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Original Research Article

ANTIMICROBIAL AND ANTITRYPANOSOMAL ACTIVITIES OF METHANOL ROOT EXTRACT AND FRACTIONS OF *BRENANIA BRIEYI* (DE WILD) PETIT (RUBIACEAE)

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ABSTRACT

Brenania brieyi (Rubiaceae) is used in ethnomedicine for the treatment of malaria, microbial infections, trypanosomal disease, infertility and inflammatory disorders. The study determined antitrypanosomal and antimicrobial activities of extract and fractions of *B. brieyi*. Cold maceration and solvent-solvent partitioning were used to obtain the extract and respective fractions in order of their increasing polarity: *n*-hexane, dichloromethane, ethylacetate and absolute methanol. Phytochemical analysis, acute toxicity test and haematological parameters (Packed Cell Volume, parasitemia count) were carried out using standard procedures. Rapid matching and Agar dilution methods were used for antitrypanosomal and antimicrobial studies respectively. The extract (100, 200 and 400 mg/kg), fractions (200 mg/kg) and diminazine aceturate (3.5 mg/kg) were administered per oral. The extract was found to be safe up to 5000 mg/kg. The phytoconstituents present in the plant include tannins, terpenoids, flavonoids, phlobatannins, alkaloids, saponins, cardiac glycosides, quinones, steroids and phenols. The methanol extract of *Brenania brieyi* (MEBB) root showed significant ($p < 0.05$) dose-dependent inhibition of parasitemia. Antitrypanosomal activity of the fractions showed that the fractions possess varying degree of parasitemia clearance at 200 mg/kg dose. Methanol fraction produced the highest parasitemia clearance (6.98 ± 0.08 %), followed by *n*-hexane (7.13± 0.23 %), dichloromethane (7.43± 0.14 %) while ethylacetate fraction (9.00± 0.00 %) caused the least parasitemia clearance when compared with the positive control (0.00± 0.00 %). Haematological parameters and survival time were significantly ($p < 0.05$) increased, *n*-hexane fraction exhibited significant ($p < 0.05$) increase in survival time at 200 mg/kg (after 26 days). Methanol fraction at MICs of 7 to10 mg/ml showed inhibitory activity against *Bacillus subtilis*, *Salmonella typhi, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus mutant, Candida albicans* and *Aspergillus niger*. The results obtained revealed that the extract and fractions possess antitrypanosomal and antimicrobial activity. The study provides scientific evidences for the traditional uses of the plant in treating infectious diseases..

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INTRODUCTION

Though a major and widespread source of mortality and morbidity in sub-Saharan Africa, Human African Trypanosomiasis (HAT), often known as sleeping sickness, has been acknowledged as one of the world's most neglected illnesses [1, 2]. *Trypanosoma brucei brucei*, *Trypanosoma congolense*, *Trypanosoma brucei rhodesiense* and

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Trypanosoma brucei gambiense cause trypanosomiasis [3, 4]. According to Pereira *et al*. [5], there are three primary phases to the clinical presentations of human African trypanosomiasis: cutaneous, hemolymphatic, and meningoencephalic. Currently, only certain conditions must be met in order to effectively diagnose human African trypanomiasis. Microscopy is required for a conclusive diagnosis in symptomatic patients that have parasites in their body fluids [6]. The major challenge in the treatment of this disease is that the registered drugs are usually toxic, requires lengthy parenteral administration, minimal efficacy and are expensive. Therefore, there is need to complement these existing drugs with new, safe, effective and cheaper alternative drugs [7]. In Nigeria there is diverse flora of wide spectrum with unique medicinal plants [8]. Futhermore, 54 chemicals from roughly 90 plants were shown to exhibit antitrypanosomal activity between 1990 and 2014 [9, 10]. Several contemporary medicinal plants are sources of bioactive natural ingredients, either in their native forms or as derivatives created from the natural lead compounds [11].

Brenania brieyi is in the family of Rubiaceae. Common name of *Brenania brieyi* is "Bokyi kalang" (Nigeria) while the local/indigenous name is "Mgbunsi" (Igbo) [12]. *Brenania brieyi* is found in African countries like Gabon, Ghana, Chad and Cameroon, Nigeria [13]. Ethno-traditional uses of the plant include remedy for parasitic infections, pain, impotency, endocrine disorders, fever, and swelling [14, 15]. The bark of *B. brieyi* is also used to treat gastrointestinal disorders, rib and heart ailments [16]. The fruit is also used as an emetic while the bark of the plant is used as vermifuges, genital stimulants/depressants and febrifuges. Other uses of the plant include its use as fish poisons (bark), carpentry applications and forestry [12].

The research aims to assess their antitrypanosomal and antimicrobial potentials of *B. brieyi*.

MATERIALS AND METHODS

Plant Material Collection, Processing and Identification

B. brieyi roots were procured in November 2021 from Abagana, Njikoka Local Government Area of Anambra, Nigeria. (It is located between 6° 11' 3" North, 6° 58' 36" East). Mr. Felix Nwafor, taxonomist at the Department of Pharmacognosy and Environmental Medicines, University of Nigeria Nsukka, authenticated the plant (Voucher bearing the number: PCG/UNN/0327). The roots were cleaned before being brought to the laboratory, where they were cut and allowed to dry for seven days. After being mechanically ground into powder, the dried powdered root was extracted using standard analytical grade methanol.

Extraction of Plant Material

Powdered root (800.6 g) of *B. brieyi* was extracted with portions (2.5L x 2) of aqueous methanol (95%) using cold maceration for 72 hours. Whatman filter paper No.1 was used for filtration. For exhaustive extraction of the plant to get a high yield, another round of 72-hour extractions of the marc were performed using 95 % methanol before filtering it. All the extracts (MEBB) were mixed together and dried *in vacuo*.

Successive Fractionation by Liquid-Liquid Partitioning

To get the various fractions, 76.3 g of the methanol extract was dissolved in 250 ml of 10% methanol and partitioned with *n*-hexane (*n*HF) (900 ml), dichloromethane (DCMF) (900 ml), ethyl acetate (EAF) (800 ml) and absolute methanol (MF) (800 ml).

Phytochemical Composition of the Extracts and Fractions

Extract and fractions of *B. brieyi* were screened for tannins, alkaloids, saponins, flavonoids, cardiac glycosides, steroids, phenols, terpenoids, quinones, and phlobatannins following standard methods [17].

Acute ToxicityTest

Acute toxicity (LD_{50}) of the extract of *Brenania brieyi* was determined according to the Lorke's method with slight modification [18, 19]. Thirteen (13) mice were divided into two stages. In stage one, nine mice were used, they were grouped into three groups of three (3) mice and were administered (10, 100, and 1000 mg/kg) orally of the extract (MEBB) respectively. They were observed for 24 hours for behavioural changes and death. The remaining 4 animals were given 1000, 1600, 2700 and 5000 mg/kg MEBB orally and also observed for 24 hours.

In Vivo Antitrypanosomal Screening

Experimental Animals

A total of fifty (50) Swiss albino mice (15 to 39 g) of both sexes were used for this study. Acclimatization of mice was done for seven (7) days; they were feed with standard pellets and allowed free access to water. Animal handling followed the approved protocol by the University of Nigeria Ethical Committee (FVUNN.IACUC.2019.0816).

Parasitological Studies

Parasite and Preparation of Inoculums

The parasite used for this experiment was obtained from a mouse infected with the flagellate protozoa *Trypanosoma brucei* found in an infected cattle and transported to the Parasitology Department University of Nigeria, Nsukka. Establishment of infection in the mouse was confirmed and blood was collected from the tail with a heparinized capillary tube. Phosphate buffered saline was used to dilute the blood and 0.1ml of the diluted blood was injected intraperitoneal in each mouse.

Parasite Inoculation

Animals of both sexes were divided at random into six (6) groups of five mice each. Infection was achieved using 0.1 ml of the donor mouse's blood injected intraperitoneally to infect the animals. Before administering the parasite to the mice, measurement of each mouse's body weight, packed cell volume and rectal temperature was all done. Following infection, mice were observed and their blood was monitored daily for the

presence of parasites. The level of experimental parasitemia in their blood was determined five days after infection, at which oral treatment was started.

Grouping and Dosing of Animals

Experimental mice were divided into six groups, of five mice of both sexes per group, Group 1-3 received orally 100, 200 and 400 mg/kg respectively of the crude extract (CE) whereas Groups 4-7 received 200 mg/kg each of *n*-hexane, dichloromethane, ethyl acetate and absolute methanol fraction respectively. Group 8 (Positive control) received the normal dosage of diminazene aceturate (3.5 mg/kg) while Group 9 received 0.2 ml of the vehicle (distilled water) (Negative control), Group 10 (Normal control received 0.2 ml of distilled water) were not infected and did not get treatment. The extract and fractions were administered orally to the mice daily for twelve (12) day. The following variables were recorded: body weight, parasitemia level, rectal temperature, packed cell volume (PCV) and survival time.

Determination of Parasitemia Level

Antitrypanosomal activity was determined following standard procedure as described by [20, 21]. Microscopic examinations were carried out on blood from the tail of infected mice. Microscopic counting of parasites per field was performed and parasitemia count was obtained by matching the number of parasites seen on the field with that on the standard matching paper. This test was used to check the level of parasitemia after infection. Parasitemia level was estimated using rapid matching method. The level of parasitemia was determined after infection on days 5, 7, 8, 11, 14 and 17. Equation 1 shows the formula used to calculate percentage parasitemia:

$$\text{Percentage Parasitemia (\%)} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC}} \times 100 \quad \text{Equation 1}$$

PCV (Packed Cell Volume) Determination

Packed cell volume (PCV) was determined using the micro hematocrit technique [22]. Using a heparinized micropipette that have one end sealed with plastacine, blood was drawn from each mouse's eye. The sealed micropipettes were spun at 1000 rpm for 5 minutes. The packed cell's length was read using a hematocrit reader. Each mouse had their PCV measured before being exposed to the parasite. On days 0, 5, 9, 13 and 17 of the experiment, the mice's PCVs were measured. Equation 2 shows the formula used to calculate the percentage of packed cell volume:

$$\text{PCV (\%)} = \frac{\text{Packed RBC column height}}{\text{Total blood volume height}} \times 100 \quad \text{Equation 2}$$

Rectal Temperature Determination

The rectal temperatures of the mice was determined with a digital thermometer. The rectal temperature of each mouse was taken before infecting them with the parasite. The rectal temperature of the mice was measured on days 0, 5, 7, 8, 11, 14 and 17.

Body Weight Determination

Weighing balance was used to determine the mice's weight. Animal weights were measured throughout the experiment on days 0, 5, 8, 11, 14 and 17.

Determination of Survival Time

Survival time of the mice was monitored for 31 days and used to assess the rate of recovery of the mice. Each mouse was monitored closely for possible death. The number of mice death per group as well as the number of mice that survived were recorded. Equation 3 shows the formular for calculating the mean survival time.

$$\text{Mean survival time} = \frac{\text{Number of days the animal survived}}{\text{Total number of days tested}} \quad \text{Equation 3}$$

Test Microorganisms

The test organisms were clinical isolates from the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, University of Nigeria, Nsukka. They include *Bacillus subtilis*, *Salmonella typhi, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus mutant, Candida albicans,* and *Aspergillus niger*.

Standardization of the Test Organism Suspension:

Standardization of organisms was done using 0.5 McFarland turbid equivalents.

Preparation of the Stock and Other Concentration of the Crude Extract and Fractions Used

The crude extract and fractions were prepared as a 50 mg/ml stock concentrations by dissolving 500 mg with 10 ml of a 50 % Dimethyl sulfoxide (DMSO) solvent. Various concentration prepared were 10, 9, 8, 7, 6, 5, 4, 3, 2, and 1 mg/ml. The control test (standard) were pure samples of Ciprofloxacin of concentration 15µg/ml, Fluconazole 30 µg/ml, and 50 % DMSO.

Agar Dilution Method

Agar dilution was employed to calculate the minimum inhibitory concentration (MIC) [23, 24, 25]. The following concentrations were prepared using sterile glucose-enriched molten agar on sterile Petri dishes: 10, 9, 8, 7, 6, 5, 4, 3, 2, and 1 mg/ml. According to the code assigned to each agent, the agar plates were labeled. Eight equal halves of each plate were divided with a permanent marker. Under stringent aseptic conditions, each test microorganism was inoculation by streaking on each segment of the sterile glucose-enriched agar plate and labeled. The culture plates were cultured in a bacteriological incubator at 37°C for 24 hours in an inverted position. The culture plates were examined after the appropriate incubation period, and the observations were noted and agent's MIC was recorded.

Statistical Analysis
Using SPSS version 25, One-Way ANOVA was used to analyse data, followed by Dunnett's multiple comparisons post-hoc test. Data are reported as mean and standard error of mean (SEM), statistical significance at $p< 0.05$.

RESULTS

Qualitative Phytochemical Analysis
The qualitative phytochemical screening of the methanol root extract and fractions of *Brenania brieyi* is represented in Table 1. The phytochemical screening revealed the presence of phenols, saponins, flavonoids, cardiac glycosides, tannins, alkaloids in the crude extract. Phlobatannins, terpenoids, steroids, and quinone were absent.

Antitrypanosomal Potentials
Infected mice treated with the extracts of *B. brieyi* showed a rapid reduction in parasitemia as shown in Table 2. The dose-dependent decrease in parasitemia was statistically significant ($p<0.05$). However, it was not until after day 14 (D14) that parasitemia cleared in group 2 (200 mg/kg MEBB) and group 3 (400 mg/kg MEBB), compared to Positive control (3.5 mg/kg diminazine aceturate) which cleared the parasitemia after Day 5 (D5). The parasitemia levels of negative control (NC) remained relatively same since they were infected but received no treatment while that of the normal group had no value since they were not infected.

Packed Cell Volume (PCV) estimation.
All the groups represented in Table 3**,** showed a marked decrease in PCV values on day 5 but recovered slowly throughout the rest of the treatment period. There was no significant difference between the treatment groups and positive control throughout the whole period. Only treatment CE400 mg/kg was able to restore the packed cell volume close to the value at Day 0 (D0)

Rectal Temperature
T. brucei caused a slight increase in temperature for all the groups including the controls throughout the treatment period. As presented in Table 4, there was a marked increase in temperature for all the groups including the controls but gradual fall in temperature starting from day 8 till day 17 (except for the negative control which maintained high rectal temperature throughout the study).

Body Weight
The present study showed that there was a sharp rise in weight of the animals treated with the extract on D5 ($p<0.05$) Table 5. This increase stayed almost same throughout the treatment period. The highest increase was seen in those treated with MEBB 100 mg/kg. There was no significant increase in body weight among those treated with the positive control group. There was only a slight increase in weight among the negative controls group throughout the treatment period.

Mean Survival Time
The survival time of MEBB100, 200 and 400 mg/kg were significantly ($P<0.05$) higher than those of positive (20.4 ± 3.49) and negative control (20.2 ± 3.88). Normal Control group had the highest survival time of 30 days since it was not infected, while that of ethylacetate fraction has the lowest. Survival time of *n*-hexane, dichloromethane and methanol fractions were significantly higher than those of positive control and negative control as shown in Table 6.

Antimicrobial Activity of Extract and Fractions of *B. brieyi*
At different range of MIC (1-10 mg/ml) the MEBB and fractions exhibited inhibitory effect against tested organisms when compared with the positive (ciprofloxacin 15µg/ml and fluconazole 30µg/ml) and negative control (DMSO 50 %). Table 7 showed that the crude extract exhibited a good antifungal and antibacterial potential especially moulds as seen in the lower MIC of *Aspergillus niger* of less than 1 mg/ml.

DISCUSSION

The present study evaluated the antitrypanosomal and antimicrobial potentials of the methanol root extract of *Brenania brieyi*. The acute toxicity showed that the extract is tolerable and safe even up to 5000 mg/kg. The study revealed that *B. brieyi* extract contained the following secondary metabolites: alkaloids, saponins, phenols, tannins, cardiac glycosides and flavonoids. Maikai *et al.,*[26] reported that flavonoids are effective phytoconstituents for antitrypanosomal compounds which contribute to the efficacy of the trypanocidal activities of this plant. Flavonoids target the replicating form of trypanosomes which are totally dependent on glycolysis for energy production [26, 27]. Alkaloids impact trypanosomes by DNA intercalation in conjunction with the reduction of protein synthesis. Phenols and polyphenolic substances affect the parasite through inhibition of alternative oxidase in trypanosome. Saponins have detergent capabilities that can disrupt the fluidity and function of membrane proteins as well as dissolve the parasite's biomembranes [27, 28]. Most of these phytoconstituents have active antimicrobial potentials against pathogenic microorganism [29, 30].

The anti-microbial disparity observed from this research could be due to the different phyto-constituents present in each fraction. However, anti-microbial efficacy depends on the concentration and interactions with other components as well as the secondary metabolites present in the extract and fractions [31]. The anti-microbial potentials of tannins may be attributed to its ability to react with proteins to form stable water insoluble component [32]. Alkaloids have the ability to intercalate with the nucleic acid component and interfere with cell division [33, 34]. Flavonoids and steroids bind with the bacterial cell wall which causes leakage of the cell contents thereby leading to cell death [35, 36].

The root extract and fractions demonstrated a considerable and quick decrease in the parasitemia load during *in-vivo* screening. Trypanosome motility is completely eliminated or reduced as a

Table 1: Phytochemical screening of the root extract and fractions of *B. brieyi*.

Phytoconstituents	MEBB	nHF	DCMF	EAF	MF
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	-	+	+	-	-
Cardiac Glycosides	+	_	+	+	+
Steroids	-	+	-	-	-
Phlobatannins	-	-	-	-	-
Phenols	+	+	+	+	+
Quinones	-	-	-	-	-

Keys: MEBB= Methanol extract of *B. brieyi*; *n*HF = *n*-hexane fraction; DCMF = Dichloromethane fraction; EAF = Ethyl acetate fraction; MF = Methanol fraction; - = absent; + = present.

Table 2: Effect of root extract and fractions of *B. brieyi* on the parasitemia level of infected mice

Groups (mg/kg)	Parasitemia Count (%)					
	Day 5	Day 7	Day 8	Day 11	Day 14	Day 17
MEBB 100	8.52 ± 0.073^{a}	8.34 ± 0.11^{a}	7.44 ± 0.112^{a}	7.32 ± 0.18	6.90 ± 0.00^{ab}	4.14 ± 1.69^{ab}
MEBB 200	8.58 ± 0.073^{ab}	7.68 ± 0.18^{a}	7.44 ± 0.112^{a}	7.20 ± 0.075^{b}	4.14 ± 1.69^{b}	0.00^{b}
MEBB 400	8.64 ± 0.06^{ab}	7.92 ± 0.22^{ab}	5.52 ± 1.38^{ab}	4.14 ± 1.69^{b}	4.14 ± 1.69^{b}	0.00^{b}
*n*HF 200	8.64 ± 0.06	8.22 ± 0.12^{ab}	8.10 ± 0.09^{ab}	7.62 ± 0.07^{ab}	7.26 ± 0.22^{ab}	7.13 ± 0.23^{ab}
DCMF 200	8.64 ± 0.11	7.92 ± 0.12^{ab}	8.22 ± 0.07^{ab}	7.68 ± 0.07^{ab}	7.44 ± 0.11^{ab}	7.43 ± 0.14^{ab}
EAF 200	8.64 ± 0.06	7.95 ± 0.15^{ab}	8.30 ± 0.10^{ab}	8.87 ± 0.13^{a}	8.93 ± 0.07^{a}	9.00 ± 0.00^{a}
MF 200	8.46 ± 0.11	8.40 ± 0.09^{ab}	7.74 ± 0.17^{ab}	7.20 ± 0.12^{ab}	7.05 ± 0.15^{ab}	6.98 ± 0.08^{ab}
PC	8.28 ± 0.07	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00b	0.00 ± 0.00^{b}
NC	8.33 ± 0.19	8.82 ± 0.07^{a}	9.00 ± 0.00^{a}	8.93 ± 0.08^{a}	8.93 ± 0.08^{a}	8.90 ± 0.10^{a}
N	0.00 ± 0.00^{ab}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Keys: Day 0=the day treatment commenced; Day 5= the 5th day of treatment, Day 7= the 7th day of treatment, Day 8= the 8th day of treatment, Day11= the 11th day of treatment, Day 14= the 14th day of treatment, Day 17= the 17th day of treatment. MEBB=Methanol extract of B. brieyi, nHF = n-hexane fraction; DCMF = Dichloromethane fraction; EAF = Ethyl acetate fraction; MF = Methanol fraction; N = Normal control received 0.2 ml of distilled water, PC= Positive Control received diminazine aceturate 3.5 mg/kg, NC=negative control received 0.2 ml of distilled water, All superscripts indicate significance at p<0.05 (a=compared to positive control; b=compared to NC) (Mean ± SEM, n = 5).

Table 3: Effect of root extract and fractions of *B. brieyi* on Packed Cell Volume (PCV) of infected mice

Groups (mg/kg)	PCV (%)				
	Day 0	Day 5	Day 9	Day 13	Day 17
MEBB 100	45.00 ± 1.67	32.40 ± 1.91	33.60 ± 1.33^{ab}	30.40 ± 1.29	38.80 ± 3.44
MEBB 200	46.40 ± 0.51	31.80 ± 0.86	39.00 ± 1.00^{b}	39.25 ± 1.55^{b}	36.75 ± 1.49
MEBB 400	43.60 ± 0.87	33.25 ± 0.85	40.20 ± 0.8^{b}	38.00 ± 2.19^{b}	41.25 ± 2.84
*n*HF 200	42.40 ± 1.03	29.00 ± 1.68	31.20 ± 0.97^{ab}	30.25 ± 2.46^{a}	36.25 ± 1.70
DCMF 200	39.80 ± 1.69	25.00 ± 3.65	30.60 ± 1.08^{ab}	37.00 ± 4.22^{a}	34.67 ± 2.40
EAF 200	45.80 ± 0.37	29.00 ± 5.02	25.67 ± 0.33^{a}	35.00 ± 6.93^{a}	37.33 ± 8.25
MF 200	41.60 ± 0.93	30.80 ± 0.73	42.60 ± 0.87^{b}	31.75 ± 2.32^{a}	37.67 ± 2.60
PC	43.80 ± 2.22	30.40 ± 1.50	41.00 ± 0.58^{b}	37.00 ± 0.58^{a}	34.67 ± 6.36
NC	42.20 ± 2.92	31.00 ± 3.56	22.50 ± 1.89^{a}	26.00 ± 1.00^{a}	34.33 ± 3.38
N	47.00 ± 1.30	33.20 ± 0.80	30.40 ± 0.51^{ab}	48.00 ± 3.08^{b}	53.50 ± 0.96^{ab}

Keys: Day 0=the day treatment commenced; Day 5= the 5th day of treatment, Day 7 =the 7th day of treatment, Day 8= the 8th day of treatment, Day 11= the 11th day of treatment, Day 14= the 14th day of treatment, Day 17= the 17th day of treatment. MEBB=Methanol extract of *B. brieyi*, *n*HF = *n*-hexane fraction; DCMF = Dichloromethane fraction; EAF = Ethyl acetate fraction; MF = Methanol fraction; N = Normal control received 0.2 ml of distilled water, PC= Positive Control received diminazine aceturate 3.5 mg/kg, NC=negative control received 0.2 ml of distilled water, All superscripts indicate significance at p<0.05 (a=compared to positive control; b=compared to NC) (Mean ± SEM, n = 5).

Table 4: Effect of root extract and fractions of *B. brieyi* on rectal temperature of infected mice

Groups (mg/kg)	Rectal Temperature (°C)						
	Day 0	Day 5	Day 7	Day 8	Day 11	Day 14	Day 17
MEBB 100	36.06 ± 0.25	36.1 ± 0.11	36.88 ± 0.28	38.1 ± 0.21	37.32 ± 0.23	37.2 ± 0.36	37.06 ± 0.23
MEBB 200	36.82 ± 0.33	36.06 ± 0.20	37.52 ± 0.26	37.26 ± 0.20	37.4 ± 0.17	37.22 ± 0.25	37.78 ± 0.12
MEBB 400	36.22 ± 0.34	36.54 ± 0.23	37.1 ± 0.38	37.96 ± 0.15	37.96 ± 0.29	37.1 ± 0.22	37.06 ± 0.23
*n*HF 200	36.28 ± 0.21	37.38 ± 0.19^{ab}	37.42 ± 0.25	37.98 ± 0.21	37.10 ± 0.29	36.16 ± 0.73	37.00 ± 0.24
DCMF 200	36.48 ± 0.25	36.38 ± 0.15	36.98 ± 0.33	37.62 ± 0.39	37.14 ± 0.37	36.60 ± 0.41	36.48 ± 0.24
EAF 200	36.16 ± 0.22	37.02 ± 0.28^{ab}	36.38 ± 0.45	37.93 ± 0.17	37.57 ± 0.26	36.57 ± 0.38	36.53 ± 0.68
MF 200	36.30 ± 0.07	37.60 ± 0.29^{ab}	37.62 ± 0.40	37.90 ± 0.35	37.83 ± 0.09	35.85 ± 0.32	36.77 ± 0.22
PC	36.72 ± 0.33	35.60 ± 0.44	37.46 ± 0.24	37.17 ± 0.58	37.83 ± 0.29	37.20 ± 0.25	36.73 ± 0.72
NC	35.88 ± 0.29	35.80 ± 0.43	37.43 ± 0.40	37.55 ± 0.31	37.90 ± 0.18	36.65 ± 0.53	37.60 ± 0.29
N	35.80 ± 0.56	36.88 ± 0.25^{a}	37.38 ± 0.27	37.32 ± 0.14	37.48 ± 0.31	36.08 ± 0.16	36.54 ± 0.24

Keys: Day 0=the day treatment commenced; Day 5= the 5th day of treatment, Day 7= the 7th day of treatment, Day 8= the 8th day of treatment, Day 11= the 11th day of treatment, Day 14= the 14th day of treatment, Day 17= the 17th day of treatment. MEBB=Methanol extract of B. brieyi, nHF = n-hexane fraction; DCMF = Dichloromethane fraction; EAF = Ethyl acetate fraction; MF = Methanol fraction; N = Normal control received 0.2 ml of distilled water, PC= Positive Control received diminazine aceturate 3.5 mg/kg, NC=negative control received 0.2 ml of distilled water, All superscripts indicate significance at p<0.05 (a=compared to positive control; b=compared to NC) (Mean ± SEM, n = 5).

Table 5: Effect of root extract and fractions of *B. brieyi* on the body weight of infected mice

Groups (mg/kg)	Body weight of animals (g)					
	Day 0	Day 5	Day 8	Day 11	Day 14	Day 17
MEBB 100	25.2 ± 2.60	28.0 ± 2.85^{a}	28.4 ± 2.78^{a}	28.40 ± 2.78^{a}	28.60 ± 2.77^{a}	27.40 ± 2.40^{a}
MEBB 200	18.6 ± 0.93	22.0 ± 0.55^{a}	21.6 ± 0.93^{a}	21.4 ± 0.93^{a}	21.80 ± 0.97^{a}	21.60 ± 0.87^{a}
MEBB 400	23.0 ± 1.26	24.6 ± 1.17^{a}	24.8 ± 0.73^{a}	24.8 ± 1.24^{a}	25.0 ± 1.18^{a}	25.0 ± 1.30^{a}
*n*HF 200	21.80 ± 1.66	23.80 ± 1.39	24.00 ± 1.67	22.80 ± 1.53	22.40 ± 1.08	22.25 ± 1.18
DCMF 200	19.00 ± 0.71^{a}	20.20 ± 1.74	22.80 ± 1.74	21.80 ± 1.88	21.80 ± 1.83	23.75 ± 1.03
EAF 200	17.20 ± 0.6^{a}	19.40 ± 1.08	20.67 ± 1.33	18.67 ± 1.86	19.00 ± 1.73	20.67 ± 1.45
MF 200	18.00 ± 1.10^{a}	21.20 ± 0.80	19.80 ± 0.86	21.25 ± 0.85	20.25 ± 0.85	20.33 ± 0.33
PC	24.60 ± 1.69	24.20 ± 0.80	24.67 ± 0.88	24.67 ± 0.67	24.00 ± 0.58	25.33 ± 0.33
NC	20.80 ± 0.66	21.00 ± 0.71	22.25 ± 1.25	22.25 ± 1.25	22.25 ± 1.65	23.33 ± 2.33
N	32.40± 1.78^{ab}	33.40± 1.75^{ab}	33.20± 1.77^{ab}	34.40± 1.57^{ab}	32.80± 1.11^{ab}	31.60 ± 1.50^{ab}

Keys: Day 0=the day treatment commenced; Day 5= the 5th day of treatment, Day 7= the 7th day of treatment, Day 8= the 8th day of treatment, Day 11= the 11th day of treatment, Day 14= the 14th day of treatment, Day 17= the 17th day of treatment. MEBB=Methanol extract of B. brieyi, nHF = n-hexane fraction; DCMF = Dichloromethane fraction; EAF = Ethyl acetate fraction; MF = Methanol fraction; N = Normal control received 0.2 ml of distilled water, PC= Positive Control received diminazine aceturate 3.5 mg/kg, NC=negative control received 0.2 ml of distilled water, All superscripts indicate significance at p<0.05 (a=compared to positive control; b=compared to NC) (Mean ± SEM, n = 5).

Table 6: Effect of methanol root extract and fractions of *B. brieyi* on the survival time of infected mice

Groups (mg/kg)	Survival Time (days)
MEBB 100	30.0 ± 0.00[b]
MEBB 200	28.2 ± 0.82
MEBB 400	30.0 ± 0.00[ab]
*n*HF 200	26 ± 2.53
DCMF 200	25.4 ± 2.82
EAF 200	16.2 ± 3.75
MF 200	24.6 ± 3.17
PC	20.4 ± 3.49
NC	20.2 ± 3.88
N	30.0 ± 0.00

Keys: MEBB=Methanol extract of *B. brieyi*, *n*HF = *n*-hexane fraction; DCMF = Dichloromethane fraction; EAF = Ethyl acetate fraction; MF = Methanol fraction; N = Normal, PC= Positive Control; NC=negative control, All superscripts indicate significance at p<0.05 (a=compared to positive control; b=compared to NC) (Mean ± SEM, n = 5).

Table 7: Minimum Inhibitory Concentration (MIC) of crude extracts and fractions of *B. brieyi* root against test organisms (mg/ml).

Sample	Minimum Inhibitory Concentration (MIC: mg/ml)							
	B. subtilis	***S. typhi***	***E. coli***	***K. pneumonia***	***S. aureus***	***S. mutant***	***C. albicans***	***A. niger***
MEBB	2.0	2.0	2.0	2.0	5.0	6.0	7.0	1.0
n-HF	6.0	6.0	6.0	6.0	9.0	9.0	9.0	1.0
DCMF	2.0	4.0	4.0	7.0	9.0	10.0	10.0	1.0
EAF	5.0	7.0	5.0	7.0	8.0	9.0	9.0	1.0
MF	5.0	5.0	5.0	6.0	5.0	8.0	7.0	1.0

MEBB = Crude extract, *n*-HF = *n*-hexane fraction, DCMF = Dichloromethane fraction, EAF = Ethylacetate fraction, MF = Methanol fraction, *B. subtilis* **=** *Bacillus subtilis, S. typhi* **=** *Salmonella typhi, E. coli = Escherichia coli, K. pneumonia = Klebsiella pneumonia, S. aureus = Staphylococcus aureus, S. mutant = Streptococcus mutant, C. albicans = Candida albicans, A. niger = Aspergillus niger.*

result of the parasites' ability to serve as a reasonably correct measure of the trypanosomes' vitality [28]. Diminazene aceturate elicits its action by selectively blocking the replication of kinetoplast DNA, though its primary target is not known [37]. Mice treated with 200 mg/kg each of *n*-hexane, dichloromethane, methanol fractions all gave decrease in the parasite count, although none totally cleared the parasite from the blood throughout the experiment. Ethyl acetate fraction gave similar reading with the negative control, showing that it has no activity. Methanol fraction gave the best decline in number of the parasite in blood. The reduction in parasitemia level in all the groups can be summarized as follow starting from the highest reduction to the lowest: Normal control > Positive control > Methanol fraction > *n*-hexane fraction > Dichloromethane fraction > Ethylacetate fraction > Negative control. The observed activity of *B. brieyi* could be attributed to the presence of alkaloids, phenols and flavonoids found in the root of plant. These secondary metabolites may exert their effects by acting at one or more target sites involved in the physiological process of the trypanosomes in an additive or synergistic manner [27, 38].

The parasite trypanosome, generate reactive oxygen species that attack red blood cells' membrane inducing oxidation and subsequently haemolysis which leads to one of the classical symptoms in trypanomiasis which is anaemia [39]. The present study showed that, the extract at 400 mg/kg significantly ameliorated the level of anaemia when compared to the negative control. Also, it was able to restore the packed cell volume of the infected mice close to the value at day 0 and this suggest a good antitrypanosomal potentials of the plant. Rise in packed cell volume could be attributed to the reduction of the proliferating parasite load, neutralization of the toxic metabolites and free radicals produced by trypanosomes [40, 38]. During the treatment, the packed cell volume of all groups was significantly decreased on day 5 as a result of destruction of red blood cell by invading parasites. On day 17 the PCV of all groups were restored, but not to the initial values. The PCV for the mice in the normal group also showed similar pattern (which should not be); this result shows some underlying effect which cannot be explained, further studies are therefore recommended.

There was also a siginificant (p< 0.05) rise in weight of animals treated with extract. This increase stayed almost the same throughout the treatment period. Since human African trypanosomiasis is associated with decreased appetite and a rapid weight loss which progresses to extreme emaciation [39], mice treated with *B. brieyi* extract and fractions seemed to have increased body weight. There was no significant increase

among animals treated with diminazene aceturate (3.5 mg/kg) and there was only a slight increase in weight among the negative control group throughout the treatment period.

Rectal temperature measurement was used to determine the level of pyrexia or fever present in the mice. As presented in Table 5, the mean rectal temperature of the mice were elevated at Day 5 after manifestation of the infection which indicated the presence of fever or pyrexia. But throughout the treatment period, there was no significant decrease in temperature for all groups including the controls. Since the fluctuations in temperature were observed across all groups including the controls (positive, negative and normal) it could be attributed to stress-induced hyperthermia (SIH) in the mice. Olivier *et al.* [41] reported that in mice, a unique cage stress raises body temperature by 2 to 2.5 degrees Celsius and lasts longer than a short-term stress such as taking a rectal temperature measurement or receiving a medication injection. [41, 42].

Survival time of the mice was also evaluated in the present study. Administration of different doses of crude extract and fractions resulted in prolongation of survival time of mice ($p< 0.05$) when compared to positive and negative controls. The result agreed with Tadesse *et al.* [27] that medicinal plants (*B. brieyi* inclusive) prolong survival time of trypanosome infected mice [27]. Additionally, the amelioration of anaemia and fever, which is responsible for deaths of mice have shown the ability of the extract and fractions to improve packed cell volume and decreased rectal temperature which may have improved the survival time of the mice. These indicated that the extract and fractions has an antitrypanosomal potentials.

CONCLUSION

The current investigation showed that *B. brieyi* root extract and fractions exhibited antitrypanosomal activity *in vivo* and anti-microbial potentials in a dose-dependent manner. This finding could be the initial point for the creation of new antitrypanosomal and antimicrobial medications. To ascertain the plant's mode of action and to identify the precise metabolite in charge of its activity, additional research on antitrypanosomal activity is required.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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AUTHORS' DECLARATION

Authors declare that the research presented in this article is original and liability for claims relating to the content of this article will be borne by them.

AUTHORS' CONTRIBUTIONS

ICE, CUO and CIA: did the animal experiment, literature review, phytochemical studies, and analysis of result; ICE and CME: carried out the antimicrobial studies; ICE and PFU: wrote and revised the first manuscript, and NJN: worked on data analysis.

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