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Original Research Article

Extracts of *Telfeiria occidentalis* as an Alternative Treatment for *Trypanosoma brucei* Infection in Mice

Omowaye O. Stephen[1], Oche J. Otorkpa[2], Maji A. Emmanel[3], Abuka V. Amichi[1], Attah Friday[4], Ayodele P. Folorunsho[5], Oludare T. Tayo[6] and Olubiyo C. Kehinde[7]

[1]Department of Microbiology, Federal University Lokoja, Kogi State, Nigeria..
[2]Department of Public Health,National Open University of Nigeria., Lokoja, Nigeria.
[3]Department of Biosciences, College of Natural and Applied Sciences, Salem University, Lokoja, Kogi State, Nigeria
[4]Department of Microbiology, Confluence University of Science and Technology, Osara, Kogi State, Nigeria.
[5]Department of Biological Sciences, Thomas Adewumi University, Oko-Irese, Kwara State, Nigeria.
[6]Department of Biology, Confluence University of Science and Technology,Osara, Kogi State Nigeria.
[7]Department of Biological Sciences, Kogi State University, Kabba.

Corresponding Author's email:jesuniyi4wealth@gmail.com Contact: 08037696091

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Abstract

Purpose: Historically, plants have been used to cure parasite illnesses, such as infections caused by *Trypanosoma brucei*. Using *in vivo* techniques, this study evaluated the antitrypanosomal properties of methanolic extracts of *Telfairia occidentalis* (Family Cucurbitaceae).
Method: Mice were infected with 0.1 ml of blood containing roughly 1×10^3 trypanosomes for the *in vivo* study. Blood samples were drawn, smeared, stained with 10% Giemsa dye, and seen under a microscope over the course of seven days. Extracts were added, incubated for 10 to 15 minutes, and then observed at concentrations of 0.1, 0.01, and 0.001 mg/ml. Before, during, and after a seven-day oral treatment, haematological parameters were tracked. With no discernible negative effects ($P > 0.05$).
Results: The methanolic extract of *Telfairia occidentalis* (TOM) shows remarkable efficiency in maintaining and re-establishing normal physiological functioning in the infected mice. Fifteen bioactive components were found in the methanolic extract of *Telfairia occidentalis* leaves using gas chromatography-mass spectrometry (GC-MS) analysis. Among the other bioactive compounds discovered, oleic acid accounted for the largest percentage at 42.26 per cent, followed by propanoic acid (12.98 per cent) and docosanoic acid (1.18 per cent). Because of its efficacy and few side effects.
Conclusion: The study emphasi*ses Telfairia occidentalis* methanolic extract as a possible treatment for trypanosomiasis. These results provide credence to its possible use in the treatment of this parasite illness. To guarantee safety and therapeutic success, it is crucial to optimise extraction techniques and dosages, as evidenced by the toxicity and reduced efficacy seen in the moderate dosage concentration.

Keywords:*Trypanosoma brucei, Telfairia occidentalis,* Alternative *drug, induced mice,*

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INTRODUCTION

Trypanosomiasis is still one of the most difficult tropical diseases to treat in Nigeria and throughout Africa. Conventional treatments have had minimal effectiveness in eliminating the disease-causing *Trypanosoma brucei* parasite, despite multiple attempts to do so[4]. Human African Trypanosomiasis (HAT), a disease with two clinical stages, is caused by *Trypanosoma brucei*. The parasite lives in the bloodstream and lymphatic system during the first stage, known as the haemolymphatic phase, and produces symptoms like fever, headaches and anaemia.

The condition of lymphadenopathy [9]. Neuropsychiatric symptoms, such as sleep difficulties, are present in the second stag[e7]. Because the symptoms of the various stages overlap, misdiagnosis with other neuropsychiatric and febrile disorders is still frequent. [17]. The complicated life cycle of *Trypanosoma bruce*i, which is spread by Glossina (tsetse flies), makes the parasite difficult to eradicate. Disease control efforts are made more difficult by the parasite's capacity to elude host immunity and the resistance of certain tsetse species to infection. [15, 16];

The abundance of medicinal plants found in Africa's great biodiversity can be used to treat a variety of illnesses. [2]. Nigerian native *Telfairia occidentalis* is utilised for its antibacterial and antiparasitic qualities, among other medicinal uses. Research on the effectiveness of T. occidentalis in treating various illnesses is scarce, but nothing is known about how it works against *Trypanosoma bruce*i. [1]. The use of *T. occidentalis* for trypanosomiasis, especially in animal models, has received little to no investigation despite advancements in the study of medicinal plants. By examining the effectiveness of *T. occidentalis* extracts as a substitute therapy for *Trypanosoma brucei* in experimentally infected mice, this work aims to close this gap. This study tackles important problems, such as the growing resistance to traditional medications and the negative consequences they cause, and it suggests a possible remedy in the form of plant-based substitutes.

MATERIALS AND METHODS

Plant collection and authentication:

T. occidentalis (fluted pumpkin) leaves were purchased from Pata market, Lokoja, Kogi State, and identified and authenticated at the Department of Plant Biology and Biotechnology Herbarium Unit, Faculty of Life Science, University of Benin City, Edo State, using Voucher Number UBH-T187.

Plate 1:*Telfairia occidentalis* [19]

Preparation of plant extracts

The leaves of *T. occidentalis* were gathered, cleaned with water, allowed to air dry or shade dry, manually crushed to the appropriate size, and then ground into a fine powder. Following three days of maceration with intermittent shaking, this powder was mixed with 80% methanol in an Erlenmeyer flask and filtered through thick layers of 40 mesh gauze and a Whatman paper filter (No. 1). The second extraction was performed again after 72 hours of residual maceration, and an additional 72 hours later, the same procedure was repeated to increase the yield.

A rotary evaporator was used to concentrate the filtrate at 40°C in order to remove the methanol. The concentrated filtrate was then lyophilised to produce a dry powder by removing the water. The final quantity was weighed and stored in an airtight bottle in the refrigerator prior to use.

Phytochemical screening

According to standard practice, phytochemical screening of the different extracts was carried out to see if phytoconstituents like steroids, alkaloids, flavonoids, tannins, glycosides, and saponins were present.

Qualitative phytochemical screening of *Telfairia occidentalis* leaf extracts

Test for Phenols

Singleton noted that the test was conducted using the [14]. technique. Two millilitres of the extract were placed in a beaker. Then, a few drops of a ferric chloride solution were added. A deep bluish-green solution was a sign that phenols were present

Test for Terpenoids
The Salkowski test was conducted using the [5]. method. Five millilitres of aqueous extract were mixed with two millilitres of chloroform. Then, three millilitres of sulphuric acid concentrate were added to form a layer. The reddish-brown colour of the interface indicated the presence of terpenoids.

Test for Saponins
The 3rd approach was used to conduct the test. When a few drops of distilled water were added to 2.5 millilitres of the filtrate and stirred very well to make a stable, long-lasting froth, a thick layer of lather formed, which showed that saponins were present.

Test for Flavonoids (Shindo's Test)
The 3. approach was used to conduct the test. After mixing 0.5 g of magnesium with a third millilitre of the extract, the mixture was heated in a steam bath at 40 to 50 degrees Celsius for five minutes. The colour changed from orange to red, indicating the presence of flavonoids.

Test for Alkaloids
The Ajuru [3.] approach was used to conduct the test. We measured out one millilitre of the extract and placed it in a test tube. Mayer's reagents and 0.5 millilitres of diluted hydrochloric acid were then added to the mixture. The presence of a white precipitate gave the samples a positive score.

Test for Glycosides
The 10th method was employed to carry out the Kellar-Kiliani test. Two millilitres of the filtrate were combined with one millilitre of glacial acetic acid. Then, one millilitre of ferric chloride and one millilitre of strong sulphuric acid were added. The green-blue colouration of the solution indicated the presence of a glycoside.

Test for Tannins
We conducted the test using technique [8]. Three millilitres of methanolic extract (1:1) were mixed with a 10% alcoholic ferric chloride solution. The solution's dark blue colouration development suggested the presence of tannins.

Test for steroids.
Steroid identification was accomplished using the [5.] approach. The presence of steroids was indicated by the change in colour from blue to dark green that occurred when 1 millilitre of extract was mixed with 2 millilitres of concentrated sulphuric acid and 2 millilitres of acetic anhydride.

Quantitative Phytochemical Screening of *Telfairia occidentalis*'s Extract
Total Phenol Determination The [13.] technique was used to determine the extract's total phenol concentration. After dissolving 0.01 g of the extract in 10 mL of distilled water, 2.5 mL of 10% Folin-Ciocalteu's reagent oxidised 0.5 mL of the extract, which was subsequently neutralised with 2 mL of 7.5% sodium carbonate. We incubated the reaction mixture at 45°C for forty minutes. The double beam Shimadzu UV. A spectrophotometer, UV-1800, was used to measure absorbance at 765 nm. We created the calibration curve using standard garlic acid.

Total Flavonoids Determination
The method of [16, 18]. was used to ascertain the total flavonoid content of the extract. 0.5 ml of the extract was put into a test tube containing 1.5 ml of 100% methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M sodium acetate, and 2.8 ml of distilled water. Next, we incubated the tube at room temperature (28°C) for 30 minutes. The absorbance was measured at 415 nm using a double beam UV spectrophotometer (Shimadzu, UV-1800 Japan). The calibration curve was created using standard quercetin.

Total Alkaloid Determination
The 11th method was used to determine the extract's total alkaloid content. This procedure was used to weigh 0.5 g of the crude extract, dissolve it in 5 ml of a 96% ethanol:20% H_{2SO_4} (1:1) mixture, and filter the liquid. A test tube containing five millilitres of 60% H_{2SO_4} was then filled with one millilitre of the filtrate, and it was let to stand for five minutes. After adding five millilitres of 0.5% formaldehyde, the mixture was allowed to stand at room temperature for three hours.The absorbance was read at a wavelength of 565 nm. Vincristine extinction coefficient (E296, ethanol {ETOH} = 15136 M^{-1} cm^{-1}) was used as a reference alkaloid.

Determination of Tannins
The method of AOAC (1984) was used to determine the crude extract's tannin concentration. A 50 ml beaker with 0.2 g of the extract was filled with 20 ml of 50% methanol, covered with parafilm, and heated for an hour at 80 °C in a water bath. To guarantee homogeneity, the reaction mixture was briskly stirred. After filtering the extract into a 100 ml volumetric flask, 10 ml of sodium carbonate, 2.5 ml of Folin-Denis' reagent, and 20 ml of distilled water were added and thoroughly mixed. We then let the reaction mixture stand at room temperature for 20 minutes to develop a bluish-green hue.The absorbance was

taken at 760 nm using a double beam UV spectrophotometer (Shimadzu, UV-1800, Japan). Standard tannic acid was used to prepare the calibration curve.

Determination of Saponins

We determined the extract's saponin content using the 12-step methodology. After weighing and dissolving 0.5 g of the crude extract in 20 ml of 1N HCl, it was heated for 4 hours at 80 °C in a water bath. After cooling, the reaction mixture was filtered. We added 50 millilitres of petroleum ether, gathered the ether layer, and dried it by evaporation. n. Thereafter, two millilitres of concentrated sulphuric acid, six millilitres of ferrous sulphate, and five millilitres of acetone-ethanol (1:1) were added, and the mixture was let to stand for ten minutes. At 490 nm, the absorbance was measured. The calibration curve was created using standard saponins.

GC-MS Analysis (Gas Chromatography-Mass Spectroscopy)

The plant extract can be taken to Abuja for GCMS analysis to evaluate the potential antimicrobial activity. GC-MS: *We used the EI-MS spectrum to analyse and characterise the leaf extract of Telfairia occidentalis.* GC-MS is a combination of two analytical techniques into a single method of analysing the mixtures of chemical compounds. Gas chromatography separates the components of the mixture, and mass spectrometry analyses each of the components separately. We We compared the spectra of the unknown and known components. We used pharmaceutical databases to identify and characterise the molecule that the spectra revealed. es**.** *GC-MS identified the components present in the T. occidentalis extracts.* The active principles with their retention time (RT), molecular weight (MW) and percentage composition in the methanolic extract of *T. occidentalis* are presented in Table 3.

Plant Extract Sterility

The sterility of the extract was confirmed by the standard laboratory procedure, which involved incubating *Telf*airia occidentalis leaves extract on sterile nutrient agar for 24 hours at 37°C. The absence of microbiological growth during incubation proved the extract's sterility. [16 18.]

Test organs and experimental animals

Trypanosoma brucei was gotten from the Nigeria Institute for Trypanosomiasis in Evom, Jos, and the donor mice were used to keep the parasite alive during the study's real protocol. We purchased both-sex and pellet-fed mice. We kept the animals at room temperature, fed them a pellet diet, and provided them with free access to water in cages designed to resemble a typical animal home. We also maintained them in a 12-hour natural light-dark cycle. They spent a week getting acquainted with the test environment prior to the experiment. The handling and care of the mice adhered to international criteria for the use and upkeep of experimental animals.

Experimental Design

We randomly selected twenty-four 16-week-old mice into six groups, A, B, C, D, E, and F, each containing four mice. e. Group A was uninfected and untreated (control); Group B was uninfected but receiving treatment (0.1, 0.01 & 0.001 mg/ml bwt TOM); Group C was infected but not receiving treatment (*Trypanosoma brucei)*; Group D was infected and receiving treatment *(Trypanosoma brucei)*; Group E was infected and receiving treatment *(Trypanosoma brucei)*; Group F was infected and receiving treatment (0.1, 0.01, 0.001 mg/ml bwt TOM). Each mouse in the infected groups received an intraperitoneal injection of 0.1 ml of blood containing roughly $1x10^3$ Trypanosomes to infect the experimental mice. The trial ran for 7 days.

Paras: The number of parasitised red cells is five million.

The number of parasitised red cells multiplied by the number of white cells counted equals the number of parasites per litre.

Data analysis

The data were expressed as the mean ± SE.EM. Tabulation of information was employed with data obtained from samples. We used frequency distribution percentages and bar charts to address the formulated research questions. While descriptive and inferential statistics (regression analysis) were used to test the relationship between the variables and the effect of the independent on the dependent variables

RESULT AND DISCUSSION

In methanol extract of Telfairia occidentalis, there is presence of six phytochemical compounds. No presence of Terpenoids and Steroidswhile the remaining phytochemicals were in appreciable amount. Table 1

Table 1: Showing Qualitative Photochemical Analysis Qualitative phytochemical analysis of methanol extract of *Telfairia occidentalis* Leaves

Phytochemical	*Telfairia occidentalis* methanol
Phenols	+
Flavonoids	+
Tannins	+
Saponins	+
Alkaloids	+
Terpenoids	-
Cardiac glycosides	+
Steroids	-

Keys: "+" = present and "–'' absent

In table 2 results represent Mean ± Standard deviation Mean of duplicate determinations. Values with the same subscript on the same row are not significantly different at (P < 0. GC-MS analysis

Table 2: Showing Quantitative Phytochemical Analysis of Methanol Extract of Leaves

Phytochemical ***Telfairia occidentalis***	*Telfairia occidentalis*,methanol extract (mg/100 g)
Phenols	535.71 ± 0.55^{e}
Flavonoids	102.36 ± 084^{b}
Tannins	202.28 ± 0.63^{d}
Saponins	129.90 ± 0.41^{c}
Alkaloids	38.41 ± 7.04^{a}

The compounds were oleic acid (42.26%) as the major component followed by propanoic acid (12.98%), 9-Tetradecenal (10.17%), octane octanol (5.64%), 2-hexadecen-1-ol (4.39%), octadecanoicacid (4.29%), 1,2-ethane diyl ester (3.13), 3,5 octadecanoic acid (2.86%), methyl 11-octadecanoate (2.82%), Hexadecanoic acid (2.76%), 1,3 dimethyl ether (2.59%), 2-methoxy-4-vinyl (2.39%), Benzene (1.30%), 3,7-octadiene-2,6-diol (1.25%), Docosanoic acid (1.18%)(Table 3) Looking at figure1,above,various groups had almost the same weight before infection while there was weight gained immediately when plant extract was administered .it can also be seen that weight before and weight after treatment are almost recorded the same measurement in group DEF (Figure 1)

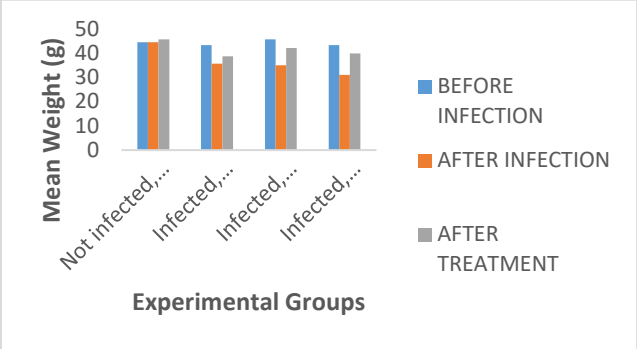

Figure 1: Showing Effect of Extracts of *Telfeiria occidentalis* On Mean Weight of Mice

According table 4, since therapy began on day three after infection, the temperature fluctuated within the normal range even though a fever was observed during the post-infection phase. However, the temperature increased above normal for the mice in the negative control group. None of the extract-treated mice's average daily temperature increased significantly

Looking at figure 2 above, groups ,E,F had almost the same temperature range immediately after treatment while groups D temperature range are lower compared to groups ,E,F (Figure 2)

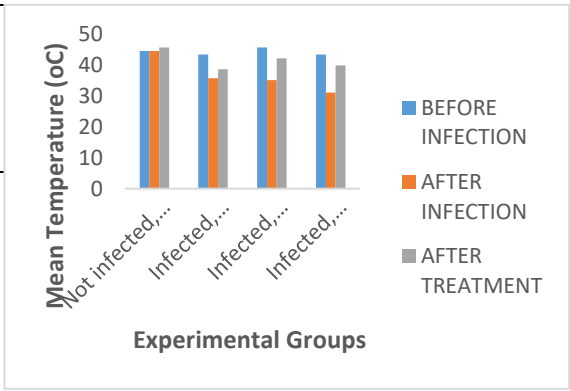

Figure 2: Showing Effect of Extracts of *Telfeiria occidentalis* On Mean Temperature Changes of Mice

During the duration of the illness, haemoglobin levels decreased, but after *T. occidentalis* methanol extracts were administered, they gradually began to rise. Following treatment, haemoglobin levels stabilised. This suggests that the drug may have helped to cure the disease and that the drop in Hb levels was a transient reaction to the infection or therapy. After the illness was treated, haemoglobin levels returned to normal. (Table 5)

Looking at figure 3 above, groups ,E,F had similar hemoglobin levels compared to groups D immediately after treatment. (Figure 3)
In table 6 above, PCV values decreased following 3 days post infection. However, following treatments with the methanol *T. occidentalis* extracts the PCV values increased. Only the negative control had a further decrease in the mean PCV value.(Table 6)

Looking at figure 4 above, groups D ,E, had similar Packed Cell Volume compared to groups F that had reduced Packed Cell Volume immediately after treatment. (Figure 3)

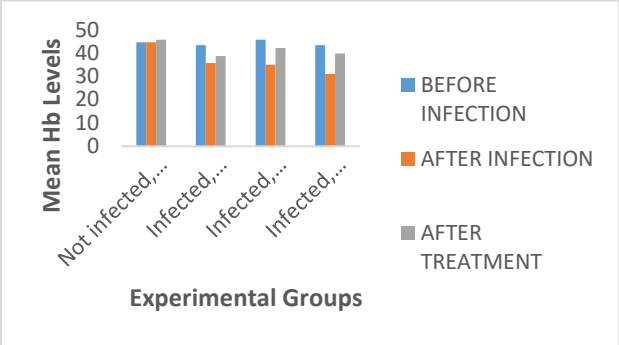

Figure 3: Showing Effect of Extracts of *Telfeiria occidentalis* On Mean Hemoglobin Changes

In table 7 above, Parasitemia count in Methanolic regardless the dosage given was lower in number compared to Parasitemia count in TWEEN 80 (PC) regardless the dosage given.(Table 6)

Table 3: Showing Components Detected in Methanol Extract of *Telfeiria occidentalis* Leaves

Peak	Compound name	Molecular formular	Molecular weight	Retention time	Peak height	Peak percentage	Ret index
1	Propanoic acid	$C_4H_8O_3$	104	3.68	1813303	12.98	867
2	Octane octanol	$C_{16}H_{36}O$	272	5.40	787295	5.64	1677
3	Benzene	C_8H_8O	120	7.51	181361	1.30	959
4	2-methoxy-4-vinyl	$C_9H_{10}O_2$	150	8.40	333782	2.39	1293
5	1,3-dimethyl ether	$C_8H_{10}O_3$	154	8.91	361856	2.59	1279
6	3,7-Octadiene-2,6-diol	$C_{10}H_{18}O_2$	170	12.24	174754	1.25	1197
7	3,5-Octadienoic acid	$C_9H_{14}O_3$	170	14.60	399398	2.86	1386
8	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.10	386177	2.76	1968
9	Methyl 11-octadecanoate	$C_{19}H_{36}O_2$	296	18.10	393517	2.82	2085
10	2-Hexadecen-1-ol	$C_{20}H_{40}O$	296	19.21	612709	4.39	2045
11	Docosanoic acid	$C_{23}H_{46}O_2$	354	19.31	164558	1.18	2475
12	Oleic acid	$C_{81}H_{34}O_2$	282	20.30	5903120	42.26	2175
13	Octadecanoic acid	$C_{39}H_{76}O_5$	624	21.70	599786	4.29	4395
14	9-Tetradecenal	$C_{14}H_{26}O$	210	23.54	1420548	10.17	1609
15	1,2-ethanediyl ester	$C_{37}H_{74}NO_8P$	691	23.74	437678	3.13	0
TOTAL	----------	---------	---------	------	13969842	100.0	-------

Table 4: Showing Average Temperature (T ^{O}C) Of Mice throughout Experiment

GROUPS	TREATMENT	BEFORE INFECTION	AFTER INFECTION	AFTER TREATMENT
A	Not infected, not treated	36.2	37.7ºC	36.6ºC
B	Not infected, treated	36.4ºC	36.9ºC	36.7ºC
C	Infected, not treated	35.7ºC	36.3ºC	36.4ºC

D	Infected, treated	35.9ºC	36.8ºC	36.9ºC
E	Infected, treated	36.6ºC	35.7ºC	36.9ºC
F	Infected, treated	36.5ºC	35.4ºC	36.4ºC

Table 5: Showing Average Hemoglobin of Mice throughout Experiment

GROUPS	TREATMENT	BEFORE INFECTION	AFTER INFECTION	AFTER TREATMENT
A	Not infected, not treated	15.2	15.6	15.6
B	Not infected, treated	14.9	14.9	15.2
C	Infected, not treated	14.9	10.9	8.4
D	Infected, treated	14.5	11.9	12.9
E	Infected, treated	15.2	11.7	14.1
F	Infected, treated	14.5	10.4	13.3

Table 6: Showing Average Packed Cell Volume of Mice throughout Experiment

GROUPS	TREATMENT	BEFORE INFECTION	AFTER INFECTION	AFTER TREATMENT
A	Not infected, not treated	45.7	46.8	46.8
B	Not infected, treated	44.6	44.6	45.7
C	Infected, not treated	44.6	33.2	28.9
D	Infected, treated	43.4	35.7	38.7
E	Infected, treated	45.7	35.1	42.2
F	Infected, treated	43.4	31.1	39.9

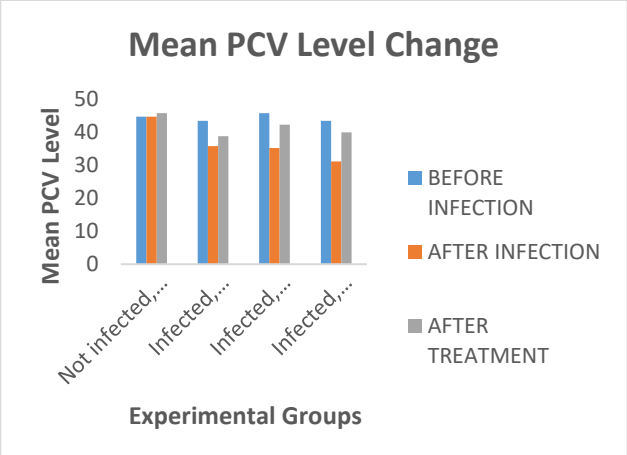

Figure 4: Showing Effect of Extracts of *Telfeiria occidentalis* on Mean Packed Cell Volume

It is imperative to find new chemicals to treat trypanosomiasis because the few approved anti-trypanosomal medications have numerous negative side effects, including toxicity and parasite resistance. Because plants are traditionally used to treat African diseases, they present potential opportunities for the development of innovative, affordable, and safe anti-trypanosomal medications. With the results of the methanolic extract of *Telfairia occidentalis* in this study, these facts had proved positive on *Trypanosoma brucei* and *Telfairia occidentalis* as safe anti-trypanosomal. North Central Nigerian trypanosomiasis [4, 12.] discovered that a large number of parasites of this kind were present in market fruit and vegetables in the middle zone of Nigeria, respectively, which calls for public intervention.

Table 7: Parasitemia count (PC) Comparison after Methanolic *Telfeiria occidentalis* Treatment and TWEEN 80 during the study

Different Dosage in Rats (mg/ml bwt)	Parasitemia count in Methanolic *Telfeiria occidentalis(*TOM)	Parasitemia count in TWEEN 80 (PC)
0.1	-	12
0.01	-	12
0.001	4	16
R2		
0.1	-	8
0.01	-	8
0.01	-	12
R3		
0.1	-	8
0.01	-	16
0.001	-	16
R4		
0.1	-	12
0.01	-	8
0.001	-	12

KEY:TOM: TELFEIRIA OCCIDENTALIS METHANOL extract,Parasitemia count (PC)

In the current study, a phytochemical screening of *Telfairia occidentalis* revealed the presence of a number of bioactive compounds, including flavonoids, alkaloids, and tannins. This is in line with research by [9.] that discovered alkaloids, flavonoids, tannins, and saponins in *T. occidentalis* methanol extracts. Flavonoids have been shown in earlier studies to be effective anti-trypanosomal drugs against a variety of trypanosome species. Because of the phytochemicals they contain, medicinal plants can both produce their therapeutic effects and serve as building blocks for the production of useful pharmaceuticals. Terpenoids and steroids were absent from the T. occidentalis methanol extracts.

.Thus,*Telfairia occidentalis* methanolic (TOM) may be another widely available anti-trypanosomal drug with minimal or no adverse effects on mice, as it was more dependable and effective in this experiment than in others. This is in line with the findings of 6, which previously reported that plant extracts were effective in suppressing parasite activities but not in the trypanosomal species of this study.

CONCLUSION

The study looked at the methanolic extract's antitrypanosomal qualities in an in experiment. The bioactive components need to be further purified and characterised to fully realise this extract's potential. *In vitro* testing is also required to evaluate the safety and efficacy of these extracts in treating *Trypanosoma brucei* infections in mice. *Telfairia occidentalis* methanolic extracts (TOM) demonstrated strong anti-trypanosomal effects, specifically a concentration-dependent reduction in trypanosome counts and an effective normalisation of haemoglobin (Hb).

CONFLICT OF INTEREST

The authors declare no conflict of interest

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AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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ETHICAL APPROVAL

The approval of the departmental animal ethical committee(FNAAB) Dated 20th November,2019 .All the protocols and experiments were conducted according to the guidelines approval.

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