



ANTIPLASMODIAL ACTIVITY OF ALKALOID FRACTION OF THE METHANOL ROOT EXTRACT OF *ANDROPOGON SCHIRENSIS* HOCHST (POACEA) IN *PLASMODIUM BERGHEI* INFECTED MICE

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ABSTRACT

Malaria is one of the major health problems in Nigeria and the increasing number of drug-resistant *Plasmodium* species continue to be a major concern. *Andropogon schirensis* is a perennial plant that is used traditionally in the treatment of malaria and dysentery. This study evaluated the *in-vivo* antiplasmodial activity of the alkaloid fraction of the methanol root extract of *Andropogon schirensis* in *Plasmodium berghei* infected mice. Gas chromatography-mass spectrometry analysis was carried out to identify the compounds present in the alkaloid fraction. Acute oral toxicity was conducted using OECD 425 guideline. The alkaloid fraction at tested doses of 250, 500 and 1000 mg/kg was evaluated for *in-vivo* antiplasmodial activity using suppressive, curative and prophylactic tests in *Plasmodium berghei* infected mice. Packed cell volume of each mouse was measured after the curative test and the mice were observed for twenty-eight days to determine the mean survival time. Gas chromatography-mass spectrometry analysis revealed the presence of seventeen bioactive compounds. The oral median lethal dose (LD₅₀) of the alkaloid fraction was estimated to be greater than 5,000 mg/kg. The alkaloid fraction at the tested doses of 250, 500 and 1,000 mg/kg showed significant ($p < 0.001$) parasitaemia suppression of 51.18, 72.25 and 72.90% respectively in the suppressive test and 61.63, 67.71 and 70.49% respectively in the curative test. The fraction prevented malaria induced changes in packed cell volume and the mean survival time of the mice at all the tested doses after the curative test was over twenty-eight days. The fraction showed significant ($p < 0.001$) parasitaemia suppression of 43.19, 49.50 and 65.61% respectively in the prophylactic test. The study showed that the alkaloid fraction has good suppressive and curative antiplasmodial activity with moderate prophylactic activity. These results suggest that the plant possesses antimalarial activity which may be due to the presence of bioactive compounds.

Keywords: *Andropogon schirensis*; Antiplasmodial; malaria; Parasitemia

INTRODUCTION

Malaria is a serious health condition caused mostly by *Plasmodium falciparum*, with children under the age of 5 and pregnant women being among the most vulnerable (WHO, 2023). Globally, 249 million new malaria cases and 608,000 deaths were reported in the year 2022, with Africa

accounting for 94% of global case and 95% global death (WHO, 2023). Malaria is endemic Nigeria and it is a leading cause of illness and death (CDC, 2024). Presently, Artemisinin based combination therapy (ACT) are mostly used for the treatment of malaria due to the emergence and spread of *Plasmodium falciparum* resistance to

sulphadoxine-pyrimethamine and chloroquine (Baird, 2004; WHO, 2023). However, there have been reported cases on the emergence and spread of *Plasmodium falciparum* resistance to ACT's (Arjen *et al.*, 2009; Abdissa *et al.*, 2018). It is therefore necessary to search for safe and effective novel antiparasmodial agents.

Medicinal plants have been used in the treatment of malaria in various parts of the world (Suleiman *et al.*, 2018). The bioactive components in medicinal plants need to be identified for scientific validation or discovery of lead compounds for therapeutic use (Vuorela *et al.*, 2004). Gas chromatography-mass spectrometry (GC-MS) is an important technique for the identification of bioactive constituents with high accuracy (Anekwe *et al.*, 2023). It involves separation of components from a mixture and analysis of each component individually (Gomathi *et al.*, 2015).

Andropogon schirensis Hochst. ex A. Rich., is a perennial plant belonging to the family Poaceae. It is called “Yambiu” or “Rumiya” or “Yaman gar gari” in Hausa language and “Lawrehe” or “Hahaendenoh” in Fulfulde language. It is up to 2 m height and grows on shallow soils over rocks or ironstone (Burkill 1985; Boisson *et al.*, 2016). The plant is found in Nigeria, Guinea, Senegal, Sierra Leone, Burkina Faso, Ivory Coast, Ghana, Togo, Burundi, Zimbabwe, Benin, Rwanda, and Cameroon (Hutchinson and Dalziel 1972). In the North-eastern part of Nigeria, the aqueous root extract of *Andropogon schirensis* is used traditionally in the treatment of malaria and dysentery (Adamu *et al.*, 2005). Previous study has reported the antibacterial activity of the aqueous root extract of the plant against *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Adamu *et al.*, 2005). This present study aims at evaluating the *in-vivo*

antiplasmodial activity of the alkaloid fraction of the methanol root extract of *Andropogon schirensis*.

MATERIALS AND METHODS

Collection of Plant Materials

The plant, *Andropogon schirensis* was collected from Guga Forest Reserve, Zaria, Kaduna State, Nigeria. It was identified by Dr. Namadi Sunusi, a Botanist at the Herbarium unit, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria, and voucher specimen number: ABU04634 given for further reference.

Parasite

Chloroquine-sensitive *Plasmodium berghei* (NK65) was obtained from National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

Animals

Swiss Albino mice (16–26 g) of both sexes were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, A.B.U., Zaria, Nigeria. The animals were kept in dry clean cages in a well-ventilated room and fed on standard rodent feed and water *ad libitum*. The animals were handled according to the standard set by the international guideline for animal care and use. Ethical approval for the study was obtained from the Ethical Committee on Animal Use and Care, Ahmadu Bello University, Zaria, Kaduna State, Nigeria with approval number: ABUCAUC/2023/014.

Preparation of Plant Extract

The roots of *Andropogon schirensis* were washed, air dried at room temperature and grounded into fine powder. About three kilograms of the powder was macerated with 70%^{v/v} methanol for five days with occasional shaking. The extract was filtered

and concentrated on a water bath at a temperature of 40°C to obtain a crude methanol root extract.

Preparation of Alkaloidal Fraction

A one-hundred (100 g) portion of the crude methanol root extract of the plant was dissolved in water and partitioned by mixing with diethyl ether in a separating funnel. The pigments and lipids were removed and the aqueous layer was made alkaline by the addition of dilute ammonia solution. Chloroform was then added and the content was shaken gently and allowed to separate. The lower chloroform layer was then run-off into another container and concentrated to dryness to give the alkaloid fraction (Evans, 2009).

The percentage yield of the alkaloid fraction was calculated as:

$$\% \text{ yield} = \frac{\text{weight of dried alkaloid fraction (g)} \times 100}{\text{weight of crude extract (g)}}$$

Gas Chromatography-mass spectrometry (GC-MS) Analysis

The analysis of the alkaloid fraction of the methanol root extract of *Andropogon schirensis* was carried out on a GC-MS equipment by Agilent 19091S-433UI in accordance to the protocol described by Ibrahim *et al.* (2023). The fraction was dissolved in a solvent, ratio of 1:10 v/v , then filtered with micro filter Nylon 0.45 μ m and about 2 μ l of the sample was transferred into a sample vial. It was then injected at 250°C in the injection port and splitters at a rate of 5:1 before reaching the Gas Chromatographic column which was conditioned at an Oven set initially at 70°C hold for 0mins at rate of 5°C/min raised to 250°C hold at 1min, then at rate of 30°C/min to 300°C and finally hold for 0min. The sample was volatilized and the relative quantity of the chemical compounds present in the fraction were expressed as a

percentage based on the peak area produced on the chromatogram.

Acute Toxicity Study

The median oral lethal dose (LD₅₀) of the alkaloid fraction of the methanol root extract of *Andropogon schirensis* was determined in accordance with OECD 425 guidelines (2001). The animals were monitored for signs of toxicity including weight loss and death for fourteen days.

Inoculation of *Plasmodium berghei* Parasite

Blood was collected into a heparinized capillary tube from a donor mouse previously infected with *Plasmodium berghei* (parasitaemia level of about 20-25%) through cardiac puncture and then transferred into a sterile plain bottle. Ten millilitres (10 ml) of normal saline were mixed with two millilitres (2 ml) of the blood, so that 0.2 ml will contain approximately 1X10⁷ infected RBC. Parasitaemia was induced by administration of 0.2 ml of infected erythrocytes into fresh mice and maintained by continuous re-infection of fresh mice intraperitoneally every 5-6 days. Percentage parasitaemia was calculated using the formula below:

$$\% \text{ Parasitemia} = \frac{\text{Number of infected RBC} \times 100}{\text{Total number of RBC}}$$

Suppressive test

Thirty (30) mice were weighed and randomly divided into 5 groups of 6 mice each. The mice were inoculated with 0.2 ml of infected erythrocytes containing approximately 1x10⁷ *Plasmodium berghei* via the intraperitoneal route. After 4 hrs, the mice in group 1 were treated with 10 ml/kg distilled water, groups 2, 3 and 4 received 250, 500 and 1,000 mg/kg of the alkaloid fraction respectively. Group 5 mice were treated with 5 mg/kg chloroquine. All doses were administered orally and the treatment

was repeated for 3 consecutive days. The level of parasitaemia was determined by preparing slides of thin films with blood obtained from the tail of each mouse. The films were allowed to dry and fixed with absolute methanol for ten minutes. The film was then stained with 3% Giemsa solution for 30 minutes. The slides were removed and dried overnight on a drying rack. One to two drops of immersion oil were placed on the area of blood film and 5 immersion fields were viewed under the microscope using x100 objective lens and the number of parasites were counted (Peters, 1975).

The percentage parasitaemia suppression was determined using the formula below:

$$\% \text{ Parasitemia Inhibition} = \frac{\text{MPC} - \text{MPT}}{\text{MPC}} \times 100$$

MPC = mean parasitaemia in control; MPT = mean parasitaemia in treated groups

Curative (established infection) Test

Thirty (30) mice were weighed and inoculated with 0.2 ml of infected erythrocytes containing approximately 1×10^7 *Plasmodium berghei* intraperitoneally. After 72 hrs, the mice were divided and treated (as described under suppressive test). The treatment was repeated for 3 consecutive days according to the method of Ryley and Peters (1970). Percentage parasitaemia level was determined as earlier described under suppressive test. In addition, the packed cell volume (PCV) of each mouse was measured after the curative test. Blood from the ophthalmic venous plexus was drawn and measurements were done as follows:

$$\text{PCV (\%)} = \frac{\text{Volume of erythrocyte in a given volume of blood}}{\text{Total volume of the blood}} \times 100$$

Mortality was monitored daily for a period of 28 days and the number of days from the time of inoculation of the parasite up to

death was recorded for each mouse in the treatment and control groups. The mean survival time (MST) for each group was calculated using the equation:

$$\text{MST} = \frac{\text{Sum of the survival times of all the mice in a group (days)}}{\text{Total number of mice in that group (n = 6)}}$$

Prophylactic test

Thirty (30) mice were weighed, randomly divided and treated (as described under suppressive test). The treatment was repeated for 3 consecutive days. On the 5th day, the mice were inoculated with 0.2 ml of infected erythrocytes containing approximately 1×10^7 *Plasmodium berghei* (*i.p*) and were left for 72 hrs (Peters, 1965). The percentage parasitaemia level was determined as earlier described under suppressive test.

Statistical analysis

The statistical analysis was done using SPSS software version 27. Data were presented as mean \pm SEM and analysed using one-way ANOVA, compared by Dunnett's *post hoc* test, with $p < 0.05$ considered statistically significant compared to the control.

RESULT

Yield of the Alkaloidal Fraction of the Methanol root Extract of *Andropogon schirensis*

Fractionation of 100 g of the methanol root extract of *Andropogon schirensis* yielded 3.74 g Alkaloid fraction and the percentage yield was 3.4%.

GC-MS Analysis of the Alkaloidal Fraction of *Andropogon schirensis*

A total of seventeen compounds were identified from the GC-MS analysis of the alkaloid fraction of *Andropogon schirensis* including two likely alkaloids: Acetylacetone, monoxime and Bicyclo[10.1.0]trideca-4,8-diene-13-carboxamide, N-(3-chlorophenyl)-, with 9-

Octadecenoic acid (Z)-, methyl ester having the highest peak area (Table 1). The reported

biological activities were presented in Table 2 and the chromatogram in Figure 1.

Table 1: Compounds found in Alkaloid Fraction of the Methanol Root Extract of *Andropogon schirensis*

| S/N | RT (min) | Compound | Molecular formula | Molecular weight (g/mol) | Peak area % |
|-----|----------|--|---|--------------------------|-------------|
| 1 | 13.20 | 11-(2-Cyclopenten-1-yl)undecanoic acid, (+)- | C ₁₆ H ₂₈ O ₂ | 252.39 | 0.29 |
| 2 | 13.37 | 2-Pentyn-1-ol | C ₅ H ₈ O | 84.12 | 0.11 |
| 3 | 14.51 | 7-Octenoic acid | C ₈ H ₁₄ O ₂ | 142.20 | 1.67 |
| 4 | 15.41 | Methyl 11-oxo-9-undecenoate | C ₁₂ H ₂₀ O ₃ | 212.28 | 0.17 |
| 5 | 17.34 | Acetylacetone, monooxime | C ₅ H ₉ NO ₂ | 115.13 | 0.82 |
| 6 | 18.00 | Bicyclo[10.1.0]trideca-4,8-diene-13-carboxamide, N-(3-chlorophenyl)- | C ₂₀ H ₂₄ ClNO | 329.90 | 1.12 |
| 7 | 18.19 | 3-Isopropyl-4-methyl-1-pentyn-3-ol | C ₉ H ₁₆ O | 140.22 | 0.77 |
| 8 | 18.56 | 1-(Methoxymethoxy)-3-methyl-3-hydroxybutane | C ₇ H ₁₆ O ₃ | 148.20 | 1.29 |
| 9 | 19.39 | 1,5-Heptadien-3-yne | C ₇ H ₈ | 92.14 | 4.90 |
| 10 | 20.31 | 1,5-Hexadiene, 2,5-dimethyl- | C ₈ H ₁₄ | 110.20 | 2.07 |
| 11 | 20.68 | Pentadecanoic acid, 14-methyl-, methyl ester | C ₁₇ H ₃₄ O ₂ | 270.50 | 18.40 |
| 12 | 22.12 | 1-Octyn-3-ol | C ₈ H ₁₄ O | 126.20 | 0.51 |
| 13 | 22.71 | Pentanoic acid | C ₅ H ₁₀ O ₂ | 102.13 | 12.42 |
| 14 | 23.97 | 9-Octadecenoic acid (Z)-, methyl ester | C ₁₉ H ₃₆ O ₂ | 296.50 | 37.30 |
| 15 | 24.56 | 7-Nonenoic acid, methyl ester | C ₁₀ H ₁₈ O ₂ | 170.25 | 3.56 |
| 16 | 25.88 | Cyclopentane, 1-methyl-2-(2-propenyl)-, trans- | C ₉ H ₁₆ | 124.22 | 14.01 |
| 17 | 31.35 | Pentafluoropropionic acid, octyl ester | C ₁₁ H ₁₇ F ₅ O ₂ | 276.24 | 0.59 |

Table 2: Reported Biological Activity of some of the Compounds found in Alkaloid Fraction of Methanol Root Extract of *Andropogon schirensis*

| Compound | Reported bioactivity |
|---|---|
| 11-(2-Cyclopenten-1-yl) undecanoic acid, (+)- | Antimicrobial, Anti-inflammatory and antinociceptive (Lima <i>et al.</i> , 2005; Sahoo <i>et al.</i> , 2014) |
| 7-Octenoic acid | Anticancer (Wisitpongpun <i>et al.</i> , 2020) |
| Methyl 11-oxo-9-undecenoate | Antibacterial, antibiofilm, antioxidant (Ghareeb <i>et al.</i> , 2022) |
| 3-Isopropyl-4-methyl-1-pentyn-3-ol | Antimicrobial, antioxidant (Saleh-e-In <i>et al.</i> , 2010) |
| 1-(Methoxymethoxy)-3-methyl-3-hydroxybutane | Antimalaria, antimicrobial, antioxidant, anticancer (Jha <i>et al.</i> , 2022) |
| 1,5-Heptadien-3-yne | Antibacterial (Idris <i>et al.</i> , 2019) |
| Pentadecanoic acid, 14-methyl-, methyl ester | Antioxidant, antifungal and antimicrobial (Han <i>et al.</i> , 2020) |
| 1-Octyn-3-ol | Antifungal, antibacterial (Ayyandurai <i>et al.</i> , 2022) |
| Pentanoic acid | Anticancer, sedative, anxiolytic, antidepressant, anti-alzheimer, hypotensive (Han <i>et al.</i> , 2020; Onyszkiewicz <i>et al.</i> , 2020; Khatkar <i>et al.</i> , 2021) |
| 9-Octadecenoic acid (Z)-, methyl ester | Anti-inflammatory, antiandrogenic cancer preventive, anemiagenic, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor (Krishnamoorthy and Subramaniam, 2014) |

Acute toxicity study

All the mice that were administered the alkaloid fraction of *Andropogon schirensis* orally at a dose of 5,000 mg/kg, did not show any signs of toxicity and death in the first four hours and after twenty-four hours of administration. All the mice survived during the two weeks observation period and there were no significant changes in their weights (Table 3). The oral median lethal dose (LD₅₀) in mice was estimated to be greater than 5,000 mg/kg.

Suppressive Activity of Alkaloid Fraction of Methanol Root Extract of *Andropogon Schirensis* in Mice Infected with *Plasmodium Berghei*

There was dose dependent and statistically significant ($p < 0.001$) reduction of parasitaemia in mice treated with the alkaloid fraction at doses of 250 mg/kg, 500 mg/kg and 1,000 mg/kg when compared with the negative control group (treated with distilled water). There was no significant difference in the reduction of parasitaemia level between chloroquine 5 mg/kg (80.76%) and the alkaloid fraction at 500 mg/kg (72.25%) and 1,000 mg/kg (72.9%). The highest percentage inhibition of the fraction was at the dose of 1,000 mg/kg (Table 4).

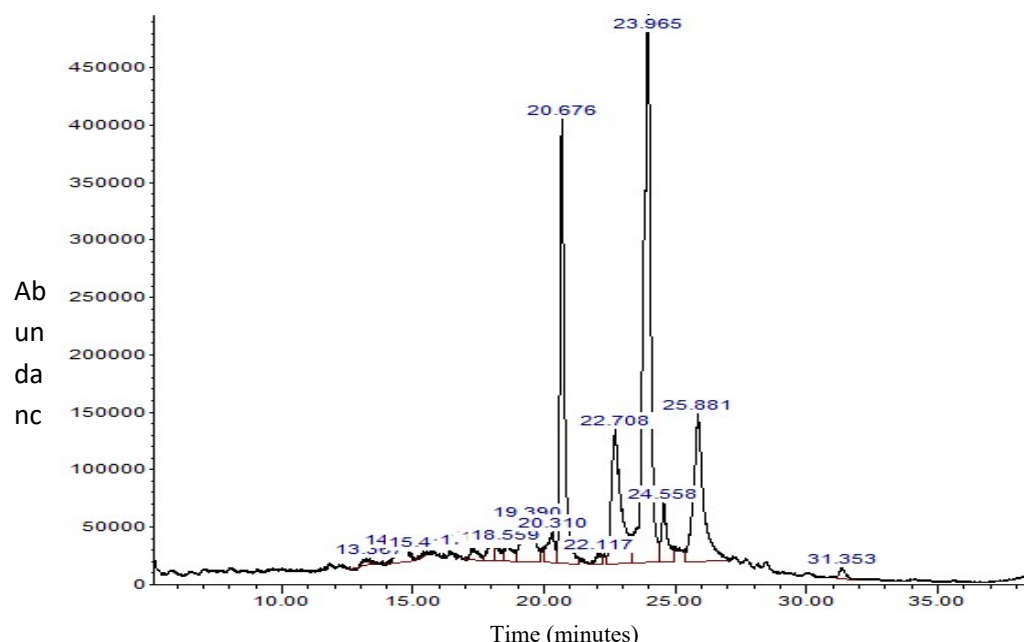


Figure: 1. GC-MS Chromatogram of Alkaloid Fraction of Methanol Root Extract of *Andropogon schirensis*

Table 3: Effect of Alkaloid Fraction of Methanol Root Extract of *Andropogon Schirensis* on Body Weight Changes After Oral Acute Toxicity Study in Mice

| Treatment at 5000 mg/kg | Body weight (g) | | | Mortality | Median oral lethal dose (LD ₅₀) (mg/kg) |
|-------------------------|-----------------|------------|------------|-----------|---|
| | Day 0 | Day 7 | Day 14 | | |
| AFAS | 22.00±3.51 | 20.67±2.67 | 21.67±3.17 | 0.00 | >5,000 |

Data presented as Mean ± SEM, n=3, AFAS= Alkaloid fraction of methanol root extract of *Andropogon schirensis*, route of administration=oral

Table 4: Effect of the Alkaloid Fraction of Methanol Root Extract of *Andropogon Schirensis* on Suppressive Activity in *Plasmodium Berghei* Infected Mice

| Treatment | Dose (mg/kg/day) | Mean Parasitaemia ± SEM | % Inhibition |
|-----------|------------------|-------------------------|--------------|
| DW | 10 ml/kg | 30.56 ± 2.20 | - |
| AFAS | 250 | 14.92 ± 0.68* | 51.18 |
| AFAS | 500 | 8.48± 1.10* | 72.25 |
| AFAS | 1000 | 8.28± 0.78* | 72.90 |
| CQ | 5 | 5.88± 1.06* | 80.76 |

Data presented as Mean ± SEM, Analysed by One-Way ANOVA followed by Dunnett's *post hoc* test, **p*<0.001, compared to the negative control, DW = Distilled water, AFAS= Alkaloid fraction of methanol root extract of *Andropogon schirensis*, CQ = Chloroquine, n=5, route of administration=oral, % = percentage

Curative Activity of Alkaloid Fraction of Methanol Root Extract of *Andropogon Schirensis* in Mice Infected with *Plasmodium Berghei*

The alkaloid fraction of methanol root extract of *Andropogon schirensis* produced a significant ($p<0.001$) dose dependent reduction in the levels of parasitaemia in mice when compared with the distilled water (negative control) group. However, the parasitaemia suppression of chloroquine was higher than that of the alkaloid fraction at all the tested doses (Table 5).

In addition, administration of the alkaloid fraction of methanol root extract of

Andropogon schirensis dose dependently prevented the reduction in PCV levels compared to the negative control group. There was significant difference in PCV at 500 and 1,000 mg/kg of the alkaloid fraction ($p<0.001$) and 5 mg/kg chloroquine ($p<0.001$), compared with the negative control group (Table 5).

Mice that were administered with the alkaloid fraction and chloroquine survived throughout the observation period of 28 days (Table 5). However, two mice that were given distilled water both died on the 17th day of the study.

Table 5: Effect of the Alkaloid Fraction of Methanol Root Extract of *Andropogon Schirensis* On Parasitaemia Level, Mean Survival Time and Packed Cell Volume (PCV) in *Plasmodium Berghei* Infected Mice Using Curative Model

| Treatment (mg/kg/day) | Mean Parasitemia ± SEM | % Inhibition | Mean survival time (days) | PCV (%) |
|-----------------------|------------------------|--------------|---------------------------|-------------|
| DW 10ml/kg | 23.04 ± 0.31 | - | 24.67±2.11 | 29.00±0.71 |
| AFAS 250 | 8.84± 0.58* | 61.63 | 28.00±0.00 | 36.60±1.83 |
| AFAS 500 | 7.44± 0.64* | 67.71 | 28.00±0.00 | 43.60±0.40* |
| AFAS 1000 | 6.80± 0.52* | 70.49 | 28.00±0.00 | 45.20±2.27* |
| CQ 5 | 2.44± 0.42* | 89.41 | 28.00±0.00 | 46.80±1.98* |

Data presented as Mean ± SEM, Analysed by One-Way ANOVA followed by Dunnett's *post hoc* test, * $p<0.001$, compared to the negative control, DW= Distilled water, AFAS= Alkaloid fraction of methanol root extract of *Andropogon schirensis*, CQ= Chloroquine, n=5, route of administration=oral, % = percentage

Prophylactic Activity of The Alkaloid Fraction of Methanol Root Extract of *Andropogon Schirensis* in Mice Infected with *Plasmodium Berghei*

The alkaloid fraction dose dependently and significantly ($p<0.001$) reduced the levels of parasitemia in mice when compared with the negative control group (Table 6).

DISCUSSION

Alkaloids are among the secondary metabolites that are found in plants which have been reported to have numerous biological activities such as antiplasmodial, anti-inflammatory, antibacterial, antifungal, antidiarrheal, antihelminthic, antitumor, antiparasitic, antiplatelet, antiviral, antidiabetic, neuroprotective and cardioprotective activities (Gutierrez-Grijalva *et al.*, 2020; Doctor and Manuel, 2014; Zhang *et al.*, 2021).

Table 6: Effect of the Alkaloid Fraction of Methanol Root Extract of *Andropogon Schirensis* on Prophylactic Activity in *Plasmodium Berghei* Infected Mice

| Treatment | Dose (mg/kg/day) | Mean Parasitaemia ± SEM | % Inhibition |
|-----------|------------------|-------------------------|--------------|
| DW | 10 ml/kg | 24.08± 0.34 | - |
| AFAS | 250 | 13.68± 0.19* | 43.19 |
| AFAS | 500 | 12.16± 0.61* | 49.50 |
| AFAS | 1000 | 8.28± 0.22* | 65.61 |
| PY | 1.2 | 5.32± 0.98* | 77.91 |

Data presented as Mean ± SEM, Analysed by One-Way ANOVA followed by Dunnett's *post hoc* test, * $p < 0.001$, compared to the negative control, DW= Distilled water, AFAS= Alkaloid fraction of methanol root extract of *Andropogon schirensis*, PY= Pyrimethamine, n=5, route of administration=oral, % = percentage

In the GC-MS analysis, the compound 9-Octadecenoic acid (Z)-, methyl ester, covered the highest percentage area of 37.30%, followed by Pentadecanoic acid, 14-methyl-, methyl ester, with 18.40%, indicating their dominance in the fraction. These compounds have been previously reported to have several pharmacological activities (Table 3). In addition, two likely alkaloids were identified and several studies have reported that alkaloids possess antiplasmodial activity (Grellier *et al.*, 1996; Campbell *et al.*, 1998; Addae-Kyereme *et al.*, 2001; Uzor, 2020). Moreover, the commonly available antimalarial drugs including artemisinin derived from *Artemisia annua* plant (Weathers *et al.*, 2011) and quinine derived from Cinchona tree (Achan *et al.*, 2011) are alkaloids. Thus, these compounds might be among the bioactive compounds that are responsible for the antimalarial activity of *Andropogon schirensis*.

The acute toxicity study of the alkaloid fraction of methanol root extract of *Andropogon schirensis* was conducted prior to antiplasmodial studies to determine the LD₅₀ and possible therapeutic doses to be employed in the main study. According to globally harmonised classification system (GHS) for toxicity, substance with LD₅₀ > 5,000 mg/kg are non-toxic and are categorised as class VI (Bernerjee *et al.*,

2024). Hence, the current finding showed that the LD₅₀ of the alkaloid fraction was above 5,000 mg/kg and non-toxic. In a recent study, Ibrahim *et al.* (2023) similarly reported the LD₅₀ of the methanol root extract of *Andropogon schirensis* to be above 5,000 mg/kg.

The *in-vivo* antiplasmodial study of the alkaloid fraction of methanol root extract of *Andropogon schirensis* provided evidence of its antimalarial potential. The extract showed good suppressive effect against early infection, curative effect against established infection and prophylactic effect against residual infection in *Plasmodium berghei* infected mice. In the suppressive test, the extract significantly reduced the level of parasitaemia, and the highest parasitaemia inhibition was observed at highest dose. Thus, the extract exerts an antimalarial activity in the early infection test in mice. A previous study classified *in-vivo* antimalarial activity as moderate, good, or very good if the percentage parasitaemia suppression is 50% or more at doses of 500, 250, and 100 mg/kg, respectively (Deharo *et al.*, 2001). Consequently, this study showed that the alkaloid fraction has good antimalarial activity in early infection. There was no significant distinction observed in the reduction of parasitaemia between chloroquine and the fraction at the two highest doses. This suggests that at these

dosage levels, the alkaloid fraction is equally effective as chloroquine in lowering parasitaemia levels during early infection caused by the plasmodium parasite.

In the curative test, the extract significantly reduced the level of parasitaemia, with the highest parasitaemia inhibition observed at the highest dose. Also, the fraction extended the mean survival time compared to the negative control group. In antimalarial studies, the ability of a compound to extend the survival time beyond 12 days is an indication of good parasite suppressing activity (Peter and Anantoli, 1998). Thus, suppression of parasitaemia and increase in survival time produced by the fraction are indicators of good antimalarial activity in established infection. High parasitaemia level in human and rodent malaria infection leads to reduction in PCV levels as a result of rapid destruction of parasitized and uninfected erythrocytes, suppression of erythropoietin, and dyserythropoiesis which may develop into severe anaemia (Lamikanra *et al.*, 2007; Jaureguiberry *et al.*, 2015). In this current study, the PCV level of all the mice administered the alkaloid fraction at all the tested doses, were higher than those in the negative control group. The antimalarial efficacy of the fraction was supported by its ability to prevent the reduction in PCV which is an indication of reduction in parasitaemia level. This showed that the extract is effective and has good antimalarial activity in an established infection. In the prophylactic test, the alkaloid fraction significantly reduced the level of parasitaemia, and the highest parasitaemia inhibition observed at the highest dose. This showed that the extract is effective and has moderate antimalarial activity against residual infection.

CONCLUSION

The study demonstrated that the alkaloid fraction extracted from the methanol root

extract of *Andropogon schirensis* exhibits good suppressive and curative antiplasmodial effect, along with moderate prophylactic activity. Analysis via GC-MS revealed the presence of bioactive compounds within the fraction, possessing therapeutic properties that likely contribute to the plants' antimalarial efficacy.

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