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Evaluation of trypanosomal activity of *Tapinanthus globiferus* and *Gongronema latifolium* on *Trypanosoma congolense*

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This investigation compared the various extracts obtained from different parts of *Tapinanthus globiferus* and *Gongrenema latifolium* for trypanocidal activity, *in vitro* and *in vivo* at different concentration on mice infected with *Trypanosoma congolense*. The methanolic extracts of stem bark of *Gongrenema latifolium* and *Tapinanthus globiferus* leaves had highest activity *in vitro* thus they were evaluated for *in vivo* activity. The albino mice were treated with extracts ranging from 100-400mg/kg body weight intraperitoneally for 7 consecutive days. The group treated with 400mg/kg of *Tapinanthus globiferus* had significant reduction In parasiteamia and their life's were prolong up to the 24th day before they culminated in death compared with those treated with the same dose of *Gongrenema latifolium* but all died by day 9th post treatment. The result also showed that, the group inoculated with parasite and extract of *Tapinanthus globiferus* simultaneously, did not develop parasiteamia while the experiment lasted. Groups treated with 100-200mg/kg and the control had increased in parasiteamia and they all died by day 9th post treatment. However the groups treated with 200mg/kg of *Tapinanthus globiferus,* had reduced parasiteamia by day 4 but was followed by a relapse causing the death of animals by day 10th. Phytochemical screening showed appreciable amount of alkaloids and flavonoids in the extract of Tapinanthus globiferus compared to that found in Gongrenema *latifolium* which are only in traces. Further fractionation of this extracts will be required in order to isolate the active component.

Key words: *in vitro, in vivo,* trypanosomal activity, *Trypanosoma brucei brucei,* phytochemical screening.

African Trypanosomiasis, also known as African sleeping sickness is a disease syndrome that affects man and domestic animals. It is a parasitic disease caused by different species of protozoan blood parasite (Genus Trypanosoma) one of the major obstacles to livestock production in Africa (Antia *etal*., 2009; Steverding and Tyler 2005). It is estimated that over 60 million people are at risk of the disease in thirty-six countries in sub-Saharan Africa 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 and chemoterapy, however the seasonality of their abundance and patchy distribution of the flies makes it difficult to adapt to efficient programme. Another method is the use of trypanotolerant cattle.The current chemotherapy relies on four main drugs suramin, pentamidine, melarsoprol and eflornithine) are currently in use for the treatment of Human African Trypanosomiasis (Kuzoe,1993). However these drugs display undesirable toxic side effect, limited and expensive nature of the drugs (Onyeyili and Egwu, 1995; Osma *et al*., 1992). The search for vaccination against African

Trypanosomiasis remains elusive due to the phenomenon of antigenic variation exhibited by the parasite (Donald, 1994; Anene, *et.al*., 2001)These problems of current treatment methods and other factors increase the need for urgent search of more effective and less toxic chemotherapeutic agents from natural origin. Herbal plants are considered to be less toxic with little or no side effect and therefore potential source of development of alternative therepeutics. O'Neil and Lewis (1992) stated that close to half selling pharmaceuticals were either natural products or their derivatives therefore, investigation of natural remedies as a source of new drugs gain great interest in recent years. However, a number of African medicinal plants were evaluated for their *in vitro* trypanocidal activity (Abegaz *et al*., 2002; Asres etal., 2001; Atawodi *et al*., 2003; Freiburghaus *et al*., 1997; Hoet et al., 2007; Igweh and Onabanjo, 1989; Kamanzi *et al*., 2004; Ogbunugafor *et al*., 2007; Owolabi *et al*., 1990; Nok *et al*., 1993; Wosu and Ibe, 1989; Wurochekke and Nok, 2004). Furthermore, several plant extracts or plant derivatives were also investigated *invivo* for there antitrypanosomal efficacies (Asuzu and Chineme,1990; Asuzu and Ugwuja, 1989; Youan et al., 1997).

Here we present comparative studies of *in vitro* and *in vivo* antitrypanosomal activity of the extracts of *Gongronema Latifolium* and *Tamianthus globeferus* on mice infected with *Trypanosoma congolense. Gongronema Latifolium* belongs to the family of Asclepiadaceae. The plant is commonly known in English as amaranth globe. Parts commonly use 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. while *Tamianthus globeferus* (Family- Loranthaceae) known by other names as mistletoe (English), Afomo onisana (Yoruba), Kauci 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 the Tropical 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

Collection of plant material

The plant materials of *Gongronema latifolium* was collected from a farm land in Akwa local government area of Anambra state, while *Taminathus globeferus* was collected in Zaria metropolis and were both identified at the herbarium unit of Biological Sciences Department, Ahmadu Bello University Zaria.The plant materials were air dried after which 200g were powdered and sieved (British Pharmacopoiea grade sieve No 44) to remove coarse material. The sieved material was kept in an enclosed container in a cool dried place.

Extraction of plant materials.

Two hundred grams of each powdered material was defatted in 500 ml of Petroleum ether for 24 hours. The recovered extract from the filtrate, was concentrated under Rotary evaporator. The air dried residue was macerated with 500ml of methanol for 24hours, it was filtered and also concentrated under a rotary evaporator. The aqueous extract was obtained by maceration of the dried residue obtained from methanolic extract, in 500ml of distilled water for 24hrs. It was then filtered and the filtrate concentrated on water bath at 80°C for 10hours.

Test organism and parasite count

Trypanosoma congolense was obtained from stabilates maintained at the Nigerian Institute for Trypanosomiasis Research, (NITR) Kaduna and was subsequently maintained through serial passage in laboratory mice.

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 Hurberts and Lumsden (1976) was converted to antilog to provide absolute number of trypanosomes per ml of blood. When the parasite count is such as 35-40 per field, the animal was sacrificed and

blood recovered by cardiac puncture was collected in heparinised tubes to be used for *in vitro* studies or for inoculation of animals for *in vivo* studies

***In vitro* Trypanocidal Activity**

Determination of the Trypanosomal activity was performed in duplicate in a round bottom 96 well micro titre plates (Flow laboratories Inc., McLean, Virginia 22101, USA) as described by Atawodi *etal*.,(2002) as follows: 10mg of each hydrophilic extract were weighed and dissolved in 1ml of phosphate buffer saline while the all lipohilic extracts were dissolve in 5% DMSO. Serial dilution of stock extract solution with concentration ranging from 2.5mg, 4mg and 10mg/ml were constituted. Control wells were also included containing parasite suspension in 5% DMSO only, without extract while the other contain *diminal*^{R} a trypanocidal drug (*445mg diminazene diaceurate*+555mg phenazone/g, Eagle Chemical Company LTD, Ikeja, Nigeria) with the same concentrations. Blood containing parasite collected in heparinised tubes 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 20µl of the constituted extract to give a final volume of 70µl. After 5min. incubation in covered micro titre plate maintained at 37^{o}C, a drop of the test mixtures were placed on separate slides and Parasites motility were observed every 5min. 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 of trypanocidal activity.

Phytochemical screening

Chemical tests were carried out on the powdered specimen of methanolic stem bark extract of *Gongrenema latifolium* and methanolic leaf extract of *Tamianthus 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.

***In vivo* experiment**

Thirty albino mice weighing between 20-25kg of both sexes were used for the *in vivo* experiment for each plant extract. The animals were obtained from the animal colonies of Nigerian Institute for Trypanosomiasis Research Kaduna They were kept in well ventilated clean cages, fed with growers mash and water *ad libitum.* The care and handling of the animals were in accordance with the national regulations for Animal Research and the International Animal Welfare Guidelines.

***In vivo* experiment**

Twenty five mice were infected with 10^{4} as described earlier. At the height of parasiteamia such as 10^{7}, the animals were divided into five groups of five mice each (ABCD and E) they were treated with methanolic extract of both plants as follows:

Group A infected and treatment simultaneously with 200mg/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
Group E infected control (negative control)
Group F not infected control(positive control)

Crude extract were constituted in normal saline administered via intraperitoneally route at 0.2ml.

RESULTS

Table 1 shows the activity of nine extracts recovered from plant materials of *Tapinanthus globiferus* and *Gongrenema latifolium* on *Trypanosoma congolense in vitro.* At various concentration of : 2.5, 5 and 10mg/ml. The methanolic leaf extract of *Tapinanthus globiferus* showed highest activity by ceasing the motility of the parasite within 5 minutes, followed by methanolic stem bark extract of *Gongrenema latifolium*, which ceased motility at 10 minutes. Petroleum ether extract of both plants did not show any *in vitro* activity. Diminal^{R} ceased trypanosomal motility within 30min. of incubation.

Phytochemical screening of methanolic extract of stem bark *Gongrenema latifolium* and leaf extract of *Tapinanthus globiferus* is presented in table 2. Treatment commenced on the 7th day post infection. The result presented in table 3 and 4 showed that untreated groups (control), had progressive increase in parasiteamia from day 7th post infection up to day 12th when they all died. Also, groups treated with extract of both plants at 100mg/kg body weight develop parasiteamia before they all died by day 7th

Table 1. Trypanocidal activity of extracts of *Tapinanthus globiferus* and *Gongrenema latifolium* on *Trypanosoma congolense*

Plant	Plant part	Time in (min.) in which trypanosome motility was observed in suspension with different effective concentrations of extracts (mg/ml).								
		Petroleum ether			Methanol			Aqueous		
		10	5	2.5	10	5	2.5	10	5	2.5
Gongrenema Latifolium	leaves	NA	NA	NA	30min***	NA	NA	NA	NA	NA
	Stem bark	NA	NA	NA	10min*	20min*	55min***	NA	NA	NA
Tapinanthus globiferus	leaves	NA	NA	NA	5min*	10min*	20min**	25min*	30min*	70min***
	Stem bark	NA	NA	NA	15min**	20min*	45min**	40min***	NA	NA

NA= parasite highly motile after 120min; *= 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	-	-

post treatment. While those that were treated with 200mg of *Tapinanthus globiferus,* had reduced parasiteamia from day 4 post treatment. But was followed by relapse leading to increase in parasiteamia and subsequent death of the animals by day 10th post treatment. Similarly the group treated with the same dose but with *Gongrenema latifolium* had progressive increase in parasite, which lead to the death of the animals by the 6th day post treatment. However the group treated with methanolic extract of *Tapinanthus globiferus* at 400mg/kg, showed significant reduction in parasiteamia and their lives were prolong up to the 24th day post treatment. Compared to the group treated with *Gongrenema latifolium* with the same dose (400mg/kg) but died by the 9th day post treatment. Interestingly, the group that were inoculated and treated simultaneously with 200mg /kg body weight of methanolic extract of *Tapinanthus globiferus* did not develop parasiteamia up to the time the experiment was terminated. On the other hand, the group that was inoculated with extract of *Gongrenema latifolium* simultaneously with parasite, developed parasiteamia by the 10th day post inoculation and died by days7 th post treatment.

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, 1997; Nok *etal.*, 1993). In our present study we evaluated two plants for Trypanosomal activity *in vitro* and *in vivo*, against *Trypanosoma congolense*. All the extract obtained from both plants were trypanocidal except the aqueous extracts of Leaf, stem bark of *Gongronema latifolium* and stem bark *Tapinanthus globeferus* . While the lowest concentration of extract which resulted in complete elimination of motility (Minimum Lethal Concentration, M I C) was methanolic leaf extract of *Tapinanthus globiferus*. Our results agree with previous studies

Table 3: Trypanocidal activities of methanolic leaf extract of *Tapinanthus globiferus* on *Trypanosoma congolense*

Days post Infection	A 200mg	B 100mg	C 200mg	D 400mg/kg	E positive control	F Negative control
7	NIL	3.50 ± 0.2	4.00 ± 0.2	3.60 ±03	3.20± 0.0	NIL
8	NIL	4.60 ± 0.8	5.0.0 ± 0.6	6.60 ± 0.7	6.10 ± 0.0	NIL
9	NIL	6.30 ± 0.0	6.70 ± 0.0	6.30± 0.0	7.20 ± 2.0	NIL
10	NIL	6.90 ± 0.8	5.50 ± 0.2	6.0 0± 0.0	8.60 ± 00	NIL
11	NIL	7.20 ± 0.2	3.20 ± 2.0	5.80 ± 0.8	9.00 ±20	
12	NIL	8.60 ± 0.0	5.0 ± 2.0	5. 50 ±0.8	DEAD	
13		DEAD	6.70 ± 0.3	5.30 ±0.2		
14			8-70 ± 0.4	4.80 ±0.0		
15			8.90 ± 07	4.70 ±2.0		
16			DEAD	4.50 ±0.3		
17				4.30 ±0.2		
18				4.00 ±0.4		
19				3.90 ±0.2		
20 -21				3.70 ±0.0		
22 -23				3.50 ±0.0		
24- 25				3.50 ±0.2		
23 -27				3.00 ±0.3		
24 - 29				DEAD		
30						

Group A infected and given 200mg extract simultaneously , group B infected treated with 100mg, group C, treated with 200mg, group D infected treated with 400mg, group E infected but not treated and group F not infected not treated given normal saline.

Table 4: Trypanocidal activities of methanolic stem bark extract of *Gongrenema latifolium Trypanosoma congolense*

Days post Infection -	A 200mg	B 100mg	C 200mg/kg	D 400mg	E positive control	F Negative control
7	NIL	3.20 ±1.3	3.40 ± 0.0	3.60 ± 1.0	4.00 ± 1.0	NIL
8	NIL	5.00 ± 0.0	4.70 ± 1.0	4.80 ± 0.0	6.80 ± 0.0	NIL
9	NIL	6.07 ± 0.7	5.80 ± 0.3	5.20 ± 2.0	8.00 ± 0.0	NIL
10	3.50± 0.0	8.60 ± 0.3	6.50 ± 0.2	6.80 ± 0.3	8.40 ± 0.2	NIL
11	6.2 ± 0.8	8.00 ± 0.0	8.70± 0.4	7.1 0± 0.0	8.90 ± 0.2	NIL
12	8.40 ±.20	8.80 ± 0.2	DEAD	7.60 ± 0.0	DEAD	NIL
13	DEAD	DEAD		8.00 ± 0.2		NIL
14				8.50 ± 0.0		NIL
15				DEAD		NIL
16						NIL
17						NIL
18						NIL
19						NIL
20-21						NIL
22-23						
24-25						
26-27						
28-29						
30						NIL

Group A infected and given 200mg extract simultaneously with 10^3 trypanosomes, group B infected treated with 100mg, group C infected treated with 200mg, group D infected treated with 400mg, group E infected but not treated and group F was not infected but given normal saline.

(Wurochekke and Nok, 2004; Ogbadoyi *et al*., 2007; Mbaya *et al*., 2010) that reported the *in vitro* trypanocidal activity of some medicinal plants. The highest level of activity displaced by the methanolic extract suggest that, methanol could be use to extract the biological active principle(s) responsible for the Trypanosomal activity of the plant. 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* did not result in infection. 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 extended up to the 24th day post treatment. This suggests that plant extract might clear parasite from the blood if the concentration is increased or the active compound is isolated and use.

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 9th day post treatment. 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 et al*., 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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