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Research Article

Comparative Studies of *In vitro, In vivo* Trypanocidal Activity and Phytochemical Screening of *Tapinanthus globiferus* and *Gongronema latifolium*

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Abstract: The present study investigates the trypanocidal activity of Tapinanthus *globiferus* and *Gongrenema latifolium* on *Trypanosoma brucei brucei in vitro* and *in vivo.* Various extracts obtained from these plants, were compared for trypanocidal activity *in vitro* and *in vivo* at different concentration. The methanolic extract of *Tapinanthus globiferus* and *Gongrenema latifolium* had the highest activity *in vitro* and were further evaluated for *in vivo* activity on mice infected with *Trpanosoma brucei brucei*. The groups were treated with extract concentration ranging from 100-400mg/kg of body weight intraperitoneally for 7 consecutive days. The group treated with 400mg/kg of *Tapinanthus globiferus* had significant reduction in parasiteamia and their life span was prolonged up to the 20-21st day, in comparison to those treated with the same dose of *Gongrenema latifolium* and all other groups treated with other doses, including the control, all died between day 5th-8th post infection. The group treated with extract of *Tapinanthus globiferus* and inoculated with parasite simultaneously did not develop parasite until the 9th day with low level of parasiteamia, with appreciable life span ranging between 15-16 days post infection. The Phytochemical screening showed appreciable amount of alkaloids and flavonoids in the extract of *Tapinanthus globiferus* compared to that found in *Gongrenema latifolium* which were only in traces.

Keywords: *Gongrenema latifolium*, *in vivo* and *in vitro*, phytochemical screening, *Tapinanthus globiferus*, trypanosomal activity, *Trypanosoma brucei brucei*

INTRODUCTION

African Trypanosomiasis is a disease that affects man and domestic animals. It is a parasitic disease caused by different species of protozoan blood parasite (Genus Trypanosoma). It is one of the major obstacles to livestock production in Africa (Antia *et al*., 2009). Direct losses in meat production, milk yield and the cost of programmes that attempt to Control Trypanosomiasis are estimated to cost between $600 million and 1.2 billion each year (Gutteridge, 1985; Aldhous, 1994; Kamuanga, 2003). It is estimated that over 60 million people are at risk of the disease, of which only 3.5 million are under surveillance in endemic countries (WHO, 2004). The management of the disease is principally based on vector control, the use of trypanotolerant cattle and chemotherapy. Four main drugs (suramin, pentamidine, melarsoprol and eflornithine) are currently in use for the treatment of Trypanosomiasis (Kuzoe, 1993). However these are beset with so many problems such as toxicity, limited and expensive nature of the drugs (Onyeyili and Egwu, 1995; Osma *et al*., 1992). Furthermore Production of vaccine in the near future has been dwarfed due to the phenomenon of antigenic variation exhibited by the parasite (Donald, 1994; Anene *et al*., 2001) and vector control strategy is faced with difficulties. These problems of current treatment methods and other factors increase the need for urgent search for more effective and less toxic chemotherapeutic agents from natural origin.

Recent studies revealed several plants as potent tryponocides (Asuzu and Chineme, 1990; Freiburghaus *et al*., 1996; Nok *et al*., 1996; Atawodi *et al*., 2003; Ogbunugafor *et al*., 2007; Nwodo *et al*., 2007; Maikai *et al*., 2008). These reports suggest the possibility of producing potent trypanocides from medicinal plants.

Here we present comparative studies of *in vitro* and *in vivo* antitrypanosomal activity of the extracts of *Gongronema latifolium Tapinanthus globiferus. Gongronema Latifolium* belongs to the family of Asclepiadaceae. The plant common name is amaranth globe. The parts commonly used are leaves, stem and root. The origin of the plant is traced to Nigeria in West Africa. *Gongronema latifolium* is called Madumaro by Yoruba ethnic group in Nigeria. It is a rainforest plant which has been traditionally used in the South Eastern part of Nigeria over the ages for the management of diseases such as diabetes, high blood pressure etc. And *Tapinanthus globeferus* (Family-Loranthaceae) known

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by other names such as mistletoe (English), Afomo onisana (Yoruba), Kauchi Doruwa (Hausa) is a parasitic plant growing on a large number of tree species such as Kola, Citrus, Combretum, Acacia, Aloe,Pakia and Terminalia as host plants (Waterberg *et al*., 1989). It is wide spread and has been known to be very common in North Central Namibia and theTropical rainforest of Nigeria. The aqueous extract of the leaves of *Tapinanthus globiferus* have been used in traditional medicine in the management of hypertension, epilepsy, relief pain, tinnitus and trypanosomiasis.

MATERIALS AND METHODS

Plant material: *Gongronema latifolium* was collected from a farm land in Akwa local government area of Anambra state, while *Tapinanthus globiferus* was collected in Zaria metropolis. Both plants were identified at the herbarium unit of Biological Sciences Department, Ahmadu Bello University Zaria.

Extraction: Exactly 200 g of the powdered leaf was defatted in 500 mL of Petroleum ether for 24 h. The recovered extract was concentrated under a Rotary evaporator. The Mac was macerated with 500ml of methanol for 48 h; it was filtered and also concentrated under a rotary evaporator. Furthermore, the aqueous extract was obtained by maceration of the Mac in 500 mL of water for 24 h, it was then filtered and concentrated on water bath at 50°C for 12 h.

Trypanosome: *Trypanosoma brucei brucei* (Federe strain) was obtained from the Nigerian Institute for Trypanosomiasis Research, (NITR) Kaduna and was maintained in the laboratory through serial passage in laboratory animals.

Inoculation of mice and parasite count: Parasites were monitored from blood obtained from the tail of previously inoculated donor mice. Briefly the trypanosome count was determined by wet mount microscopic at x 40 magnification using the rapid matching method of Herbert and Lusmden (1976). This method involves the counting of parasite per field in pure blood or blood appropriately diluted with phosphate buffer saline. The logarithm of this count obtained by matching with the table of Herbert and Lumsden (1976) is converted to antilog to provide absolute number of trypanosomes per ml of blood. When the parasite count is such as $\log 10^8$ per ml, the animal was sacrificed, blood recovered by cardiac puncture and collected in heparinised tubes to be used for *in vitro* studies or for inoculation of animals for *in vivo* studies.

***In vitro* trypanocidal activity:** Trypanocidal activity was performed in duplicate in 96 well micro titre plates (Flow laboratories Inc., McLean, Virginia 22101, USA) as described by Atawodi *et al*. (2002) as follows:10mg of each extract was weighed and dissolved in 1ml of phosphate buffer saline serial dilutions with concentration ranging from 2.5mg, 4mg and 10mg/ml were obtained also by using PBS. (Control wells were also included containing parasite suspension in 5% DMSO only without extract. *Diminal*^{R} a trypanocidal drug (*445mg diminazene diaceurate*+555mg phenazone/g, Eagle Chemical Company LTD, Ikeja, Nigeria) was also included in the set of controls at different concentration.

At the height of Parasiteamia, such as 10^8 the donor mice was sacrificed and the blood was collected in heparinised tubes which was further dispensed into a solution of glucose phosphate buffer saline at the ratio of 1:2.

Fifty micro litres of blood was dispensed into a well of the micro titre plate and was mixed with 20 µL 0f the constituted extract to give a final volume of 70 µL. After 5 min incubation in covered micro titre plate maintained at 37°C, a drop of each test mixtures was placed on separate microscope slides and covered with cover slips and the Parasites observed every 5 minutes for a total duration of two hours. Cessation or complete elimination of motility of the parasites in extract-treated blood compared to that of parasite-loaded control blood without extract was taken as an indication f or trypanocidal activity.

Phytochemical screening: Chemical test were carried out on the powdered specimen of methanolic Stem bark extract of *Gongrenema latifolium* and methanolic leaf extract of *Tapinanthus globiferus*, using standard procedure to identify the constituents as described by Odebiyi and Sofowora (1978). This is to identify the presence of tannins, resin, glycosides, flavonoids, alkaloids, saponins among others.

Experimental animals: Thirty healthy Albino mice were used for the *in vivo* experiment. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations of the World Health Organization (WHO). The animals were obtained from the animal colonies of Nigerian Institute for Trypanosomiasis Research Kaduna they were made of both sexes weighing about 20-25 g. They were fed growers mash and water *ad libitum.*

Mice were infected with 10^4 as described earlier, at the height of parasiteamia such as 10^7 the animals were divided into six groups of five each ABCDE and F. They were treated with methanolic extracts from both plants as follows:

Group A: Infected and treatment simultaneously with 200 mg/kg/day
Group B: Infected treated with 100mg/kg/day
Group C: Infected treated with 200mg /kg/day
Group D: Infected treated with 400mg/kg/day

Table 1: Trypanocidal activity of extracts of *Tapinanthus globiferus* and *Gongrenema latifolium* on *Trypanosoma brucei brucei*

Plant name		Time in (min) in which trypanosome motility was observed in suspension with different effective concentrations of extracts (mg/mL)								
		Petroleum ether			Methanol			Aqueous		
Gongrenema latifolium	Planr part	10	5	2.5	10	5	2.5	10	5	2.5
Tapinanthus globiferus	Leaves	NA	NA	NA	45 min***	NA	NA	NA	NA	NA
	Stem bark	NA	NA	NA	15 min*	30 min*	75 min***	NA	NA	NA
	Leaves	NA	NA	NA	10 min*	15 min*	25 min*	25 min*	30 min*	80 min*
	Stem bark	NA	NA	NA	25 min*	30 min*	45 min**	40 min***	NA	NA

NA: Parasite highly motile active 120 min; *: Motility ceased; **: Motility reduced drastically; ***: Slightly reduce

Table 2: Phytochemical screening of methanolic extract of Stem bark *Gongrenema latifolium* and Leaf extract of *Tapinanthus globiferus*

Compound tested	*Gongrenema latifolium*	*Tapinanthus globiferus*
Alkaloids	+	++
Saponins	+	+
Cardiac glycosides	+	+
Antraquinones	-	
Flavonoids	+	-
Terpenoids	+	++
Phlobabtanins	-	+
Tannins	+	-
Sterols	-	-

+present, ++highly present and - absent

Group E: Infected control (negative control)
Group F: not infected control(positive control)

Crude extract were constituted in normal saline and administered intraperitoneally at 0.3 mL.

RESULTS

The Petroleum ether, methanolic and aqueous extract of *Tapinanthus globiferus* and *Gongrenema latifolium* were tested for *in vitro* activity on *Trypanosoma brucei brucei* at 2.5, 5 and 10mg/ml Table 1. The highest activity was observed with methanolic leaf extract of *Tapinanthus globiferus* which ceased the motility of the parasite within 10 min, followed by methanolic stem bark extract of *Gongrenema latifolium*, which ceased motility at 15 min of incubation. Petroleum ether extract of both plants did not show any *in vitro* activity. Diminal^{R} ceased Trypanosome motility within 30 min of incubation.

The result of the Phytochemical screening of *Tapinanthus globiferus* and *Gongrenema latifolium* revealed the presence of alkaloids, saponins, tannins among others (Table 2).

The result of the *in vivo* studies presented in Fig. 1 and 2 showed that the extract activity was dose dependant. Parasiteamia developed by day three post infection when treatment also commenced. There was significant reduction in parasiteamia with groups treated with methanolic extract of *Tapinanthus globiferus* at 400 mg/kg body weight. Their lives were prolong up to the 20th-21st day post infection compared to the group that was treated with *Gongrenema latifolium* with the same dose (400 mg/kg) but died between the 7th and 8th days post infection. Furthermore parasiteamia was delayed up to the 9th day with the group inoculated and treated simultaneously with methanolic extract of *Tapinanthus globiferus;* as such their lives were extended up to the 16th day post infection. Also those that were given extract of *Gongrenema latifolium* simultaneously with parasite, developed parasiteamia by the 5th day and died between days 8-9th Post infection. Similarly a progressive increase in parasiteamia was observed from day three in the other groups treated with extract of both plant at 100-200 mg/kg and the infected untreated groups (control).

DISCUSSION

The search for an active Trypanocides from original plants is a concern for many researchers. Several researchers have reported investigations carried out on Plants of various species to have promising trypanocidal activity (Freiburghaus *et al*., 1996, 1998; Nok *et al*., 1993). This study gave indications of two plants with variations *in vitro* and *in vivo* trypanosomal activity. The methanolic Leaf extract of *Gongronema latifolium*, showed significant activities *in vitro* compared to other extracts obtained from the same plant. While the methanolic extract of *Tapinanthus globeferus*, showed highest activity in the entire *in vitro* test carried out on both plants. As such it was tested for *in vivo* activity. Parasite motility constitutes a relatively reliable indicator of viability of most zooflagelates parasites (Kaminsky *et al*., 1996). Cessation or drop in motility of trypanosomes, may therefore serve as a measure of anti–trypanosomal potential of the crude extract when compared to the control. The quantitative difference in *in vitro* antitrypanosomal activities among the plant parts could be attributed to the variation (s) in concentration and composition of Phytochemical in the different parts. Since distinct function (s) is performed by all the parts and hence tend to produce slightly different chemical constituents. The results obtained in the *in vivo* experiment was very interesting as the groups that were infected simultaneously with the methanolic extract of *Tapinanthus globiferus and Gongorema latifolium* delayed development of parasites in the blood between days 9th and 5th days respectively this implies that the former gave a better

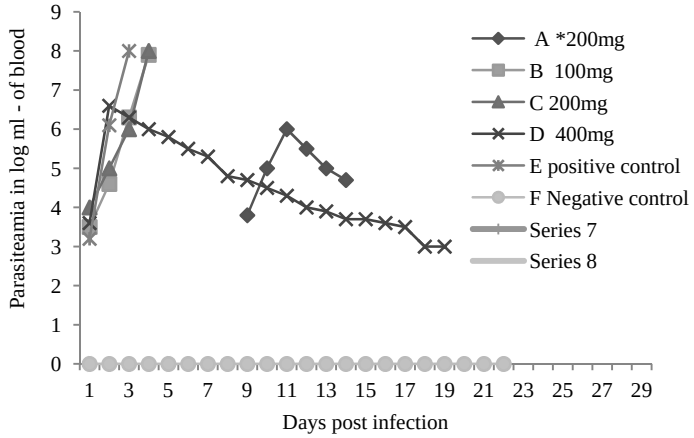

Fig. 1: Trypanocidal activity of various doses of methanolic leaf extract of *Tapinanthus globiferus* on infected mice *200 mg /kg of extract was given to mice simultaneously with 10^4 trypanosomes

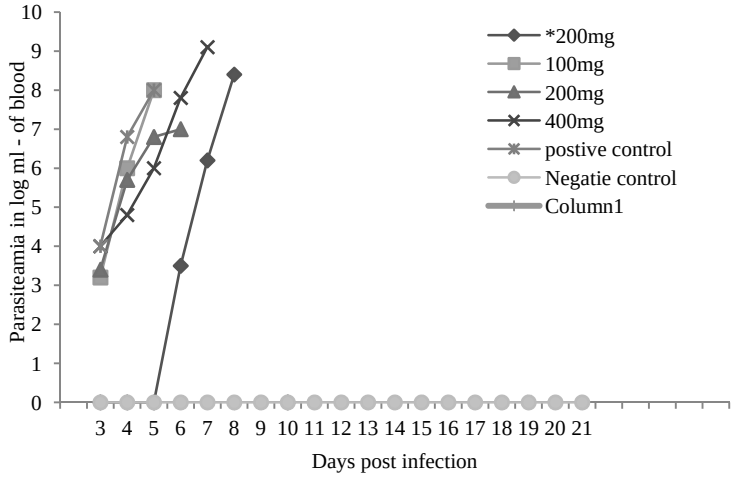

Fig. 2: Trypanocidal activity of various doses of methanolic stem bark extract of *Gongrenema latifolium* on infected mice *200 mg /kg of extract was given to mice simultaneously with 10^4 trypanosomes

result and perhaps if the dose is increased it may lead to the clearance of the parasite. Furthermore the groups treated with 400mg/kg of extract of *Tapinanthus globiferus* showed a much slower increase and reduction of parasiteamia and their lives were prolonged up to the 21st days post infection. On the other hand, progressive increase in parasiteamia was observed in the group treated with the same dose but with *Gongorenema latifolium*, causing the death of the animals between the 7th and the 8th days post infection. However, this is not surprising since a plant with high *in vitro* anti trypanosomal activity may have no *in vivo* activity and vice versa, due to peculiarities in the metabolic disposition of the plant chemical constituent. This findings may also agree with the finding of Atawodi (2005), which states that some extract belong to groups that act by static action, affecting growth and multiplication rather than eliminating them. The Phytochemical screening (Table 1) showed that the extract of *Tapinanthus globiferus* contains an appreciable amount of flavonoids, alkaloids and tannins amongst others, may suggest that these group of bioactive compounds may play a role in antitrypanosomal action. This study has shown that the methanolic Leaf extract of *Tapinanthus globiferus* had anti-trypanosomal activity by suppressing the establishment of parasiteamia. Thus the study supports the traditional usage of this plant in the management of several diseases.

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