mortality [2]. Antimalarial drug resistance has become one of the greatest challenges against malaria control. Resistance to antimalarial drugs has been described for two of the four species of malaria parasites that naturally infect humans, *P. falciparum* and *P. vivax*. *P. falciparum* has developed resistance to nearly all antimalarial drug in use [3]. With the problems of increasing levels of drug resistance, alternative medicine could be an important and sustainable source of treatment [6]. In Africa, the use of Indigenous medicinal plants with traditional reputation still plays an important role in malaria treatment serving as interesting sources for the detection of novel antiplasmodial compounds [7]. There is so much contribution in the area of plant study in search for potent antimalarial agents [8-11]. In furtherance of these efforts, this research was designed to examine the antimalarial efficacy of ethanolic leaf extract of *V. amygdalina* on the strain of *Plasmodium berghei* infection in albino infected mice. *P. berghei* infection of laboratory mouse strains is frequently used in research as a model for human malaria [12].

Vernonia amygdalina commonly known as bitter leaf in English, Oriwo in Edo, Ewuro in Yoruba, Shuwaka/Chusadoki in Hausa, and Olubu/Onugbu in Igbo, is a tropical shrub that grows up to 3 meters high in the African tropics and other parts of Africa particularly Nigeria, Cameroon, and Zimbabwe. The leaves are dark green coloured with a characteristics odour and a bitter taste. It is reputed to have several health benefits. It is effective against amoebic dysentery, gastrointestinal disorder and has antimicrobial and anti-parasitic activity [13]. *V. amygdalina* is a perennial herb belonging to the Asteraceae family. The species is indigenous to tropical Africa and is found wild or cultivated all over sub Saharan African [14]. The leaves are eaten after crushing and washing thoroughly to remove the bitterness [15]. However, almost all parts of the plant are pharmacologically useful, both the root and the leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort among others [16]. *V. amygdalina* has been ascertained to provide various culinary and medicinal properties, these medicinal properties exert bacteriostatic and bacteriocidal effect on some bacteria [17]. Antimalarial properties [18] as well as antitumourigenic properties [19] have also been reported for extracts from the plant. Furthermore, Ogbuagu *et al*. [20] have demonstrated the effect of methanolic extract of its leaves on glycemic and lipidaemic indexes in Wistar rats. Its prophylactic propensity against acute ethanol-induced oxidative stress has also been reported [21]. Many herbalist and native doctors in Africa recommend its aqueous extract for their patients for the treatment of varieties of ailment ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexual transmitted diseases among others [9].

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh plants of *V. amygdalina* free from diseases were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory

space for 14 days and milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion *et al*. [22, 23]. About 25 g of the powder was packed into the thimble of the soxhlet extractor and 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35 ^{o}C with a yield of 2.07 g which represents a percentage yield of 8.28%. The extract was preserved in the refrigerator for further analysis.

2.2 Parasite Inoculums

Plasmodium berghei NK65 strain infected erythrocytes were obtained from a donor-infected mouse maintained at the *Department* of Veterinary Microbiology *and* Parasitology, Federal University of Agriculture, Abeokuta, Nigeria. The inoculum was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and then diluting with normal saline.

2.3 Experimental Animal and Curative Test

Thirty (30) Swiss albino mice weighing between 20 and 25 g were obtained from the Animal House of Federal University of Agriculture, Abeokuta, Nigeria. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into six groups of five mice each. In order to evaluate the curative potential of the crude extract, methods described in literature were adopted [24, 25]. Each mouse in the treatment group (groups 2 to 6) was inoculated intraperitoneally with infected blood suspension (0.2 ml) containing about $1x10^{7}$ *Plasmodium berghei* parasitized red blood cells on day zero while those in group 1 were not infected and this served as the normal control group. Animals in group 2 were administered 0.2 ml normal saline (negative control), those in group 3 were administered Chloroquine diphosphate (standard antimalarial drug) at 5 mg/kg body weight (positive control), those in groups 4, 5 and 6 were administered 100, 200 and 400 mg/kg of the ethanolic leaf extract of *V. amygdalina* respectively. All treatments were orally done once per day for five consecutive days from when parasites were first seen in the infected animal blood. Four days after the treatment was stopped, the animals were weighed and sacrificed.

2.4 Parasitemia Count

On each day of treatment and post treatment, a drop of blood was collected from each mouse for parasitemia screening by tail nip. The blood collected was placed on a slide and smeared to make a thick film, fixed with ethanol and stained with Giemsa stain. When dried, the film was microscopically viewed by adding a drop of immersion oil and viewing it under x100 magnification of the microscope. The parasitemia density was examined by counting the parasitized red blood cell [24, 25].

2.5 Determination of Packed Cell Volume

Capillary tubes were filled with blood to about 1 cm or two-third (2/3) of its length and the vacant end of each tube was sealed with plasticin to protect the blood from spilling. The tubes were placed in haematocrit centrifuge with sealed side towards the periphery and then centrifuge for 5-6 minutes. The packed cell volume was read directly from haematocrit reader [24, 25].

2.6 Statistical Analysis

Data were subjected to analysis using Microsoft Excel. Values are presented as mean±standard deviation.

3. RESULTS AND DISCUSSION

The development of an affordable Artemisinin-based Combination Therapy (ACT) or an alternative cost-effective antimalarial drug is imperative in the rural areas where majority of the people are poor. Many scientists are now even turning towards herbs to seek for answers to drug resistance. Plants used in treatment of diseases are said to contain active phytochemicals some of which are responsible for the plants' characteristic adours, pugencies and color while others give virtues as food, medicinal or poisonous [26].

The result of the effect of ethanolic leaf extract of *V. amygdalina* on body weight of *Plasmodium berghei*-infected mice is shown in Figure 1. The body weight of the infected untreated mice (negative control) and infected treated with 100 mg/kg of *V. amygdalina* showed significant weight lost after 4 days post

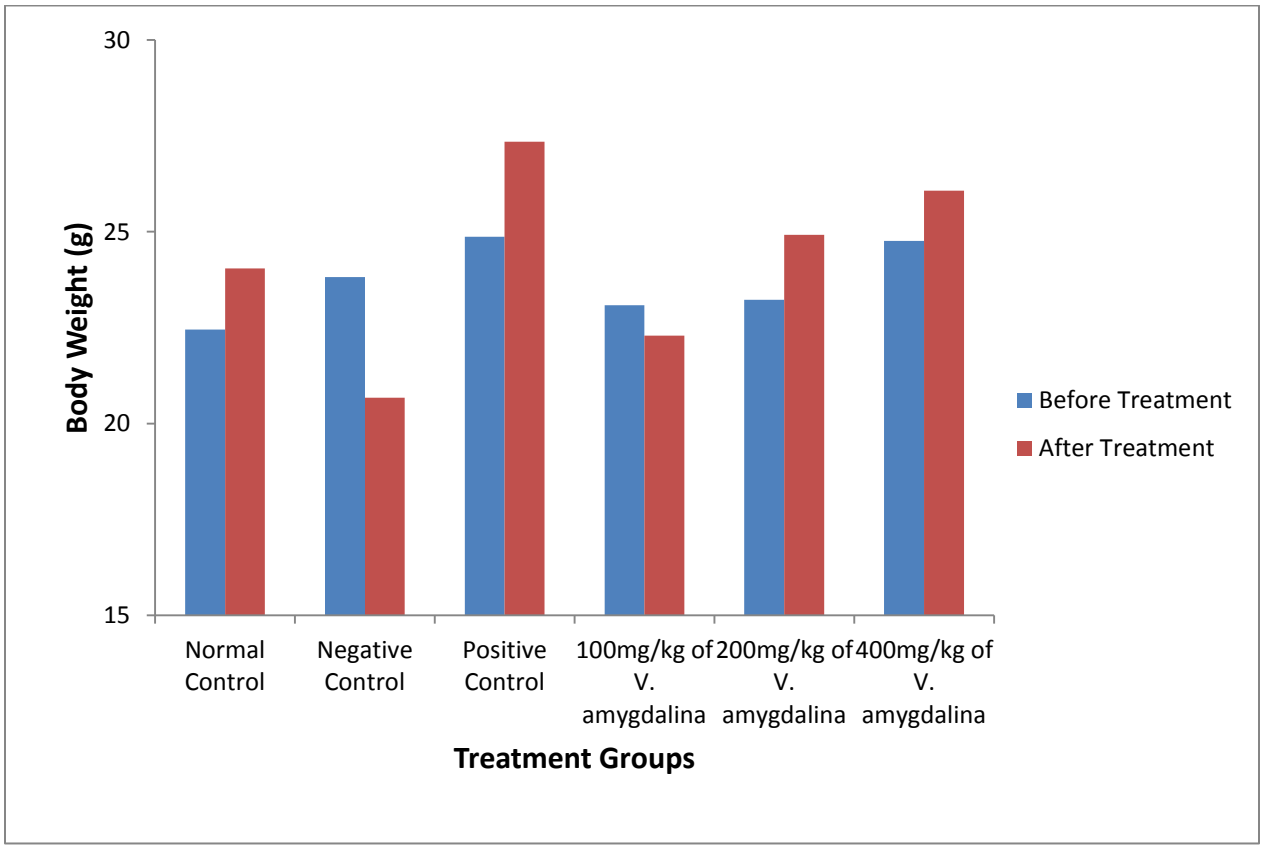

Figure 1: Effect of ethanolic leaf extract of *V. amygdalina* on body weight of *P. berghei*-infected mice. Values are presented as mean with n = 5

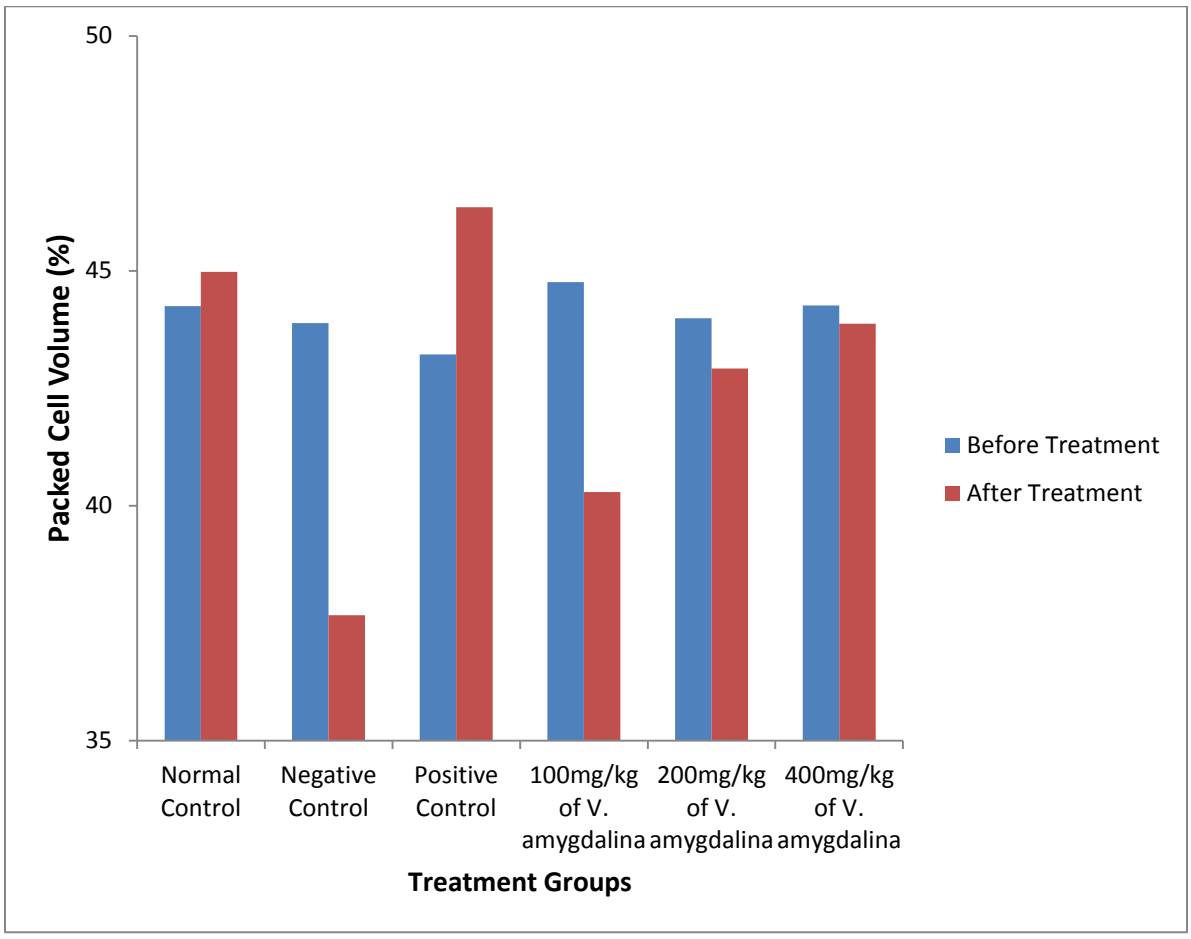

Figure 2: Effect of ethanolic leaves extract of *V. amygdalina* on Packed Cell Volume of *P. berghei*-infected mice

treatment. On the other hand, the infected mice treated with 200 mg/kg, 400 mg/kg of *V. amygdalina* as well as those treated with 5 mg/kg chloroquine (positive control) showed weight gain after 4 days of treatment. The weight gain in these groups is not different when compared with uninfected animals (normal control). Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice [27]. So an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in infected mice [28]. In the present study, extract of *V. amygdalina* significantly prevented weight loss associated with increase in parasitemia level.

The effect of ethanolic leaf extract of *V. amygdalina* on packed cell volume (PCV) of *Plasmodium berghei*-infected mice is shown in Figure 2. The PCV of animals in the normal control and positive control was observed to increase after four days post-treatment when compared with those of the same groups before the commencement of treatment. *P. berghei* infected untreated mice (negative control) had their PCV significantly reduced which is an indication that malarial infection could be a risk of anaemia. Although *P. berghei* infected mice treated with varying doses of *V. amygdalina* leaf extract had increased PCV when compared with those infected and untreated but their PCV was lower when compared with animal in the same respective groups

before infection with *P. berghei*. This collaborate the study of Airaodion *et al*. [29] who reported that *V. amygdalina* leaves decreased the PCV of animals thereby being a potent anaemic agent.

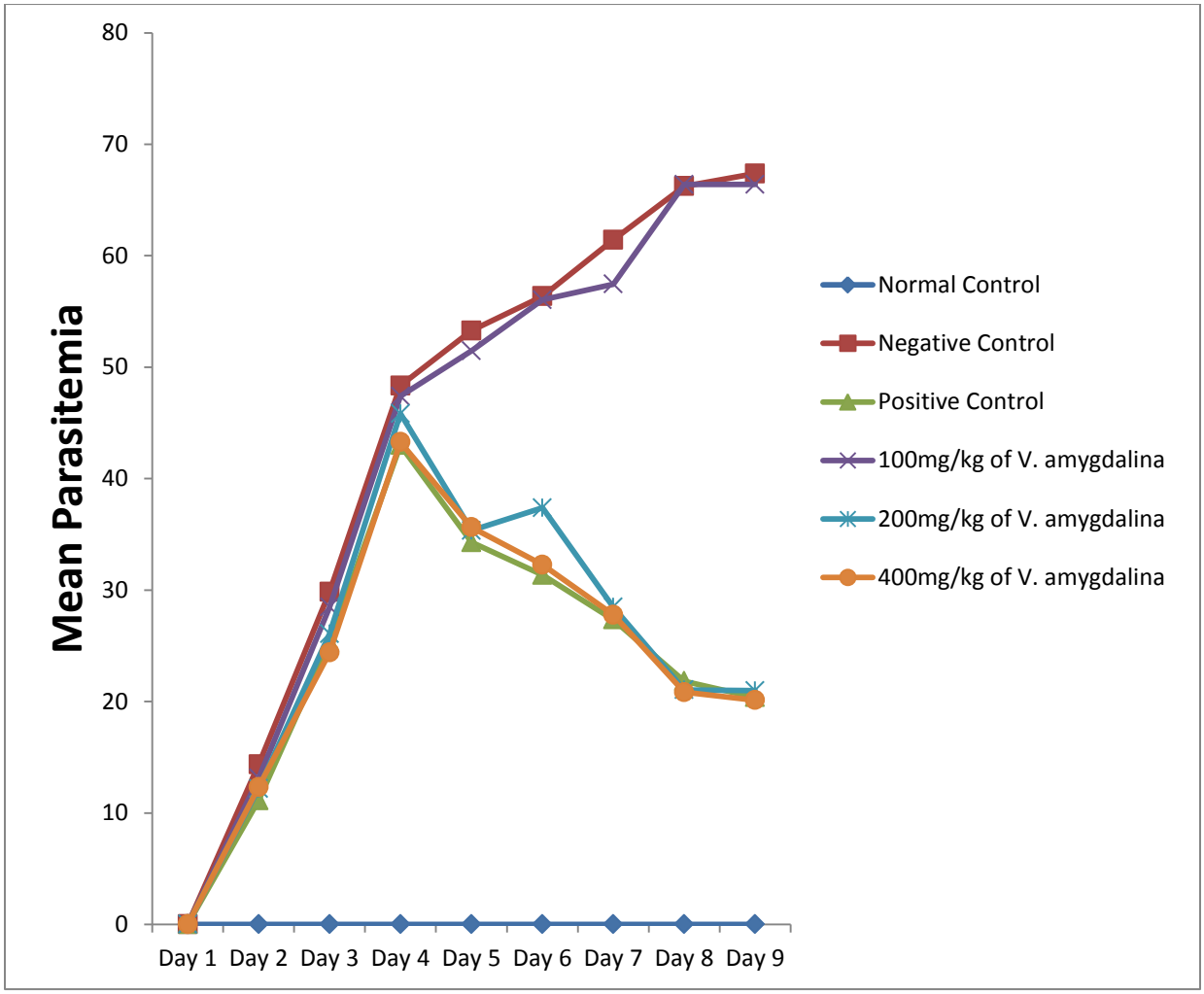

Figure 3: *In vivo* antiplasmodial activity of ethanolic leaf extract of *V. amygdalina* against *Plasmodium berghei* in infected mice: Each point is a Mean±SD with n=5

The average daily parasitaemia level of the *P. berghei* in infected mice treated with ethanolic leaf extract of *V. amygdalina* is shown in Figure 3. The average daily parasitaemia of infected mice treated, respectively, with chloroquine, 400 mg/kg and 200 mg/kg leaf extract of *V. amygdalina* significantly ($P<0.05$) reduced when compared with control group. However there is no significant ($P<0.05$) difference in the level of parasitaemia in infected mice treated with 100 mg/kg leaf extract of *V. amygdalina* as compared with the control group. This is in agreement with the study of Longdet and Adoga [30] who reported the effect of methanolic leaf extract of *Vernonia amygdalina* on *Plasmodium berghei* infection in albino mice. Several studies of the qualitative phytochemical analysis of *V. amygdalina* leaves showed the presence of alkaloid, flavonoid, Saponin, Tannin and Glycosides [31]. Therapeutic effect of some plants has been attributed to the presence of phytochemicals [32-36]. Flavonoids have also been reported to have exhibited significant *in vitro* antimalarial activity against *P. falciparum* [37]. This could justify the antimalarial activities exhibited by the plant extract.

The 400 mg/kg, 200 mg/kg and 100 mg/kg ethanolic leaf extracts of *V. amygdalina* showed a dose dependent and progressive reduction in parasitaemia with time. This is a very promising feature in the potentials of *V. amygdalina* as an antimalarial agent. Good enough, the antimalarial effect demonstrated by *V. amygdalina* leaf extract compete well with chloroquine treatment. Chloroquine has been used as the standard antimalarial drug because of its established activities on *P. berghei* [38, 39].

4. CONCLUSION

Increasing the global spread of multi-drug resistant malaria parasite showed that there is a need for new chemotherapeutic agents to combat malaria. Development of new active and safe drugs for the community is therefore an urgent need. Towards this goal, research into new antimalarial drugs from natural products, traditional healers use parts of many plants for the treatment of several pathologies, including malaria, and have done so for centuries. The result of this present study confirm that extracts from leaves of *V. amygdalina* which displayed good activities against *P. berghei* are suitable for their traditional use in the treatment of malaria fever.

5. ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

6. CONFLICT OF INTERESTS

Authors wish to declare that no conflict of interest exist in the publication of this paper.

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