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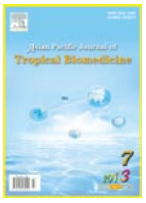

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Anti-trypanosomal effect of *Peristrophe bicalyculata* extract on *Trypanosoma brucei brucei*-infected rats

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PEER REVIEW

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Comments
The study has gone a step further in the search of anti-trypanosomiasis drug candidate. The potential of using the plant has also been established, making it a ground-breaking research for more works to be done to further ascertain the use of the plant for trypanosomiasis treatment.
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ABSTRACT

Objective: To investigate the *in vitro* and *in vivo* effect of whole plant extracts of *Peristrophe bicalyculata* on *Trypanosoma brucei brucei*-infected rats. **Methods:** The experiment was divided into two phases: In the first phase, the anti-trypanosomal activity of the hot water, cold water, methanol and butanol extracts of the whole plant were determined by incubating with *Trypanosoma brucei brucei*. The cold water extract was partially-purified and the anti-trypanosomal activity of the fractions determined. In the second phase, *Trypanosoma brucei brucei*-infected rats were treated with fraction 2c for nine days. Packed cell volume (PCV), high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC), triacylglycerol (TAG), aspartate aminotransferase, alanine aminotransferases (ALT), alkaline phosphatase (ALP), total and direct bilirubin levels were determined at the end of the experiment. **Results:** Cold water extract immobilized 90% of the parasites after 60 min of incubation, and fraction 2c completely immobilized the parasites after 35 min. It significantly increased PCV in *Trypanosoma brucei brucei*-infected rats. Decreased TC, TAG, HDL and LDL levels of infected rats increased significantly when rats were treated with the fraction, while elevated levels of total bilirubin and ALT also decreased. The difference in urea, direct bilirubin and ALP was not significant when infected rats were compared to rats in other groups. **Conclusions:** The ability of the plant to ameliorate the infection-induced biochemical changes calls for detailed investigation of the potentials of the plant for antitrypanosomiasis drug delivery.

KEYWORDS
Anti-trypanosomal activity, *Trypanosoma brucei brucei*, *Peristrophe bicalyculata*, Lipoproteins

1. Introduction

African trypanosomiasis is a parasitic disease that affects both humans and animals. It is caused by the protozoan parasite *Trypanosoma brucei* (*T. brucei*) which is transmitted by tsetsefly (*Glossina* genus). Although the number of reported cases in 2009 and 2010 dropped below 10 000, the disease still poses a significant health challenge, especially in sub-saharan Africa (World Health Organization report, 2012). African animal typanosomiasis causes serious losses in Cattle, sheep, goats, pigs, horses and many other host animals[1], thus reducing the source of animal protein[2], and ultimately contributing to food insecurity in the region. The development of a vaccine for the treatment of the disease is becoming increasingly difficult due to the problem of antigenic variation[3], hence the dependence on chemotherapeutic agents to control the disease.

A vast majority of people from less developed countries, including Nigeria depend on medicinal plants for the treatment of various diseases, due to the high cost of synthetic drugs. Most of these plants have proved to be useful sources of treatment of various diseases[4-6]. Indeed,

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there have been efforts to discover new anti-trypanosomal agents from plants, especially with information obtained from ethnomedical data, whereby a plant is selected based on prior knowledge of its use in folk medicine[7]. However in the case of trypanosomiasis, the use of such data alone may be inadequate, as chemotherapy of trypanosomiasis is presently confronted with problems of unavailability of drugs, resistance to available ones, unacceptability, toxicity and long treatment protocols[8,9]. Therefore, it is important to search for cheaper, more effective, easily available and less toxic chemotherapeutic agents for the treatment of the disease. Several studies have demonstrated the trypanocidal activity of various plants[7,10,11], but there has been no available data on the trypanocidal effect of *Perisrophe bicalyculata* (*P. bicalyculata*).

P. bicalyculata is a plant native to warm tropical region of Africa, in the Sahel parts of Mauritania, Niger and Nigeria as well as Burma and Thailand[12]. It has been reported to have anti-inflammatory and anti-bacterial properties[13], antihypertensive[14] and anticancer properties[15]. It is also used in the treatment of skin diseases, and serves as an antidote for snake poison, diabetes among others[16]. The present study was designed to evaluate the anti-trypanosomal activity of different extracts of *P. bicalyculata* and the possibility of its use as a potential anti-trypanosomal drug candidate. Also, since studies have demonstrated that drugs, whether synthetic or herbal may have significant effect on kidney and liver functions, as well as blood lipid concentration[17-19]; it is pertinent to evaluate these parameters after treatment.

2. Materials and methods

2.1. Plant material

The whole plant of *P. bicalyculata* were harvested at maturity in the month of August, 2012 at Ibadan (70 26' N and 3° 54' E), Oyo State, Nigeria. It was identified and authenticated by the botanist, in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

The whole plant was air dried in the laboratory and made into powder by grinding. About 50 g was extracted in distilled water (100 mL) by stirring (Harmony Hot Plate Stirrer, Japan) for 30 min to obtain cold water extract. Another 50 g was boiled for 30 min in 100 mL water to obtain hot water extract. The methanol and butanol extracts were obtained by soaking 100 g of extract in 500 mL methanol and butanol respectively, for 48 h. The extracts were sieved using a muslin cloth and then filtered under suction pressure with a Whatman's filter paper. They were then concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland), lyophilized (Christ Alpha 1-2 LD, Germany) and stored at 40 ℃ until needed.

2.1.1. Partial purification of cold water extract of *P. bicalyculata*

The cold water extract was partially purified after thin layer chromatographic separation and fractionated on silica gel packed a column. The column was eluted with acetone (100%), acetone:methanol (15:0.5), acetone:methanol (1:1). Three fractions were obtained (fractions 1, 2a and 2c).

2.2. Parasites

T. brucei brucei was obtained from stabilates maintained at the Nigerian institute for trypanosomiasis research, Kaduna State, Nigeria in the month of July, 2012; and was thereafter maintained in the Department of Parasitology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, by continuous passage of infected blood into the rats.

2.3. Animals

Thirty six apparently healthy male Wistar rats of approximately 8 weeks old weighing between 200-250 g were purchased from the National Institute of Trypanosomiasis Research, Kaduna State, Nigeria. They were housed under standard laboratory conditions, fed normal rat chow (PLS feeds, Zaria) and given access to clean water *ad libitum*. They were allowed to acclimatize for a period of 2 weeks before the commencement of the experiment.

The rats were grouped into 6 groups of 6 rats each: Group 1 (Normal; neither infected nor treated), Group 2 (Infected, not treated), Group 3 (Infected, treated with 10 mg/kg diminazene aceturate), Group 4 (Not infected, treated with 200 mg/kg), Group 5 (Infected, treated with 100 mg/kg fraction 2c), Group 6 (Infected, treated with 200 mg/kg fraction 2c).

2.4. Trypanosome infection and treatment

Blood from a highly parasitized mouse was obtained by cardiac puncture, collected into an EDTA-coated sample bottle, and diluted appropriately with physiological saline to serve as inoculum. Rats from groups 2, 3, 5 and 6 were infected intraperitoneally with 0.1 mL of the inoculum containing about 10^3 trypanosomes/mL. Treatment began a day after the parasites were first detected in the blood stream (day 4) and lasted until the thirteenth day (*ie.* 9 days of treatment). All experimental protocols were approved and conducted with strict adherence to guidelines of the Institutional Animal Care and Use Committee of Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

2.5. In vitro anti-trypanosomal screening of extracts and fractions of *P. bicalyculata*

Infected blood obtained from a rat at peak parasitaemia by cardiac puncture was collected in Eppendorf tubes containing 0.2 mL of 1% EDTA prepared with phosphate

buffered saline (PBS) and glucose. All extracts were prepared at three concentrations (25, 50 and 100 mg/mL), while 10 mg/mL of diminazeneaceturate was used. Aliquot of 30 μL of the extracts and standard was incubated with 40 μL of the infected blood containing about 32-64 parasites per field[20] in wells of microtitre plates (Flow laboratories Inc., Mclean, Virginia 22101, USA). For the control, phosphate buffer saline (pH 7.4) was used. After 5 min incubation in the wells, about 2 μL of test mixture was placed on microscope slides and the motility of the parasites was observed under the microscope (Olympus CK 40, Japan) at 5 min intervals for 60 min. The procedure was carried out separately for all extracts. A cessation or drop in motility of the parasites in treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of anti-trypanosomal activity, since motility constitutes a relatively reliable indicator of the viability of most zooflagellate parasites[21]. The shorter the time of cessation of motility of the parasite, the more active the extract was considered to be[22].

2.5. Sample collection

Blood was collected at four days interval from the tail of each rat to determine packed cell volume (PCV). At the end of the experiment, rats were sacrificed under anaesthesia to collect blood by cardiac puncture. Blood was allowed to coagulate and serum obtained for biochemical analysis.

2.6. Determination biochemical parameters

PCV was determined by the microhaematocrit method. Low density lipoprotein, high density lipoprotein, triacylglycerol, total cholesterol levels, alanine amino-transferase, aspartate aminotransferase, alkaline phosphatase and bilirubin were determined using assay kits (Randox Laboratories, County Antrim, United Kingdom). Urea and creatinine were determined using Randox kits (Randox Laboratories, County Antrim, United Kingdom).

2.7. Statistical analysis

Data obtained were expressed as mean±standard error of the mean (mean±SEM) and analysed using SPSS 17. Data were subjected to one way analysis of variance and LSD *post-hoc* test was applied for multiple comparisons. Values of $P < 0.05$ were regarded as statistically significant.

3. Results

Table 1 shows the yield and percentage yield of extracts using different solvents. The cold water extract yielded 9.03 g, while the hot water extract yielded 7.79 g. The methanol and butanol extracts yielded 18.14 and 10.61 g, respectively.

Table 1
Percentage yield of different extracts of *P. bicalyculata*.

Extract	Yield (g)	Percentage (%)
Cold water	9.03	18.06
Hot water	7.79	15.58
Methanol	18.14	18.14
Butanol	10.61	10.61

Result of the *in vitro* activity of the cold water extract of *P. bicalyculata* is presented in Figure 1. From the results, the extract at 25 mg/kg did not have effect on the parasites, as all parasites were still motile even after 60 min of incubation. When used at 50 mg/kg, about 50% of parasites were immobilized, while 100 mg/kg of the extract immobilized 90% of the parasites, the response of the cold water extract of *P. bicalyculata* was dose-dependent. The standard drug, diminazine aceturate completely immobilized the parasites after 20 min incubation.

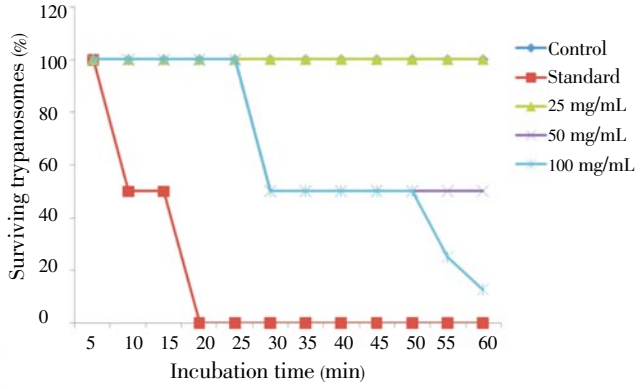

Figure 1. The *in vitro* anti-trypanosomal activity of cold extract of *Peristrophe bicalyculata* at different concentrations.

The hot water extract of *P. bicalyculata* at 100 mg/kg immobilized over 70% of the parasites after 40 min of incubation, and the percentage immobilized parasites did not increase even after 60 min, whereas no parasite was motile 25 min after incubation with the standard drug. Sixty min after incubation with the 25 mg/kg extract, all parasites were still motile, while at 50 mg/kg, 50% of the parasites were immobilized, showing a dose-dependent effect (Figure 2).

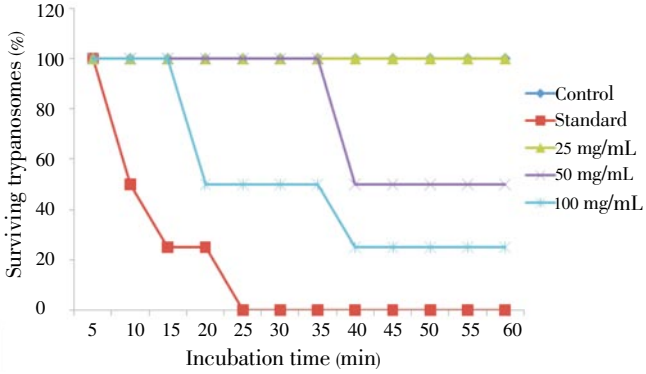

Figure 2. The *in vitro* anti-trypanosomal activity of hot water extract of *Peristrophe bicalyculata* at different concentrations.

Figure 3 shows the *in vitro* anti-trypanosomal activity of methanol extract of *P. bicalyculata*. From the results, the standard drug completely immobilized the parasites after 25 min of incubation, while 20% of the parasites were motile 60 min after incubating with the methanol extract at 100 mg/mL. The extract at 50 mg/mL immobilized 50% of the parasites after 30 min and remained unchanged after 60 min (Figure 3).

The butanol extract of *P. bicalyculata* at 25 and 50 immobilized about 50% of the parasites after 45 and 60 min of incubation. Increasing incubation time to 60 min did not increase the percentage of immobilized parasites treated with the extract at 50 mg/kg. However, at 100 mg/kg, only about 30% of the parasites were motile after 60 min of incubation (Figure 4).

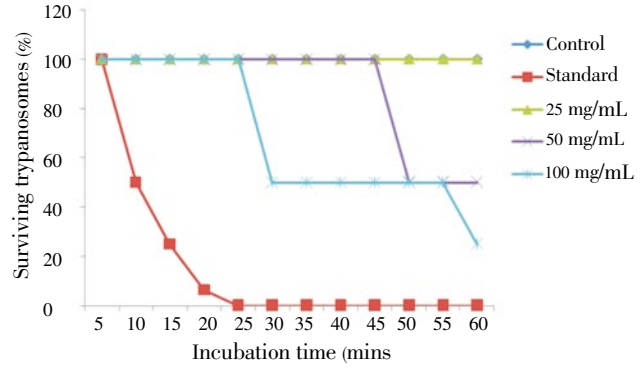

Figure 3. The *in vitro* anti-trypanosomal effect of methanol extract of *Peristrophe bicalyculata* at different concentrations.

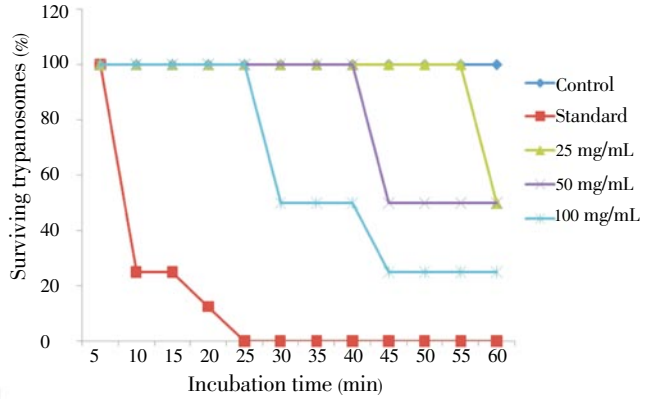

Figure 4. The *in vitro* anti-trypanosomal effect of butanol extract of *Peristrophe bicalyculata* at different concentrations.

Table 2 shows the yield obtained after partially purifying the cold water extract. Fraction 1 was obtained from acetone (100%), fractions 2 and 3 were obtained from acetone:butanol (7:3).

Table 2

Fractions and yields obtained from column chromatographic separation of aqueous extract of *P. bicalyculata*.

Solvents	Fractions	Yield (g)	Yield (%)
Acetone (100%)	Fraction 1	0.56	28.0
Acetone:methanol (7:3)	Fraction 2a	0.72	36.0
Acetone:methanol (1:1)	Fraction 2c	0.34	17.0
Total yield		1.62	81.0

The effect of the partially-purified fraction (fraction1) of *P. bicalyculata* is presented in Figure 5. From the results, the effect of the fraction on the mobility of the parasites was dose-dependent. The fraction at 25 mg/kg was inactive, while at 50 mg/kg, over 80% of the parasites were immobilized after 50 min of incubation. However, the 100 mg/kg of the fraction immobilized all the parasites after 50 min of incubation.

The second fraction obtained after partial purification (fraction 2a), also exhibited a dose-dependent effect (Figure 6). The fraction at 25 and 50 and 100 mg/kg immobilized 50%, 90% and 100% of the parasites after 55, 60 and 45 min, respectively. The standard drug completely immobilized the parasites after 25 min of incubation.

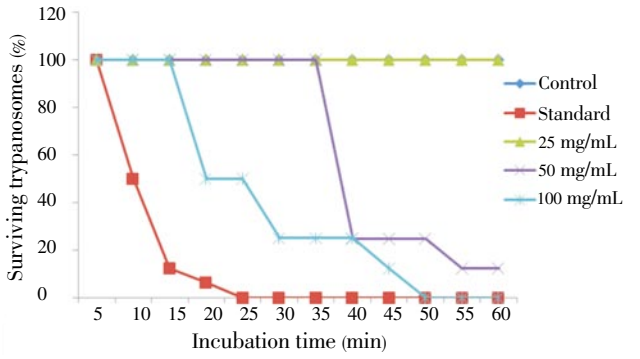

Figure 5. The *in vitro* anti-trypanosomal activity of partially-purified extract of *Peristrophe bicalyculata* (fraction 1).

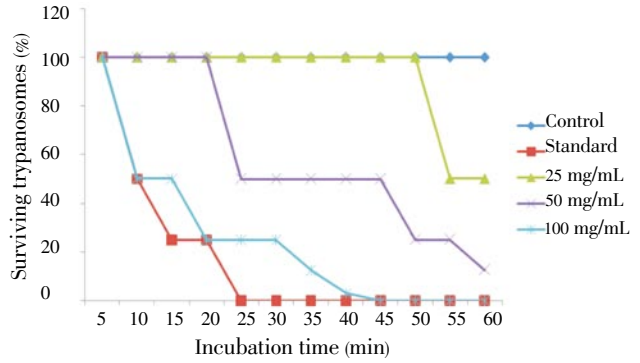

Figure 6. The *in vitro* anti-trypanosomal activity of partially-purified extract of *Peristrophe bicalyculata* (fraction 2a).

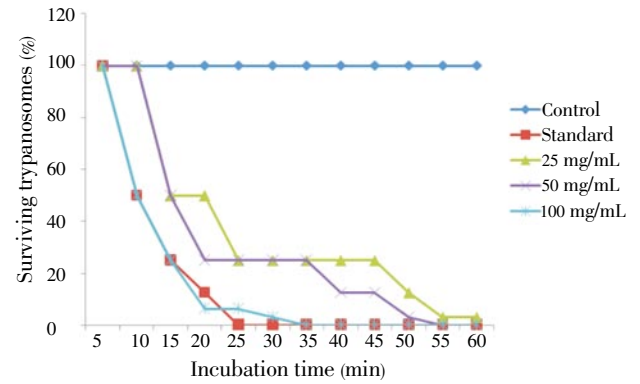

Figure 7. The *in vitro* anti-trypanosomal activity of partially-purified extract of *Peristrophe bicalyculata* (fraction 2c).

From Figure 7, it was observed that the diminazene aceturate completely immobilized the parasites after 25 min of incubation, while, fraction 2c at 100 mg/mL immobilized the parasites after 35 min. At 25 and 50 mg/mL, the fraction immobilized all the parasites after 55 and 60 min of incubation, respectively.

Results of PCV of rats treated with the partially-purified fraction of *P. bicalyculata* is presented on Table 3. There was a significant (P<0.05) decrease in PCV of *T. brucei brucei* infected rats when compared to normal rats throughout the treatment period. The PCV of infected rats given the partially-purified extract at 100 and 200 mg/kg decreased significantly (P<0.05) by the 8th day (that is 4th day of treatment) when compared with normal rats and rats treated with the standard drug. Although the PCV of normal rats given the extract decreased by 21% after 4 days of treatment, it was significantly (P<0.05) higher than that of infected rats treated with the extract at the same dose (33%). By the 12th day, PCV of infected rats given the extract at 200 mg/kg increased significantly.

The levels of total cholestero (TC), triacylglycerol (TAG), high density lipoprotein and low density lipoprotein cholesterol decreased significantly (P<0.05) in *T. brucei brucei*-infected rats compared to normal rats, but increased significantly (P<0.05) when rats were treated with the standard drug and partially purified extract (Table 4). Normal rats given the partially-purified extract had significantly (P<0.05) lower levels of TC and TAG when compared to untreated normal rats. Also, serum levels of TC, TAG and high density lipoprotein in infected rats treated with the partially-purified extract were significantly (P<0.05) lower than that of normal rats, but higher than infected rats. There was no significant difference in low density lipoprotein levels of normal rats and rats treated with both the standard drug and partially-purified extract (Table 4).

Table 5 shows the effect of partially-purified extract of *P. bicalyculata* on some parameters of liver function in *T. brucei brucei*-infected rats. The activity of alanine aminotransferases (ALT) increased significantly (P<0.05) in infected rats compared to normal rats. Administration of standard drug and the partially-purified extract at both 100 and 200 mg/kg reduced it significantly. Infected rats had significantly (P<0.05) lower level of sspartate aminotransferase than normal rats; and administration of the standard drug and the partially-purified extract at 100 and 200 mg/kg did not increase the levels. There was no significant difference in levels of alkaline phosphatase in rats of all groups.

The level of total bilirubin increased significantly (P<0.05) in *T. brucei brucei* infected rats compared to normal rats and all treated rats (Table 6). There was no significant difference in total bilirubin levels between normal and

Table 3

Effect of the partially purified fraction of *P. bicalyculata* on packed cell volume of *T. brucei brucei*-infected rats (n=36).

Groups	Day 0	Day 8	Day 12
Normal	39.20±3.13^{a}	40.00±2.10^{a}	43.00±2.14^{a}
Infected untreated	38.40±1.09^{a}	28.00±1.26^{b}	21.70±2.15^{c}
Infected+Standard drug	40.51±1.92^{a}	38.00±1.92^{a}	42.35±2.00^{a}
Normal+Extract (200 mg/mL)	41.20±2.30^{a}	32.56±2.06^{c}	38.78±3.45^{a,b}
Infected+Extract(100 mg/mL)	40.28±3.66^{a}	25.30±2.76^{b}	27.50±3.82^{b}
Infected+Extract(200 mg/mL)	39.42±1.54^{a}	26.67±1.54^{b}	35.43±1.48^{c}

Values with different superscripts within the same column are significantly different at P<0.05.

Table 4

Effect of *P. bicalyculata* on serum lipid profile of *T. brucei brucei*-infected rats (n=36).

Groups	TC (mg/dL)	TAG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Normal	235±37.2^{a}	246±69.6^{a}	200±18.1^{a}	205±51.1^{a}
Infected untreated	89±20.9^{c}	110±24.2^{c}	168±15.1^{c}	179±30.2^{d}
Infected+Standard drug	211±37.1^{ab}	222±60.9^{ab}	191±17.6^{b}	196±47.3^{a}
Normal+Extract (200 mg/mL)	203±22.9^{d}	192±22.3^{ab}	203±21.5^{a}	184±28.6^{a}
Infected+Extract(100 mg/mL)	173±28.9^{b}	187±56.7^{b}	177±19.3^{b}	192±39.9^{a}
Infected+Extract(200 mg/mL)	182±25.8^{b}	206±57.1^{ab}	184±16.3^{ab}	195±41.5^{a}

Value with different superscript within the same column are significantly different at P<0.05.

Table 5

Effect of *P. bicalyculata* on some parameters of liver function in *T. brucei brucei*-infected rats (n=36).

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal	0.15 ± 0.04^{a}	0.24 ± 0.03^{b}	0.24 ± 0.03^{a}
Infected untreated	0.23 ± 0.02^{b}	0.19 ± 0.01^{ab}	0.29 ± 0.03^{a}
Infected+Standard drug	0.13 ± 0.01^{a}	0.20 ± 0.01^{ab}	0.24 ± 0.03^{a}
Normal+Extract (200 mg/mL)	0.14 ± 0.02^{a}	0.26 ± 0.02^{b}	0.25 ± 0.03^{a}
Infected+Extract(100 mg/mL)	0.12 ± 0.01^{a}	0.18 ± 0.02^{ab}	0.28 ± 0.02^{a}
Infected+Extract(200 mg/mL)	0.12 ± 0.01^{a}	0.15 ± 0.02^{a}	0.28 ± 0.02^{a}

Values with different superscripts within the same column are significantly different at P<0.05.

Table 6

Effect of *P. bicalyculata* on bilirubin levels in *T. brucei brucei*-infected rats.

Groups	Total bilirubin (U/L)	Direct bilirubin (U/L)
Normal	0.10 ± 0.04^{ab}	0.07 ± 0.01^{a}
Infected untreated	0.18 ± 0.04^{b}	0.11 ± 0.01^{a}
Infected+Standard drug	0.08 ± 0.03^{ab}	0.09 ± 0.01^{a}
Normal+Extract (200 mg/mL)	0.09 ± 0.03^{ab}	0.12 ± 0.03^{a}
Infected+Extract(100 mg/mL)	0.07 ± 0.02^{ab}	0.09 ± 0.02^{a}
Infected+Extract(200 mg/mL)	0.08 ± 0.02^{ab}	0.10 ± 0.01^{a}

RValues with different superscripts within the same column are significantly different at P<0.05.

infected rats treated with the extract, infected rats treated with the standard drug and normal rats. However there was no significant ($P>0.05$) difference in the levels of direct bilirubin in rats of all groups.

There was a significant ($P<0.05$) increase in serum creatinine levels of *T. brucei brucei* infected rats when compared to normal rats and normal rats treated with the partially-purified extract (Table 7). This decreased significantly ($P<0.05$) after administration of standard drug, but the partially purified extract at both 100 and 200 mg/kg, did decrease creatinine levels when compared to normal rats. The levels of urea were not significantly different in rats of all groups.

Table 7

Effect of *P. bicalyculata* on kidney function in *T. brucei brucei*-infected rats.

Groups	Urea (mg/dL)	Creatinine (mg/dL)
Normal	0.68 ± 0.07^{a}	0.22 ± 0.03^{a}
Infected untreated	0.64 ± 0.09^{a}	0.31 ± 0.03^{ab}
Infected+Standard drug	0.67 ± 0.10^{a}	0.24 ± 0.03^{a}
Normal+Extract (200 mg/mL)	0.65 ± 0.08^{a}	0.22 ± 0.09^{a}
Infected+Extract(100 mg/mL)	0.66 ± 0.10^{a}	0.28 ± 0.02^{ab}
Infected+Extract(200 mg/mL)	0.66 ± 0.10^{a}	0.28 ± 0.02^{ab}

Value with different superscripts within the same column are significantly different at $P<0.05$.

4. Discussion

Parasites motility constitute a relatively reliable indicator of viability of most zoo flagellate parasites and an arrest or drop in motility of trypanosomes may serve as a measure of anti-trypanosomal activity of plant extracts when compared to the control, phosphate-glucose buffer saline[23]. Similarly, it has been reported that a complete elimination or reduction of motility of parasites when compared to the control could be taken as index of trypanocidal activity[21]. From the results in the present study, all crude extracts of *P. bicalyculata*: cold water, hot water, butanol and methanol extracts exhibited *in vitro* anti-trypanosomal activity against *T. brucei brucei*, however, the cold water extract was considered most active as it inhibited the 90% of the parasites after 60 min of incubation compared to other extracts. Partial purification of this extract yielded three fractions (1, 2a and 2c), with fraction 2c being most active as it completely immobilized the parasites within the shortest time (35 min) when compared with other extracts.

Studies have shown that there are times when plants with *in vitro* anti-trypanosomal activity may have no effect *in vivo*, probably due to peculiarities in the metabolic disposition of the chemical constituents within the plant[11], thus, the *in vivo* effect of the fraction was determined to ascertain its anti-trypanosomal activity.

Anaemia is a constant feature of trypanosome infections whose severity is linked to the level of parasitaemia[24], a measurement of anaemia provides information on the severity of a disease. The significant decrease in PCV of *T. brucei brucei* infected rats is in consonance with earlier reports[4,25]in trypanosome-infected animals. This has been attributed to the release of hemolytic factors into the animals blood by dead trypanosomes causing destruction of erythrocytes and hence, reduction in PCV[22,26]. It has also been reported that anaemia may be caused by erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition[27]. The increase in PCV after treatment with fraction 2c at 100 and 200 mg/kg may be due to its ability to eliminate parasites from the blood, probably by reaching the site of action or rapid metabolization[4,28]. Also, the antioxidant activity of the plant[13] may have contributed to the increase, as studies have demonstrated the ability of vitamins to ameliorate anaemia in trypanosome-infected rats[29,30].

Reports have shown that lipids play important roles in the pathogenesis of trypanosomiasis[31,32]. From the findings in this study, it is reasonable to infer that *T. brucei* infection causes significant decrease in the serum levels of cholesterol, high density lipoprotein, triacylglycerol and low density lipoprotein. The findings in this study are in conformity with other reports[33-36]. Although we cannot ascertain the reasons for the sudden drop, some pathophysiological mechanisms may be involved. It has been reported that lipids serve as an important source of energy for *T. brucei*[31,32], thus, the lowering of the serum lipids and cholesterol as observed in the present study could, partly, be the result of trypanosomal utilization of the molecules. Thus, the continuous utilization of these molecules from the blood stream could be a contributory factor to lowering of the serum levels of lipids and cholesterol. Also, it is known that blood-stream trypanosomes scavenge blood glucose for energy[31], which could cause hypoglycemia in the trypanosome-infected animal. Although blood glucose level was not determined in this study, hypoglycemia can undoubtedly result in increased catabolism of lipids and cholesterol in order to meet some strategic energy needs in the body of the host animal[31,32]. Consequently, this could lead to decrease in serum levels of these molecules as observed in the present study. Also, studies have demonstrated that low feed intake

associated with trypanosomiasis[34] may affect blood levels of the triglyceride, high density lipoprotein and cholesterol in infected animals.

In this study, serum ALT levels increased, while there was no difference in levels of alkaline phosphatase in infected rats when compared to normal rats. This agrees with studies where ALT was elevated in *Trypanosomaevansi*-infected[37] and *T. brucei brucei*-infected[38] animals. Several other studies have reported elevated serum enzymes[29,39,40]. The elevation of these enzymes is usually indicative of liver damage, being the major liver maker enzymes; or partly to cellular damage caused by lysis or destruction of the trypanosomes[38].

The high levels of total bilirubin in infected rats in this experiment supports earlier observations in several trypanosome-infected animals[41-43]. The increase in bilirubin is suggestive of haemolytic anaemia which may be due to the activity of proliferating parasites. It could also be associated to the inability of the liver to conjugate bilirubin[42]. The liver detoxifies harmful substances secretes bile into the intestine synthesizes and stores up important material, hence, it is common in clinical practice to screen for liver disease, monitor the progression of a known disease and monitor the effect of potentially hepatotoxic drugs[44]. Administration of the standard drug, diminazine aceturate and the partially-purified fraction of *P. bicalyculata* prevented, to a significant extent, the disease-induced increases in serum ALT. This suggests that the fraction protected the liver against some oxidative species generated during the disease, probably by virtue of its antioxidant property, providing greater protection to plasma membrane and other susceptible cellular structures against oxidative agents[29,30].

The elevated creatinine level in the *T. brucei*-infected rats agrees with previous findings[39,43] and could be due to destruction of kidney cells resulting in the inability of the kidneys to excrete creatinine[43]. The decrease in creatinine levels when rats were given fraction 2c indicated that the ability of the fraction to provide some degree of protection to the kidneys during the course of the disease[11].

In conclusion, this study has established the *in vitro* and *in vivo* anti-trypanosomal activity of *P. bicalyculata*, and its potential as a possible drug candidate in the management of trypanosomiasis. Although the exact mechanism of action is unknown, previous reports have attributed the trypanocidal activity of a number of tropical plants to their flavonoids, alkaloids and other phytochemicals constituents[45-47]. Also, it has been suggested that many natural products exhibit their trypanocidal effect by interfering with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress[11]. Thus, the anti-trypanosomal activity of *P. bicalyculata* may be attributed to its high antioxidant activity and its phenol, flavonoids and alkaloid contents[13].

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The chemotherapy of African trypanosomiasis is besieged with numerous problems as most trypanocides are toxic, require lengthy parenteral administration, lack efficacy and are unaffordable. Also, a suitable vaccine is yet to be developed. Thus, there is a need for alternatives that are safe, effective and affordable. Emphasis is now shifting to medicinal plants and other natural products in search of such molecules that could be further developed into new antitrypanosomal agents

Research frontiers

This study was done to investigate the *in vitro* and *in vivo* effect of whole plant extracts of *P. bicalyculata* on *T. brucei brucei*. A lot of research is being undertaken in this field as a result of the persistent problem of Trypanosomiasis.

Related reports

The result of this study agrees with Faremi and Ekanem (2011), Wurochekke and Anyanwu (2012) demonstrating that plants with antitrypanosomal activity result in increased PCV. Also, the present study agrees with Biryomumaisho *et al* (2003), Adamu *et al* (2008) and Taiwo *et al* (2003) that *T. brucei* infection causes significant decrease in serum

lipoprotein levels, which increased on treatment with antitrypanosomal plants.

Innovations and breakthroughs

P. bicalyculata, the plant used in this study has been shown to possess many medicinal properties, however, there has been no research done to determine its antitrypanosomal activity. Results from the current study has demonstrated the antitrypanosomal activity of the cold water extract of the plant. Also, partial purification of the extract yielded a very active fraction.

Applications

This study has established the *in vitro* and *in vivo* anti-trypanosomal activity of *P. bicalyculata*, and its potential as a possible drug candidate in the management of trypanosomiasis.

Peer review

The study has gone a step further in the search of anti-trypanosomiasis drug candidate. The potential of using the plant has also been established, making it a ground-breaking research for more works to be done to further ascertain the use of the plant for trypanosomiasis treatment.

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