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Full Length Research Paper

Evaluation of the extracts of *Morinda lucida* and *Tridax procumbens* for anti-trypanosomal activity in mice

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A major problem besetting the chemotherapy of African Trypanosomiasis is parasite resistance to the few available drugs, and a known strategy is to use combination of drugs to overcome parasite drug resistance. An attempt has been made in this study to explore the potential of *Tridax procumbens* and *Morinda lucida* individually as single therapy and as combination therapy in the treatment of trypanosomiasis. The effective dosage of the *T. procumbens* and *M. lucida* extracts were combined at ratio 1:1, 1:2 and 2:1(w/w) respectively. All treatments were by intraperitoneal and commenced after the establishment of infection. The methanol extract of *M. lucida* stem bark and leaves gave significant mean survival of 7.0 ± 3.3 and 9.7 ± 3.7 days respectively when compared to the untreated control (P<0.05). The ethyl acetate and methanol extracts of *T. procumbens* gave a mean survival of 11.7 ± 5.4 and 14.3 ± 10.2 days respectively (P<0.05). The combination of *Tridax procumbens* and leaves of *Morinda lucida* methanol extracts at 1:2 gave significant mean survival of 10±2.2 days (P<0.05) at 200 mg/kg body weight, while the least was when combined in ratio 1:1. Phytochemical screening revealed the presence of steroids, saponins, tannins, alkaloids, flavonoids, phenols and carbohydrate in the crude methanol extract and phenols, flavonoids and steroids in the crude ethyl acetate extract of *T. procumbens* while *M. lucida* showed the presence of alkaloids, tannins and saponins. The methanol extracts of the two plants exhibited significant anti-trypanosomal activity and when combined, the plant extracts showed synergy in their activity. The plant extracts therefore have the potential for use as antitrypanosomal phytomedicine which could provide antitrypanosomal drug leads.

Key words: *Tridax procumbens, Morinda lucida,* antitrypanosomal activity, combination therapy, Rubiaceae, Asteraceae.

INTRODUCTION

In tropical Africa, protozoan parasites cause several diseases of social and economic importance. One of the most devastating is trypanosomiasis which is caused by infection with trypanosomes. Trypanosomes are transmitted by tsetse flies to people, domestic livestock and wildlife. The disease constrains agricultural

development on over a third of the African continent by causing livestock production losses due to poor weight gains, stunted growth, poor milk production, reproductive failure and finally death (Olukunle et al., 2010)

Morinda lucida Benth (Rubiaceae) is a medium-sized tree about 15 m tall (Yinusa et al., 2005). It is known as Sangogo or Bondoukou alongua in Cote d' Ivoire; Twi, kon kroma or Ewe amake in Ghana; Ewe amake or atak ake in Togo and Oruwo in South Western Nigeria (Adeneye and Agbaje, 2008). In many countries different parts of the plants are used in different ways. Cold decoction of the plant leaves is used for the treatment of fever in Cameroon; the bitter water decoction of the plant bark, root and leaf are used as bitter tonic and as astringent for dysentery, abdominal colic and intestinal worm infestation (Adeneye and Agbaje, 2008). Oliver-Bever (1986) reported the use of weak decoction of the stem bark in the treatment of jaundice. There are documented *in vitro* antimalarial activity of *M. lucida* leaf extract Koumaglo et al. (1992) and stem bark extract (Bello et al., 2009) against *Plasmodium falciparum* and antimalarial activity of *M. lucida* against *Plasmodium berghei berghei* in mice (Obih et al., 1985). Methanolic extract of *M. lucida* leaf extract have been reported to possess trypanocidal activity (Asuzu and Chineme, 1990) and aortic vasorelaxant effect (Ettarh and Emeka, 2004). Oliver-Bever (1986) documented the use of a weak decoction of the stem bark to treat severe jaundice.

Tridax procumbens (Asteraceae) is known for several potential therapeutic activities like antiviral, antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity (Suseela et al., 2002). Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding. The plants has been extensively used in Ayurvadic system of medicine for various ailments and is shown to possess significant antiinflamatory, hepatoprotective, wound healing and antimicrobial properties (Diwan et al., 1989; Pathak et al., 1991; Saraf et al., 1991; Udupa et al., 1991; Taddei and Rosas, 2000).

The entire plant is used by indigenous people in Gautemala for the treatment of protozoal infections (malaria, leishmaniasis, vaginitis, dysentry) and gastrointestinal disorders (colic/stomach pains, gastritis/ enterocolitis) (Caceres et al., 1998; Berger et al., 1998). Ethnobotanically, in Gautemala the whole plant of *Tridax procumbens* is used by the population for topical applications to treat chronic ulcers caused by leishmaniasis (Caceres et al., 1998).

The present study is designed to evaluate anti-trypanosomal activities of *M. lucida* (leaf and stem bark) extract and *T. Procumbens* singly and in combination using various solvents.

MATERIALS AND METHODS

Parasites (*Trypanosoma brucei brucei)*

A stabilate of *Trypanosoma brucei brucei*, a parasite originally isolated from cattle in Lafia, Nasarawa State and kept in liquid nitrogen at the Nigerian Institute for Trypanosomiasis Research Vom, Plateau State was used. It was maintained in rats by serial passaging.

Plant materials

The *T. procumbens* were collected in Kaduna Vom of Plateau state and Bida and Minna of Niger state while *M. lucida* was from Osogbo in Osun state of Nigeria. The aerial part of *T. procumbens* were collected in the months of May and June. Similarly, the leaves, flowers as well as the local names of *M. lucida* were collected in March at Oba Ile, Olorunda Local Government Area, Osogbo, Osun State, Nigeria.

Identification of plants materials

All the plant materials were identified at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. The Plant, *Tridax procumbens* with voucher number NIPRD/H/6155 and *Morinda lucida* with voucher number NIPRD/H/6289 were deposited at the herbarium of NIPRD, Idu, Abuja.

Preparation of plant materials

About 1 kg each of the *T. procumbens* and *Morinda lucida (*stem and leaves*)* were freshly obtained washed with running tap water and dried at room temperature to a constant weight. The dried plant samples were grinded into powder form using mortar and pestle. The powdered samples were stored in clean polythene bags until required for use.

Preparation of crude extracts

The extraction and screening carried out using the method described by Ogbadoyi et al. (2007). In this method, fifty grams (50 g) of the dried powdered samples of each of the leaves, stem of the *M. lucida* and whole plant of *T. procumbens* were extracted sequentially under reflux with 400 ml of hexane, ethyl acetate, methanol and water in that order for 2 h in each case. Extracts were filtered hot using muslin cloth and solvent was removed using rotatory evaporator for organic solvents and freeze-drier for water extracts. The dried extracts were finally transferred into sterile sample bottles for storage at refrigerated temperature until when required for use. The residue was dried after each extraction for the next extraction process.

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Table 1. Phytochemical constituents of *T. procumbens* crude methanol and ethyl acetate extracts.

Phytochemical	Methanol	Ethyl acetate
Carbohydrates	+	-
Steroids	+	+ +
Saponins	+	-
Flavonoids	+ +	+ + +
Tannins	+	-
Alkaloids	+	-
Anthraquinones	-	-
Resins	-	-

++, Highly present; **+**, fairly present; _, absent.

Phytochemical analysis

The crude methanol and ethyl acetate extracts of *T. procumbens* was subjected to phytochemical analysis using standard analytical methods described by Sofowora (1979) (Table 1).

Infection of animals

Blood from heavily infected donor mouse was obtained by cardiac puncture and collected with EDTA coated syringe to avoid clotting. The blood was immediately diluted with physiological saline to give 1.0×10^7 parasites per ml to obtain inoculums. Healthy mice were then infected intraperitoneally with 0.1 ml of the inoculum containing about 10^6 trypanosomes (Herbert and Lumsden, 1976).

Administration of extracts

Crude extracts dissolved in (physiological buffered saline PBS) for aqueous extract or in little Dimethyl sulphoxide (DMSO) and made up with PBS for organic solvent extract in varied concentrations were administered on infected animals via the intraperitoneal route. Parasitaemia in the blood of infected animals was monitored daily by obtaining blood from the tail end of mice and observing the wet smear under light microscope set at 40X magnification for parasite per field. The number of parasites per ml of blood is then estimated using “Rapid Matching Method” described by Herbert and Lumsden (1976).

Initial screening of the extracts for antitrypanosomal activities

In *T. procumbens,* the ethyl acetate, methanol and aqueous extracts of the whole plants were subjected to the screening against *T. b. brucei* infected mice. For each extract, there are 7 groups (A - G) of 3 mice each. Groups A - F were inoculated with *T. b. brucei* and with the appearance of parasitaemia, animals in Groups A - D were each treated intraperitoneally with the extract at 100, 200, 300 and 400 mg/kg body weight respectively for 14 consecutive days respectively. Mice in Group E were treated once intraperitoneally with berenil at 3.5 mg/kg body weight while Groups F was untreated and Group G was neither infected nor treated and served as control. Similarly in *M. lucida,* the hexane, ethyl acetate, methanol and aqueous extracts of the stem bark and leaves were screened for antitrypanosomal activity against *T. b. brucei* in mice. For each extract of leaves and stem bark, there are 7 groups (A - G) of 3 mice each. Groups A - F were inoculated with the parasite and with the appearance of parasitaemia, animals in Groups A - D were each treated intraperitoneally with the extract at 100, 200, 300 and 400 mg/kg body weight respectively for 14 consecutive days respectively. Mice in Group E were treated once intraperitoneally with berenil at 3.5 mg/kg body weight while Groups F was untreated and Group G was neither infected nor treated and served as control.

Confirmatory screening with effective doses of *T. procumbens* and *M. lucida*

In order to ascertain the efficacy and reproducibility of the doses that demonstrated appreciable antitrypanosomal activities in the initial screening, eight groups of mice (A - H) each containing three mice, were set up. Group A, B and C were administered with methanolic leaves, methanolic stem bark and ethyl acetate extracts of *M. lucida* at 400, 200 and 100 mg/kg body weight respectively. Groups D, E and F were administered with ethyl acetate, methanol and aqueous extracts of *Tridax procumbens* at 200, 300 and 300 mg/kg body weight respectively. All the administration was through intraperitoneal route for 14 consecutive days. Groups G was infected untreated while Group H was neither infected nor treated and served as controls. Parasitemia was monitored three times weekly.

Combination therapy

The possibility of synergistic action of different extracts in varying combinations was investigated using a modified method of Gerardo et al. (2007). To establish this, different extracts that gave highest antitrypanosomal activity in the initial screening were combined in varying ratios and was screened against *T. b. brucei* infected mice. Thus, *T. procumbens* ethyl acetate extract at 200 mg/kg body weight and *M. lucida* methanolic leaves extract at 400 mg/kg body weight were combined in ratios 1:1, 2:1 and 1:2. Each ratio was used treat 3 groups of *T. b. brucei* infected mice comprising of 3 animals each at 200, 300 and 400 mg/kg body weight respectively. In all cases, parasitaemia was monitored daily and means (±SD), maximum / minimum survival was calculated.

RESULTS

Weight of extracts

When 100 g of the crude *M. lucida* stem bark was extracted, hexane gave 0.37 g; ethyl acetate = 0.42 g, methanol = 1.19 g and aqueous = 5.14 g extracts. Similarly, 100 g of crude *M. lucida* leaf extraction gave 1.27 g with hexane, 5.12 g for ethyl acetate, absolute methanol 3.92 g and aqueous = 10.62 g extracts. The weight of *T. procumbens* extracts using 100 g of the crude is as follows: Ethyl acetate = 3.31 g; Methanol = 4.5 g and Aqueous = 7.12 g.

Screening of *M. lucida* leaf extract

The result for the screening of leaf extract of *Morinda lucida* is presented in Figures 1 to 4. The parasitaemia

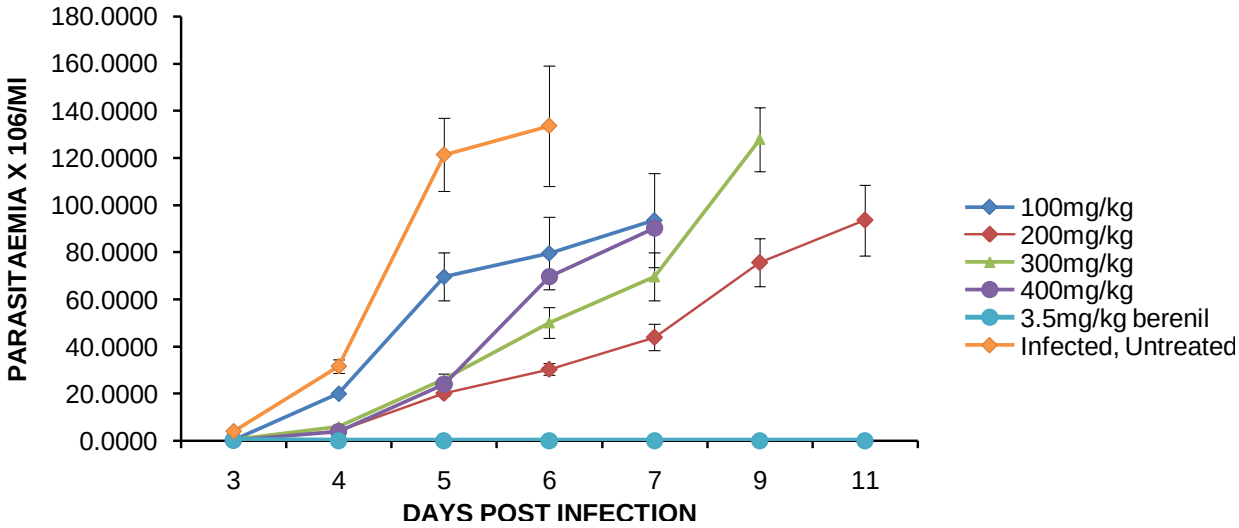

Figure 1. Effect of different doses of *Morinda lucida* hexane leaves extract on *T. b. brucei* infected mice.

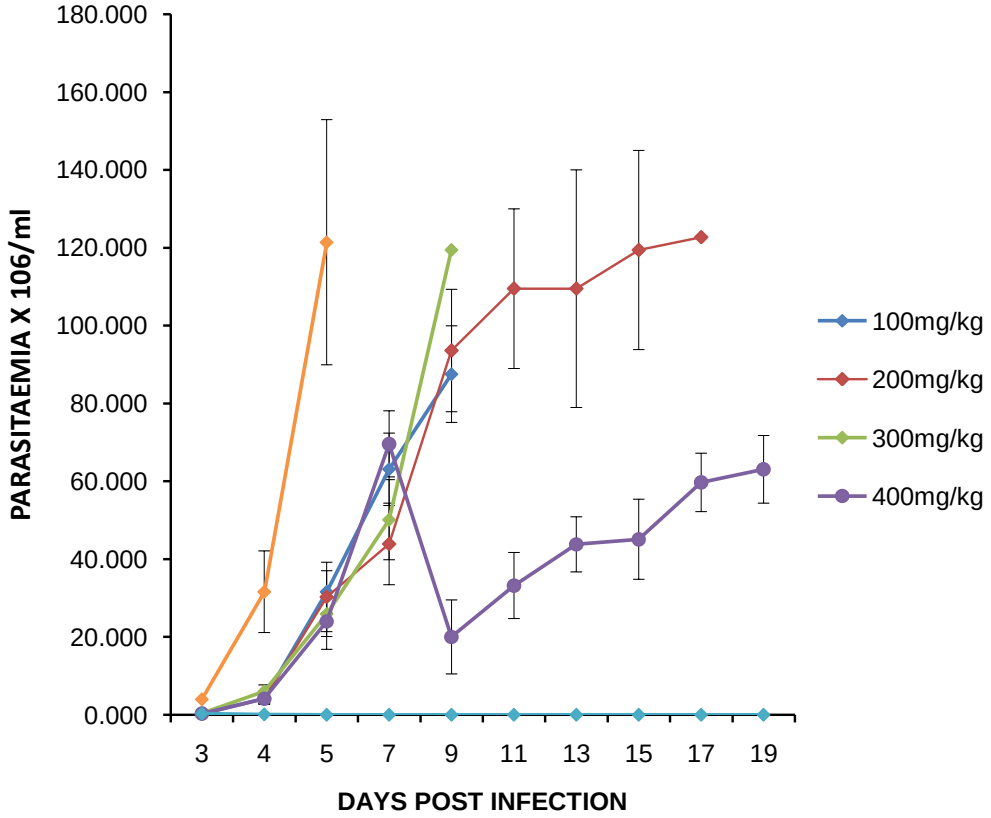

Figure 2. Effect of different doses of *M. lucida* ethyl acetate leaves extract on *T. b. brucei* infected mice.

was lowered with methanol extract (Figure 3) having a means prolongation of life by 9.7 days at 400 mg/kg body weight (Table 2). Other solvent extracts also extend the life of treated groups and the least was the hexane

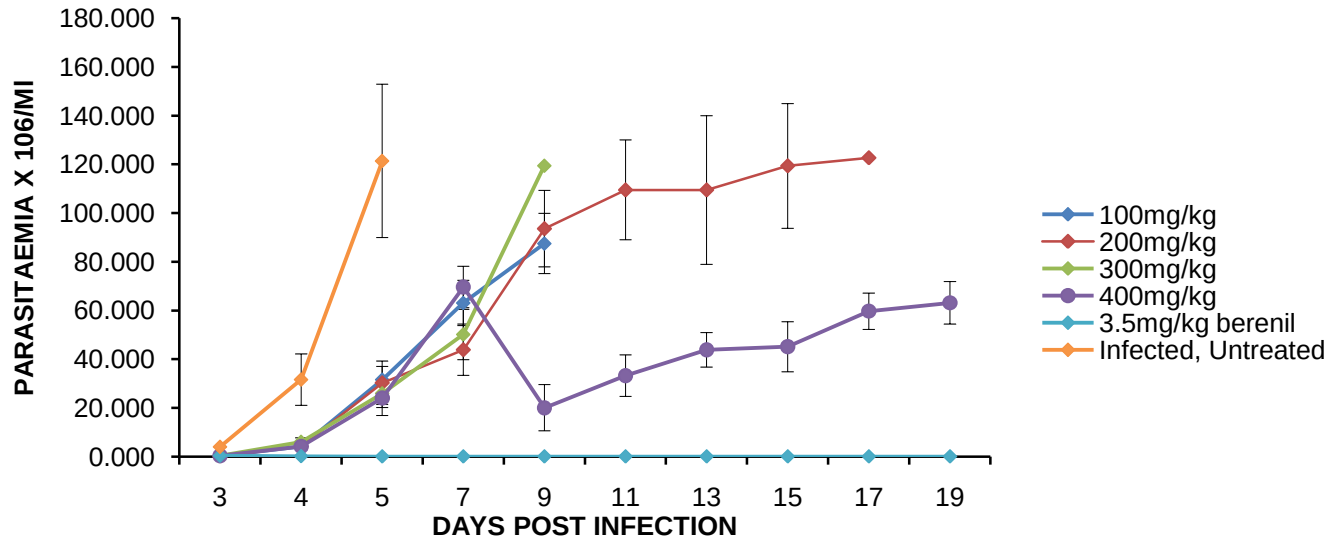

Figure 3. Effect of different doses of *M. lucida* Methanolic leaves extract on *T. b. brucei* infected mice.

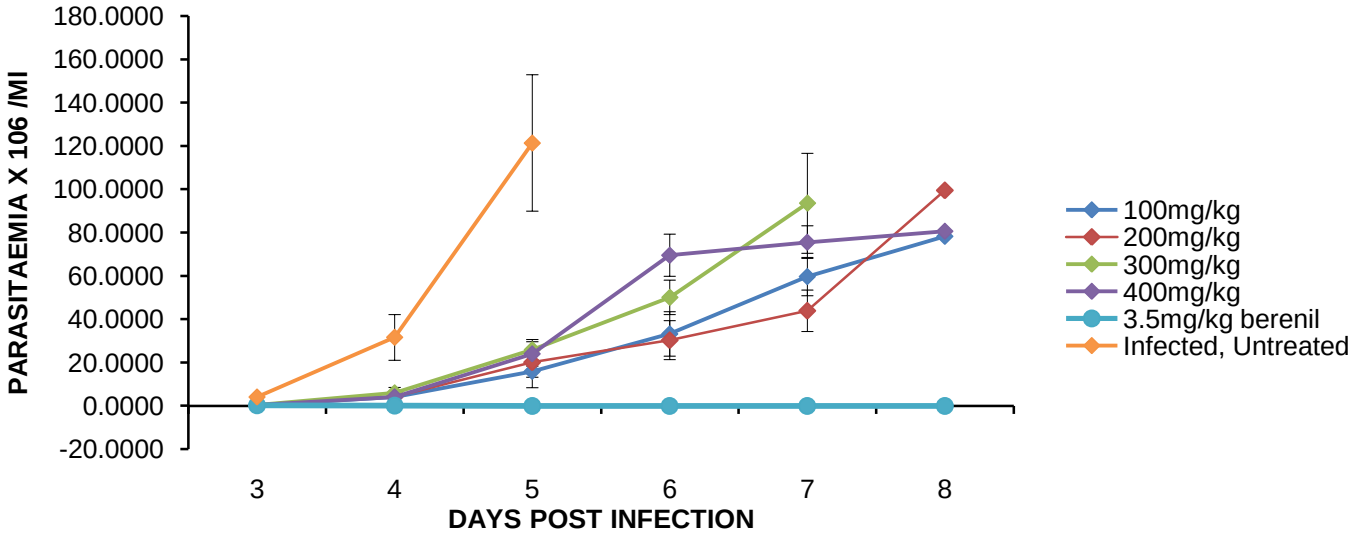

Figure 4. Effect of different doses of *M. lucida* aqueous leaves extract on *T. b. brucei* infected mice.

extract.

Screening of *M. lucida* stem bark extract

The result for screening stem extract of *M. lucida* was presented on Figures 5 to 8. The methanol extract at a dose of 200 mg/kg recorded the longest mean life prolongation by 7.0 days beyond the infected untreated control. The active principle may be less shielded and hence the desire effect was observed at low dosage. The least was the animals treated with ethyl acetate extract (Table 3). In all, the parasitaemia kept fluctuating until the animals died of acute infection.

Screening of *T. procumbens* extracts

Treatment with the *T. procumbens* at all the dose levels resulted to lowering of the parasitaemia leading to prolongation of life (Figures 9 to 11). Ethyl acetate and methanol extract have the best trypanostatic effect resulting to significant means prolongation life by 11.7 and 14.3 days respectively ($P<0.05$). Aqueous extract

Table 2. Summary of screening *M. lucida* leaves extract.

Extraction solvent	Dose (mg/Kg)	Survival range (Days)	Survival beyond control (Days)		Means survival (±SD)
			Min	Max	
Hexane	100	6-8	0	2	1.0±0.8
	200	9-11	3	5	4.0±0.8
	300	7-9	1	3	2.0±0.8
	400	7-8	1	2	1.3±0.5
Ethyl acetate	100	9-16	3	10	6.7±2.9
	200	8	2	2	2.0±0.0
	300	6-9	0	3	1.3±1.2
	400	7-11	1	5	3.0±1.6
	Infected, untreated	6	-	-	-
Methanol	100	6-10	2	5	4.3±0.5
	200	9-17	5	12	7.6±3.3
	300	6-7	2	2	1.5±0.5
	400	10-19	6	14	9.7±3.7
Aqueous	100	7-8	3	3	2.3±0.5
	200	6-8	2	3	2.0±0.8
	300	6-7	2	2	1.6±0.5
	400	6-8	2	3	2.0±0.8
	Infected, untreated	4-5	-	-	-

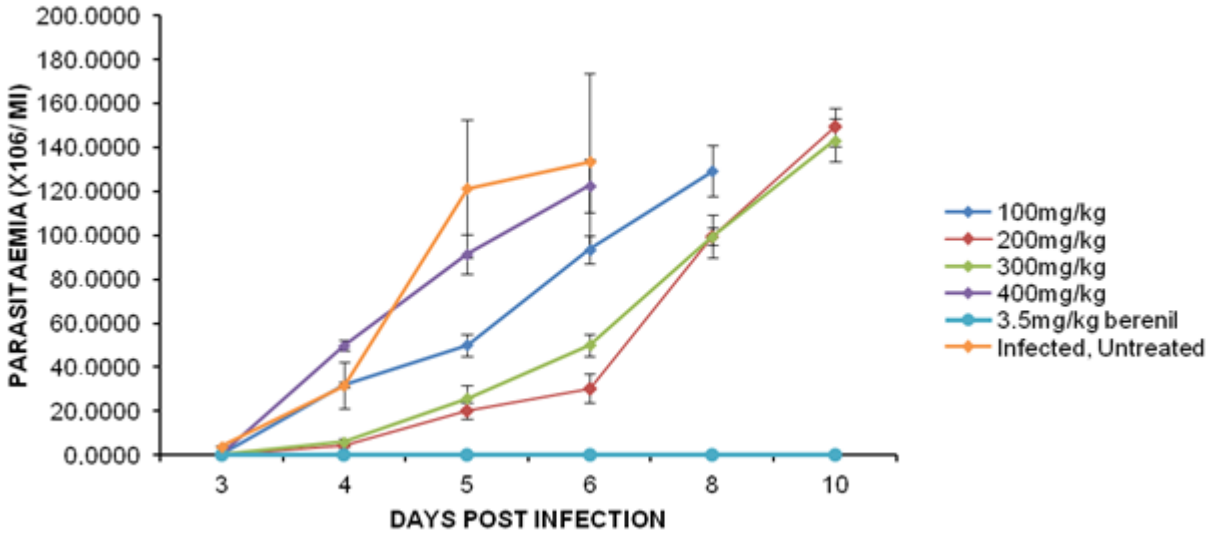

Figure 5. Effect of different doses of *M. lucida* hexane stem bark extract on *T. b. brucei* infected mice.

has the least effect (Table 4).

Confirmatory test

Confirmatory test for the initial screening with the extracts at different doses and a subsequent repeated screening showed that 400 mg/kg body weight of methanol extract of *M. lucida* leaves and 200 mg/kg body weight of *T. procumbens* ethyl acetate extracts gave consistent antitrypanosomal activities. The means prolongation of life was almost the same with the result of the initial

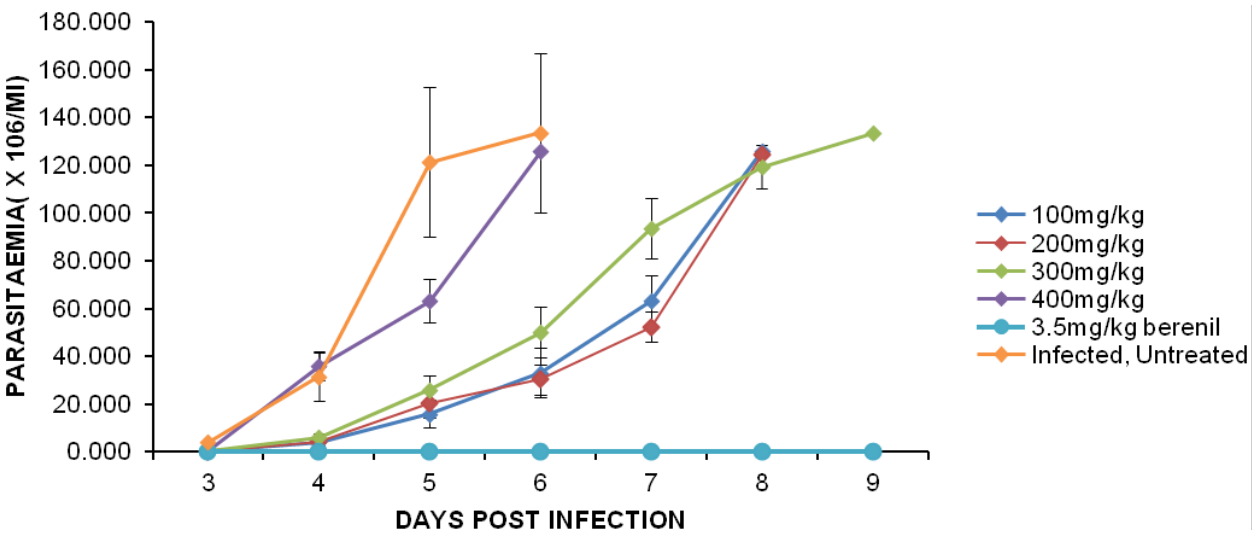

Figure 6. Effect of different doses of *M. lucida* ethyl acetate stem bark extract on *T. b. brucei* infected mice.

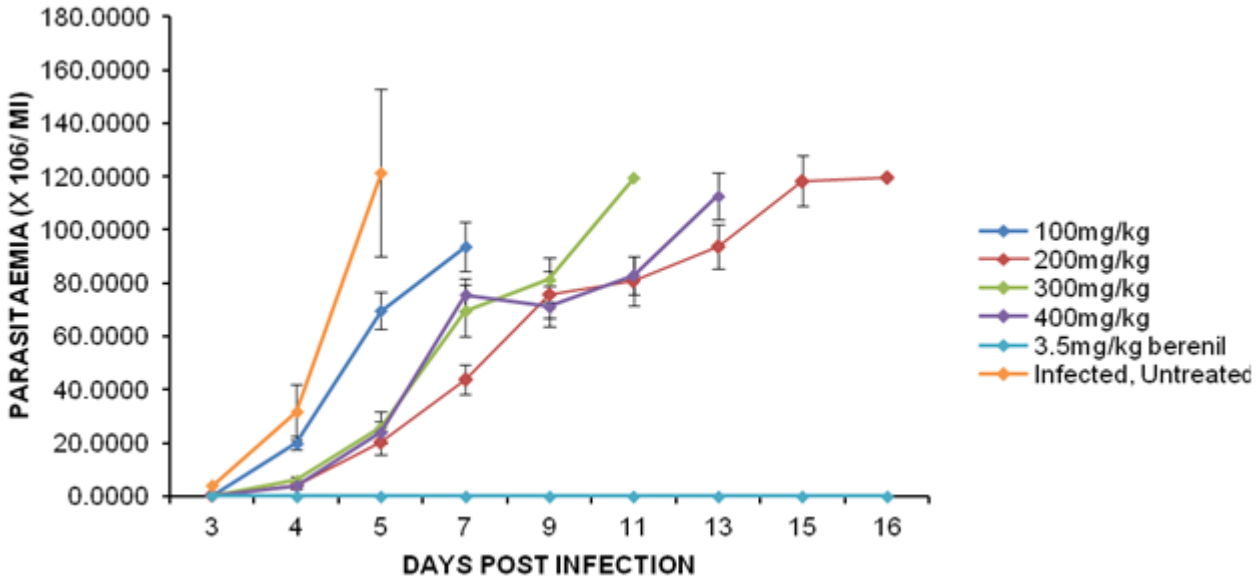

Figure 7. Effect of different doses of *M. lucida* methanolic stem bark extract on *T. b. brucei* infected mice.

screening. However, the methanol stem bark extract of *M. Lucida* and methanol extract of *T. procumbens* at 200 mg/kg body weight could not reproduce the result of the initial screening (Table 5). The control animals that were untreated died one week post infection.

Combination of extracts

The combination of *T. procumbens* and *M. lucida* at 1:2 gave significant means survival of 10 days ($P<0.05$) at 200 mg/kg body weight, while the least was when combined in ratio 1:1 (Figures 12 to 14). It is possible that there is less interference of other compounds with active principle in the extract, hence the observed effect at low dosage.

DISCUSSION

As a medicinal plant, all the various solvent extract of the dried leaves, in order of increasing polarity, has shown

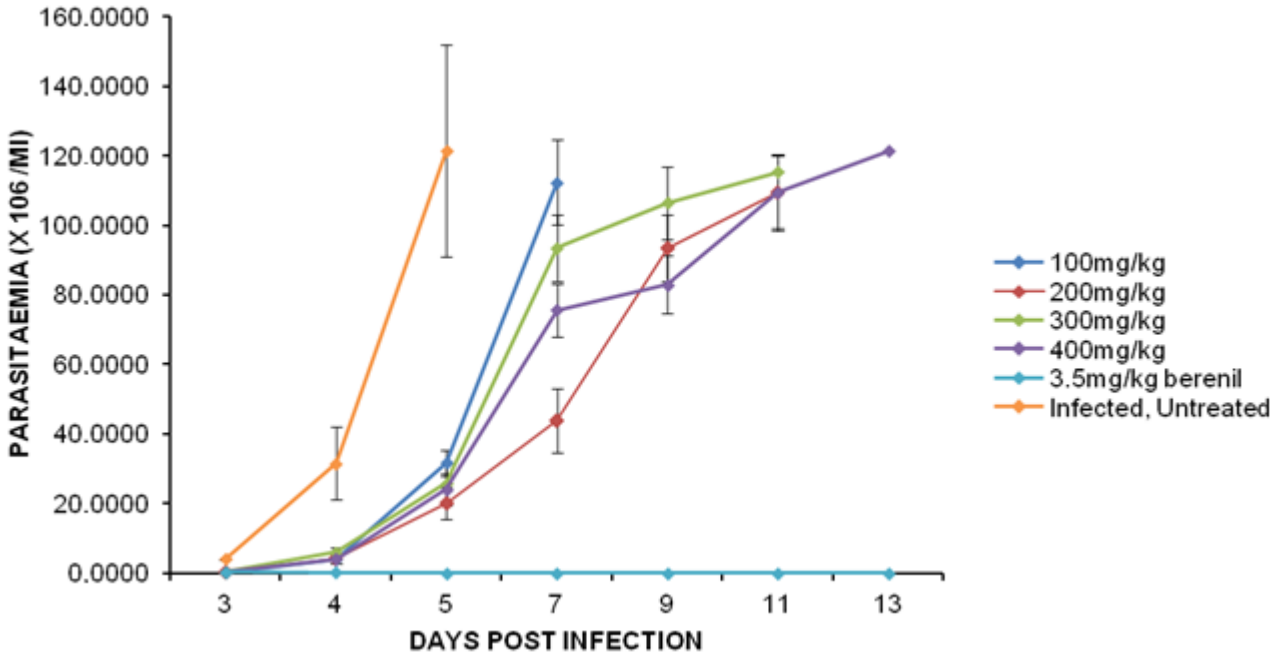

Figure 8. Effect of different doses of *M. lucida* aqueous stem bark extract on *T. b. brucei* infected mice.

Table 3. Summary of screening *M. lucida* stem bark extract (Table 2).

Extraction solvent	Dose (mg/Kg)	Survival range (Days)	Survival beyond Control (Days).		Means survival (±SD)
			Min	Max	
Hexane	100	6-9	0	3	1.3±1.2
	200	6-10	0	4	2.3±1.7
	300	7-10	1	4	2.6±1.2
	400	6-7	0	1	0.6±0.5
Ethyl acetate	100	6-8	0	2	1.0±0.8
	200	6-8	0	2	1.0±0,8
	300	9	3	3	3.0±0.0
	400	4-6	-2	0	0.0±0.0
	Infected, untreated	6	-		-
Methanol	100	6-8	2	3	2.0±0.8
	200	8-16	4	11	7.0±3.3
	300	7-12	3	7	4.3±2.1
	400	9-13	5	8	6.0±1.6
Aqueous	100	4-7	0	2	1.5±0.5
	200	5-12	1	7	4.0±2.9
	300	4-12	0	7	6.0±1.0
	400	9-13	5	8	6.0±1.6
	Infected, untreated	4-5	-	-	-

some level of sporadic antitrypanosomal activity. The most promising activity was recorded with groups treated with more polar methanolic leaves extract. In methanol extract of *M. lucida* leaf extract, the highest dose level of 400 mg/kg body weight recorded highest means survival of 9.7 days with maximum life prolongation of 14 days.

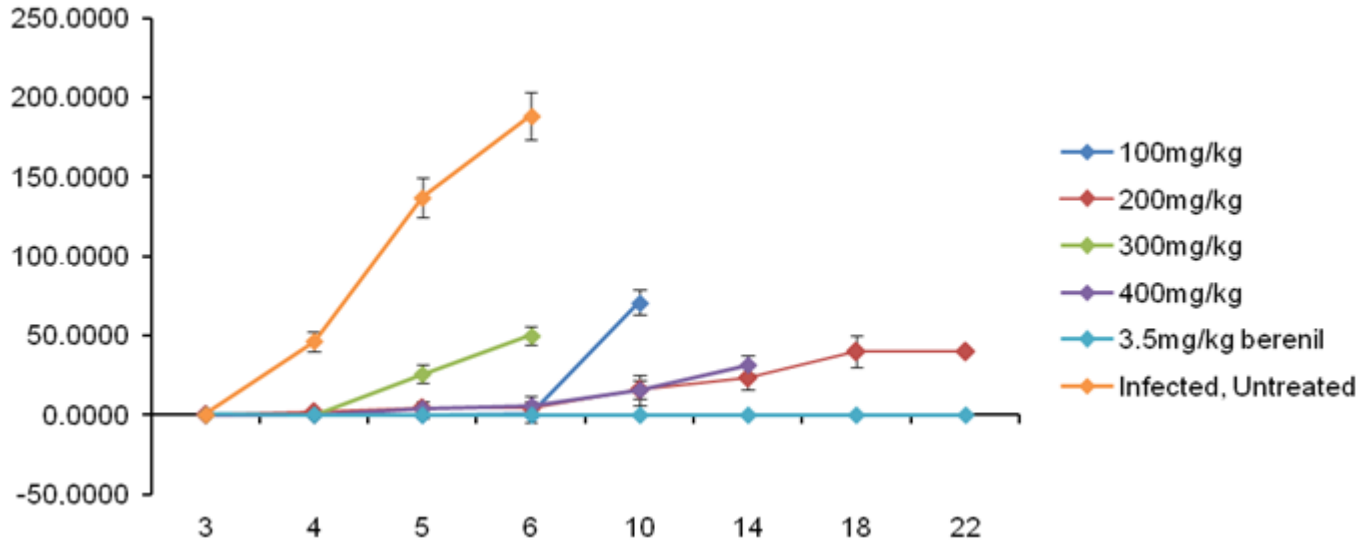

Figure 9. Effect of different doses of *Tridax procumbens* ethyl acetate extract on *T. b. brucei* Infected Mice.

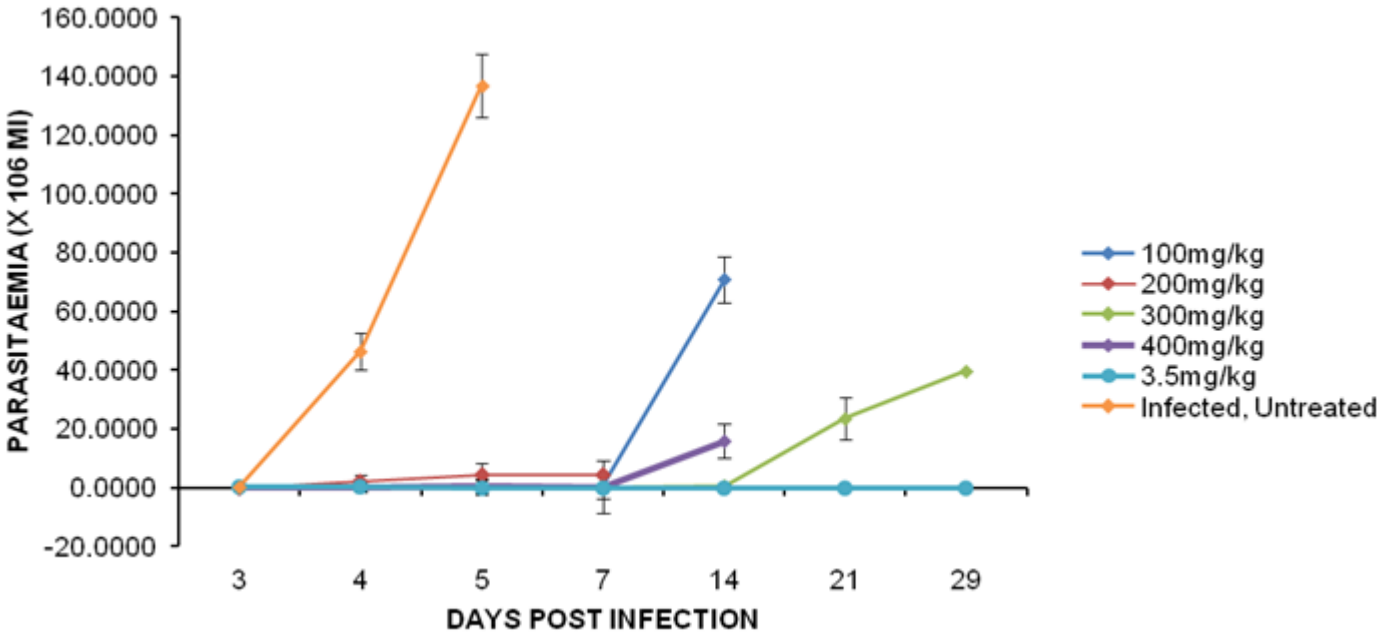

Figure 10. Effect of different doses of *T. procumbens* methanolic extract on *T.b. brucei* infected mice.

This was possible because there was suppression of parasitaemia which was more in group treated at 400 mg/kg body weight. The ethyl acetate treated group was the second best in terms of mean survival and parasitaemia. The ethyl acetate extract is more polar than hexane extract and less than methanol. The effect of hexane and aqueous extract on the parasitaemia and prolongation of life beyond the control are minimal ($P>0.05$).

The stem bark extract showed a similar activity with leaves extract although to a lesser extent. Therefore, the antitrypanosomal property of leaves methanol extract supersede that of the stem bark methanol extract both in term of mean survival and prolongation of life beyond the untreated control.

The transient trypanocidal activity of methanol extracts

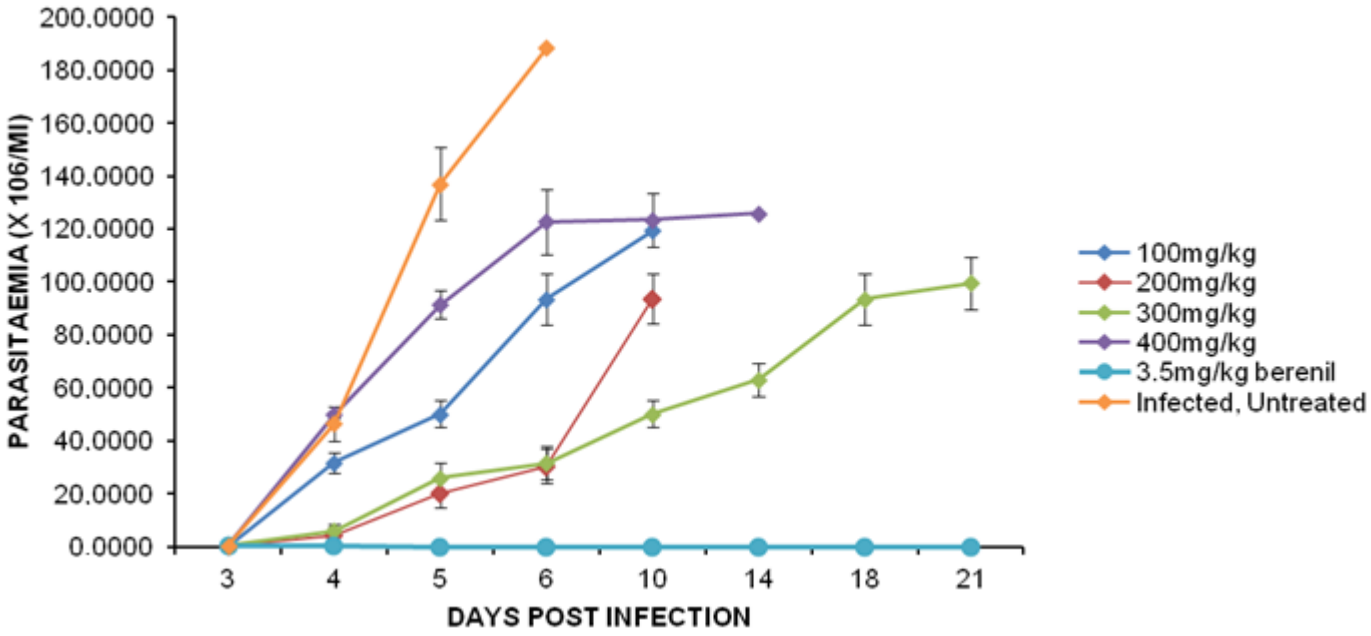

Figure 11. Effect of different doses of *T. procumbens* aqueous extract on *T. b. brucei* infected mice.

Table 4. Summary of screening *T. procumbens* extracts (Table 2).

Extraction solvent	Dose (mg/Kg)	Survival range (Days)	Survival beyond Control (Days).		Means survival (±SD)
			Min	Max	
Ethyl acetate	100	8-10	3	4	3.6±0.5
	200	9-22	4	16	11.7±5.4
	300	7-9	2	3	2.7±0.8
	400	6-16	1	10	6.3±3.8
Methanol	100	6-17	1	11	7.0±4.3
	200	6-10	1	4	2.6±1.2
	300	7-30	2	24	14.3±10.2
	400	5-15	0	10	5.6±4.2
	Infected, untreated				
Aqueous	100	8-10	3	4	3.7±0.5
	200	7-12	2	6	4.3±1.7
	300	7-21	2	15	8.0±5.4
	400	8-16	3	10	7.3±3.1
	Infected untreated	5-6	-	-	-

of *Morinda lucida* leaves and stem bark is in agreement with the earlier studies by Asuzu and Chineme (1990). They reported the suppression of the *T. b. brucei* infected animal when treated with 50% methanol dry leaves extract and 1000 mg/kg produced the highest suppression. In this study, the extracted leaves of *M. lucida* were in absolute methanol and the dose level that *gave* highest suppression of parasite was 400 mg/kg body weight. Furthermore, Asuzu and Chineme (1990) obtained the best trypanocidal activity when treatment with *M. lucida* extract commenced simultaneously with trypanosome inoculation. In this study the best anti-trypanosomal activity was observed when treatment commenced at 24 h post parasite inoculation. Therefore

Table 5. Summary of screening combined *T. procumbens* and *M. lucida* methanolic extracts.

Combination type	Dosage (Mg/Kg)	Survival range	Survival beyond control (Days)		Means survival (±SD)
			Min	Max	
TP:ML(1:1)	200	9-11	4	5	4.0±0.8
	300	13	-	7	7.0±0.0
	400	9-11	4	5	4.0±0.8
TP:ML (1:2)	200	13-19	8	13	10.0±2.2
	300	9-14	4	8	5.3±2.1
	400	9-11	4	5	4.0±0.8
TP:ML (2:1)	200	13-15	8	9	8.0±0.8
	300	12-17	7	11	8.3±2.1
	400	8-13	3	7	4.7±2.1
	Infected, Untreated	5-6	-	-	

TP, *T. procumbens;* ML, *M. lucida.*

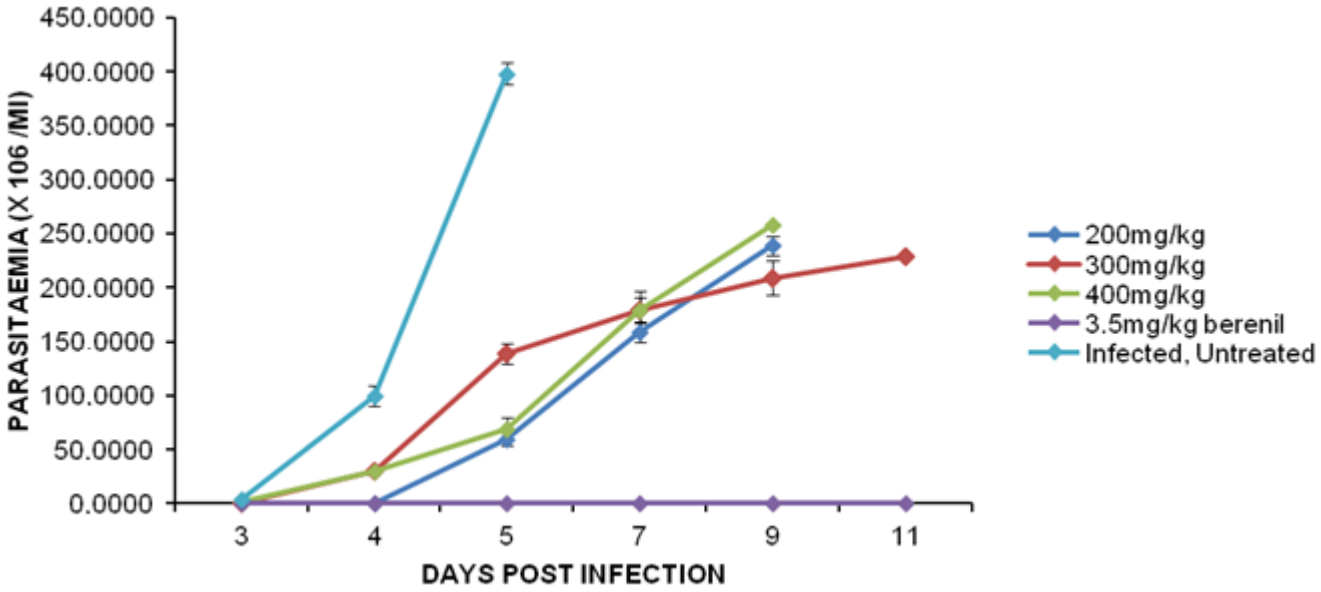

Figure 12. Effect of different doses of combined *T. procumbens* and *M. lucida* methanol extract (1:1) on *T. b. brucei* infected mice.

the efficacy of treament is dependent on the number of parasite inoculated and time the treatment commenced. This studies and many other investigators (Asuzu and Chineme, 1990; Aguiyi et al., 1999; Onyekwelu and Okwuasaba, 2006; Ogbadoyi et al., 2011) all observed that the trypanocidal activity of the extracts from the various plants was not sustained and parasites were not cleared from the peripheral circulation. The phyto-chemicals present in *M. lucida* was earlier reported to be alkaloids, saponins, tannins. Doughari (2012) reported that plant-derived alkaloids in clinical use include the analgesics, the muscle relaxant, the antibiotics, the anticancer agent, the antiarrythmic, the pupil dilator, and the sedative.

Tannin rich medicinal plants are used as healing agents in a number of diseases. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhea (Doughari, 2012). Extracts of *T. procumbens* has shown antitrypanomal potential against *T. b. brucei* infected mice. Both the prolongation of life and suppression of parasitaemia in infected animals was possible probably because *T. procumbens* was earlier reported to have immunomodulatory activity which suggest its therapeutic

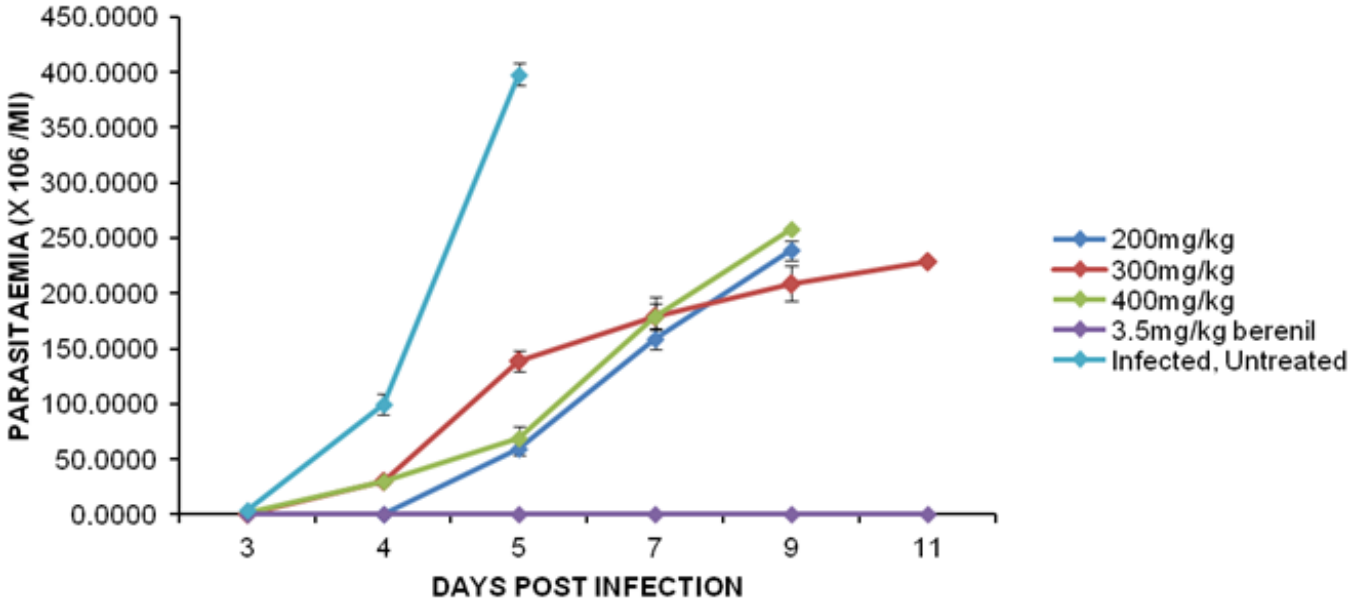

Figure 13. Effect of different doses of combined *T. procumbens* and *M. lucida* methanol extract (1:2) on *T. b. brucei* infected mice.

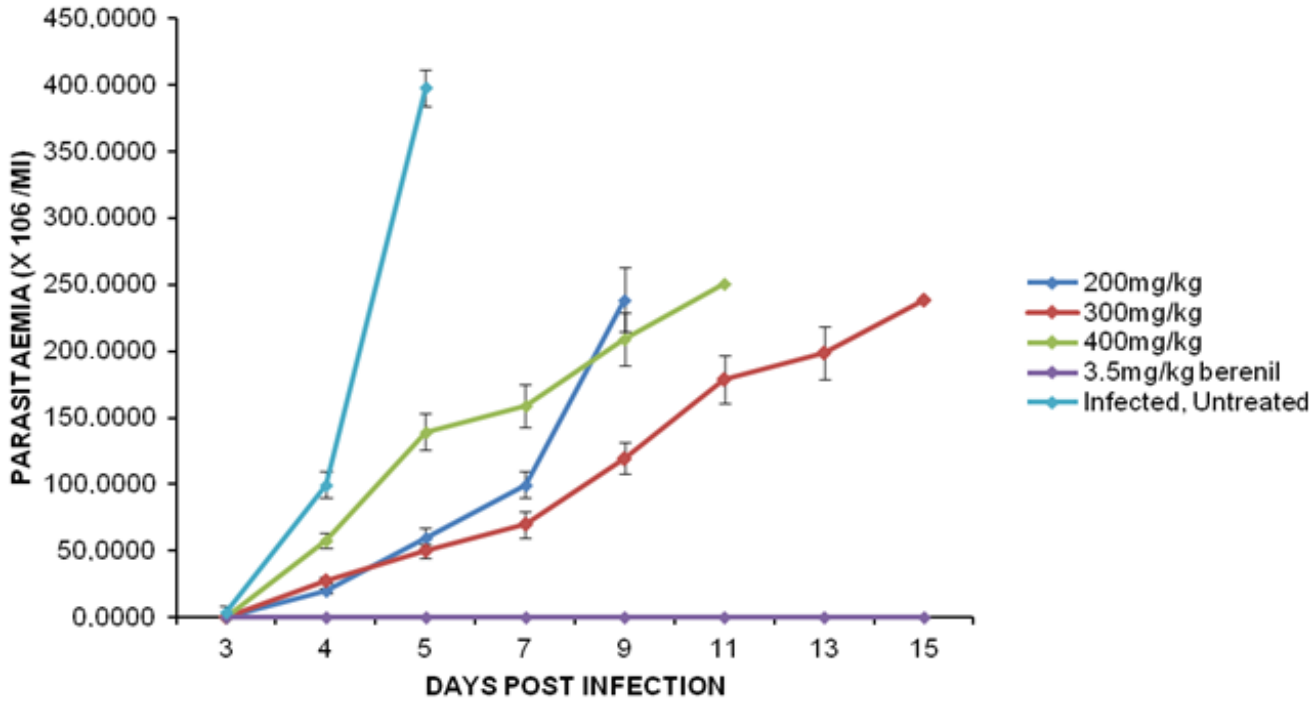

Figure 14. Effect of different doses of combined *T. procumbens* and *M. lucida* methanol extracts (2:1) on *T. b. brucei* infected mice.

usefulness (Tiwari et al., 2004). *T. procumbens* has been demonstrated to stimulate both humoral as well as cell mediated immune system vis-a-vis assists in genesis of improved antibody response against specific clinical antigen (Tiwari et al., 2004). Since trypanosome infection causes immunosuppression, any herbal preparation that

is immune boosting may have a significant effect on trypanosomes. Infection with trypanosomes has been known to impair the immune system of the host, cause anaemia, weight loss, reproductive disorders and death of animals if not treated (Olukunle et al., 2010). Consequently in the presence of antigen (parasites) in the blood circulation, the administration of *T. procumbens* therefore may have activated the lymphocytes to increase the effectiveness of antigen clearance by phagocytosis or to secrete various immune effector molecules.

This resulted to low parasitaemia and prolongation of life beyond the untreated control of all this plant extract investigated. The immunostimulative effect of *T. procumbens* may have occurred in all the extract and methanolic extract recorded highest means survival followed by ethyl acetate, while aqueous extract was the least. Furthermore, the presence of various phyto-chemicals particularly, flavonoid in this plant could be responsible for the observation recorded. The phytochemical analysis of the methanol and ethyl acetate extract of the plant revealed the presence of flavonoid in high amount in ethyl acetate than methanol extract. An important effect of flavonoids is the scavenging of oxygen-derived free radicals. *In vitro* experimental systems also showed that flavonoids possess anti-inflammatory, antiallergic, antiviral, and anticarcinogenic properties (Middleton, 1998). Any anticarcinogenic plant could serve as a good source of trypanocide since trypanocide currently in use to treat sleeping sickness are known to have some level of anticancer activities (Barrett and Barrett, 2000).

All the extracts with antitrypanosomal activities are polar and most likely the active compound could therefore be polar in nature. In addition to its reported immuno-modulatory effect, *T. procumbens* Linn (compositae) is also employed as indigenous medicine for a variety of ailments, including jaundice (Saraf et al., 1991). The plant has been extensively used in traditional medicine as anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery (Ali et al., 2001). Moreover, it also possesses wound healing activity and promotes hair growth (Saraf et al., 1991). *Tridax procumbens* is also dispensed as 'Bhringraj', which is well known Ayurvedic medicine for liver disorders (Pathak et al., 1991). Antioxidant properties (Ravikumar et al., 2005), have also been reported.

In Africa, Nigeria in particular, most of herbal preparations used for the treatment of illnesses is usually a combination of two or more herbs. Combinations of medicinal herbs in medicinal prescriptions may not only affect a balance of active components, but also undergo a mutual synergy which improves efficacy, safety, and minimizes side-effects. The combination that gave highest mean survival was *T. procumbens* and *M. lucida* (1:2) treated at 200 mg/kg body weight. When compared with the activity of all the single extracts, the combination at 200 mg/kg body weight gave the best antitrypanosomal effect by having higher prolongation of life. From the result of phytochemical analysis, *M. lucida* has high amount of alkaloid while *T. procumbens* is rich in flavonoids. This implies that the synergistic effect of alkaloids present in *M. lucida* and antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties of flavonoids present in *T. procumbens* could be responsible for the observed activity. Another advantage of this combination studies is that the dose level that gave highest activity was lower than those that gave activity singly. Dosage reductions of each drug combined may reduce the overall toxicity while maintaining good efficacy (Gerardo et al., 2007).

A major problem besetting the chemotherapy of African Trypanosomiasis is parasite resistance to the few available drugs (DeKoning, 2001). One major benefit of combination therapies therefore is that they reduce development of drug resistance, since a pathogen is less likely to have resistance to multiple drugs simultaneously. Drugs that has different mode of action can be combined to achieve desirable effect. In these studies an attempt was made to explore the potentials of combination therapy using *T. procumbens* and *M. lucida* leaves extract in different combinations to treat *T.b.brucei*-infected mice. The only combination that has antitrypanosomal activity from the infected mice was the combination of the methanol extracts of the leaves of the two plants (Figures 12 to 14). Though the mean survival rate was not very high as compared to when treated singly, but there was prolongation of life particularly at 200 mg/kg. This also provided evidence that the combination of plants has some efficacy as practise in tradomedicine. Sometimes combination chemotherapy is used not to cure but to reduce severe symptoms and prolong life.

Conclusion

M. lucida and *T. procumbens* have trypanostatic effect and prolonged the life of *T. b. brucei* infected, treated animals. There is synergy in combination of the 2 plants extracts which result in significant prolongation of life than when used singly.

Conflict of Interests

Authors have not declared any conflict of interest.

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