

Pharmacological Evaluation of Whole Plant Ethanol Extract of *Thesium viride* Against *Plasmodium berghei* and *Trypanosoma brucei* Inoculated into BALB/c Mice

Mustapha, F. J. ¹, Luka, S. A. ¹, Ella, E. E. ², Wada, Y. A. ^{1*}

¹Department of Zoology,
Faculty of Life Sciences,
Ahmadu Bello University,
Zaria

²Department of Microbiology,
Faculty of Life Sciences,
Ahmadu Bello University,
Zaria

* E-mail: yunuwad@yahoo.com ; jummykale@gmail.com

Abstract

Malaria and African trypanosomiasis are major public health issues in Africa, and urgent efforts are needed to identify new anti-protozoal drugs with high effectiveness and low toxicity. This study was designed to determine the pharmacological activities of whole plant ethanol extract of *Thesium viride* on a *Plasmodium berghei* and *Trypanosoma brucei* in BALB/c mice model. The whole dried plant sample was collected from Zaria Local Government Area in Kaduna State, and the plant was processed according to standard methods. Forty (40) adult BALB/c mice (*Mus musculus*) were randomly allocated to two major groups, A and B, to determine the anti-plasmodial and anti-trypanosomal activities of the plant extract, respectively. In group A, each mouse in subgroups I to V was inoculated with 0.1 mL containing 10^6 *P. berghei* merozoites. In group B, each mouse in subgroups I to V was inoculated with 0.1 mL containing 10^6 *T. brucei* trypomastigotes. Following patency of 3 days, all mice in groups A and B were either non-treated, treated with standard drug or treated at varying dosages of the ethanol extract for four days. Parasitaemia level, packed cell volume, rectal temperature, and body weight of the experimental mice were measured. Results generated indicate established that whole plant ethanol extract of *Thesium viride* showed a significant ($p < 0.01$) suppressive ability against *Plasmodium berghei* in all the mice administered 200, 400 and 800 mg/kg/day, compared to *Trypanosoma brucei* in mice administered similar dosages. Additionally, the extract showed better anti-weight loss ability in *Plasmodium berghei*-infected mice compared to *Trypanosoma brucei*-infected mice. The study concludes that the ethanol extract of *Thesium viride* has anti-protozoal prospects and should be explored for further pharmacological investigation.

Keywords: *Thesium viride*, Malaria, African trypanosomiasis, Mouse model, Ethanol extract

*Author for Correspondence

INTRODUCTION

Malaria parasites (*Plasmodium* sp.) and African trypanosomes (*Trypanosoma* sp.) cause major morbidity and mortality in humans, animals, and wildlife (Kotepui *et al.*, 2021; Sanches-Vaz *et al.*, 2019; Ungogo *et al.*, 2020). They have detrimental public health and economic effects in developing countries, especially in Nigeria (Ungogo *et al.*, 2020). To treat malaria or trypanosomiasis, chemoprophylactic or chemotherapeutic medicines are commonly utilized. Drug shortages, drug resistance, high costs, unpleasant side effects, and toxicity make treating malaria and African trypanosomiasis difficult (Matovu *et al.*, 2001; Toya, 2010; Barrett *et al.*, 2011). Drug resistance is a major concern that threatens to undermine global efforts to control or eradicate malaria and African sleeping sickness (Kotepui *et al.*, 2021; Sanches-Vaz *et al.*, 2019; Ungogo *et al.*, 2020; Yun *et al.*, 2021). The progress of multidrug resistance is an alarming feature that further hampers an effective control strategy (Ungogo *et al.*, 2020). Additionally, fast access to safe and effective pharmaceuticals is the primary concern of the general public, patients, and customers (Ayawa *et al.*, 2021).

Considering this growing problem, urgent efforts are needed to identify new anti-protozoal drugs with high effectiveness and low toxicity for treating these parasitic protozoans. As a result, it's critical to look for chemotherapeutic drugs that are less expensive, more effective, more readily available, and less toxic to treat the condition. Medicinal plants are an important part of Africa's traditional healthcare system (Mahomoodally, 2013). They include chemicals that can be employed for medicinal purposes or serve as templates in synthesizing beneficial pharmaceuticals in some or all of their parts (Sofowora *et al.*, 2013). In Nigeria, *Thesium viride* is called "Huntu" by the Hausa ethnic group (Moore *et al.*, 2010). The plants have been proven traditionally effective against several ailments (Bosch, 2008). The leaves are boiled in water as a decoction to treat jaundice and fever and as a laxative for intestinal worms (Belakhdar *et al.*, 2014). The extract and fractions of *T. viride* have been reported to demonstrate antimicrobial, antibacterial, and antiulcer and protect and improve the liver's antioxidant enzymes against tetrachloromethane (CCl₄)-induced liver damage (Kamaruding *et al.*, 2020; Shehu *et al.*, 2016, 2022). *Thesium viride* has interesting pharmacological prospects yet to be explore. Hence, there is a need to scientifically explore the prospect of whole plant extract of *Thesium viride* as a remedy for treating malaria or trypanosomiasis using mouse models. Hence, this study was conducted to evaluate the pharmacological activities of whole plant ethanol extract of *Thesium viride* on a *Plasmodium berghei* and *Trypanosoma brucei* BALB/c mice model.

MATERIALS AND METHODS

Ethical Consideration

The study protocol and permission to conduct the study were obtained with the approval number ABUCAUC/2021/078 from the Ethical Committee on Animal Use and Care at Ahmadu Bello University in Zaria, Nigeria.

Study Location

The research was conducted in the Parasitology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University (ABU) in Zaria, Nigeria.

Collection and Identification of the Plant Material

The whole dried plant (2000 g) sample of *T. viride* was collected from Zaria Local Government Area in Kaduna State, Nigeria, around February 2020. The Taxonomist identified the plant at the herbarium section of the Department of Botany, ABU, Zaria (voucher number ABU06986).

Preparation of Extract

The dried powder plant (400 g) was macerated with 1200 mL of aqueous ethanol (70% v/v) for 72 hours at room temperature and filtered. The filtrate obtained was concentrated in a water bath (75 °C) and allowed to dry at room temperature. The extract was collected and transferred to an evaporating dish. As done by Ayawa *et al.* (2021), it was subsequently placed in a water bath to allow the methanol to evaporate (at 100 °C) so as to obtain a concentrated extract.

Acute Oral Toxicity Study

Acute toxicity studies were carried out on BALB/c mice weighing 17–23 g and maintained in standard laboratory conditions following recommendations from the Organization for Economic Cooperation and Development (OECD) (423; OECD guideline, 2002). The LD₅₀ value was calculated using the equation:

$$LD_{50} = \sqrt{D0 \times D100}$$

where D0 = the highest dose that gave no mortality

D100 = lowest dose that produced no mortality

Experimental Animal

Forty (40) healthy adult male and female mice weighing 17 to 23g were obtained from the animal house, Department of Physiology, Faculty of Medicine, ABU, Zaria, Nigeria. They were allowed to acclimatize for two weeks in the animal house, Department of Veterinary Parasitology and Entomology, ABU in Zaria, Nigeria. They were housed in clean plastic cages with wood shavings as bedding, which were changed twice a week. The mice were fed standard feed and access to clean water *ad libitum*.

Experimental Parasites

Plasmodium berghei (NK45) employed in this study was sourced from the Nigerian Institute of Medical Research, Lagos state, Nigeria. The parasites were maintained in rats by continuous passage and transported by road to Zaria, Kaduna state.

Trypanosoma brucei used for this experiment was obtained from the Department of Veterinary Parasitology and Entomology, ABU, Zaria. The parasites were maintained in the experimental mice by continuous passage. Each cycle of passage was done when parasitaemia was in the range of 35 to 40 parasites per field of a prepared wet mount.

Experimental Design, Parasite Inoculation and Treatment

Following toxicity studies and establishing safe lethal doses for ethanol extract, the experimental design was set up in a simple complete randomized design (CRD). Forty (40) adult BALB/c mice (*Mus musculus*) of both sexes weighing 17-23g were randomly allocated to two major groups, A and B, of twenty (20) mice each. Mice in group A were further subdivided into five groups I (negative control), II (positive control), III (E200 mg/kg), IV (E400 mg/kg), and V (E800 mg/kg) in a simple complete randomized design to determine the anti-plasmodial activity of the ethanol extract of *Thesium viride*, on *Plasmodium berghei*-infected

mice (Figure 1A). Each mouse in groups I to V was inoculated with 0.1 mL containing 10^6 *P. berghei* merozoites. Following patency of 3 days, mice in groups I, II, III, IV, and V were either treated with a normal saline (NC-negative control), treated with standard drug (PC-positive control, Chloroquine 25 mg/Kg) or treated at varying dosages of ethanol extracts III (E200 mg/kg), IV (E400 mg/kg), and V (E800 mg/kg) (Figure 1A).

Mice in group B were also subdivided into five groups I (negative control), II (positive control), III (E200 mg/kg), IV (E400 mg/kg), and V (E800 mg/kg) in a simple complete randomized design to determine the anti-trypanosomal activity of the ethanol extract of *Thesium viride*, on *Trypanosoma brucei*-infected mice (Figure 1B). Each mouse in groups I to V was inoculated with 0.1 mL containing 10^6 *T. brucei* trypomastigotes. Following patency of 3 days, mice in groups I, II, III, IV, and V were either treated with a normal saline (NC-negative control), treated with standard drug (PC-positive control, Diminazene aceturate 3.5 mg/kg) or treated at varying dosages of ethanol extracts III (E200 mg/kg), IV (E400 mg/kg), and V (E800 mg/kg) (Figure 1B).

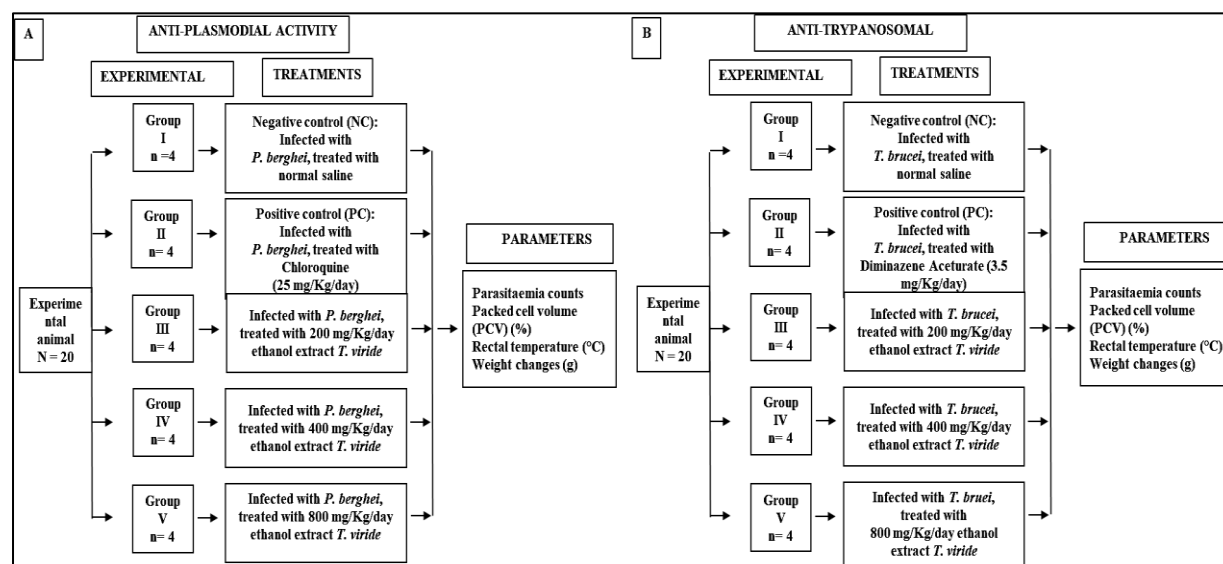


Figure 1: Simple complete randomized experimental design to assess the effect of ethanol whole plant extract against (A)- *Plasmodium berghei* (B)- *Trypanosoma brucei* in a mouse model

Pharmacological Evaluation of Extracts

Observation of clinical signs

Clinical signs investigated during the study include rectal temperature, feed intake, body condition, weakness and dullness, rough hair coat and weight changes, as described by Ayawa *et al.* (2021).

Determination of parasitaemia: *Plasmodium berghei*

Thin smears of blood from *Plasmodium berghei*-infected mice were made from the tail of each mouse. The smears were fixed with absolute methanol and stained with 10% Giemsa's stain at pH 7.2 for 15 min. The stained slides were washed gently using distilled water and were air-dried at room temperature. Two stained slides for each mouse were examined under

a light microscope using a ×100 objective lens with oil immersion. Ten different fields on each slide were examined at random, and the average percentage parasitaemia was determined by counting the number of parasitised RBCs out of 500 erythrocytes and thus calculated using the formula reported by Mekonnen (2015):

$$\% \text{ Parasitaemia} = \frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \times 100$$

Determination of parasitaemia: *Trypanosoma brucei*

Parasitaemia was monitored in blood obtained from the tail of *Trypanosoma brucei*-infected mice. A wet film mount was prepared daily, covered with a coverslip, and slides were examined under a × 40 objective lens, and parasites were counted. The number of fields counted depends on the abundance of parasites per field. The sample was considered negative if no parasite was found in 20 fields. The number of trypanosomes per ml of blood was counted microscopically at × 400 magnification under a compound microscope (Herbert and Lumsden, 1976).

Evaluation of Packed Cell Volume

Heparinised capillary tubes were $\frac{3}{4}$ filled with blood by capillary action. Excess blood was cleaned with a ball of cotton wool. After that, the dry end of the tube was sealed with a Bunsen flame. It was centrifuged using a microhaematocrit centrifuge for four minutes at 12,000 revolutions per minute (rpm) for five minutes. The spun tubes were after that read and expressed as a percentage with a microhematocrit reader, as described by Ochei and Kolhatkhar (2000).

Determination of Rectal Temperature

The rectal temperature for each experimental animal was assessed daily in the morning between 7.00 am - 8.00 am using a clinical digital thermometer (KRIS-ALOY CE 0197). The thermometer was inserted into the rectum and tilted to touch the rectal mucosa. After a beep, the thermometer was removed, and the body temperature changes were read and recorded in degrees centigrade (°C).

Determination of Live Body Weight

The live body weight of mice was measured using a sensitive digital weighing balance throughout the study to monitor the variation in weight in gram (g).

Data Analyses

For each experimental setup, descriptive analyses of pre-treatment and post-treatment data on clinical parameters, parasitaemia, packed cell volume, rectal temperature, and weight changes were presented as means with standard errors (SE) with bar charts. Student paired t-Test was used to compare the pre-treatment and post-treatment means clinical parameters with each treatment. One-way analysis of variance was used to compare the clinical indices across the treatments. The Tukey post-hoc test was used to determine whether any group means or set of treatment conditions significantly differed from each other, with a significance level of $p= 0.05$. Statistical analysis was conducted with Microsoft Excel 2019 and the IBM SPSS statistical software version 20.

RESULTS AND DISCUSSION

All mice given lower and higher dosages of *Thesium viride* ethanol extracts displayed no toxic effects; their behavioural responses were normal, and there were no reported fatalities. As a result, the median lethal dose was calculated to be greater than 5000 mg/kg. According to Loomis and Hayes' (1996) toxicity categorization, the extract is classified as non-toxic and has a high safety level when supplied orally to mice. This is in similar with the findings of Shehu *et al.* (2017) for an oral administration of an ethanol extract of *Thesium viride* in rats.

Following inoculation of the parasites and the three days pre-treatment period, all infected mice developed acute malaria and trypanosomiasis three days after infection, with clinical signs and symptoms ranging from intermittent pyrexia, weakness, lethargy, dull and rough coats, slight weight losses, and reduced packed cell volumes, which are the classical symptoms reported by various researchers in *Plasmodium* and *Trypanosoma* sp. infections in animals (Oluyemi *et al.*, 2020; Ungogo *et al.*, 2020; Ayawa *et al.*, 2021). Figure 2 shows the effect of a crude ethanol extract of *Thesium viride* on *Plasmodium berghei* and *Trypanosoma brucei* parasitaemia in mice. For the anti-plasmodial activity, the percent parasitaemia suppression following the four days of treatment was highly significant ($p < 0.01$) for the positive control (-63.94%), E400 mg/kg (-28.00%), E800 mg/kg (-17.48%), and E200 mg/kg (-15.25%) in comparison to the negative control, which showed an increase in parasitaemia level by 156.95% (Figure 2A). In terms of anti-trypanosomal activity, the positive control resulted in a significant ($p < 0.01$) percent reduction in parasitaemia (-77.45%), followed by extract doses of E400 mg/kg (-12.63%) and E800 mg/kg (-7.25%). Compared to the negative control (27.21%), no parasite suppressions were observed with E200 mg/kg (2.63%) (Figure 2B). Figure 3 shows the comparative mean percent change in parasitaemia level of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated with a crude ethanol extract of *Thesium viride* was significantly higher ($p < 0.01$) for *Plasmodium berghei* in all mice administered 200 mg/kg/day (-15.25%), 400 mg/kg/day (-28.00%) and 800 mg/kg/day (-17.48%), compared to its activity against *Trypanosoma brucei* in mice administered 200 mg/kg/day (2.63%), 400 mg/kg/day (-12.63%) and 800 mg/kg/day (-7.25%), respectively (Figure 3).

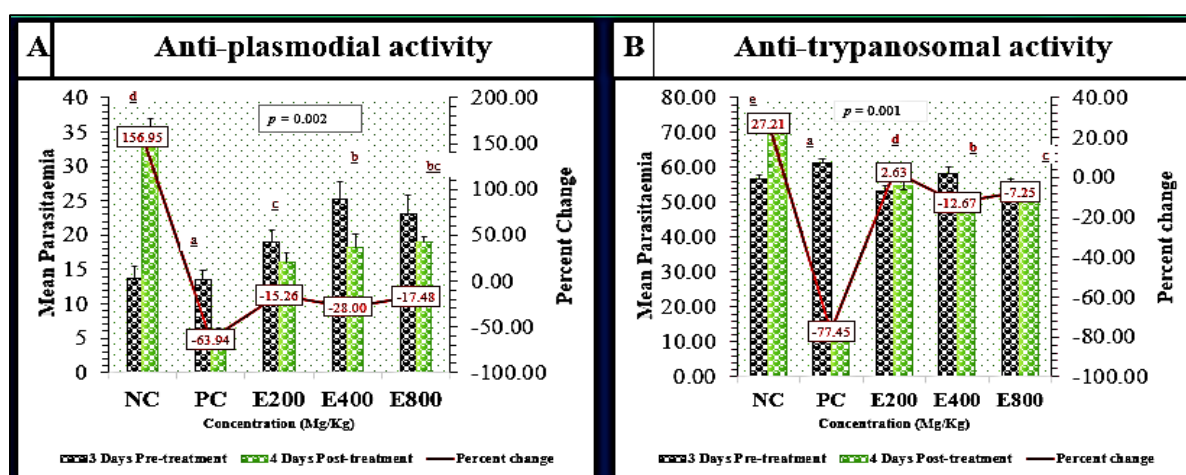


Figure 2: Effect of a crude ethanol extract of *Thesium viride* on the parasitaemia level of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), E = Crude Ethanol Extract.

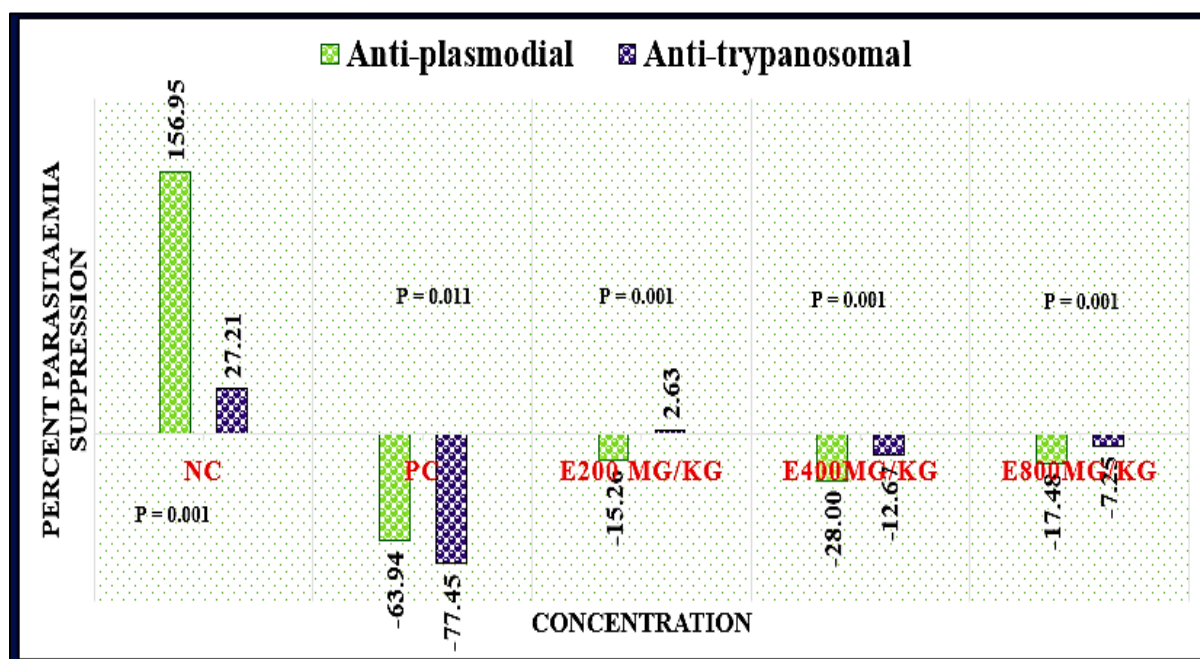


Figure 3: Student-t test for comparing the mean percent change in the parasitaemia level of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with crude ethanol extract *Thesium viride*. NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), E = Crude Ethanol Extract.

The non-clearance of parasitaemia completely, as observed for all the positive controls in all the treatment groups, may be attributed to the short duration of the treatment period, which was not enough for the treatment regime to be completed. Furthermore, the inability of the plant extract in this study to remove the parasite from the blood could very well be explained by the oral route of administration of the plant extract, as the active chemicals in the extracts may not have reached the site of action or undergone rapid metabolism within the host (Wurochekke *et al.*, 2004, 2005). It is also likely that the secondary metabolites in the extracts underwent biotransformation in the liver and gastrointestinal tract, preventing the parasites from being completely removed (Ayawa *et al.*, 2021). Generally, in this study, parasitemia significantly reduced, especially in all groups that administered the ethanol extracts against *Plasmodium berghei* compared to *Trypanosoma brucei*. This implies that the whole plant ethanol extracts of *Thesium viride* displayed better suppressive activity against *Plasmodium berghei* than *Trypanosoma brucei* in the mouse model. Interestingly, this is the first pharmacological study of the *Thesium viride* whole plant extract against *Plasmodium berghei* and *Trypanosoma brucei* in mice model.

Figure 4 shows the effect of a crude ethanol extract of *Thesium viride* on the packed cell volume (PCV) of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. In the anti-plasmodial and anti-trypanosomal activities of the crude extract, there was a decrease in the PCV across all the doses administered. However, a student t-test paired analysis for each of the paired doses revealed a non-significant difference ($p > 0.05$) between the pre-treatment and post-treatment PCV values, except for the E200 mg/kg dosage administered against *Trypanosoma brucei*, which was statistically significant ($p < 0.05$) (Figure 4B).

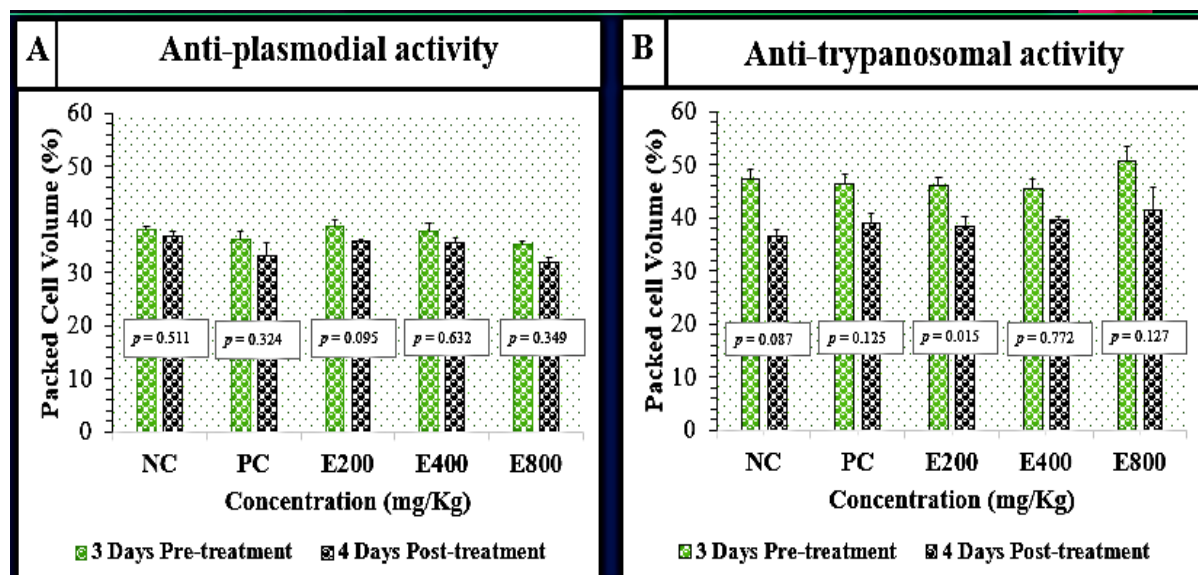


Figure 4: Effect of a crude ethanol extract of *Thesium viride* on the packed cell volumes (PCVs) of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), E = Crude Ethanol Extract

Table 1 shows the mean percent change in packed cell volume (PCV) of *Plasmodium berghei* or *Trypanosoma brucei*-infected mice treated daily for four days with a crude ethanol extract of *Thesium viride*. After a four-day treatment with ethanol fractions of whole plant extract of *Thesium viride* at 200, 400, and 800 mg/kg/day, there was a 6.88, 6.01, and 9.28% decrease in PCVs of *Plasmodium berghei*-infected mice. Similar trends were observed with *Trypanosoma brucei*-infected mice. However, the percent decrease in PCV was higher at doses of 200 mg/kg/day (16.67%), 400 mg/kg/day (13.16%), and 800 mg/kg/day (18.31%) in comparison to the values obtained for *Plasmodium berghei* (Table 1).

Table 1: Mean percent change in packed cell volume (PCV) of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with crude ethanol extract of *Thesium viride*

| Pathogen | Concentration | 3 Days Pre-treatment | 4 Days Post-treatment | Percent change |
|---------------------------|---------------|----------------------|-----------------------|----------------|
| <i>Plasmodium berghei</i> | NC | 38.00±0.71 | 36.93±0.81 | -2.82 |
| | PC | 36.25±1.32 | 33.15±2.41 | -8.55 |
| | E200 (mg/Kg) | 38.50±1.26 | 35.85±0.28 | -6.88 |
| | E400 (mg/Kg) | 37.75±1.49 | 35.48±1.11 | -6.01 |
| | E800 (mg/Kg) | 35.25±0.75 | 31.98±0.93 | -9.28 |
| <i>Trypanosoma brucei</i> | NC | 47.20±2.08 | 36.40±1.36 | -22.88 |
| | PC | 46.40±1.72 | 39.00±1.98 | -15.95 |
| | E200 (mg/Kg) | 46.20±1.36 | 38.50±1.85 | -16.67 |
| | E400 (mg/Kg) | 45.60±1.57 | 39.60±0.68 | -13.16 |
| | E800 (mg/Kg) | 50.80±2.76 | 41.50±4.21 | -18.31 |

NC = Negative Control (Normal saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene aceturate 3.5 mg/Kg), E = Crude ethanol extract

None of the dosages of the extract demonstrated increased PCV. The reduced values of the PCVs observed for *Plasmodium berghei* and *Trypanosoma brucei*-infected mice may be attributed to immune-mediated hemolysis due to high parasitaemic load, leading to the destruction of erythrocytes by parasites (Chamond *et al.*, 2010), which will lead to acute anaemia, a characteristic feature of malaria and African trypanosomiasis, indicating the severity of both diseases as reported by Wada *et al.* (2016a, 2016b). The inability of the whole plant extract of *Thesium viride* to arrest the decrease in PCV implies that they have little or no haematinic activity against *Plasmodium* and *Trypanosoma* parasites in mice.

Figure 5 shows the effect of a crude ethanol extract of *Thesium viride* on the rectal temperature of *Plasmodium berghei* or *Trypanosoma brucei*-infected mice. In anti-plasmodial and anti-trypanosomal activities of the crude extract, there was a general decrease in the rectal temperatures between the pre-treatment and post-treatment values across all the doses administered, except for the negative control for *Trypanosoma brucei* (Figure 5B). The mean percent change in rectal temperature of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with a crude ethanol extract of *Thesium viride* is presented in Table 2. Following a four-day treatment at doses of 200, 400, and 800 mg/kg/day, a percent decrease in rectal temperature was observed from the pre-treatment values of 2.98, 2.31, and 1.81%, respectively, for *Plasmodium berghei* infection. While for the anti-trypanosomal activity of *Trypanosoma brucei*, a percent decrease of 2.11, 3.15, and 4.07% was observed at 200, 400, and 800 mg/kg/day doses, respectively (Table 2).

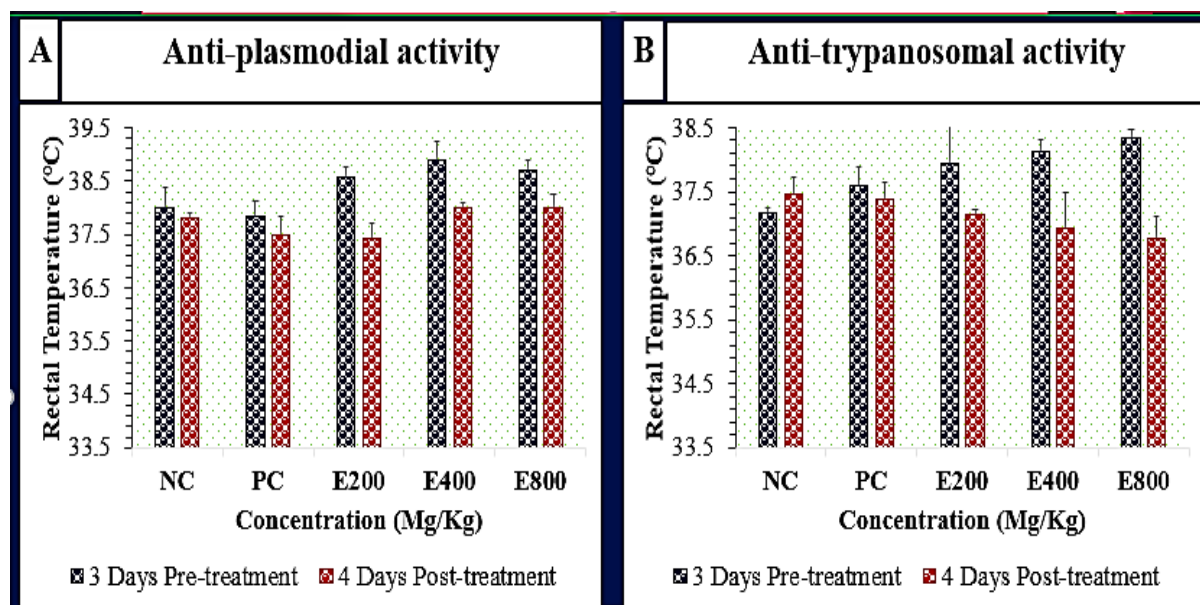


Figure 5: Effect of a crude ethanol extract of *Thesium viride* on the rectal temperature of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), E = Crude Ethanol Extract

Table 2: Mean percent change in rectal temperature of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with crude ethanol extract of *Thesium viride*.

| Pathogen | Concentration | 3 Days Pre-treatment | 4 Days Post-treatment | Percent change |
|---------------------------|---------------|----------------------|-----------------------|----------------|
| <i>Plasmodium berghei</i> | NC | 38.00±0.37 | 37.80±0.10 | -0.53 |
| | PC | 37.85±0.26 | 37.50±0.33 | -0.92 |
| | E200 (mg/Kg) | 38.58±0.19 | 37.43±0.27 | -2.98 |
| | E400 (mg/Kg) | 38.90±0.33 | 38.00±0.11 | -2.31 |
| | E800 (mg/Kg) | 38.70±0.20 | 38.00±0.24 | -1.81 |
| <i>Trypanosoma brucei</i> | NC | 37.17±0.09 | 37.47±0.26 | 0.81 |
| | PC | 37.60±0.27 | 37.38±0.28 | -0.59 |
| | E200 (mg/Kg) | 37.93±0.72 | 37.13±0.89 | -2.11 |
| | E400 (mg/Kg) | 38.13±0.19 | 36.93±0.55 | -3.15 |
| | E800 (mg/Kg) | 38.33±0.15 | 36.77±0.34 | -4.07 |

NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), E = Crude Ethanol Extract

There was no significant change in all treated mice mean daily rectal temperature. Increased body temperature is a characteristic sign and symptom of malaria fever and African trypanosomiasis in susceptible animals (Wada *et al.*, 2016a, 2016b; Mekonnen, 2015; Ayawa *et al.*, 2021). Despite the undulating parasitaemia in mice treated with ethanol extract of *Thesium viride*, the mice maintained a rectal temperature that fluctuated within the normal range following the treatment. According to studies, mice infected with *Trypanosoma brucei* or *Plasmodium berghei* had lower rectal temperatures. This could be because the host's immune system suppressed the high parasitaemia, causing an abrupt drop in body temperature, or hypothermia (Mekonnen, 2015; Ayawa *et al.*, 2021).

Figure 6 shows the effect of a crude ethanol extract of *Thesium viride* on the live weight of *Plasmodium berghei* or *Trypanosoma brucei*-infected mice. Regarding anti-plasmodial activity, the ethanol extract caused a general increase in the mean weights of all mice given doses of 200, 400, and 800 mg/kg/day (Figure 6A). In contrast, the anti-trypanosomal effect showed a rather general decrease in the mean weights of mice administered 200, 400, or 800 mg/kg/day for *Trypanosoma brucei* (Figure 6B). Table 3 shows the mean percent change in the live weight of *Plasmodium berghei* or *Trypanosoma brucei*-infected mice treated daily for four days with a crude ethanol extract of *Thesium viride*. *Plasmodium berghei*-infected mice treated with an ethanol extract of whole *Thesium viride* at 200, 400, and 800 mg/kg/day showed increases in weight of 2.95%, 1.24%, and 0.73%, respectively, in comparison to the positive control (3.37%). However, for *Trypanosoma brucei*-infected mice, there was a percent decrease in the mean weights of mice treated with 200 mg/kg (11.11%) and 800 mg/kg (15.84%) of the ethanol fractions, with no observed percent weight changes in mice administered 400 mg/kg (Table 3).

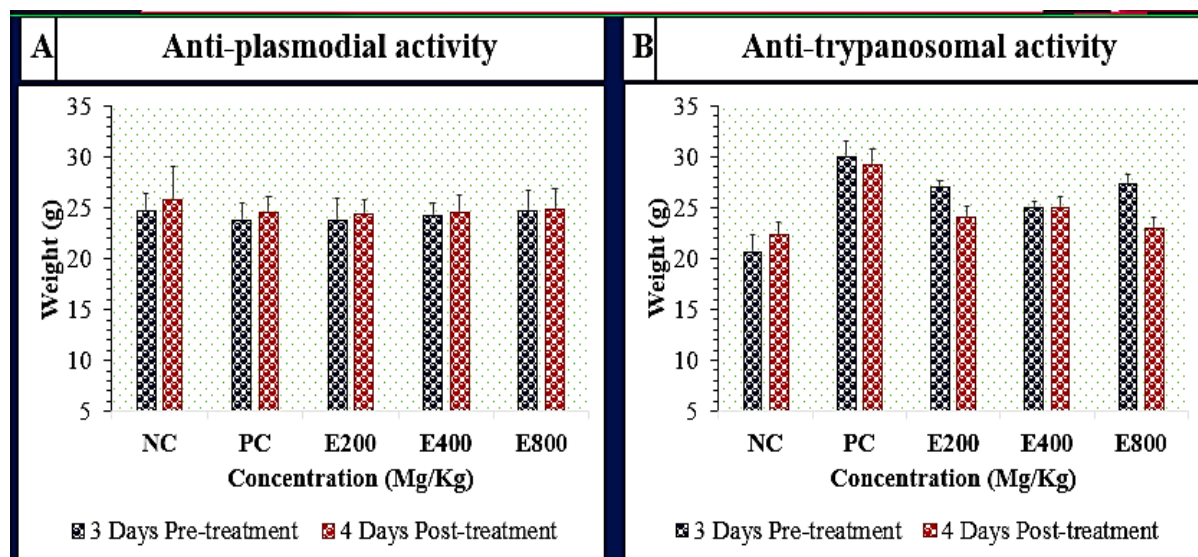


Figure 6: Effect of a crude ethanol extract of *Thesium viride* on the live weight of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice. NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), E = Crude Ethanol Extract

Table 3: Mean percent change in live weight of *Plasmodium berghei* and *Trypanosoma brucei*-infected mice treated daily for four days with crude ethanol extract of *Thesium viride*

| Pathogen | Concentration | 3 Days Pre-treatment | 4 Days Post-treatment | Percent change |
|---------------------------|---------------|----------------------|-----------------------|----------------|
| <i>Plasmodium berghei</i> | NC | 25.87±3.20 | 24.75±1.75 | -4.33 |
| | PC | 23.75±1.80 | 24.55±1.62 | 3.37 |
| | E200 (mg/Kg) | 23.75±2.14 | 24.45±1.37 | 2.95 |
| | E400 (mg/Kg) | 24.25±1.32 | 24.55±1.78 | 1.24 |
| | E800 (mg/Kg) | 24.75±1.93 | 24.93±2.01 | 0.73 |
| <i>Trypanosoma brucei</i> | NC | 22.33±1.20 | 20.67±1.67 | -7.43 |
| | PC | 30.00±1.58 | 29.25±1.55 | -2.50 |
| | E200 (mg/Kg) | 27.00±0.58 | 24.00±1.16 | -11.11 |
| | E400 (mg/Kg) | 25.00±0.58 | 25.00±1.16 | 0.00 |
| | E800 (mg/Kg) | 27.33±0.88 | 23.00±1.00 | -15.84 |

NC = Negative Control (Normal Saline), PC = Positive Control (*Plasmodium berghei*, Chloroquine 25 mg/Kg), PC = Positive Control (*Trypanosoma brucei*, Diminazene Aceturate 3.5 mg/Kg), E = Crude Ethanol Extract

Generally, the extract showed better activity inhibiting the weight loss associated with *Plasmodium berghei* infection than *Trypanosoma brucei* in the experimental mice (Table 3). The loss in body weight, especially in *Trypanosoma brucei*-infected mice, may be attributed to increased body temperatures and the host's extravascular nature of *Trypanosoma brucei*. Additionally, weight loss in the *Trypanosoma brucei*-treated mice could be attributed to the progressive increase in parasitemia with resultant muscular degeneration, which reflects the inability of the ethanol extract to clear the parasites and restore the weight loss effects. Several studies have reported weight loss in *Plasmodium berghei*-infected mice (Toma et al., 2015; Oluyemi et al., 2020; Ungogo et al., 2020; Yun et al., 2021) and also in *T.brucei*-infected mice (Trindade et al., 2016; Ayawa et al., 2021).

CONCLUSION

The ethanol extract of *Thesium viride* showed significant suppressive potential against *Plasmodium berghei* than *Trypanosoma brucei* in the studied infected mice, with better activity in inhibiting weight loss. The study has established scientific insights on the pharmacological prospect of the whole plant ethanol extract of *Thesium viride* in treating malaria and African trypanosomiasis. However, the anti-plasmodial and anti-trypanosomal evaluation were limited to only four days treatment period, which is not enough for the treatment regime to be completed. This is a limitation of the current study. Hence, further study is encouraged to consider a complete treatment regime.

REFERENCES

- Ayawa, N. G., Ramon-Yusuf, S. B., Wada Y. A., Oniye, S. J. and Shehu, D. M. (2021). Toxicity study and anti-trypanosomal activities of aqueous and methanol extracts of *Brillantaisia owariensis* on *Trypanosoma brucei*-induced infection in BALB/c mice. *Clinical Phytoscience*, 7(39): 1-11.
- Barrett, M. P., Vincent, I. M., Burchmore, R. J., Kazibwe, A. J., and Matovu, E. (2011). Drug resistance in human African trypanosomiasis. *Future Microbiology*, 6(9):1037-1047.
- Belakhdar, G., Benjouad A., Kessabi, M. and Abdennebi, E. H. (2014). Identification of the pyrrolizidine alkaloid 1-hydroxymethylpyrrolizidine from *Thesium humile* Vahl. *Journal of Material and Environmental Science*, 5 (3) 811-814.
- Bosch, C. H. (2008) *Thesium viride* A. W Hill. In: Schmelzer, G. H and Gurib-Fakim, A. (Editors). *Plant Resources of Tropical Africa*, 11 (1): Medicinal Plants 1. PROTA Foundation, Wageningen, Netherlands.
- Chamond, N., Cosson, A., Blom-Potar, M.C., Jouvion, G., D'Archivio, S., Medina, M., Droin-Bergère, S., Huerre, M., Goyard, S. and Minoprio, P. (2010). *Trypanosoma vivax* Infections: Pushing Ahead with Mouse Models for the Study of Nagana. I. Parasitological, Hematological and Pathological Parameters. *PloS Neglected Tropical Diseases*, 4(8): e792.
- Herbert, W. J. and Lumsden, W. H. R. (1976). *Trypanosoma brucei*: A rapid "matching" method for estimating the host's parasitemia. *Experimental Parasitology*, 40:427-431.
- Kamaruding, N. A., Ismail, N., and Sokry, N. (2020). Identification of antibacterial activity with bioactive compounds from selected marine sponges. *Pharmacognosy Journal*, 12(3), 493-502. <https://doi.org/10.5530/pj.2020.12.76>
- Kotepui, K. U., Masangkay, F. R., De Jesus Milanez, G., and Kotepui, M. (2021). Prevalence and outcomes of malaria as co-infection among patients with human African trypanosomiasis: a systematic review and meta-analysis. *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-021-03295-8>
- Loomis TA, Hayes AW. *Loomis's essentials of toxicology*. 4. California: Academic press; 1996. pp. 208-245.
- Mahomoodally, M. F. (2013) Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. *Evidence-Based Complementary Alternative Medicine*, 1-14.
- Matovu, E., Seebeck, T., Enyaru, J.C.K. and Kaminsky, R. (2001). Drug resistance in *Trypanosoma brucei* spp., the causative agents of sleeping sickness in man and nagana in cattle. *Microbes and Infections*, 3: 763-770.
- Mekonnen, L. B. (2015). In vivo antimalarial activity of the crude root and fruit extracts of *Croton macrostachyus* (Euphorbiaceae) against *Plasmodium berghei* in mice, *Journal of*

- Traditional and Complementary Medicine*. <http://dx.doi.org/10.1016/j.jtcme.2014.07.002>
- Moore, T. E, Verboom, G. A and Forest, F. (2010). Phylogenetics and biogeography of the parasitic genus *Thesium* L. (Santalaceae), with an emphasis on the Cape of South Africa. *Botanical Journal of the Linnean Society*, 162, 435–452.
- Ochei, J. and Kolhatkhar, A. (2000). *Medical laboratory science, theory and practice*. Tata Mac Graw.
- OECD (2002). Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 423: Acute oral toxicity- acute toxic class method. Paris: Organization for Economic Cooperation and Development.
- Oluyemi, W. M., Samuel, B. B., Kaehlig, H., Zehl, M., Parapini, S., D'Alessandro, S., Taramelli, D., and Krenn, L. (2020). Antiplasmodial activity of triterpenes isolated from the methanolic leaf extract of *Combretum racemosum* P. Beauv. *Journal of Ethnopharmacology*, 247, 112203. <https://doi.org/10.1016/J.JEP.2019.112203>
- Sanches-Vaz, M., Temporão, A., Luis, R., Nunes-Cabaço, H., Mendes, A. M., Goellner, S., Carvalho, T., Figueiredo, L. M., and Prudêncio, M. (2019). *Trypanosoma brucei* infection protects mice against malaria. *PLoS Pathogens*, 15(11). <https://doi.org/10.1371/journal.ppat.1008145>
- Shehu, S., Abdurahman, E. M., Danmalam, U. H., Mohammed, A., Shehu, S., and Iliyasu, U. (2022). Effects of *Thesium viride* Extract and Fractions on Some Liver Biochemical Parameters in CCl₄-Induced Damage in Wistar Rats. *Tropical Journal of Natural Product Research*, 6(6), 1004–1009. <https://doi.org/10.26538/tjnpr/v6i6.30>
- Shehu, S., Abubakar, M., Ibrahim, G., and Iliyasu, U. (2016). Phytochemical and Antibacterial Studies on Aqueous Ethanol Extract of *Thesium viride* (Santalaceae). *British Journal of Pharmaceutical Research*, 11(2), 1–6. <https://doi.org/10.9734/bjpr/2016/23046>
- Shehu, S., Ibrahim, G., Iliyasu, U., Shehu, S., Nuhu, A., and Abubakar, M. S. (2017). Evaluation of antiulcer activity of aqueous ethanol extract of *Thesium viride* on ethanol and aspirin induced models in rats. *Bayero Journal of Pure and Applied Sciences*, 9(2), 82. <https://doi.org/10.4314/bajopas.v9i2.16>
- Sofowora, A., Ogunbodede, E., and Onayade, A. (2013). The role of medicinal plants in the strategies of disease prevention, *African Journal of Traditional Medicine, complementary and alternativemedicine*, 10(5), 210–229.
- Toma, A., Deyno, S., Fikru, A., Eyado, A., and Beale, A. (2015). *In vivo* antiplasmodial and toxicological effect of crude ethanol extract of *Echinops kebericho* traditionally used in treatment of malaria in Ethiopia. *Malaria Journal*, 14(1). <https://doi.org/10.1186/s12936-015-0716-1>
- Toya, N.B. (2010). Immunobiology of African trypanosomes: Need of alternative interventions. *Journal of Biomed Biotechnology*, Doi: 10.1155/2010/389153.
- Trindade, S., Rijo-Ferreira, F., Carvalho, T., Pinto-Neves, D., Guegan, F., Aresta-Branco, F., Bento, F., Young, S. A., Pinto, A., van den Abbeele, J., Ribeiro, R. M., Dias, S., Smith, T. K., and Figueiredo, L. M. (2016). *Trypanosoma brucei* Parasites Occupy and Functionally Adapt to the Adipose Tissue in Mice. *Cell Host and Microbe*, 19(6), 837–848. <https://doi.org/10.1016/j.chom.2016.05.002>
- Ungogo, M. A., Ebiloma, G. U., Ichoron, N., Igoli, J. O., de Koning, H. P., and Balogun, E. O. (2020). A Review of the Antimalarial, Anti-trypanosomal, and Antileishmanial Activities of Natural Compounds Isolated From Nigerian Flora. In *Frontiers in Chemistry* (Vol. 8). Frontiers Media S. A. <https://doi.org/10.3389/fchem.2020.617448>

- Wada, Y. A., Oniye, S. J., Rekwot, P. I., and Okubanjo, O. O. (2016a). Single and mixed interaction of experimental *Trypanosoma brucei brucei* and *Trypanosoma evansi* on the semen collection reaction time and spermatozoa morphology of Yankasa rams. *Journal of Advanced Veterinary and Animal Research*, 3(4). 360-367
- Wada, Y. A., Oniye, S. J., Rekwot, P. I. and Okubanjo, O. O. (2016b). Testicular pathology, gonadal and epididymal sperm reserves of Yankasa rams infected with experimental *Trypanosoma brucei brucei* and *Trypanosoma evansi*. *Veterinary World*, 9: 759-765.
- Wurochekke, A. U and Nok, A. J. (2004). In vitro anti trypanosomal activity of some medicinal plants used in the treatment of trypanosomiasis in Northern Nigeria. *African Journal of Biotechnology*. 3(9): 481-483.
- Wurochekke, A.U., James, D.B., Bello, M.I. and Ahmodu, A. (2005). Trypanocidal activity of the leaf of *Guiera senegalensis* against *Trypanosoma brucei brucei* infection in rats. *Journal of Medical Science*, 5:1-4.
- Yun, H. S., Dinzouna-Boutamba, S. D., Lee, S., Moon, Z., Kwak, D., Rhee, M. H., Chung, D. il, Hong, Y., & Goo, Y. K. (2021). Antimalarial effect of the total glycosides of the medicinal plant, *Ranunculus japonicus*. *Pathogens*, 10(5). 532.