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# Antitrypanosomal Activity of *Piliostigma thoningii* and *Calotropis procera* Ethanol Lea Crude Extracts Singly and in Combination in Mice

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## ABSTRACT

Combination therapy with medicinal plants has been explored as a potential approac treat African trypanosomiasis. This study involved screening *Piliostigma thonningii* (PT) *Calotropis procera* (CP) aqueous ethanol extract and their combination antitrypanosomal activities. The plants were separately extracted with 70% ethanol and extract obtained were subjected to acute toxicity determination and phytochemical anal For *in vivo* study, *T. brucei brucei* infected mice were grouped into A-E of three (3) ani each. Groups A-C were treated orally with PT, CP and combined PT/CP (1:1) respecti Group D infected mice were treated with standard berenil drug, Group E was infected untreated while Group F was neither infected nor treated. All the extract treated groups v dosed at 250 mg/kg bodyweight for 15 days consecuively while the standard control gr was treated at 3.5 mg/kg berenil. Parasitaemia, bodyweight and PCV were monitored days interval from all the groups. The results showed that CP and PT ethanol extract tre groups recorded clearance of parasitaemia for groups treated with CP and combined extr at days 12 and 9 respectively compared to untreated control group ($p<0.05$). Similarly, treated animals with such extracts did not suffer weight loss and increased PCV. T combination of CP and PT extracts has trypanocidal effect.

**Keywords**: *Piliostigma thonningii* (PT), *Calotropis procera* (CP), Antitrypanosomal, Parasitaemia, *Trypanosoma brucei brucei*

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## INTRODUCTION

The use of herbal medicines has gained popularity worldwide due to their long history of traditional use, their cost-effectiveness, availability, accessibility and reportedly lower incidence of adverse effects (Mullaicharam, 2011). About 75 - 90 % of the world population still relies on plants and plant extracts as a source of primary health care (Benzie and Watchtel-Galor, 2011). The widespread use of medicinal plants derived extracts in disease management has led to an increasing desire for the identification and characterization of the active compounds responsible for the extracts' therape potentials hence, providing ideal leads drug development (Gangwar *et al.*, 20 The plant *Piliostigma thonningii* (PT) species of flowering plants in the legu family, Fabaceae. Its common names camel's foot tree, monkey bread, monk biscuit tree, Abefe in Yoruba and Kalg Hausa. *Calotropis procera (CP)* is als species of flowering plant in the fam Apocynaceae. Its common names are ap of sodom, tumpapia in Hausa and E Bomubomu in Yoruba. PT and CP are us alone or with other herbs to treat comm diseases such as fevers, cold, indigesti

rheumatism, eczema, and diarrhea (Raghubir *et al.*, 1999).

African trypanosomiasis is one of the neglected tropical infectious diseases that affect human and animal, transmitted by the bite of tsetse flies. The World Health Organization has targeted African Trypanosomiasis for elimination as a public health problem by 2030. (Wang *et al,* 2016).. The most widely accepted method of managing the disease involved the use of trypanocidal drugs. The few available drugs, however, are faced with problems ranging from high cost, toxicity, to prolonged duration of administration (Onyekwelu,1999). This study is therefore carried out to screen *Piliostigma thonningii* and *Calotropis procera* singly and in combination for antitrypanosomal effect in mice. .

## MATERIALS AND METHODS

### Sourcing of the Plant Materials/Identification

Fresh and healthy leaves of *Piliostigma thoningii* and *Calotropis procera* were harvested from Paiko, Niger State, Nigeria. They were identified and authenticated by a Botanist in the Department of Plant Biology, Federal University of Technology, Minna, Nigeria with a voucher number, FUT/PLB/FAB/062 and FUT/PLB/ASC/001. All chemicals and reagents used in this study were of analytical grade.

### Preparation of Crude Extracts of *Piliostigma thonningii* (PT) and *Calotropis procera (CP)*

The air-dried and powdered *Piliostigma thonningii* and *Calotropis procera* was extracted with 70 % ethanol for 24 hours via a maceration process with intermittent stirring. The crude extracts were obtained after the evaporation of the 70 % ethanol to complete dryness using steam bath. (What equipment was used? Rotary evaporator or water bath?).

### Extraction Procedure of Plant Material

The leaves of *Piliostigma thonningii* and *Calotropis procera* were harvested within the premises of General Hospital, Paiko, Niger State in December 2023. The leaves were washed under running tap and dried at room temperature (25±3°C) for seven days and pulverized using a mortar and pestle, the powdered of the leaves were extracted, then dried by heating in a water bath set at 40ºC for 24 hours and percentage yield was then calculated.

Percentage Yield (%) = (Dry weight of extract ÷ Dry weight of pulverized plant material) × 100

### Determination of Qualitative and Quantitative Phytochemical Composition

The following phytochemicals: saponins, flavonoids, alkaloids, phenols, terpenoids, glycosides, steroids, carotenoids and tannins were determined qualitatively and quantitatively from the crude extracts of *Piliostigma thonningii* (PT) and *Calotropis procera (CP)* as described by Sofowora (1993) and Harbone (1973)

### Determination of Acute Toxicity of Ethanol Extract of the 2 plants

The acute toxicity of each plant extracts obtained above were subjected to acute toxicity studies as describe by Lorke (1983). Briefly, extracts were separately administered orally at 10 mg/kg, 100 mg/kg, 1000 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg bodyweight respectively. Mice were observed for behavioral manifestation of acute toxicity or death within 24 hours post administration. Animals were also observed again for death as the index of toxicity. The $LD_{50}$ was calculated by taking

the square root of the product of the highest dose that recorded no death and the lowest lethal dose respectively.

**Screening of Extracts for Antitrypanosomal Activity**

The extracts were screened for antitrypasomal activity using eighteen mice which were grouped into A - F of three animals each. All animals in group A - E were infected with 0.2 ml containing $1x10^6$ parasites/ml. Mice in groups A and B were treated orally with *Piliostigma thonningii* and Calotropis *procera* extracts respectively, groups C and D mice were treated with combined extracts (1 : 1) and berenil at 3.5 mg/kgbw respectively while groups E and F were infected untreated and uninfected, untreated controls. All the extract treated groups were carried out at 250 mg/kg bodyweight based on the outcome of result of acute toxicity assay for 15 consecutive days. The parasitaemia, PCV and bodyweight were monitored at days intervals as described by Abdulraza *et al.* (2021).

**Data Analysis**

The data obtained from this study we statistically analysed using Analysis Variance (ANOVA).

## RESULTS

The percentage yield after the ethar extraction of *CP and PT* was 16.44 % a 13.48 % respectively.

**Phytochemical Contents Present**

The highest phytochemical present in C flavonoid while that present in PT w phenols as seen from table 2. T phytochemicals present in the pl extracts are presented below.

**Table 1: Qualitative Phytochemical Composition of CP and PT**

| Phytochemical | CP | PT |
|---|---|---|
| Saponins | + | + |
| Flavonoids | + | + |
| Alkaloids | + | + |
| Phenols | + | + |
| Terpenoids | + | - |
| Glycosides | - | + |
| Steroids | - | - |
| Carotenoids | + | + |
| Tannins | + | + |

| | | |
|---|---|---|
| Anthocyanins | - | - |
| Free reducing sugars | - | - |

Keys: (+) present, (−) absent.

**Table 2: Quantitative Phytochemical Composition of CP and PT**

| Phytochemical | CP | PT | P value |
|---|---|---|---|
| Saponins | $10.20\pm0.06^{a}$ | $10.18\pm0.04^{a}$ | 0.76 |
| Flavonoids | $16.10\pm0.00^{b}$ | $10.01\pm0.01^{a}$ | 0.00 |
| Alkaloids | $8.82\pm0.00^{b}$ | $4.85\pm0.03^{a}$ | 0.00 |
| Phenols | $4.02\pm0.00^{a}$ | $16.48\pm0.01^{b}$ | 0.00 |
| Terpenoids | $3.85\pm0.00^{b}$ | $0.00\pm0.00^{a}$ | 0.00 |
| Glycosides | $0.00\pm0.00^{a}$ | $9.97\pm0.01^{b}$ | 0.00 |
| Carotenoids | $2.87\pm0.01^{a}$ | $8.61\pm0.01^{b}$ | 0.00 |
| Tannins | $7.66\pm0.01^{a}$ | $8.12\pm0.01^{b}$ | 0.00 |

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts across each row are significantly different at $p < 0.05$.

## Acute Toxicity Test

There was no mortality and sign of toxicity observed in all the animals dosed during the acute toxicity studies. According to Lorke (1983) method, the extracts are considered safe since the extracts are well tolerated and no mortality was recorded at 5000 mg/kg.

## Bodyweight (g) Changes of *T. b. brucei* Infected and Treated Mice

The table 3 below showed that the bodyweight of animals in groups treated with CP, PT and combined increased significantly ($p<0.05$) during post treatment compared to the day zero of tratment

Table 3. Changes in Bodyweight of Treated Mice

| Group | Bodyweight (g) | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 | Day 15 |
| 250 mg/kg PT | 25.27±5.49$^{a}$ | 23.93±5.26$^{a}$ | 25.20±5.55$^{ab}$ | 28.26±5.90$^{b}$ | 24.87±3.15$^{b}$ | 30.00±4.16$^{b}$ |
| 250 mg/kg CP | 27.50±3.97$^{a}$ | 25.60±3.61$^{a}$ | 28.73±3.46$^{b}$ | 32.53±3.21$^{b}$ | 39.00±2.08$^{c}$ | 42.47±2.34$^{c}$ |
| 250 mg/kg Combined | 26.53±5.87$^{a}$ | 25.27±6.08$^{a}$ | 28.50±6.60$^{b}$ | 32.33±6.80$^{b}$ | 38.20±5.94$^{c}$ | 42.93±6.32$^{c}$ |
| 3.5 mg/kg Berenil | 28.57±3.77$^{a}$ | 27.63±4.11$^{a}$ | 32.73±4.06$^{b}$ | 36.90±3.83$^{b}$ | 42.60±3.67$^{c}$ | 47.27±4.27$^{c}$ |
| Negative control | 20.37±2.70$^{a}$ | 16.63±2.57$^{a}$ | 13.23±2.72$^{a}$ | 10.50±2.41$^{a}$ | 10.20±2.12$^{a}$ | 8.33±1.04$^{a}$ |
| Normal control | 23.77±2.40$^{a}$ | 26.10±2.40$^{a}$ | 30.80±3.25$^{b}$ | 34.57±3.42$^{b}$ | 39.43±3.47$^{c}$ | 44.70±3.79$^{c}$ |

Values are presented as mean ± standard error of mean of three replicates. Values with different superscripts along eac column are significantly different at $p < 0.05$.

## PCV of Infected and Treated Mice

The mean percentage Packed Cell Volume (PCV) of *T. b. brucei* infected mice treated with CP and PT ethanol crude extract is presented in Table 5. At day fifteen po treatment, the PCV of animals in all t treated groups (CP, PT and combine increased significantly ($p<0.05$) wh compared to day zero values.

**Table 4: Changes in PCV of *T. brucei* Infected and Treated Mice**

| Group | PCV (%) | | | | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 3 | Day 6 | Day 9 | Day 12 | Day 1 |
| 250 mg/kg PT | 23.00±1.15$^{a}$ | 25.00±1.15$^{ab}$ | 25.67±0.88$^{b}$ | 29.00±1.15$^{b}$ | 31.00±0.58$^{b}$ | 34.67± |
| 250 mg/kg CP | 23.67±1.45$^{a}$ | 26.67±1.76$^{bc}$ | 29.67±2.73$^{bc}$ | 34.67±2.19$^{c}$ | 39.00±1.15$^{c}$ | 40.33± |
| 250 mg/kg Combined | 23.67±0.88$^{a}$ | 27.67±1.20$^{bc}$ | 33.00±1.15$^{cd}$ | 39.00±0.58$^{d}$ | 41.33±1.20$^{c}$ | 40.33± |
| 3.5 mg/kg Berenil | 22.33±1.20$^{a}$ | 30.00±1.15$^{c}$ | 37.00±0.58$^{de}$ | 39.00±0.58$^{d}$ | 40.00±0.58$^{c}$ | 40.33± |

| | | | | | | |
|---|---|---|---|---|---|---|
| Negative control | 25.00±1.15[a] | 22.33±1.45[a] | 18.00±1.73a | 15.00±1.53[a] | 12.67±1.33[a] | 9.67±0.88[a] |
| Normal control | 39.00±0.58[b] | 39.33±0.88[d] | 38.67±0.88[e] | 39.33±0.88[d] | 39.67±0.33[c] | 39.33±0.88[c] |

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along each column are significantly different at $p < 0.05$.

## Parasitaemia Profile of Infected and Treated Mice

Parasitaemia started to be observed in blood circulation of the infected animals after forty-eight hours (2 days) of infection. The *Piliostigma thonningii (PT)* treated group recorded steady reduction of parasitaemia from 11.11 per field on day 3 to 0.33 by day 15 post treatment. While *Calotropis procera (CP) and combined* treated groups recorded total clearance of parasitaemia at 12 and 9 days respectively as compared to the control groups (Figure 1).

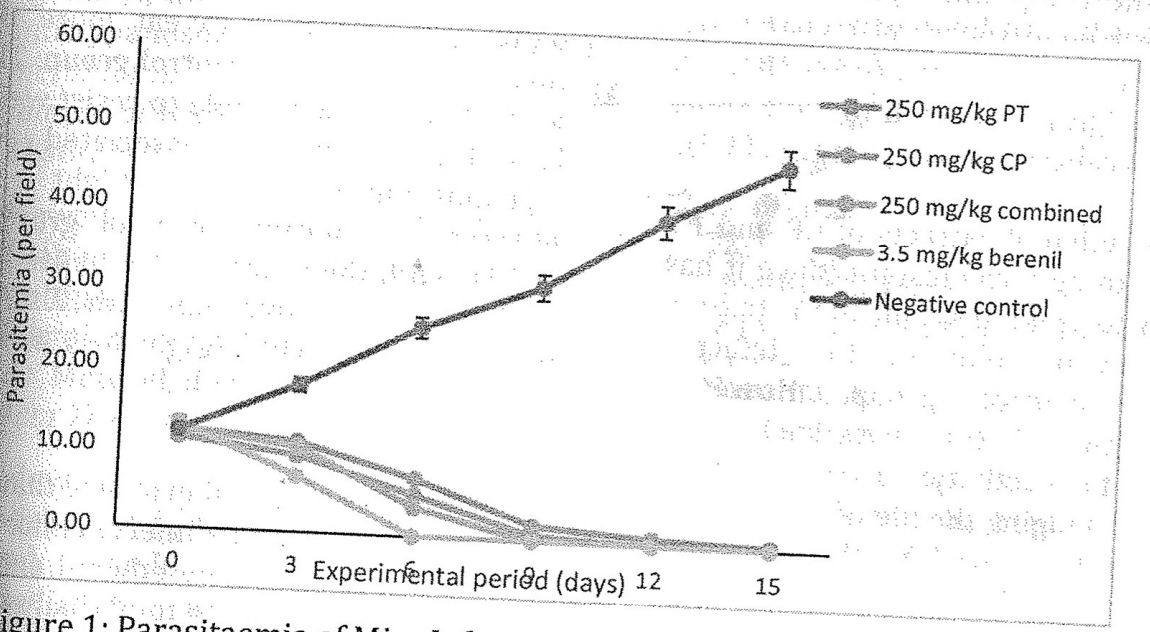


Figure 1: Parasitaemia of Mice Infected with *T. brucei* and treated with CP, PT, Combination of both Extracts and Berenil

## DISCUSSION

The use of herbal medicine plays a significant role for the treatment of disease; however, because of differences in composition of different herbs, their biological effects on the parasite differ.

The qualitative phytochemical composition of *Piliostigma thonningii (PT)* and *Calotropis procera (CP)* extracts presented in Table 1, which indicates the presence of saponins, flavonoids, alkaloids, phenols, carotenoids and tannins in which terpenoids is absent in CP but present in PT and glycosides present in PT but absent in CP. The presence of alkaloids and saponins in CP and PT shows that CP and PT contain compounds with basic nitrogen atoms and foaming characteristic which basically are reported to have trypanocidal effect (Ene *et al.*, 2009). The reduction in parasitaemia observed in the group treated with CP and

PT ethanol extract, may be due to secondary metabolites in the extracts. Some secondary metabolites have previously reported to possess antitrypanocidal properties (Ene *et al.*, 2009). Similarly, the flavonoids are also reported to possess anti-inflammatory, anti-allergic, anti-viral and anticarcinogenic properties (Scholz and Williamson 2007). Therefore, any plant with anti-carcinogenic properties may serve as a good source of trypanocide (Scholz and Williamson 2007). Therefore, the *In vivo* actitivity recorded in this study in CP extract could be due to anti-carcinogenic compounds present, which may serve a good source of trypanocide, since eflornithine currently in use to treat, sleeping sickness is known to have some level of anticancer activity (Barett, 2000).

Therefore, ethanol extracts of CP and PT were said to be trypanostatic since it has the ability to prolong the life of the treated groups beyond that of the infected untreated control group. However, trypanostatic effects are known to suppress the activity of the parasite thereby prolonging the life of the infected mice when compared to the untreated infected control. The results obtained in this study, was also similar to the work of Oluwaniyi *et al* (2019). They recorded potentiation of antitrypanosomal activity of *Vernonia amygdalina* by combination with *Azadirachta indica* extracts.

The observed trypanostatic effect of the *Piliostigma thonningii (PT)* and trypanocidal effect of *Calotropis procera (CP)* ethanolic extract was accompanied by corresponding increase in PCV (Table 4). The increase in packed cell volume observed in CP, PT and combined treated groups is in conformity with the work of (Alagbe, 2020) who had previously reported that *Piliostigma thoningii* have hematopoietic-stimulating factor. Similarly, animals in groups CP, TP and combined treated groups do not suffered anaemia as compared with the negative control group (Table 4). This study therefore, demonstrated that combining plants extracts has led to enhanced antitrypanosomal activity, improved synergistic effect and efficacy.

Furthermore, the result for the bodyweight of animals (Table 3), which increased in all the treated animals indicates that the animals in CP, PT, combined, diminazene aceturate (Berenil) and normal control groups were in a better physical state to eat more than those in the negative control group. They were therefore more able to resist weight loss that is usually associated with trypanosomiasis. The weight lost observed in negative control group is similar with the report of Abubakar *et al* (2011) in which infection with *T. brucei* was associated with weight loss in mice and rats.

## CONCLUSION

The study has provided evidence that oral treatment of *T. b. brucei* infected mice with CP and Combined crude ethanol extracts of CP and PT resulted to total clearance of parasitaemia which led to prolongation of life span. The reduced PCV and weight loss associated with experimental trypanosomiasis were significantly improved. Consequently. *Piliostigma thonningii* and *Calotropis procera* as herbal medicines have potential in the management of Africa Trypanosomiasis.

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