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Research Article

Rutin-Rich Flavonoid Subfraction of *Annona senegalensis* Mitigates *Trypanosoma brucei brucei* Infection and Hematobiochemical Changes in Infected Mice

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Trypanosomiasis is an important but neglected tropical diseases which continues to cause morbidity and mortality on a large scale in sub-Saharan Africa. In the present study, the aqueous leaf extract, flavonoid fraction, and subfractions of the flavonoid from *A. senegalensis* leaves were evaluated for their antitrypanosomal activities, effects on some hematological parameters, and some liver-based enzymes in mice infected with *Trypanosoma brucei brucei*. Mice treated with aqueous extract at a concentration range of 100–400 mg kg bw exhibited parasite inhibition range between 66.08 and 73.09% while mice treated with the flavonoid fraction (50-200 mg/kg bw) exhibited higher parasite inhibition (83.37-86.38%). Mice treated with subfractions of the flavonoids showed a significant decrease in parasite count ($p < 0.05$), with subfraction 3 completely clearing parasites in the circulation after twenty-one days of treatment. Biochemical analysis revealed that the subfraction 3 restored the alterations in the serum aspartate transaminase (AST), alanine transaminase (ALT), and total protein concentrations that were observed in the infected nontreated mice. The subfraction 3 was subsequently characterized by HPLC analysis which revealed the presence of rutin (RT: 22.26, 65.34 ppm) as the most abundant bioactive compound that could be attributed to the bioactivity of the subfraction 3. Other polyphenolic compounds identified included gallic acid (RT: 4.39, 0.578 ppm), quercetin (RT: 37.27, 0.729 ppm), kaempferol (RT: 38.33, 1.52 ppm), and luteolin (RT; 39.173, 32.77 ppm). Taken together, subfraction 3 of the flavonoid fraction from of *A.* senegalensis demonstrated potent antitrypanosomal activities and therefore represents a reserve of bioactive metabolite worthy of further exploration for the treatment of trypanosomiasis.

1. Introduction

Human African trypanosomiasis (HAT) is a protozoan parasitic disease that is endemic in sub-Saharan Africa, where millions are at risk from this disease [1]. Although trypanosomiasis is lethal if not properly treated, it is among the major neglected diseases that affect people around the world particularly in Africa. More than thirty countries in Africa are known to be a pandemic region of this disease, and the resources for treating or managing the disease are lacking [2].Trypanosomiasis is epitomized by the spasmodic presence of the parasites in the patient blood stream and recurrent fever [3]. Anemic condition normally develops in animals infected with the parasite, and this is accompanied by body weight, loss of productivity and high mortality [4]. The current treatments for early-stage infection are suramin and pentamidine while melarsoprol, eflornithine, and the combination therapy (nifurtimox and eflornithine) are the therapeutic option for the late-stage infection [5]. However, these drugs are not satisfactory, due to their toxicity and poor efficacy [6]; thus, there is a need for alternative treatment approaches.

In Nigeria and other developing countries in Africa, several low- or middle-income resident and earners rely mostly on traditional plants and natural products for the treatment of a number of diseases, including convulsion, trypanosomiasis, malaria, epilepsy, dysentery, and infertility [7]. Natural products have continually been explored as a source of healing agents against several diseases and have been subjected to scientific analysis for the validation of their efficacy against several human diseases [8]. A number of medicinal plants and secondary metabolites isolated from them have been reported for antitrypanosomal activity [9] and for the treatment of other parasitic and metabolic diseases [10]. The biological activities of these plants have been attributed to the presence of major bioactive phytochemicals including flavonoids, alkaloids, phenolics, saponins, cardiac glycosides, and terpenoids [11].

Annona senegalensis, also known as the wild custard apple, is a shrub or small tree of about 2-6 m tall but may reach 11 m under favorable conditions [7, 12]. The plant is mostly found in Nigeria particularly in the Kaduna, Nasarawa, Kano, Niger, and Plateau States. *A. senegalensis* is used as food for direct consumption and in food additives. Major parts of the plant are a rich source of important phytochemicals, annogalene, (-)-roemerine, acetogenins, annosenegalin, and kaurenoic acid [13].Traditionally, the plant has been used ethnopharmacologically as an oral remedy for tuberculosis, small pox, and yellow fever [14]. The stem bark has also been explored for the treatment of hernia and snake bite [15]. Several pharmacological properties of *A. senegalensis* include antimalarial, analgesic, anti-inflammatory, antioxidants, and antimicrobial [16–19]. The root bark of the plant has also been reported for anti-inflammatory and antiulcerogenic potential [20] and was recently characterized for anti-enteropooling compounds [21]. The present study is, therefore, aimed at evaluating the trypanocidal potency of crude extract, flavonoid fraction, and subfraction of *A. senegalensis* in experimental animals and evaluating the safety profile on liver integrity of the animals.

2. Materials and Methods

2.1. Reagent and Chemicals. Reagents and chemicals used are of analytical grade purpose and were products of Sigma Chemical Co., United States of America.

2.2. Plant Sample Collection and Extraction. Fresh leaves of *A. senegalensis* were obtained from Minna, Bosso, area of Niger State Nigeria, in the Month of February 2017. Taxonomic identification of the plant was done by Dangana, M. C. of the Biological Sciences, Department Federal University of Technology Minna, Nigeria. The leaves were rinsed to remove dust, dried at room temperature for two weeks, and pulverized into fine powder with an electrical blender. Distilled water (1500 ml) was added to 300 g of the leaf powder, vigorously shaken intermittently, and allowed for extraction for seventy-two hours. The mixture was then filtered, and the filtrate was concentrated with a freeze dryer to obtain the aqueous extract which was afterward stored for further analysis.

2.3. Experimental Animals. Healthy albino mice with an average weight of 20-35 g were purchased from the animal house of the Federal University of Technology, Minna, Nigeria. The mice were kept under the standard condition for animals handling and experimentation with adequate access to pellets and water. The in vivo animal experimentation was approved by the "Committee on Ethics for Medical and Scientific Research of Federal University of Technology Minna, Nigeria."

2.4. Analysis of Phytochemical Compositions. The crude aqueous extract of *A. senegalensis* was subjected to the qualitative and quantitative phytochemical analysis to check the presence and quantity of alkaloids, total phenols, tannins, total flavonoids, saponins, and glycoside using standard protocols [22–29]. Quantitatively, the total flavonoid contents were estimated using a spectrophotometer based on the formation of flavonoid-aluminium complex that absorb maximally at 415 nm [25]. Total phenol was estimated using the Folin-Ciocalteu reagent protocol [26]. The total alkaloids were quantitatively estimated spectrophotometrically at 565 nm using vincristine as standard [27]. The tannin content was determined using Folin-Denis reagent. The amount of tannin was calculated as tannic acid equivalent from the standard curve [28]. A gravimetric method of AOAC [29] was used for saponin determination in the samples.

2.5. Extraction of flavonoids. Flavonoids were extracted and fractionated by the method of Musa [30]. Thirty (30) grams of fine powder of *A. senegalensis* was defatted with 250 ml of N-hexane using a Soxhlet extractor. The extraction was carried out for six hours at a temperature between 65°C and 70°C. The defatted marc was further extracted with 250 ml methanol at 60°C, and the extract was then evaporated in a water bath. Methanolic extract (6 g) was chromatographed over silica gel column (200 g: 230-400 mesh) and eluted with the solvent mixture of $CH_3C1/CH_3OH/H_2O$ (70 : 30 : 1, V/V). The fraction was then dried in the water bath.

2.6. Subfractionation of Flavonoids from A. senegalensis. The flavonoid obtained was further subjected to column chromatography to separate the flavonoids into their component subfractions. Silica gel was used in packing the column while varying solvent combinations of increasing polarity (chloroform, n-hexane, ethyl acetate, and methanol) were used as the mobile phase. The TLC was carried out on the flavonoid fractions obtained using a TLC precoated plate with the solvent systems of increasing polarity. The Rf values for each fraction were calculated (Equation (1)) and recorded. The Rf values correspond to the number of constituents that may likely present in the fraction of eluent from the column.

2.7. Antitrypanosomal Screening of the Extracts and Flavonoid Fractions

2.7.1. Infection of Animals. The mice were inoculated as described by Mann and Ogbadoyi [31]. A highly infected and parasitized blood from a mouse was obtained through cardiac puncture with the aid of a syringe. The blood was appropriately diluted with physiological saline to serve as inoculum. Healthy mice of weight range 20-35 g were injected intraperitoneally with 0.1 ml of the inoculums.

2.7.2. Monitoring of the Course of Parasitemia. The presence of parasites in experimental animals was monitored at two-day intervals for 14 days by a blood smear on a glass slide and observed with the aid of a microscope set. The number of parasites in 1 ml of blood was then estimated using the method of Herbert and Lumsden [32].

2.7.3. Antitrypanosomal Screening of the Aqueous Extract of A. senegalensis Leaf. To determine the bioactivity of the aqueous extract, a preliminary study was done to provide information on the antitrypanosomal activity of the aqueous extract of *A. senegalensis*. Four groups of mice (A-D) of three mice each were orally administered the extract at doses of 100, 200, 300, and 400 mg per kilogram body weight (kg^{-1} bw). The fifth group formed the control (i.e., infected but not treated), while the infected and treated with 3.5 mg kg^{-1} bw of the standard drug Berenil formed the sixth group. Parasitemia was thereafter monitored at two-day intervals for two weeks by microscopic examination of the wet film prepared from blood samples taken from the tail of infected animals."

2.7.4. Antitrypanosomal Screening of Flavonoids of A. senegalensis. Eighteen (18) mice were used for this study. The mice were grouped into six, with three mice in each group, and they were all infected with *T. brucei brucei* from the infected mouse. Groups A–D were administered 50, 100, 150, and 200 mg kg^{-1} body weight of total flavonoid fraction of *A. senegalensis*. "Group E was infected and treated with 3.5 mg kg^{-1} bodyweight Berenil (standard drug), and group F was infected but given normal saline only to serve as a negative control. Parasitemia was thereafter monitored at every 48 h for two weeks."

2.7.5. Antitrypanosomal Screening of Flavonoid Subfractions. Twenty-one (21) mice were used for this study. They were grouped A–G, with three mice in each group, and they were all infected with *T. brucei brucei* from the infected mice. Groups A–E were administered a constant dose of 100 mg kg^{-1} bw of subfractions 1, II, III, IV, and V of the flavonoid fraction, group F was infected and treated with 3.5 mg kg^{-1} bw Berenil (standard drug), and group G was infected but given normal saline only to serve as a control. Parasitemia was thereafter monitored every 48 h for two weeks.

2.7.6. Determination of Percentage Parasite Inhibition. The percentage inhibition of parasites was calculated for each dose level by comparing the parasitemia in the infected control with that in the treated group. According to the modified method of Peters and Robinson, [33], percentage parasitemia and inhibition were calculated as

$$\%\text{parasite inhibition} = \frac{\text{Parasitemia innegative control group} - \text{parasitemia in treated group} \times 100}{\text{Parasitemia in negative control group}}. \quad (1)$$

2.7.7. Determination of Mean Survival Period (MSP). The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (postinfection) in each group over a period of 35 days (D0–D35).

$$\text{MSP} = \frac{\text{“Sum of survival time of all mice in a group (days)”}}{\text{“Total number of mice in that group”}}. \quad (2)$$

2.7.8. Determination of Packed Cell Volume (PCV). The PCV analysis was conducted as described by Dacie and Lewis [34]. The blood was collected in heparinized capillary tubes, centrifuged at 11,000 rpm for 5 minutes.

2.8. Analysis of Biochemical Parameters. All biochemical analyses were conducted using Randox Diagnostic kit (Randox Laboratories Ltd, Crumlin, UK). Alanine transaminase (ALT) was analyzed on the principle of catalytic action of ALT on alanine and α-oxoglutarate to form pyruvate and glutamate [35]. Aspartate transaminase (AST) was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4–dinitrophenylhydrazine [36]. Alkaline phosphatase (ALP) was analyzed using standard protocol [37]. Serum total protein concentration was estimated based on the principle that cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex which absorbed maximally at 546 nm [38].

2.9. High-Performance Liquid Chromatography (HPLC). The flavonoid subfraction 3 was subjected to HPLC analysis according to the method described in our previous study [39]. A hundred milligrams (100 mg) of flavonoid subfraction 3 was dissolved in five millilitres (5 ml) of an HPLC-grade methanol. The extract solution was filtered and run on the HPLC (Agilent Technologies 1200) with the following chromatographic conditions: stationary phase (Hypersil BDS C18), mobile phase (acetonitrile and 0.1% formic acid), column dimension of 250 mm × 4.0 mm, injection volume of 10 μl, a flow rate of 0.6 ml/min, detector wavelength of 280 nm, and at gradient mode of elution.

2.10. Statistical Analysis. Data were analyzed and expressed as the mean ± SEM. The significant difference in parameters among groups was determined by one-way ANOVA and Duncan multiple range test ($p < 0.05$).

3. Results

3.1. Phytochemical Composition of A. senegalensis. Results of the qualitative phytochemical analysis revealed the presence of alkaloids, total phenols, tannins, total flavonoids, saponins, and glycoside (Table 1). Quantitatively, *A. senegalensis* contains 19.48 ± 0.35 mg/g of total phenol, 15.05 ± 0.55 mg/g of total flavonoids, 18.28 ± 0.59 mg/g of tannins, 1.36 ± 0.50 mg/g of alkaloids, and 89.06 ± 0.55 mg/g of saponins (Table 2). The percentage (%) yields of total flavonoid subfraction 1 (hexane/chloroform 1 : 9), subfraction 2 (hexane/chloroform 2 : 8), subfraction 3 (chloroform/ethyl acetate 2 : 8), subfraction 4 (ethyl acetate/methanol 1 : 1), and subfraction 5 (100% methanol) are 16, 4.1, 4.7, 26.7, and 18.3%, respectively.

3.2. Antitrypanosomal Activities of Crude Extract and Flavonoid Fraction of A. senegalensis

3.2.1. Parasitemia Count. The antitrypanosomal activity of aqueous extract and total flavonoid extract of *A. senegalensis* is shown in Figures 1 and 2. Mice treated with the aqueous extract (100, 200, 300, and 400 mg/kg body weight) of *A. senegalensis* show a significant decrease ($p < 0.05$) in parasite count (Figure 1) with percentage parasite inhibition of 66.08%, 69.00%, 73.09%, and 71.34%, respectively (Table 3). Mice in the positive control group (3.5 mg/kg body weight Berenil) had the highest parasite inhibition of 78.94%. Similarly, mice treated with the flavonoid fraction (50, 100, 150, and 200 mg/kg bw) showed significant decrease ($p < 0.05$) in parasite count (Figure 2) with percentage parasite inhibition of 83.37%, 84.56%, 84.77%, and 86.38%, respectively (Table 4). However, the control mice, Berenil (3.5 mg/kg bw) in this experiment, had the highest parasite inhibition of 98.79%.

3.2.2. Mean Survival Periods. The infected untreated group (normal saline) had mean survival periods of 10.50 ± 1.11. Mice treated with aqueous leave extract of *A. senegalensis* at 100, 200, 300, and 400 mg/kg had the mean survival periods of 11.10 ± 1.88, 11.80 ± 1.09, 12.45 ± 2.72, and 15.33 ± 1.66, respectively (Table 3). Similarly, mice treated with the flavonoid fraction (50, 100, 150, and 200 mg/kg body weight) had mean survival periods of 12.50 ± 0.51, 13.50 ± 2.21, 14.00 ± 1.68, and 15.30 ± 0.33, respectively. However, mice in the positive control group, diminazene aceturate (3.5 mg/kg bw), had the highest mean survival periods of 19.65 ± 0.59 (Table 4).

3.2.3. Body Weight Changes. The effect of aqueous extract of *A. senegalensis* (100, 200 300, and 400 mg/kg body weight) on body weight changes is shown in Figure 3. All groups of animals showed a decrease in body weight on the third day (after infection). Infected mice treated with aqueous extract 300 and 400 mg/kg bw and 3.5 mg/kg bw of Berenil showed an increase in weight after treatment. However, there was a significant decrease ($p < 0.05$) in the body weight of mice in the negative control group (untreated group) when compared to the treatment groups. Similarly, infected mice treated with flavonoid fraction (50, 100, 150, and 200 mg/kg bw) showed an increase in body weight after treatment (Figure 4).

3.2.4. Changes in Packed Cell Volume (PCV). There were decreases in PCV across all experimental groups after parasite inoculation. The infected nontreated mice show a progressive and significant decrease in PCV throughout the experimental period. However, a group of mice treated with the aqueous extracts of *A. senegalensis* (100, 200, 300, and 400 mg/kg bw) showed an increase in PCV after treatment (Figure 5). Similarly, mice treated with the total flavonoid fraction (50, 100, 150, and 200 mg/kg body weight) as well as a standard drug, Berenil (3.5 mg/kg bw), demonstrated increased in PCV after treatment period (Figure 6).

3.3. Antitrypanosomal Activity of Subfractions. There was an increase in parasite levels in *T. brucei*-infected mice administered normal saline and subfractions (2 and 5). However, the mice treated with fractions 3, 4, 1, and Berenil (standard drug) showed a significant decrease ($p < 0.05$) in parasite level (Figure 7). Mice treated with subfraction 3 of the flavonoids showed the most significant decrease in parasite count ($p < 0.05$) and completely cleared the parasites from circulation after twenty-one days of treatment (Figure 7). The effect of flavonoid subfractions (1, 2, 3, 4, and 5) on PCV in mice infected with *T. b.* brucei is shown in Figure 8. On day three postinfection, there was a decrease in PCV in all groups. After treatment, there was an increase in PCV in mice treated with fractions (3 and 5) while other treatment groups were decreased in PCV posttreatment (Figure 8). Similarly, only mice treated with fractions 1, 3, 5, and the standard drug showed an increase in the body weight after treatment (Figure 9).

3.4. Effect of Flavonoid Subfraction 3 on Some Biochemical Parameters. Analysis of serum biochemical parameters revealed a significant ($p < 0.05$) increase in alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) activities and total protein concentrations when compared with the normal control mice (Figure 10). No significant differences ($p > 0.05$) in alkaline phosphatase (ALP) activity in infected nontreated mice when compared with the normal control and other experimental groups. Interestingly, treatment with subfraction 3 restored the alterations in the serum aspartate transaminase (AST), alanine transaminase (ALT), and total protein concentrations that were observed in the infected nontreated mice.

3.5. Characterization of Subfraction 3 of the Flavonoid. The current study found that subfraction 3 of the flavonoid demonstrated promising antitrypanosomal properties. Therefore, we next characterized the subfraction 3 using the HPLC analysis. Our results revealed the presence of 5 polyphenolic compounds including gallic acid (RT: 4.39, 0.578 ppm), rutin (RT: 22.26, 65.34 ppm), quercetin (RT: 37.27, 0.729 ppm), kaempferol (RT: 38.33, 1.52 ppm), and luteolin (RT; 39.173, 32.77 ppm) (Table 5; Figure 11).

Table 1: Qualitative phytochemical composition of aqueous leaf extract of *A. senegalensis*.

Phytochemicals	Inference
Total phenol	+
Total flavonoids	+
Tannins	+
Alkaloids	+
Saponins	+
Phlobatannin	-
Glycoside	+

Key: +: present; -: absent.

Table 2: Quantitative phytochemical compositions of aqueous leaf extract of *A. senegalensis*.

Phytochemicals	Concentration (mg/g)
Total phenol	19.48 ± 0.35^{b}
Total flavonoids	15.05 ± 0.55^{b}
Tannins	18.28 ± 0.59^{b}
Alkaloids	1.36 ± 0.50^{a}
Saponins	89.06 ± 0.55^{c}

Data are expressed asmean ± SEM, and different superscripts in alphabets indicate significant difference (Duncan multiple range post hoc test, $p < 0.05$).

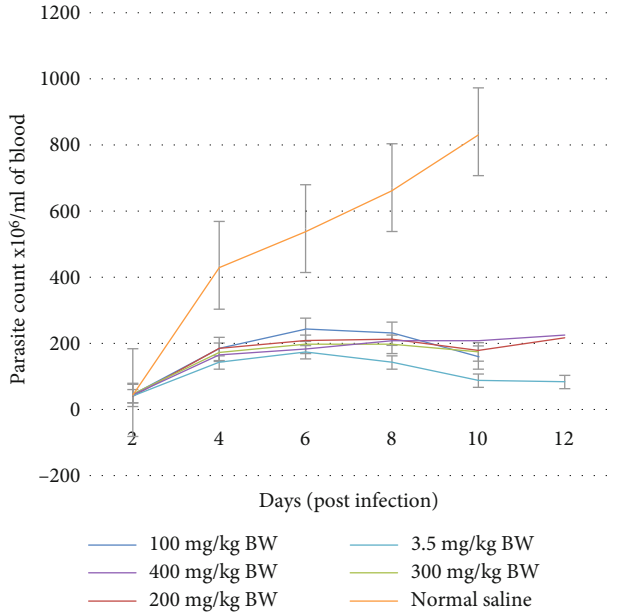

Figure 1: Effects of aqueous leaf extract of *A. senegalensis* on parasite count in *T. b. brucei*-infected mice. Results are expressed as mean ± SEM of replicate determination.

Collectively, the study revealed that rutin is the most abundant flavonoid content that could be attributed to the antitrypanosomal activities of subfraction 3 flavonoid of *A. senegalensis*.

4. Discussion

The antitrypanosomal activity of plants and plant products has been linked with the presence of several biologically active compounds [40], and there is increased interest in the identification of therapeutic compound in plants in order to justify or validate the medicinal claim by the

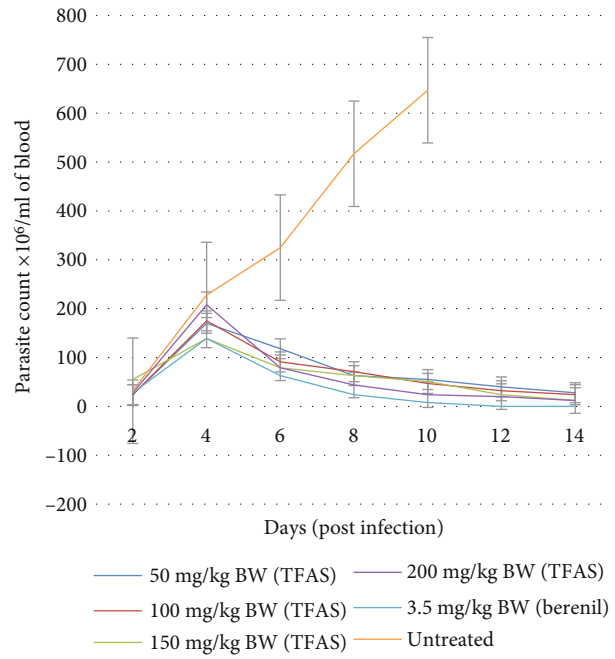

Figure 2: Effect of total flavonoid fraction of *A. senegalensis* (TFAS) on parasitemia in *T.B. brucei*-infected mice. Results are expressed as mean ± SEM of replicate determinations. Key: BW: body weight; TFAS: total flavonoid fraction of *A. senegalensis*.

Table 3: Parasite inhibition and survival period of *T. b. brucei*-infected mice treated with the aqueous extract of *A. senegalensis*.

Dose (mg/kg bw)	Parasitaemia ($\times 10^6$)	% inhibition	Mean survival period (days)
100	58.00 ± 1.42	66.08	11.10 ± 1.88
200	53.00 ± 11.31	69.00	11.80 ± 1.09
300	50.00 ± 7.17	73.09	12.45 ± 2.72
400	49.00 ± 9.07	71.34	15.33 ± 1.66
3.5 Berenil	36.00 ± 3.46	78.94	19.65 ± 1.84
Normal saline	171.06 ± 27.09	—	10.50 ± 1.11

Results are expressed as mean ± SEM.

Table 4: Parasite inhibition and survival period of *T. b. brucei*-infected mice treated with the total flavonoid fraction from *A. senegalensis*.

Dosage (mg/kg bw)	Parasitaemia ($\times 10^6$)	% inhibition	Mean survival period (days)
50	21.00 ± 2.34	83.37	12.50 ± 0.51
100	20.00 ± 1.56	84.56	13.50 ± 2.21
150	19.00 ± 2.56	84.77	14.00 ± 1.68
200	16.00 ± 2.35	86.38	15.30 ± 0.33
3.5 Berenil	2.00 ± 1.57	98.79	19.65 ± 0.59
Normal saline	166.00 ± 15.67	—	9.50 ± 2.51

Results are expressed as mean ± SEM.

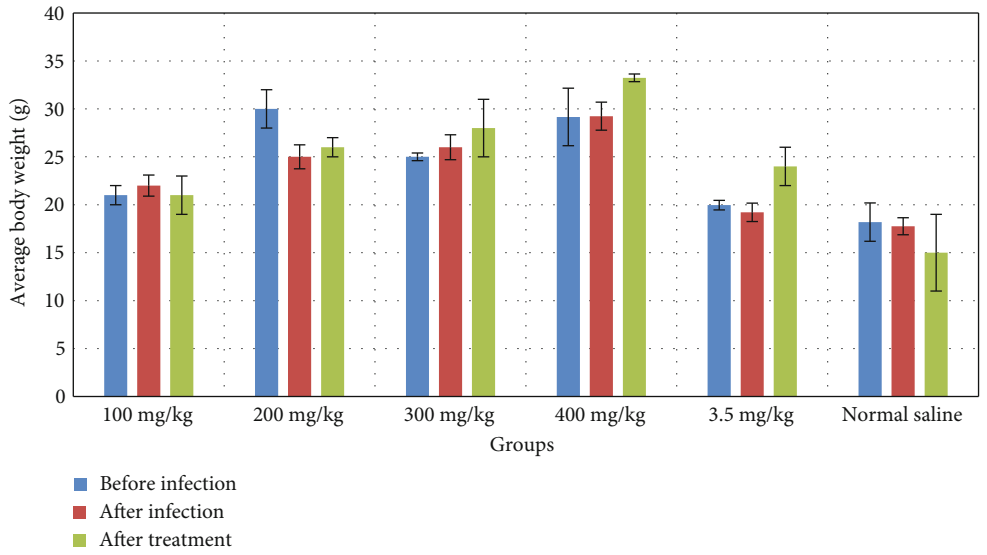

Figure 3: Effect of the aqueous extract of *A. senegalensis* on the body weight of *T. b. brucei*-infected mice. Results are expressed as mean ± SEM of replicate determination.

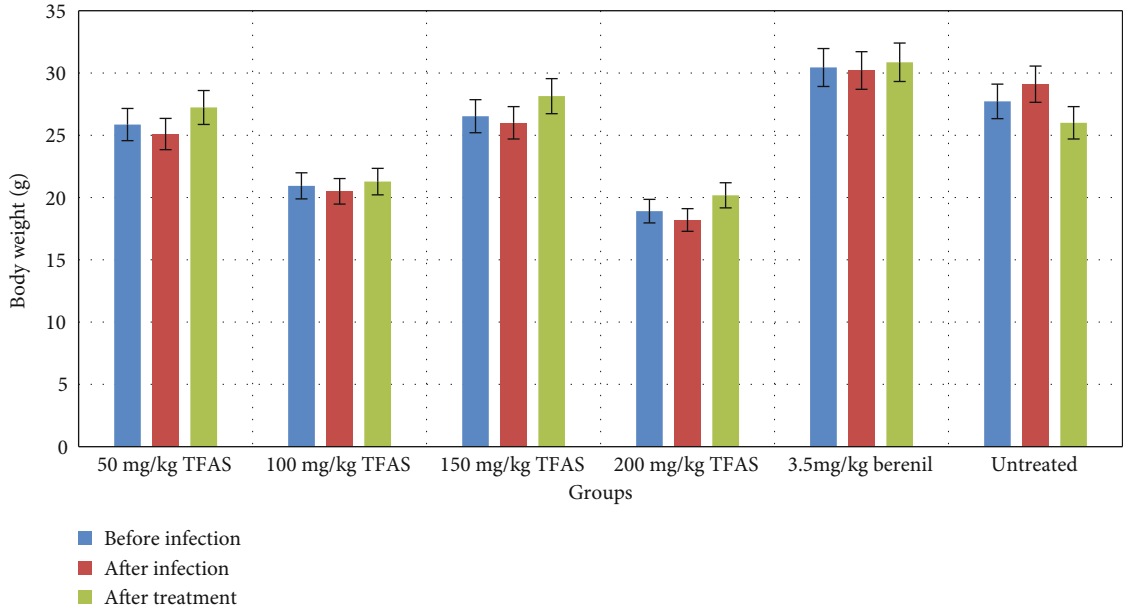

Figure 4: Effect of the total flavonoid fraction of *A. senegalensis* on the body weight of *T. b. brucei*-infected mice. Results are expressed as mean ± SEM of replicate determination.

traditional health care provider. These bioactive compounds are the major basis for the pharmacological properties of the plants [41]. Numerous plants containing a wide variety of phytochemicals as their bioactive components have shown antiplasmodial, anti-inflammatory, analgesic, and antinociception potentials [42].

The screening of phytochemical in the aqueous leaf extract of *A. senegalensis* (Table 1) revealed the presence of saponins, alkaloids, flavonoids, total phenols, glycosides, and tannins which confirms the works of other researchers on phytochemical constituents of the plants [43].

Quantitative analysis of *A. senegalensis* showed that it contains 15.05 ± 0.55 mg/g of total flavonoids, 19.48 ± 0.35 mg/g of total phenol, 18.28 ± 0.59 mg/g of tannins; 1.36 ± 0.50 mg/g of alkaloids, and 89.06 ± 0.55 mg/g of saponins (Table 2). The results of the present study also support the folkloric use of the studied plant in the treatment of diseases. The fact that phlobatannins and anthraquinones were absent in *A. senegalensis* confirmed the earlier findings that different forms and quantities of phytochemicals are found in different types and parts of medicinal plant, with great variation in solvent extraction system [24].

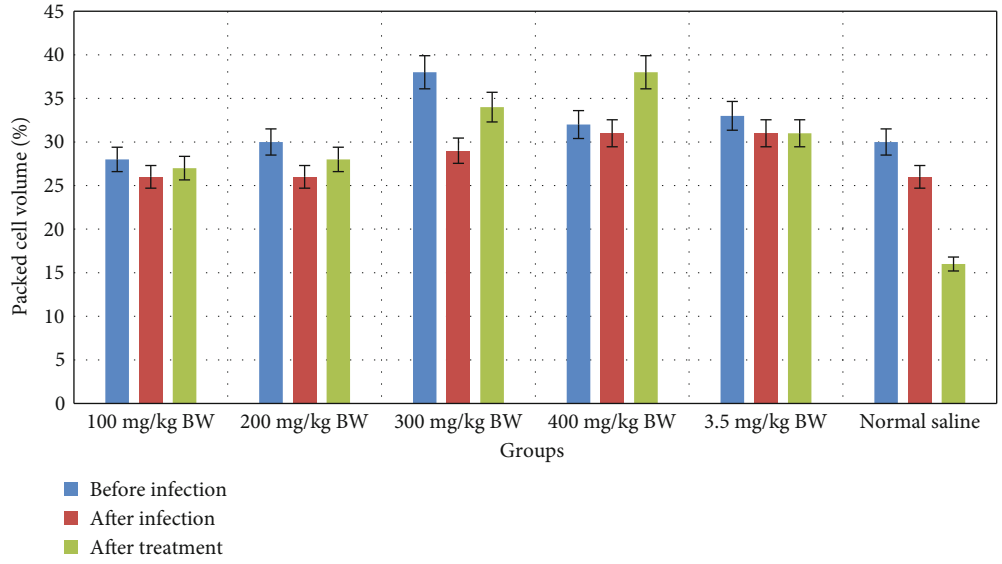

Figure 5: Effect of aqueous extract of *A. senegalensis* on packed cell volume of *T. b. brucei*-infected mice. Results are expressed as mean ± SEM of replicate determination.

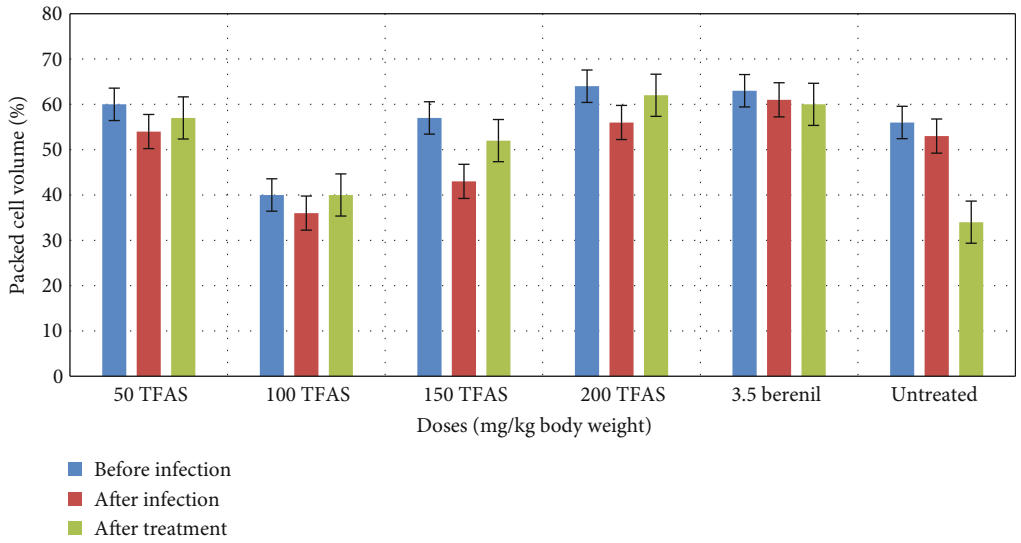

Figure 6: Effect of total flavonoid fraction of *A. senegalensis* (TFAS) on packed cell volume in *T. b. brucei*-infected mice. Results are expressed as mean ± SEM. TFAS: total flavonoid fraction of *A. senegalensis*.

Plant species have been reported to possess trypanocidal activity. During trypanosome infection and invasion of a mammalian system, a rapid parasite proliferation occurs in an infected host [44]. In this study, the administration of *A. senegalensis* extract on *T. b. brucei*-infected mice reduced the level of parasites in the blood of experimental mice (Figure 1). Therefore, the aqueous extract of *A. senegalensis* has trypanocidal properties based on its ability to reduce parasitemia and extension of life span of *T. b. brucei*-infected mice.

Also, the result obtained from the mice treated with total flavonoid fraction showed a decrease in parasitemia at the end of the treatment (Figure 2), indicating its trypanocidal potency. Flavonoids are important phytochemicals that are said to be pharmacologically active. The trypanocidal activities of certain plant extracts have been reported to be due to the flavonoids and other constituents present. In addition to the antimicrobial activities exhibited by flavonoids, they also exhibit antitrypanosomal and antileishmanial activities [45]. In the present study, flavonoid fractions of *A. senegalensis* demonstrated antitrypanosomal activities with a significant reduction in parasite multiplication Figure 10.

Although the aqueous extracts, total flavonoids, and flavonoid subfractions suppressed parasites in experimental infected mice, it is exciting to have the flavonoid fractions being clearly more efficacious than the aqueous and total flavonoid fractions. The subfraction 3 of total flavonoids at a dose of 100 mg/kg body weight cleared parasites from the

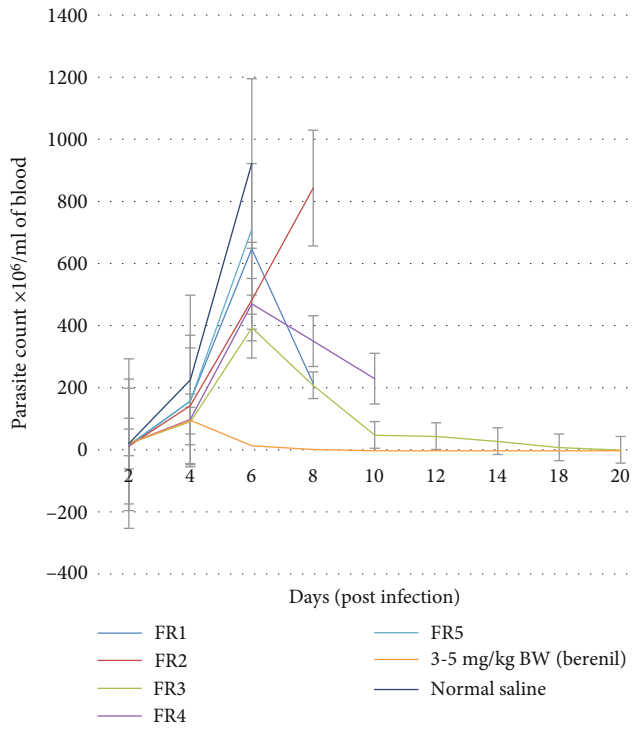

Figure 7: Effect of flavonoid subfractions (1, 2, 3, 4, and 5) of *Annona* senegalensis on parasitemia count in *T. b.* brucei-infected mice. Results are expressed as mean ± SEM of replicate determinations. FR: fraction; BW: body weight.

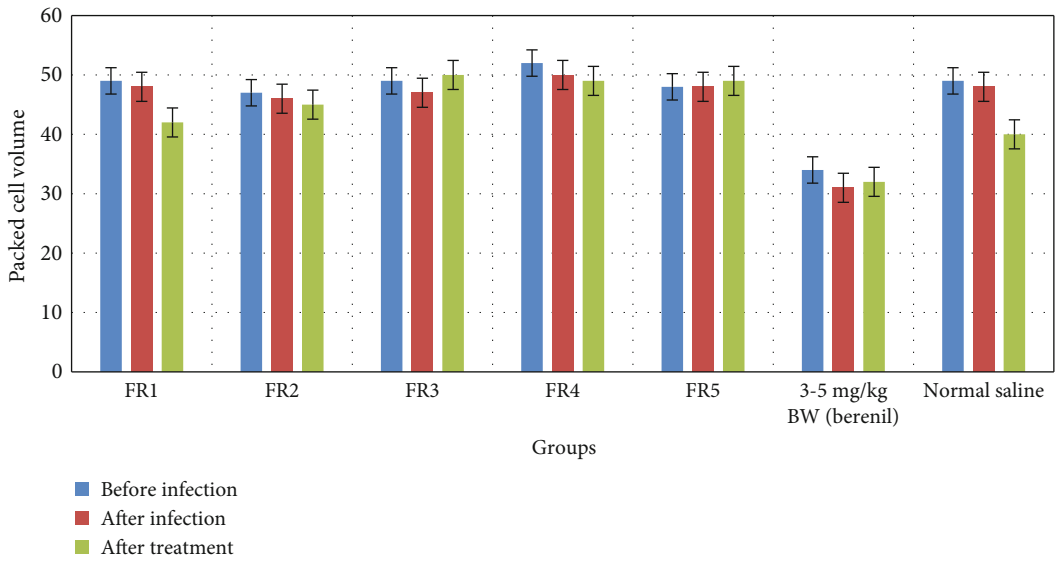

Figure 8: Effect of flavonoid subfractions (1, 2, 3, 4, and 5) of *Annona senegalensis* on packed cell volume in *T. b. brucei*-infected mice. Results are expressed as mean ± SEM of replicate determinations. FR: subfraction.

circulation within twenty-one days of treatment in contrast to the aqueous extract (Figure 1) and total flavonoid fraction (Figure 2) which could not clear the parasite completely.

Anemia is part of the manifestation of African trypanosomiasis which influences the pathogenesis of the infection. This finding is supported by the decline in packed cell volume (PCV) observed in the infected untreated group Figures 7, 8, and 10. These results confirmed that anemia is a critical feature in the pathogenesis of African trypanosomiasis [46]. However, the increase in packed cell volume observed in the infected treated and mice compared to infected untreated groups suggests that the extract reduced the anemic effect of *T. b. brucei* in mice.

Changes in biochemical enzymes are a good marker of tissue damage. Elevated enzyme levels occur as a results of trypanosomes lysis due to defense mechanisms of the host

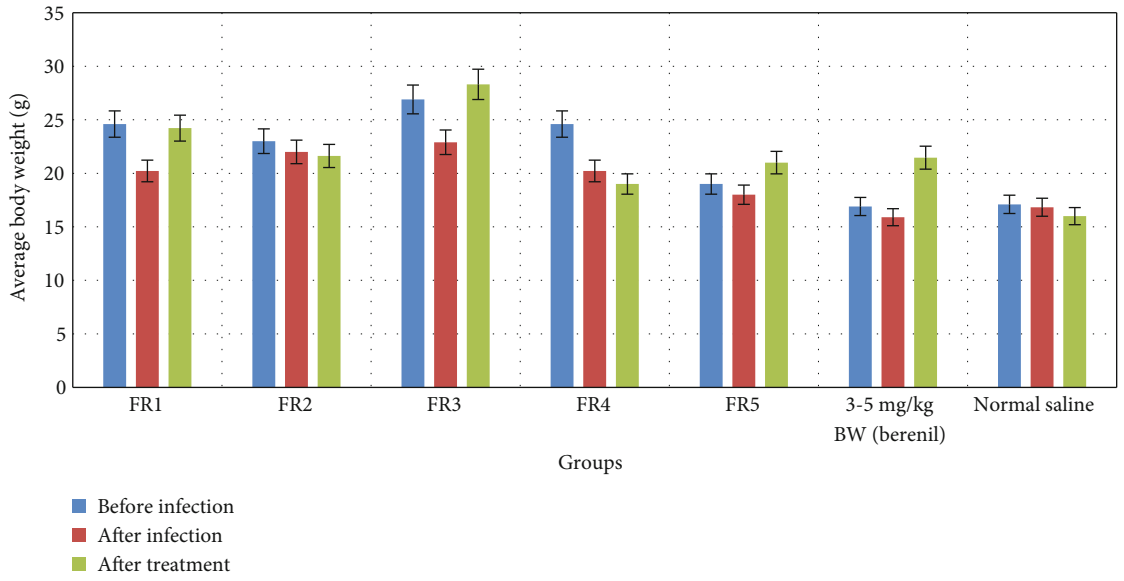

Figure 9: Effect of flavonoid subfractions (1, 2, 3, 4, and 5) of *Annona senegalensis* on body weight in *T. b. brucei*-infected mice. Results are expressed as mean ± SEM of replicate determinations. FR: subfraction.

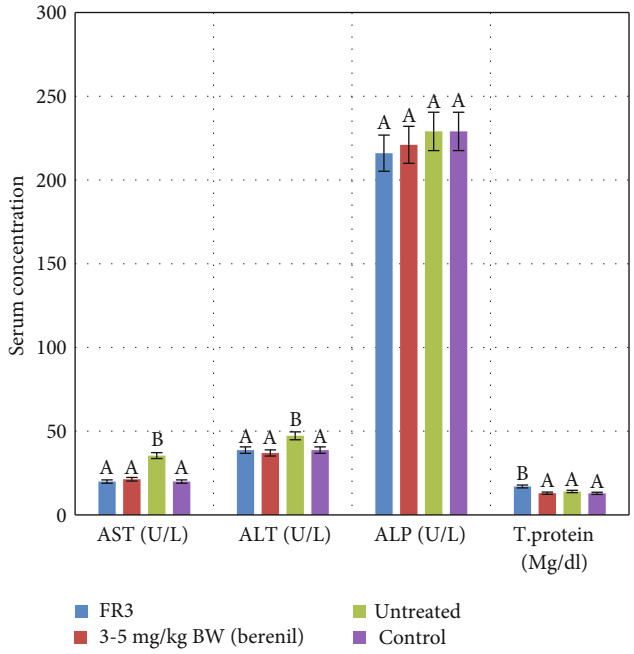

Figure 10: Effect of flavonoid subfraction 3 on liver enzymes' activities; AST: aspartate transaminase (U/l); ALT: alanine transaminase (U/l); ALP: alkaline phosphatase (U/l); T-protein: serum total protein (mg/dl). Results are expressed as mean ± SEM, and different superscripts in alphabets indicate significant difference. The superscript alphabets a and b referred to the significant differences between the treatment groups corresponding to $p < 0.05$ and $p < 0.01$, respectively.

[47]. Thus, the enzymes analyzed in this study are important biomarker enzymes of hepatic cytolysis, oxidative stress, and damage to the plasma membrane [48].

The increase in serum AST activities of the infected nontreated mice when compared with the mice treated with flavonoid subfraction 3 and Berenil(Figure 10) validates an earlier report that trypanosome infection could gradually alter enzyme levels with the extract treatment ameliorating the effect [49]. In the same trend, the serum ALT activity in the infected nontreated mice when compared with the infected mice treated with flavonoid subfraction 3 (Figure 10) suggests that there may be enzyme leakages from tissues as a result of damage to the cell membrane [50]. The fact that there are no significant differences in ALP concentration in untreated mice when compared with those treated with the subfraction 3 implies that there was no loss of membrane component into the ECF or enzyme inactivation or depletion of important molecules needed for enzyme optimum activity [51].

Table 5: Polyphenolic compounds identified from the subfraction 3 flavonoid of *A. senegalensis*.

Polyphenol	RT	ppm	Area (mAU*s)	Amount area
Gallic acid	4.398	5.78038*e*-1.	53.0048	1.09054*e*-2
Rutin	22.261	65.34865	829.70630	7.87612*e*-2
Quercetin	37.278	7.29051*e*-1	12.31526	5.91990*e*-2
Kaempferol	38.33	1.52028	23.65234	6.42762*e*-2
Luteolin	39.173	32.77736	197.31702	1.66115*e*-1

The observed increase in the total protein content only in mice treated with the fraction 3 (Figure 10) suggests the hepatic synthetic capability due to the flavonoid administration to the experimental animals. The flavonoid fraction must have altered the hepatic functionality by compromising the balance in the synthesis, destruction, and clearance of the total protein from the animal's system. Previous findings have previously reported a corroborated finding [52, 53]. The total protein increase could lead to system dehydration, and this is detrimental to homeostasis of the cell. This will induce a negative effect on hepatic metabolic activities and thus the health status of the animals [52, 53].

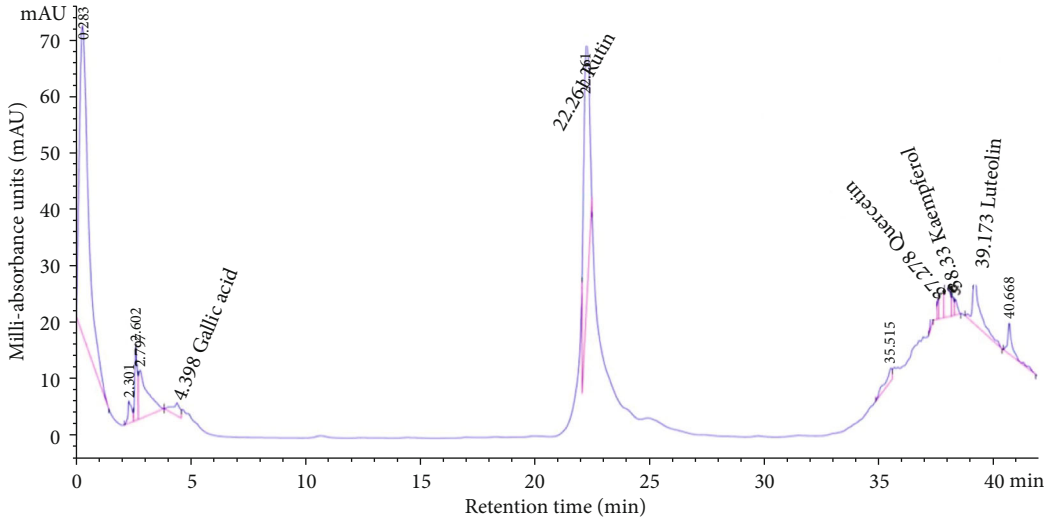

Figure 11: HPLC chromatogram of compound identified from the subfraction 3.

The current study found that subfraction 3 of the flavonoid demonstrated promising antitrypanosomal properties. Therefore, we next characterized the fraction using the HPLC analysis. Our results revealed the presence of 5 polyphenolic compounds including gallic acid (RT: 4.39, 0.578 ppm), rutin (RT: 22.26, 65.34 ppm), quercetin (RT: 37.27, 0.729 ppm), kaempferol (RT: 38.33, 1.52 ppm), and luteolin (RT; 39.173, 32.77 ppm) (Table 5 and Figure 11). Collectively, the study revealed that rutin is the most abundant flavonoid content that could be attributed to the antitrypanosomal activities of subfraction 3 flavonoid of *A. senegalensis*. Previous studies have implicated these compounds as the bioactive principles responsible for the bioactivity of several medicinal plants [39]. Taken together, flavonoid subfraction 3 of *A. senegalensis*, therefore, represents a reserve of bioactive metabolite for the management of trypanosomiasis. However, the limitation of this study lies on the isolation and identification of the specific flavonoid compound that is responsible for the significant therapeutic effects of the flavonoid subfraction 3. However, a study is ongoing in our laboratory in this regard.

5. Conclusion

In this study, it has been revealed that aqueous extract, total flavonoids, and flavonoid subfractions obtained from *A. senegalensis* leaves had significant effects against *Trypanosoma brucei brucei* in vivo by reducing parasite count in the infected mice. However, flavonoid subfraction 3 at a dose of 100 mg/kg bw was more effective against trypanosome than aqueous and total flavonoids. Both aqueous extract and total flavonoids ameliorate the effect of trypanosomiasis on PCV in experimental mice. Rutin was the most abundant bioactive compound that could be attributed to the bioactivity of the fraction. Taken together, flavonoid subfraction 3 of *A. senegalensis* can be a source of a lead molecule for the development of a new drug for the treatment of trypanosomiasis and its associated complications.

Data Availability

All raw data use in this study are available upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

All authors participate in research design, conducted the research work, and write the manuscript. All authors read and approved the final manuscript.

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References

[1] P. Büscher, G. Cecchi, V. Jamonneau, and G. Priotto, "Human African trypanosomiasis," *The Lancet*, vol. 390, no. 10110, pp. 2397–2409, 2017.

[2] D. Steverding, "The history of African trypanosomiasis," *Parasites & Vectors*, vol. 1, no. 1, pp. 3–8, 2008.

[3] B. Wellde, R. Lötzsch, G. Deindl, E. Sadun, J. Williams, and G. Warui, "Trypanosoma congolense: I. Clinical observations of experimentally infected cattle," *Experimental Parasitology*, vol. 36, no. 1, pp. 6–19, 1974.

[4] J. N. Abenga, "A comparative pathology of Trypanosoma brucei infections," *Global Advanced Research Journal of Medicine and Medical Sciences*, vol. 3, pp. 390–399, 2014.

[5] D. Legros, G. Ollivier, M. Gastellu-Etchegorry et al., "Treatment of human African trypanosomiasis–present situation and needs for research and development," *The Lancet Infectious Diseases*, vol. 2, no. 7, pp. 437–440, 2002.

[6] A. H. Fairlamb, "Chemotherapy of human African trypanosomiasis: current and future prospects," *Trends in Parasitology*, vol. 19, no. 11, pp. 488–494, 2003.

[7] A. Kabiru, A. A. Salako, and E. O. Ogbadoyi, "Therapeutic effects of Annona senegalensis Pers stem bark extracts in experimental African trypanosomiasis," *International Journal of Health Research*, vol. 3, pp. 45–49, 2011.

[8] I. Hussain, R. Ullah, M. Khurram et al., "Phytochemical analysis of selected medicinal plants," *African Journal of Biotechnology*, vol. 10, pp. 7487–7492, 2011.

[9] L. Bashir, O. Shittu, S. Sani, M. Busari, and K. Adeniyi, "African natural products with potential anti-trypanosomal properties: a review," *International Journal of Biochemistry Research & Review*, vol. 7, no. 2, pp. 45–79, 2015.

[10] G.-H. Du, *Natural Small Molecule Drugs from Plants*, Springer, 2018.

[11] M. Saxena, J. Saxena, R. Nema, D. Singh, and A. Gupta, "Phytochemistry of medicinal plants," *Journal of Pharmacognosy and Phytochemistry*, vol. 1, pp. 168–182, 2013.

[12] T. O. Ajboye, M. Yakubu, A. K. Salau, A. T. Oladiji, M. A. Akanji, and J. Okogun, "Antioxidant and drug detoxification potential of aqueous extract of Annona senegalensis leaves in carbon tetrachloride-induced hepatocellular damage," *Pharmaceutical Biology*, vol. 48, no. 12, pp. 1361–1370, 2010.

[13] S. E. Okhale, E. Akpan, O. T. Fatokun, K. B. Esievo, and O. F. Kunle, "Annona senegalensis Persoon (Annonaceae): a review of its ethnomedicinal uses, biological activities and phytocompounds," *Journal of Pharmacognosy and Phytochemistry*, vol. 5, p. 211, 2016.

[14] A. Aiyeloja and O. Bello, "Ethnobotanical potentials of common herbs in Nigeria: a case study of Enugu state," *Educational Research and Reviews*, vol. 1, pp. 16–22, 2006.

[15] S. H. Dambatta and B. Aliyu, "A survey of major ethno medicinal plants of Kano north, Nigeria, their knowledge and uses by traditional healers," *Bayero Journal of Pure and Applied Sciences*, vol. 4, pp. 28–34, 2011.

[16] J. Yisa, J. Egila, and A. Darlinton, "Chemical composition of Annona senegalensis from Nupe land," *Nigeria. African Journal of Biotechnology*, vol. 9, pp. 4106–4109, 2010.

[17] R. A. Adisa, N. Kolawole, L. A. Sulaimon, B. Brai, and A. Ijaola, "Alterations of antioxidant status and mitochondrial succinate dehydrogenase activity in the liver of Wistar strain albino rats treated with by ethanol extracts of Annona senegalensis Pers (Annonaceae) stem bark," *Toxicological Research*, vol. 35, no. 1, pp. 13–24, 2019.

[18] B. Adzu, S. Amos, M. Adamu, and K. Gamaniel, "Anti-nociceptive and anti-inflammatory effects of the methanol extract of Annona senegalensis root bark," *Journal of Natural Remedies*, vol. 3, pp. 63–67, 2003.

[19] E. O. Ogbadoyi, A. O. Abdulganiy, T. Adama, and J. Okogun, "In vivo trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei*," *Journal of Ethnopharmacology*, vol. 112, no. 1, pp. 85–89, 2007.

[20] K. Konaté, A. Sanou, R. R. Aworet-Samseny et al., "Safety profile, in vitro anti-inflammatory activity, and in vivo antiulcerogenic potential of root barks from *Annona senegalensis* Pers. (Annonaceae)," *Evidence-based Complementary and Alternative Medicine*, vol. 2021, Article ID 4441375, 12 pages, 2021.

[21] M. U. Ahmed, R. O. Arise, and I. J. Umaru, "Identification and biochemical characterization of anti-enteropooling compounds from *Annona senegalensis* root bark," *Scientific African*, vol. 15, article e01128, 2022.

[22] T. A. Ndarubu, O. S. Chiamaka, S. Alfa et al., "Phytochemicals, hypoglycemic and hypolipidemic effects of methanol leaf extract of Hibiscus sabdariffa in alloxan induced diabetic rats," *GSC Biological and Pharmaceutical Sciences*, vol. 8, no. 3, pp. 070–078, 2019.

[23] A. Tsado, L. Bashir, S. Mohammed et al., "Phytochemical composition and antimalarial activity of methanol leaf extract of Crateva adansonii in Plasmodium berghei infected mice," *Biotechnology Journal International*, vol. 6, no. 4, pp. 165–173, 2015.

[24] B. Lawal, P. Ossai, O. Shittu, A. Abubakar, and A. Ibrahim, "Evaluation of phytochemicals, proximate, minerals and anti-nutritional compositions of yam peel, maize chaff and bean coat," *International journal of Applied Biological research*, vol. 6, pp. 1–17, 2014.

[25] A. Sofowora, "Research on medicinal plants and traditional medicine in Africa," *Journal of Alternative and Complementary Medicine*, vol. 2, no. 3, pp. 365–372, 1996.

[26] A. E. Hagerman, K. M. Riedl, G. A. Jones et al., "High molecular weight plant polyphenolics (tannins) as biological antioxidants," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 5, pp. 1887–1892, 1998.

[27] G. Trease, "Trease and Evans," *Pharmacognosy, A Physician's Guide to Herbal Medicine*, vol. 13, p. 912, 1989.

[28] H. P. S. Makkar, M. Blümmel, N. K. Borowy, and K. Becker, "Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods," *Journal of the Science of Food and Agriculture*, vol. 61, no. 2, pp. 161–165, 1993.

[29] V. C. Midkiff, "The history of feed analysis, as chronicled in the development of AOAC official methods, 1884-1984," *Journal-Association of Official Analytical Chemists*, vol. 67, no. 5, pp. 851–860, 1984.

[30] Y. M. Musa, "Isolation and purification of flavonoids from the leaves of Mitracarpushirtus plant," *IOSR Journal of Applied Chemistry*, vol. 8, pp. 1–3, 2015.

[31] A. Mann and E. O. Ogbadoyi, "Evaluation of medicinal plants from Nupeland for their in vivo antitrypanosomal activity," *American Journal of Biochemistry*, vol. 2, no. 1, pp. 1–6, 2012.

[32] W. Herbert and W. Lumsden, "*Trypanosoma brucei*: a rapid "matching" method for estimating the host's parasitemia," *Experimental Parasitology*, vol. 40, no. 3, pp. 427–431, 1976.

[33] W. Peters and B. Robinson, "The chemotherapy of rodent malaria. XLVII. Studies on pyronaridine and other Mannich base antimalarials," *Annals of Tropical Medicine & Parasitology*, vol. 86, no. 5, pp. 455–465, 1992.

[34] J. Dacie and S. Lewis, "Practical Textbook of Haematology 7th Edition Edinburgh," *Church Livingstone*, vol. 7, pp. 54–79, 1991.

[35] F. De Ritis, M. Coltorti, and G. Giusti, "Serum-transaminase activities in liver disease," *The Lancet*, vol. 299, no. 7752, pp. 685–687, 1972.

[36] R. Rej, "Measurement of aminotransferases: part 1. Aspartate aminotransferase," *Critical Reviews in Clinical Laboratory Sciences*, vol. 21, pp. 99–186, 1984.

[37] P. J. Wright and D. T. Plummer, "The use of urinary enzyme measurements to detect renal damage caused by nephrotoxic compounds," *Biochemical Pharmacology*, vol. 23, no. 1, pp. 65–73, 1974.

[38] A. G. Gornall, C. J. Bardawill, and M. M. David, "Determination of serum proteins by means of the biuret reaction," *Journal of Biological Chemistry*, vol. 177, no. 2, pp. 751–766, 1949.

[39] A. S. Onikanni, B. Lawal, B. E. Oyinloye et al., "Therapeutic efficacy of *Clompanus pubescens* leaves fractions via downregulation of neuronal cholinesterases/Na^+-K^+ATPase/IL-1 β, and improving the neurocognitive and antioxidants status of streptozotocin- induced diabetic rats," *Biomedicine & Pharmacotherapy*, vol. 148, article 112730, 2022.

[40] H. O. Awobode, F. T. Fagbemi, and F. I. Afolayan, "Antitrypanosomal activity of Khaya senegalensis and Anogeissus leiocarpus stem bark on Trypanosoma brucei brucei infected rats," *African Journal of Biotechnology*, vol. 14, no. 6, pp. 525–529, 2015.

[41] R. Antia, J. Olayemi, O. Aina, and E. Ajaiyeoba, "In vitro and in vivo animal model antitrypanosomal evaluation of ten medicinal plant extracts from south west Nigeria," *African Journal of Biotechnology*, vol. 8, 2009.

[42] N. Jan, K. I. Andrabi, and R. John, "Calendula officinalis-an important medicinal plant with potential biological properties," *Proceedings of the Indian National Science Academy*, vol. 93, pp. 769–787, 2017.

[43] T. O. Johnson, A. Olatunde, and L. Nwachukwu, "Phytochemical composition of Annona senegalensis leaf and its antioxidant activity during Trypanosoma brucei brucei induced oxidative stress in mice," *Journal of Pharmacy & Bioresources*, vol. 14, pp. 219–227, 2017.

[44] J. M. Sternberg, "Human African trypanosomiasis: clinical presentation and immune response," *Parasite Immunology*, vol. 26, no. 11-12, pp. 469–476, 2004.

[45] J.-S. Shim, S.-D. Kim, T.-S. Kim, and K.-N. Kim, "Biological activities of flavonoid glycosides isolated from Angelica keiskei," *Korean Journal of Food Science and Technology*, vol. 37, pp. 78–83, 2005.

[46] O. K. Yusuf, C. O. Bewaji, and J. T. Ekanem, "Biochemical evaluation of fermented wheat germ extract on Trypanosoma brucei-infected rats," *African Journal of Biomedical Research*, vol. 13, pp. 219–224, 2010.

[47] J. T. Ekanem and O. K. Yusuf, "Some biochemical and haematological effects of black seed (Nigella sativa) oil on Trypanosoma brucei-infected rats," *African Journal of Biotechnology*, vol. 7, 2008.

[48] A. A. Jigam, F. Mahmood, and B. Lawal, "Protective effects of crude and alkaloidal extracts of Tamarindus indica against acute inflammation and nociception in rats," *Journal of Acute Disease*, vol. 6, no. 2, pp. 78–81, 2017.

[49] O. Shittu, B. Lawal, and O. Oluyomi, "Effects of methanol extract of *Musca domestica* larvae on antioxidants enzymes in T. Brucei infected rats," *Nigerian Journal of Basic and Applied Sciences*, vol. 29, pp. 1–10, 2014.

[50] H. T. Manekeng, A. T. Mbaveng, S. A. Ntyam Mendo, A.-J. D. Agokeng, and V. Kuete, "Evaluation of acute and subacute toxicities of *Psidium guajava* methanolic bark extract: a botanical with in vitro antiproliferative potential," *Evidence-based Complementary and Alternative Medicine*, vol. 2019, Article ID 8306986, 13 pages, 2019.

[51] S. A. Reid, D. B. Speedy, J. M. Thompson et al., "Study of hematological and biochemical parameters in runners completing a standard marathon," *Clinical Journal of Sport Medicine*, vol. 14, no. 6, pp. 344–353, 2004.

[52] M. A. Akanji, A. K. Salau, and M. Yakubu, "Safety evaluation of aqueous extract of Crateva adansonii leaves on selected tissues of rats," *Fountain Journal of Natural and Applied Sciences*, vol. 2, pp. 17–28, 2013.

[53] A. A. Yusuf, B. Lawal, M. A. Yusuf, A. O. Adejoke, F. H. Raji, and D. L. Wenawo, "Free radical scavenging, antimicrobial activities and effect of sub-acute exposure to Nigerian Xylopia Aethiopica seed extract on liver and kidney functional indices of albino rat," *Iranian Journal of Toxicology*, vol. 12, no. 3, pp. 51–58, 2018.