Bima Journal of Science and Technology, Vol. 8(2) June, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i2.686

Assessment of Zoochemical Constituents and Antiplasmodial Potency of Crude Methanol Extract from Millipede (Diplopoda: Pachybolidae) in *Plasmodium berghei* Infected Mice

Adeniyi Kamoru Abdulazeez[1]*, Oluwaseun Adegbola Adesoye[2], Okunlola, L. T[3]., Sherif M. A[4], D. H. Dauda[5], Israel O. Akinsete[5], Adedapo O. Adeogun[2], Blessing A. Ezeonuegbu[6], Callistus A. Akinleye[7]

[1]Department of Biological Sciences, Federal University Dutse, Dutse, Jigawa State, Nigeria
[2]Molecular Entomology and Vector Control Unit, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria
[3]Parasitology Unit, Department of Zoology, University of Ibadan, Oyo State, Nigeria
[4]Department of Hospital Service, Jigawa State Ministry of Health, Jigawa State, Nigeria
[5]Department of Animal Biology, Federal University of Technology, Minna, Niger State, Nigeria
[6]Deparment of Microbiology Technology, School of Science Laboratory Technology, University of Port Harcourt, Nigeria
[7]Department of Community Medicine, Osun State University, Osogbo, Nigeria
Corresponding Author: adeniyi.k@fud.edu.ng

ABSTRACT

Malaria infection remains a major public health problem in more than 106 countries, in spite of the aggressive control over the years. Therefore, the need for alternative drugs with better efficacy and lesser proneness to resistance. This study evaluated the zoochemical constituents and antiplasmodial efficacy of the methanol extract of Millipede in *Plasmodium berghei* infected mice. Mice were intraperitoneally infected with *Plasmodium berghei*. The presence of parasite was ascertained by microscopic examination of blood samples daily. The methanol extract of millipede was administered orally for 7 days from the day parasitaemia reach 5% of parasite inoculation. The acute oral toxicity of the millipede extract was also assessed. Chloroquine was used as a positive control The millipede-treated groups were given 2 different doses (i.e., 300mg/kg and 600mg/kg) and they showed a gradual dose dependent decrease in parasitemia as well as the control group. The result of the zoochemical analysis reveals the presence of Alkaloid, saponins, Tannins, flavonoids. Alkaloid content was significantly highest (42.89) than the other phytochemicals recorded. The acute oral toxicity shows that the Median Lethal dosage is above 5000mg/kg. The maximum antimalarial activity was observed in the mice treated with chloroquine (2.00 ± 0.00) which was closely followed by millipede extract (2.27± 0.15). Millipede bioactive metabolite is novel in malaria treatment and findings from the present study recommended that it should be considered as a promising antimalaria agent.

Keywords: Malaria, Millipedes, Parasitemia, Zoochemical, Antiplasmodial

INTRODUCTION

Malaria is a life-threatening disease caused by parasites of the genus *Plasmodium* (WHO, 2021). Five primary parasite species are responsible for causing malaria in humans: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi*, which was initially recognized as a cause of malaria in monkeys (Adeogun *et al.,* 2023). Common symptoms of malaria include fever, sweating, chills, nausea, vomiting, diarrhea, headache, body aches, and a general sense of illness or weakness, among others (Long, 2017; Sato, 2021). Malaria infections often exhibit defective immune responses, leading to poor efficacy against the infection and, in some cases, immunopathology (Khodzhaeva *et al.,* 2019). Severe malaria can lead to complications such as cerebral malaria, anemia, acidosis, jaundice, respiratory distress,

DOI: 10.56892/bima.v8i2.686

renal insufficiency, coagulation abnormalities, and hyperparasitemia (Sinden and Gilles, 2017). Among these complications, those caused by *P. falciparum* and *P. vivax* are the most significant, with *P. falciparum* being more virulent (Penet *et al.,* 2015).

Approximately 249 million malaria cases were documented worldwide in 2022, resulting in 608,000 deaths across 85 countries (WHO, 2021). Malaria claims between 1 to 3 million lives annually, primarily affecting children under 5 years old and pregnant women (Dufera *et al.,* 2020). Despite concerted efforts to control it in endemic regions, malaria remains a significant public health challenge in over 106 countries (Verra *et al.,* 2018). The disease spreads through various means, including the bite of female *Anopheles* mosquitoes, blood transfusion from infected individuals, or transmission from mother to child during pregnancy (Nakatani *et al.,* 2014). Female *Anopheles* mosquitoes are the primary vectors of malaria transmission in sub-Saharan Africa, with the *An. gambiae* complex being the principal species among 37 identified malaria vectors (Duguma *et al.,* 2022; Effiong *et al.,* 2022; Adesoye *et al.,* 2023; Adeniyi *et al.,* 2023).

Managing and treating malaria is becoming progressively more challenging due to the worldwide issue of drug resistance, stemming from the emergence of resistant strains of various malaria parasites, particularly *P. falciparum* (Penet *et al.,* 2015; Geleta and Ketema, 2016). One approach to address this challenge is the exploration of new antimalarial drugs often sourced from flora and fauna (Autino *et al.,* 20122). There are quite a number of studies that have reported efficacy of discovered novel antimalaria drugs of flora origin (Fentahun *et al.,* 2017; Belete *et al.,* 2020; Habte *et al.,* 2020; Adigo et al., 2021). For instance, Barliana *et al.* (2014) described the anti-plasmodial activity of the crude extract and solvent fractions of stem barks of *Schima wallichii* against *plasmodium berghei*-infected Mice; likewise, Dejen *et al*. (2021) reported similar property in *Gardenia ternifolia* against the malaria parasite. Although, there has been reports of medical potential of insects and other arthropods as well (Meyer-Rochow, 2017; Siddiqui *et al.,* 2023), however, there is a paucity of information regarding antimalarial therapies derived from arthropods sources. Consequently, this study aims to assess the zoochemical components and anti-Pasmodial effectiveness of a methanol extract from millipedes in mice infected with *P. berghei*.

MATERIALS METHODS

Collection and Processing of Millipedes

Following a standard procedure described by Means *et al.* (2015), live millipedes were collected from Bosso community, Niger State. The collected millipedes were placed in sterile containers, transported to the entomological laboratory of the Department of Animal Biology, School of Life Sciences, Federal University of Technology Minna, and was taxonomically identified as *Pachybolu ligulatus* (Diplopoda: Pachybolidae) using scientific keys (Means *et al.* 2015; Field Museum, 2024). The millipedes were anesthetized and then killed using chloroform inhalation, cut into pieces and dried under sunlight for 5-6 days. After complete dryness, the millipedes were then crushed into powder using mortar and pestle. The powder was weighed and placed in an air tight container to avoid the absorption of moisture (Abd Elkawy *et al*. 2013; Naturalist, 2024).

Animal Care and Use

Mice (*Rattus norvegicus)* weighing between 16 and 30g were acquired from the animal house, Federal University of Technology Minna. The mice were maintained on standard feed and water and were acclimatized to laboratory condition for two weeks prior to commencement of the experiment. Handling of experimental animals was in accordance

DOI: 10.56892/bima.v8i2.686

with generally accepted protocol (Upton and Chapman, 2010).

Extraction of the Powdered Millipedes

Fifty (50mg) of the powdered millipedes, *Pachybolu ligulatus,* was placed in 500 ml beakers then, 250 ml of methanol was added to the beaker to form separate mixtures of the solvent extracts. The mixtures were allowed to soak for 48 hours, with intermittent shaking. After the 48hrs period, the mixtures were agitated vigorously for 5 minutes, allowed to settle for 5 minutes and the supernatant was passed through a doubled muslin cloth and then followed by a Whatman No. 1 filter paper to remove solid millipede. The solvents were evaporated from the filtrates in a water bath at 60 ^{o}C. The methanol extracts were placed into a specimen bottle prior to usage.
Quantitative and qualitative zoochemical constituents screening of methanol extract of *Pachybolus ligulatus* was carried-out using the method described by Paduhilao II and Yap-Dejeto (Nishchal *et al.,* 2014).

Effect of Millipede Extract on Morphology and Behavior of Mice

Effect testing was performed with the two millipede extracts. Four mice, for each group, were given these extracts orally (i.e., 1500, 3000 and 6000mg/kg for each millipede extract) using a modified Lock method (Paduhilao and Yap-Dejeto, 2022). The mice were acclimatized and fasted overnight. Negative signs and symptoms such as death, behavioral change and change in physical appearance were recorded for 24 hours.

Collection of *Plasmodium berghei* (Test Parasite)

The test parasite (*Plasmodium berghei* strain NK 65) was obtained from National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. The test parasite was inoculated intraperitoneally into the mice after 2 weeks of acclimatization and left for 72hrs.

Parasitology Bioassay

Apart from the healthy control group, all other mice groups were infected intraperitoneally with an aliquot of 0.2 mL of standard inoculum, $1x10^7$ *P. berghei* parasitized erythrocytes. This was the followed by a daily determination of parasitemia using giemsa-stained blood smear. When the presence of parasite in the mice was established, treatment groups were assigned. The total number of mice was 24, with 6 mice per group. The first group was not treated (negative control), the second group were treated with chloroquine to serve as positive control (5 mg/kg body weight) (mg/kg b. wt), the third and fourth group were treated with *P. ligulatus extracts* (300 and 600 mg/kg body weight). Mice parasitemia count, weight, and mean survival time of mice over 30days were all monitored and recorded following standard procedure for all groups (Lorke, 1983).

Determination of Packed Cell Volume (PCV)

Packed Cell Volume (PCV) of each of the mice in all experimental groups were measured before infection, on day 4 after infection and after treatment with the extract (Habte and Assefa, 2020). For this purpose, blood was collected from the tail of each mouse into heparinized microhaematocrit capillary tubes up to 3/4th of their length. The capillary tubes were filled with blood to about 1cm or two-thirds (2/3) of its length and the vacant end of each of the capillary tubes was closed with a plastic seal to prevent the blood level from spilling. The tubes were placed in haematocrit centrifuge with the seal side towards the periphery and then centrifuged at 12,000 rpm for 5-6 minutes. Then, the tubes were taken out from the centrifuge and the percentage of Packed Cell Volume or haematocrit was read directly from haematocrit reader.

Data Analysis

The data was analysed using Statistical packages for Social Sciences (SSPS) 20th version, using Analysis of Variance (ANOVA)

DOI: 10.56892/bima.v8i2.686

and Duncan multiple range test (DMRT) at P<0.05 for significant level, results were presented as Mean ± Standard error of mean (SEM) and represented in Tables and chart.

RESULTS

The qualitative zoochemical content of millipede extract is presented in Table 1. The following bioactive chemicals including total phenols, alkaloids, tanin, saponin and flavonoids were present in the extract; while cardiac glycosides and reducing sugars were absent in the extract.

Table 1: Qualitative zoochemical constituents of methanol extract. of *Pachybolus ligulatus*

Zoochemical	Inference
Total phenol	+
Alkaloid	+
Tannins	+
Cardiac glycosides	-
Saponin	+
Flavonoid	+
Reducing sugars	-

Keys: '+'=present, '-'= absent.

In Table 2, the quantitative zoochemical analysis indicated that alkaloid was significantly highest (42.89±2.34), followed by Total phenol (3.90±0.16 g/100mg) and Flavonoid (3.81±0.87 g/100mg); while the least was recorded for saponnins (0.58±0.05 g/100mg).

Table 2: Quantitative zoochemical constituents of methanol extract of *Pachybolus ligulatus*

Zoochemicals	Content (g/100mg)
Total phenol	3.90 ± 0.16^{c}
Alkaloid	42.89 ± 2.34^{d}
Tannins	1.61 ± 0.02^{b}
Saponin	0.58 ± 0.05^{a}
Flavonoid	3.81 ± 0.87^{c}

Values with the same superscript on the column are not significantly different at P >0.05

Results of Extract Effect on Mice Morphology and Behavior

Mice show no signs or negative symptoms after 24 hours of continuous observation. Gross behavioral and physical observations revealed no involuntary urination, muscle weakness, convulsion or death. The animals were physically active. The acute toxicity of the millipede recorded no mortality up to dose of 6000 mg/kg b. wt. Thus, Lethal dose that could result in 50% mice mortality (LD_{50}) is above 6000 mg/kg b. wt. (Table 3).

Table 3: Acute toxicity of millipede (*Pachybolus ligulatus)* extract

Dose (mg/kg b. wt)	No of mice	Toxicity symptoms	mortality
1500	4	None	0
3000	4	None	0
6000	4	None	0

Antiplasmodial Study

Effect of methanol extract of millipede on parasitemia count

The results of average parasitaemia count of *Plasmodium berghei* infected mice treated with methanol extract of *Pachybolus ligulatus* is presented in Table 4. The result shows a progressive increase in the parasite load of the infected untreated group. However, the administration of the millipede extract and standard drug cause decrease in the parasitaemia. The extract with the highest dose (600 mg/kg body weight of the extract) shows a significant decrease in parasiteamia count, followed by the 300 mg/kg when compared to the negative control group.

Bima Journal of Science and Technology, Vol. 8(2) June, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i2.686

Table 4: Parasite count (per field) of *Plasmodium berghei* infected mice treated with methanol extract of millipede (*Pachybolus ligulatus*)

Treatment (mg/kg b wt)	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10
300	8.67±1.86 [b]	6.43±1.62 [a]	5.43±0.98 [a]	4.80±0.40 [a]	4.40±0.45 [a]	4.10±0.45 [a]	3.70±0.45 [a]	3.17±0.09 [a]
600	7.07±0.92 [b]	6.20±0.01 [a]	4.33±0.32 [a]	4.10±0.59 [a]	3.87±.47 [a]	3.07±0.47 [a]	2.37±0.67 [a]	2.27±0.15 [a]
Positive Control	15.25±1.25 [c]	12.80±1.20 [b]	11.30±1.00 [c]	8.00±1.00 [b]	5.90±0.40 [a]	4.35±0.35 [a]	3.00±0.50 [a]	2.00±0.00 [a]
Negative Control	5.10±2.18 [a]	6.87±2.14 [a]	7.67±0.89 [b]	10.17±2.59 [c]	19.80±4.10 [b]	24.33±3.28 [b]	26.00±2.52 [b]	29.33±2.19 [b]

Values are presented as mean ± SEM of 3 replicates.

Values with the same superscript on the same row are not significantly different at P >0.05

Effect of methanol extract of millipede on mice body weight

The results of average body weight change of *P. berghei* infected mice treated with methanol extract of *Pachybolus ligulatus* is presented in Figure 1. The result shows a progressive decrease in the weight in both infected untreated and extract treated mice. At the end of the experiment the weight recorded for the extract treated group was significantly higher than the negative control group. Only animal group treated with the standard drug significantly ameliorated the loss in weight induced by *Plasmodium* parasite invasion in the animal.

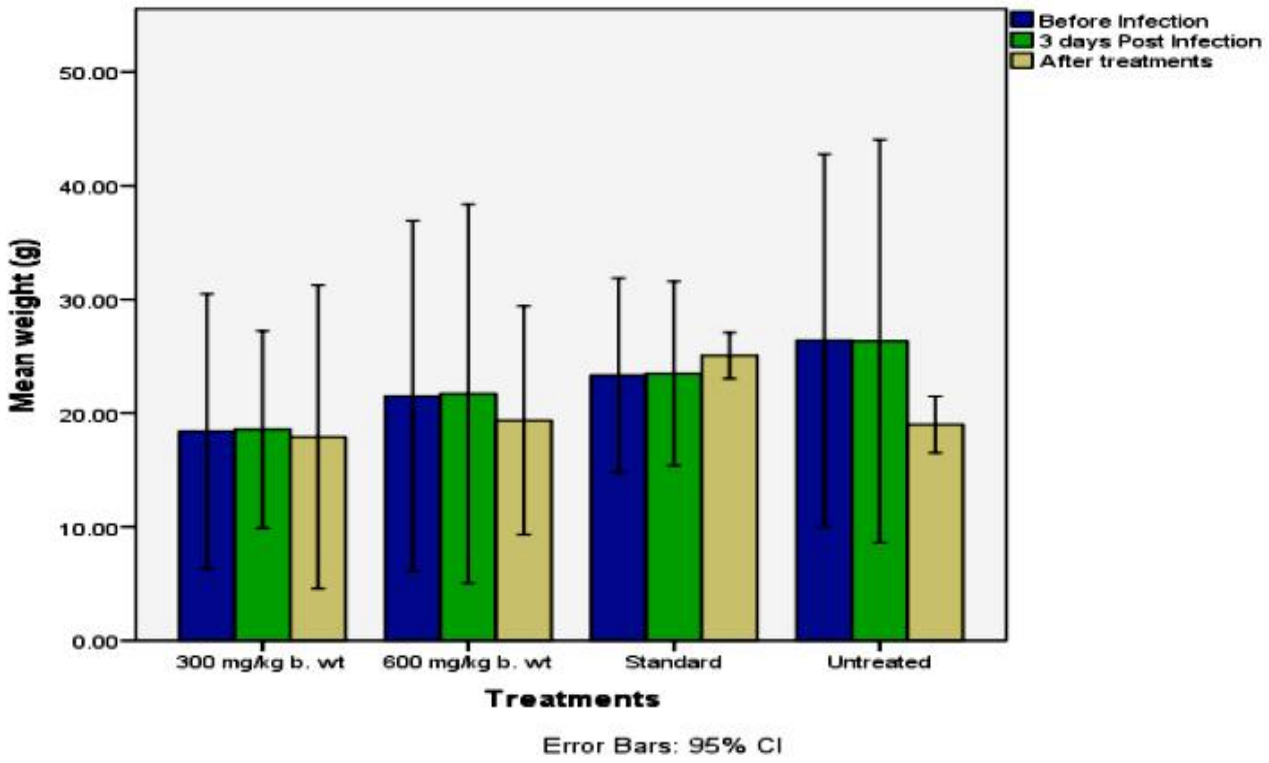

Figure 1: Mean of weight (g) of *P. berghei* infected mice treated with methanol extract of millipede

DOI: 10.56892/bima.v8i2.686

Effect of methanol extract of millipede on packed cell volume

The Packed Cell Volume test was used to measure the number of cells in the blood. There was decrease in the percentage of red blood cells after 3 days, 72 hours after inoculation, and after treatment. However, the PCV of untreated but infected group was significantly lower than those treated with the extract and standard drug groups (Figure 2).

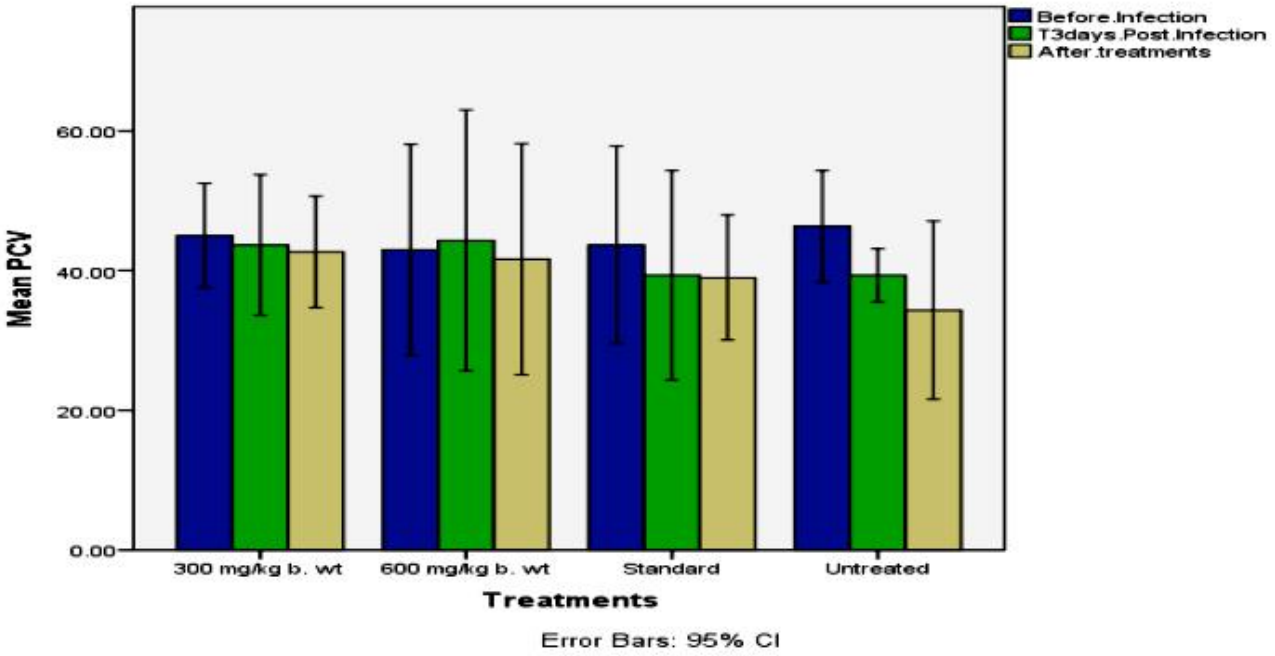

Figure 2. Mean Pack Cell Volume of *P. berghei* infected mice treated with methanol extract of millipede

Effect of methanol extract of millipede on mean survival time

The mice treated with the standard drug had a longer mean survival time compared to those treated with the millipede extract. The least mean survival time (days) was recorded for mice in infected untreated group (Table 5). The extract and standard drug prolonged the survival time (days) of the experimental mice when compared with the infected untreated groups.

Table 5: Mean survival time of mice of *P. berghei* infected mice treated with methanol extract of millipede

Sample	Survival time (days)
300mg/kg.b.wt	23.00 ± 1.53^{b}
600mg/kg.b.wt	23.33 ± 0.88^{b}
Standard	25.27 ± 0.27^{b}
Untreated	14.33 ± 0.67^{a}

Values are presented in mean ± SEM of 3 replicates

Values with the same superscript on the same row are not significantly different at P<0.05.

DISCUSSION

Studies on alternative antimalarial drugs are often traced from flora origin. The present study is therefore novel sourcing antiplasmodial medicine from arthropod (millipede) origin. The zoochemicals including total phenol, alkaloids, tannins, saponin and flavonoids recorded for the methanol extract of millipede in the current study is an indication that the animal possesses bioactive metabolites of health significance as in plants (Senguttuvan *et al.,* 2014; Kancherla *et al.,* 2019; Udu *et al.,* 2021). The presence of these important inherent zoobioactive metabolites in the

DOI: 10.56892/bima.v8i2.686

millipede methanol extract could be attributed to their breeding and possibly feeding behaviour on plant materials or immediate biome, which are rich sources of these constituents as either phytochemicals or zoochemicals (Hossain *et al.,* 2013). Besides, it is possible that they synthesis these biochemicals as chemical defense mechanisms through secondary metabolic activities (Arora *et al.,* 2023). The saponins and flavonoids are known to have antimalarial potency (Roberts *et al.,* 2012), hence, reduction in parasitemia level of mice treated with the millipede extracts in this study could be as a result of the presence of these zoochemicals. The presence of zoochemicals observed during qualitative screening for secondary metabolites suggests that *Pachybolus ligulatus* may exert some mechanisms that counter the pathological processes of *P. berghei* infection. In addition, secondary metabolites such as alkaloids have been shown to possess direct antiplasmodial effects (Bankole *et al.,* 2016). It is very important that the extract contain these active zoochemicals, accounting for their antiplasmodial effects *in vivo*.

The result of the toxicity test indicated that the methanol extract of the tested organism is very low with no mortality. The used doses of the used extract in the current study are thus safe for oral administration. This present observation is expected considering that the millipede is edible especially by the Bobo people of Burkina faso (Enghoff *et al.,* 2014). Sofowora, (1993), further explained that different extracts, from natural products, possess different levels of toxicity which majorly depends on the levels of toxico-metabolites inherent in them. The non-lethal effects produced with the high dose of this extract are an indication that the extract is relatively safe on acute oral exposure. It can therefore be concluded that millipedes are non-toxic in agreement with Nakatani *et al.* (2014), that any chemical substance with LD_{50} estimate greater than 3000-5000 mg kg-1 (oral route) could be considered of low toxicity and safe.

The current study indicated that the methanol extract showed a significant antiplasmodial effect on the test parasite. The reduction in the parasitemia level could be as a result of the presence of biologically active components such as the flavonoids and saponins. These bioactive antimalarial components have been reported to modify the essential proteins of *Plasmodium* species and inhibit parasite survival (White, 2004; Belete, 2020) and this work is a confirmation of reports who stated that zoochemicals such as benzoquinones and hydrogen cyanide in millipedes could exert antiplasmodial activities (Govinda *et al.,* 2011; Enghoff *et al.,* 2014; Nakatani *et al.,* 2014; Meyer-Rochow *et al.,* 2023; Siddiqui *et al.,* 2023).

The result of the PCV in the current study in the extract treated group compared to infected untreated group indicated that the milliped methanol extract was able to demonstrate a protective effect against the parasite-induced anaemic condition. This is an evidence and indicator that the millipede extract stimulates the erythropoietin release on the kidney, which is the humoral regulator of Red Blood Cell production (Meyer-Rochow, 2017; Bhoopalan *et al.,* 2020). Anaemia and body weight loss are the common features of *P. berghei* infected mice (Bhoopalan *et al.,* 2020). Thus, a good antimalaria agent is expected to prevent body weight loss in infected mice due to rise in parasitaemian and this is well observed in the effect of the millipede extract in the current study. Also, the extract was also observed to prolong the survival time (days) significantly compared to the infected untreated group. This indicates that the extract possesses some metabolites that are characterized with some immunomodulatory potentials that are capable of increasing the survival time (Nardos and Makonnen, 2017).

DOI: 10.56892/bima.v8i2.686

CONCLUSION

This study shows that millipede extract possesses bioactive metabolite of medical significance. The acute oral toxicity showed that the plant is safe for oral toxicity and exhibited antiplasmodial potency on the *Plasmodium berghei* infected mice. The extract ameliorated induced parasite loss in PCV and elongated the survival time of the mice. Finding from the study thus suggest more purification and exploitation of this fauna in the development of novel drug against malaria parasite.

REFERENCES

Abd Elkawy, M., Soliman, G., Abd-El E. and Abd-El R. (2013). Effect of *Solanum nigrum* Linn against Lambda Cyhalothrin-Induced Toxicity in Rats. *OSR-JPBS*, 5(5), 55-62

Adeniyi, K.A., Adesoye, O.A., Muzammil, W.S., Akinsete, I., Shuaibu, T. and Adeogun, A. (2023). Species Composition and Abundance of Indoor Adult Resting Mosquitoes in the Male Students' Hostel at Federal University. *Dujopas.*, 9(4a), 320-328

Adeogun, A., Babalola, A.S., Okoko, O.O., Oyeniyi, T., Omotayo, A., Izekor, R.T., Adetunji, O., Olakiigbe, A., Olagundoye, O., Adeleke, M., Ojianwuna, C., Adamu, D., Daskum, A., Musa, J., Sambo, O., Adedayo, O., Inyama, P.U., Samdi, L., Obembe, A., Dogara, M., Kennedy, P., Mohammed, S., Samuel, R., Amajoh, C., Adesola, M., Bala, M., Esema, M., Omo-Eboh, M., Sinka, M., Idowu, O.A., Ande, A., Olayemi, I., Yayo, A., Uhomoibhi, P., Awolola, S. and Salako, B. (2023). Spatial distribution and ecological niche modeling of geographical spread of Anopheles gambiae complex in Nigeria using real time data. *Sci Rep.,* 13(1), 13679. doi: 10.1038/s41598-023-40929-5.

Adesoye, O.A., Adeogun, A., Olagundoye, E., Oyeniyi, A.T., Izekor, T.R., Adetunji, O.O. (2023). Metabolic Resistance Mechanisms Evident in Generations of An. gambiae (Kisumu) Adults Exposed to Sub-lethal Concentrations of Permethrin Insecticide. *Pajols*., 7(3), 750– 750

Adigo, S.M., Fentahun, E. E, Mequanint, A.G. and Jemere A.T. (2021). Antimalarial Activity of Methanolic Extracts and Solvent Fractions of Combretum molle Leaves in *Plasmodium berghei* Infected Mice. *J Exp Pharmacol*., 13, 69-89. doi: 10.2147/JEP.S285117

Arora, S., Kataria, P., Nautiyal, M., Tuteja, I., Sharma, V., Ahmad, F., Haque, S., Shahwan, M., Capanoglu, E., Vashishth, R. and Gupta, A.K. (2023). Comprehensive Review on the Role of Plant Protein As a Possible Meat Analogue: Framing the Future of Meat. *ACS Omega*, 8(26),23305-23319. doi: 10.1021/acsomega.3c01373.

Autino, B., Noris, A., Russo, R. and Castelli, F. (2012). Epidemiology of malaria in endemic areas. *Mediterr J Hematol Infect Dis*., 4(1), e2012060. doi: 10.4084/MJHID.2012.060.

Bankole, A.E., Adekunle, A.A., Sowemimo, A.A., Umebese, C.E., Abiodun, O. and Gbotosho, G.O. (2016). Phytochemical screening and in vivo antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. *Parasitol Res*., 115(1), 299-305. doi: 10.1007/s00436-015-4747-x.

Barliana, M.I., Suradji, E.W., Abdulah, R., Diantini, A., Hatabu, T., Nakajima-Shimada, J., Subarnas, A. and Koyama, H. (2014). Antiplasmodial properties of kaempferol-3-O-rhamnoside isolated from the leaves of *Schima wallichii* against chloroquine-resistant *Plasmodium falciparum*. *Biomed Rep*., 2(4), 579-583. doi: 10.3892/br.2014.271

Belete, T.M. (2020). Recent Progress in the Development of New Antimalarial Drugs with Novel Targets. *Drug Des Devel Ther*.,

DOI: 10.56892/bima.v8i2.686

14, 3875-3889. doi: 10.2147/DDDT.S265602

Bhoopalan, S.V., Huang, L.J. and Weiss, M.J. (2020). Erythropoietin regulation of red blood cell production: from bench to bedside and back. *Faculty Rev*., 1153, 9 doi: 10.12688/f1000research.26648.1

Dejen, N., Sano, M., Mesfin, F., Tadesse, D. and Eyob, T. (2021). Antiplasmodial Activity of the Crude Extract and Solvent Fractions of Stem Barks of *Gardenia ternifolia* in *Plasmodium berghei*-Infected Mice. *Evidence-Based Complementary and Alternative Medicine*, 2021, 9625169. https://doi.org/10.1155/2021/9625169

Dufera, M., Dabsu, R. and Tiruneh, G. (2020). Assessment of malaria as a public health problem in and around Arjo Didhessa sugar cane plantation area, Western Ethiopia. *BMC Public Health*, 20(1), 655. doi: 10.1186/s12889-020-08784-5.

Duguma, T., Nuri, A. and Melaku, Y. (2022). Prevalence of Malaria and Associated Risk Factors among the Community of Mizan-Aman Town and Its Catchment Area in Southwest Ethiopia. *J Parasitol Res*., 2022, 3503317. doi: 10.1155/2022/3503317

Effiong, F.B., Makata, V.C., Elebesunu, E.E., Bassey, E.E., Salachi, K.I., Sagide, M.R., Abdulameed, H.T. and Uwishema, O. (2022). Prospects of malaria vaccination in Nigeria: Anticipated challenges and lessons from previous vaccination campaigns. *Ann Med Surg (Lond)*, 81, 104385. doi: 10.1016/j.amsu.2022.104385

Enghoff, H., Manno, N., Tchibozo, S., List, M., Schwarzinger, B. and Schoefberger, W. (2014). Millipedes as food for humans, their nutritional and possible antimalarial value: A first report. *Evidence-Based Comply Alter Med*: 2014, 651768.

Fentahun, S, Makonnen, E., Awas, T. and Giday, M. (2017). *In vivo* antimalarial activity of crude extracts and solvent fractions of leaves of Strychnos mitis in *Plasmodium berghei* infected mice. *BMC Complement Altern Med*., 17(1), 13. https://doi.org/10.1186/s12906-016-1529-7

Field Museum (2024). "Milli-PEET: Key to Millipede Orders". https://www.fieldmuseum.org/science/special-projects/milli-peet-class-diplopoda/milli-peet-millipedes-made-easy/milli-peet-key [Access 31/05/2024]

Geleta, G. and Ketema, T. (2016). Severe Malaria Associated with *Plasmodium falciparum* and *P. vivax* among Children in Pawe Hospital, Northwest Ethiopia. *Malar Res Treat*., 2016, 1240962. doi: 10.1155/2016/1240962

Govinda, H.V. and Asdaq, S.M. (2011) Immunomodulatory Potential of Methanol Extract of Aegle marmelos in Animals. *Indian J Pharm Sci*., 73(2), 235-40. doi: 10.4103/0250-474x.91571

Habte, G. and Assefa, S. (2020). *In Vivo* Antimalarial Activity of Crude Fruit Extract of Capsicum frutescens Var. Minima (Solanaceae) against *Plasmodium berghei*-Infected Mice. *Biomed Res Int.,* 2020, 1320952. doi: 10.1155/2020/1320952

Habte, G., Nedi, T. and Assefa, S. (2020). Antimalarial Activity of Aqueous and 80% Methanol Crude Seed Extracts and Solvent Fractions of Schinus molle Linnaeus (Anacardiaceae) in *Plasmodium berghei*-Infected Mice. *J Trop Med*., 2020, 9473250. doi: 10.1155/2020/9473250

Hossain, M.A., AL-Raqmi, K.A., AL-Mijizy, Z.H., Weli, A.M., Al-Riyami, Q. (2013). Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pac J Trop Biomed*., 3(9), 705-10. doi: 10.1016/S2221-1691(13)60142-2.

Kancherla, N., Dhakshinamoothi, A., Chitra, K. and Komaram, R.B. (2019). Preliminary Analysis of Phytoconstituents and

DOI: 10.56892/bima.v8i2.686

Evaluation of Anthelminthic Property of Cayratia auriculata (*In Vitro*). *Maedica (Bucur)*., (4), 350-356. doi: 10.26574/maedica.2019.14.4.350.

Khodzhaeva, N.M., Baranova, A.M. and Tokmalaev, A.K. (2019). The Immunological *Plasmodium falciparum* Malaria Characteristics of Children in Tajikistan Republic. J Trop Med., 5147252. https://doi.org/10.1155/2019/5147252

Long, C.A. and Zavala, F. (2017). Immune Responses in Malaria. *Cold Spring Harb Perspect*., 7(8): a025577. doi: 10.1101/cshperspect.a025577

Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of *Toxicology*, 54, 275-287.

Means, J., Francis, E., Lane, A. and Marek, P. (2015). A general methodology for collecting and preserving xystodesmid and other large millipedes for biodiversity research," *Biodiversity data journal*, 3, e5665, 2015. https://doi.org/10.3897/BDJ.3.e5665

Meyer-Rochow, V.B. (2017). Therapeutic arthropods and other, largely terrestrial, folk-medicinally important invertebrates: a comparative survey and review. *J Ethnobiology Ethnomedicine,* 13, 9. https://doi.org/10.1186/s13002-017-0136-0

Nakatani, K., Ishikawa, H., Aono, S. and Mizutani, Y. (2014). Identification of essential histidine residues involved in heme binding and Hemozoin formation in heme detoxification protein from Plasmodium falciparum. *Sci Rep*., 4, 6137. doi: 10.1038/srep06137.

Nakatani, K., Ishikawa, H., Aono, S. and Mizutani, Y. (2014). Identification of Essential Histidine Residues Involved in Heme Binding and Hemozoin Formation in Heme Detoxification Protein from *Plasmodium falciparum*. *National Academic Science*, 4, 6137.

Nardos, A, Makonnen, E. (2017). In vivo antiplasmodial activity and toxicological assessment of hydroethanolic crude extract of *Ajuga remota*. *Malar J.,* 16(1), 25. doi: 10.1186/s12936-017-1677-3

Naturalist. Key for the Identification of the Millipedes of the British Isles from Photographs," matthewvosper's Journal, https://www.inaturalist.org/posts/74349-key-for-the-identification-of-the-millipedes-of-the-british-isles-from-photographs [Accessed 31/05/2024]

Nishchal, B.S., Rai, S., Prabhu, M.N., Ullal, S.D., Rajeswari, S. and Gopalakrishna HN. (2014). Effect of Tribulus terrestris on Haloperidol-induced Catalepsy in Mice. *Indian J Pharm Sci.,* 76(6), 564-7.

Paduhilao J II. and Yap-Dejeto, L. (2022). Zoochemical Analyses and In vitro Antimicrobial Activity of Crude Methanolic Extract of *Perna viridis. ACTA Medical Phillipina*, 56(3), 82-88.

Penet, M. F., Kober, F., Confort-Gouny, S., Le Fur, Y., Dalmasso, C., Coltel, N. and Viola, A. (2007). Magnetic resonance spectroscopy reveals an impaired brain metabolic profile in mice resistant to cerebral malaria infected with *Plasmodium berghei* ANKA. *Journal of Biological Chemistry*, 282(19), 14505-14514.

Penet, M.F., Viola, A. and Confort-Gouny, S. (2015). Imaging experimental cerebral malaria *in vivo*: significant role of ischemic brain edema. *Journal of Neuroscience*, 25, 7352-7358.

Roberts, A. E., Maddocks, S. E., and Coopper, R. A. (2012). Manuka honey is bactericidal against Pseudomonas aeruginosa and results in differential expression of OprF and algD. *Microbiology*, 158(12), 3005-3013.

Sato S. (2021). Plasmodium-a brief introduction to the parasites causing

DOI: 10.56892/bima.v8i2.686

human malaria and their basic biology. *J Physiol Anthropol*., 40(1), 1. doi: 10.1186/s40101-020-00251-9.

Senguttuvan, J., Paulsamy, S. and Karthika, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for *in vitro* antioxidant activities. *Asian Pac J Trop Biomed*., 4(Suppl 1), S359-67. doi: 10.12980/APJTB.4.2014C1030

Siddiqui, S.A., Li, C., Aidoo, O.F., Fernando, I., Haddad, M.A., Pereira, J.A.M., Blinov, A., Golik, A., Câmara, J.S. (2023). Unravelling the potential of insects for medicinal purposes - A comprehensive review. *Heliyon*, 9(5), e15938. doi: 10.1016/j.heliyon.2023.e15938

Sinden, R. E. and Gilles, H. M. (2017). The malaria parasites. In Essential malariology (pp. 8-34). CRC Press.

Sofowora, J. E. A. (1993). *Medical Plants and Traditional Medicine in Africa*. Ibadan-Oweri-Kaduna-Lagos, Spectrum Bioles Limited, pp 159-176; 179-189;195-238.

Udu, R., Oyweri, J. and Gathirwa, J. (2021). Antimalarial Activity of Nigella sativa L. Seed Extracts and Selection of Resistance in *Plasmodium berghei* ANKA in a Mouse Model. *J Pathog.*, 2021,6165950. doi: 10.1155/2021/6165950

Upton, M. and Chapman, B. (2010). Methods for Collecting, Preserving and Studying Insects and Other Terrestrial Arthropods," Australian Entomological Society, 5th ed., ISBN: 978-0-646-54337-6, 2010.

Verra, F., Angheben, A., Martello, E., Giorli, G., Perandin, F. and Bisoffi Z. (2018). A systematic review of transfusion-transmitted malaria in non-endemic areas. *Malar J.*, 17(1), 36. doi: 10.1186/s12936-018-2181-0.

WHO, World Malaria Report 2021, World Health Organization, Geneva, Switzerland, 2021. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021 [Access: 30/05/2024]