

**Research Article**



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**Acute toxicity and *in vitro* trypanocidal activity of the methanolic leaves extract of *Lannea schimperi* (Hoschst. Ex Rich) Eng.**

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**Abstract**

There seems to be little or no literature about the toxicity of *Lannea schimperi* leaves, which make it important to investigate the toxicity of this plant leaves so as to establish its safety or otherwise to human handlers and animal consumers. Toxicological effects of its methanolic leaves extract were investigated in mice using lorke's method of 1983. This was conducted in two phases. In the initial phase, mice were divided into 3 groups of three mice each and treated at doses of 10, 100 and 1000 mg/kg, respectively with methanolic leaves extract of *Lannea schimperi*, intraperitoneally (i.p.). These were then observed for 24 h for signs of toxicity, including death. In the final phase, mice were divided into 4 groups of one mouse each and treated with the extract at doses of 140, 225, 370 and 600 mg/kg, respectively. The acute toxicity (LD<sub>50</sub>) of the extract was determined to be 288.53 mg/kg. Also the anti-trypanosomal activity of the extract was evaluated against *Trypanosoma brucei brucei in vitro* at concentrations of 3, 6, 12 and 24 mg/ml. Susceptibility of the organism was determined in culture medium containing 5% dextrose and 0.9% saline solution alone as control and 3, 6, 12 and 24 mg/ml of these plants extracts in the same solution. Complete mortality of the organism was observed at the concentrations of 24, 12, 6 and 3 mg/kg within 30, 60, 180, and 330 min respectively, in a dose dependent manner; the organism however survived for 6 h in the control test tube. All the tested incubated samples were assayed for infectivity in mice. The infectivity to mice of all trypanosomes incubated at various extract concentrations (24, 12, 6 and 3 mg/ml) were negative four days post inoculation, but the infectivity of the control incubated for 360 min was positive with parasitic load of  $2.5 \times 10^6$  four days post inoculation. The result suggests that methanolic leaves extract of *Lannea schimperi* possess some trypanocidal principles which may require further scientific elucidations.

**Keywords:** Acute toxicity, invitro-trypanocidal activity, incubation infectivity test, *Lannea schimperi*.

**Introduction**

*Lannea schimperi* is a small tree up to 10-15m tall; bole short, sometimes stunted and low-branching. It is distributed from Togo, northern Nigeria and Cameroon eastward to Ethiopia, southward through Uganda, Kenya, eastern Congo, Rwanda, Burundi, Tanzania and Malawi to Zimbabwe and Mozambique (Oyen, 2010).

It is a deciduous shrub or tree with a spreading crown. It can grow from 2-15m tall. The tree is valued locally for its edible fruit which is commonly harvested from the wild. It also has local medicinal uses and is a source of wood and fibre (Oyen, 2010).

*Lannea schimperi* is used in folk medicine to treat a number of illnesses. The potential pharmacologic activities of the extract from the fruit, leaf, bark and roots includes antimicrobial, anti-cough, anti-diabetic, anti-diarrhoeic, anti-vomiting and anti-fungal activities in vivo and/or in animal model (Neuwinger, 2000).

*Lannea schimperi* has been used in various areas. The bark is used to make string and rope. The wood is occasionally used in carpentry, as fuel and is valued for charcoal production. The fruit is eaten fresh by children in Nigeria during rainy season and throughout

East Africa. The seed is also eaten. The flowers are probably a source of nectar for honey bees. Branchlets and leaves are eaten by domestic animals (Oyen, 2010).

African trypanosomosis is a wasting disease of animals and man. It is caused by haemoprotozoan *Trypanosoma* species. In Africa, the most important *Trypanosoma* species are transmitted by the tsetse fly of the genus *Glossina* (Ooijen, 1993). It occurs across more than a third of Africa, and almost all animal species, except poultry, are affected. Approximately 20% of Africa's 173 million cattle are at risk of infection (Adeniji, 1993). In addition, 36 out of 52 African countries are endemic for sleeping sickness, with 55 million people at risk of contracting the infection (Cattand, 1995). The search for vaccination against African trypanosomosis remains elusive and effective treatment is beset with problems of drug resistance and toxicity (Onyeyili and Egwu, 1995; Gutteridge, 1985; Aldhous, 1994).

African trypanosomosis is one of the tropical diseases in which new and better drugs are needed. The current methods of controlling the disease include the use of trypanotolerant cattle, vector control and drug therapy. Four drugs (suramin, pentamidine, melarsoprol and eflornithine) are currently available to treat trypanosomosis (Kuzoe, 1993), with only melarsoprol and eflornithine being effective against the meningoencephalitis that develops in the late stages of the disease. In addition to emergency cases of drug resistance, all four drugs require lengthy, paraneural administration and all but eflornithine have severe toxic side effects (Onyeyili and Egwu, 1995; Gutteridge, 1985) thus, making the search for the development of more effective and safer trypanocidal agents a necessity.

Plants have always been among the common sources of medicaments. In Africa, traditional medicine in the form of herbal treatment has a long tradition and still holds a strong position in medical and veterinary care (Felerman, 1981). Several reports on the evaluation of different chemicals/drugs for trypanocidal activity have appeared (Bodley *et al.*, 1995) just as interesting reports on the antitrypanosomal effects of plant extracts and plant derivatives (Freiburghaus *et al.*, 1996, 1997, 1998; Sepulveda-Boza *et al.*, 1995; Nok *et al.*, 1993; Asuzu and Chinerne, 1990; Atawodi *et al.*, 2003; Mikail and Ajagbonna, 2007). This publication, present report on systematic *in vitro* assessment of methanolic leaves extract of *Lannea*

*schimperi* for its trypanocidal activity using *Trypanosoma brucei brucei* as test organism.

## Methodology

### Plant material

The fresh plant was collected from Gurfata, Ibwa ward of Gwagwalada area council, Federal Capital Territory (F.C.T), Abuja, Nigeria in the month of February, 2015. Botanical identification and authentication was done by U.S Gallah of National Research Institute of Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria and a voucher specimen numbered 0512 was deposited at the departmental herbarium.

### Processing and Extraction

The fresh leaves of *Lannea schimperi* were carefully separated from the other morphological parts of the plant and washed clean with water, air dried under shade for two weeks, pounded with pestle and mortar mechanically into fine particles. Fifty grams (500 g) of the pounded dried plants materials were weighed and extracted by maceration for 72 h in 100% methanol. The methanolic extracts were filtered and evaporated to dryness *in vacuo* and stored in capped bottles inside the refrigerator at 4°C until required.

### Acute toxicity study (determination of LD<sub>50</sub>)

This was conducted in two phases by using the method of Lorke (1983). In the initial phase, mice were divided into 3 groups of three mice each and treated at doses of 10, 100 and 1000 mg/kg, respectively of methanolic leaf extract of *Lannea schimperi*, intraperitoneally (*i.p.*). These were then observed for 24 h for signs of toxicity, including death. In the final phase, mice were divided into 4 groups of one mouse each and treated with the extract at doses of 140, 225, 370 and 600 mg/kg, respectively. The LD<sub>50</sub> was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e., the geometric mean of the consecutive doses with 0 and 100% survival, respectively.

### Trypanosome stock

*Trypanosoma brucei brucei* obtained from protozoology laboratory of Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria was used for this study. The organisms were maintained by serial passages in rats.

### **In vitro anti trypanosomal activity**

Solutions of 3, 6, 12 and 24 mg/ml were prepared in 5% dextrose and 0.9% saline solution from the plant extract into different glass test tubes. All the crude extracts were freshly prepared, control glass test tube containing 5% dextrose and 0.9% saline solution, without the plant extract was included. Anti-coagulated blood was collected from the infected rats. Serial dilutions of infected rat blood were made using phosphate glucose buffered saline solution. 0.4 ml of the blood was added to each of the glass test tubes. The parasitic load of the diluted blood was estimated to be  $6.3 \times 10^6$  parasites/ml (Herbert and Lumsden, 1976). The glass test tubes were closed with the aid of rubber stoppers. The solution was allowed to stand at room temperature (27°C) for 6 h. During this period, the motility or lack of motility of the parasites in the solution was checked at 30 min intervals using light microscopy (x 40 objective lens), about 2 µl of test mixtures were placed on separate microscope slides and covered with cover slips and the parasites observed.

### **Incubation infectivity test**

*Trypanosoma brucei brucei* were incubated in a liquid medium of 5% dextrose and 0.9% saline for 90, 120, 240, 360 and 360 min at 27°C in the presence of various concentrations of 24, 12, 6, 3 mg/ ml of the

extract and control respectively. The incubated samples were assayed for infectivity in mice by passing 0.3 ml of the tested samples into a mouse at 90, 120, 240, 360 and 360 minutes for test tubes containing 24, 12, 6, 3 mg/ml and control respectively. The mice were tested for presence of *T. brucei brucei* parasite using light microscopy (x 40 objective lens) on daily basis for four days.

### **Results**

Acute toxicity test indicated that the methanolic leaf extract of *Lannea schimperi* produces 100% mortality at doses of 370, 600 and 1000 mg/kg, respectively. At these doses, the animals show signs of toxicity including inactiveness, rough hair coat, dullness, depression and death. The minimum lethal dose was 370 mg/kg and the maximum non-lethal dose was found to be 225 mg/kg (Table 1 and 2). The LD<sub>50</sub> was calculated to be 288.53 mg/kg.

The extract inhibited the motility of the trypanosome in a dose dependent manner, with the highest concentration (24 mg/kg) inhibited within 30 minutes and the lowest concentration (3 mg/kg) at 330 minutes (Table 3). However, following the inoculation of mice with the tested incubated samples of *T. brucei brucei*, only the control became infected with parasitic load of  $2.5 \times 10^6$  at the fourth day post inoculation (Table 4).

**Table 1: Result of initial investigation (Phase 1 LD<sub>50</sub> determination)**

Dose (mg/kg)	Group
10	0/3
100	0/3
1000	*3/3

\*Number of animals which died/number of animals used

**Table 2: Result of final investigation (Phase 2 LD<sub>50</sub> determination)**

Dose chosen for the second test (mg/kg)	Group
140	0/1
225	0/1
370	*1/1
600	*1/1

\*Number of animals which died/number of animals used

Therefore the LD<sub>50</sub> was calculated to be the geometric mean of 225 and 370 using this formula

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where LD<sub>50</sub> = Median lethal dose

D<sub>0</sub> = Highest dose that gave no mortality (225 mg/kg body weight 0/1)

D<sub>100</sub> = Lowest dose that produced mortality (370 mg/kg body weight 1/1)

$$= \sqrt{225 \times 370}$$

$$= \sqrt{83250}$$

LD<sub>50</sub> = 288.53 mg/kg body weight

**Table 3: *In vitro* trypanocidal efficacy of different concentrations of methanolic leaves extract of *Lansea schimperi* against *T. brucei brucei*.**

Different concentrations of plant extract	Survival of trypanosomes in minutes												
	0	30	60	90	120	150	180	210	240	270	300	330	360
<i>L. schimperi</i> (leaves) 3 mg/ml	++++	+++	+++	++	+	+	+	+	+	+	+	-ve	-ve*
<i>L. schimperi</i> (leaves) 6 mg/ml	++++	++	++	+	+	+	-ve	-ve	-ve*	-ve	-ve	-ve	-ve
<i>L. schimperi</i> (leaves) 12 mg/ml	++++	+	-ve	-ve	-ve*	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>L. schimperi</i> (leaves) 24 mg/ml	++++	-ve	-ve	-ve*	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Control	++++	++++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+

- ++++ Very active and very strong parasite presence,
- +++ Active and strong parasite presence,
- ++ Sluggish and moderate parasite presence,
- + Very sluggish and mild parasite presence,
- ve No activity/ no parasite presence,
- \* Time of mice inoculation (incubation infectivity test).

**Table 4: Incubation infectivity test of tested *Trypanosoma brucei brucei* samples against different concentrations of methanolic leaves extract of *Lansea schimperi***

Different concentrations of tested solutions/samples	Days post mice inoculation/infectivity				
	1	2	3	4	5
<i>L. schimperi</i> (leaves) 24 mg/ml, 90 min post incubation	-ve	-ve	-ve	-ve	-ve
<i>L. schimperi</i> (leaves) 12 mg/ml, 120 min post incubation	-ve	-ve	-ve	-ve	-ve
<i>L. schimperi</i> (leaves) 6 mg/ml, 240 min post incubation	-ve	-ve	-ve	-ve	-ve
<i>L. schimperi</i> (leaves) 3 mg/ml, 360 min post incubation	-ve	-ve	-ve	-ve	-ve
control, 360 min post incubation	-ve	-ve	+	++	+++

+++ Strong parasite presence, ++ moderate parasite presence, + mild parasite presence, -ve no parasite presence

## Discussion

The extract induced behavioural signs like inactiveness, rough hair coat, dullness, depression and death at the doses of 370, 600 and 1000 mg/kg. In contrast, there was no death recorded in animals given the doses of 10, 100, 140 and 225 mg/kg of the extract. According to this study the minimum lethal dose of the extract was found to be 370 mg/kg and the maximum non-lethal dose of the extract was found to be 225 mg/kg. According to Buck *et al.*, (1976) a substance with toxicity range of 50-500 mg/kg is considered to be moderately toxic. Thus in this study the methanolic leaves extract of *Lannea schimperi* is considered to be moderately toxic.

The plants, at different concentrations used in this study showed considerable trypanocidal activity. This finding is in line with earlier reports (Freiburghaus *et al.*, 1996, 1997, 1998; Nok *et al.*, 1993; Asuzu and Chineme, 1990; Atawodi *et al.*, 2003; Mikail and Ajagbonna, 2007, Mikail, 2009) that clearly indicated that plants of different families could possess potent trypanocidal activity. In fact, natural products with trypanocidal activity and belonging to a variety of phytochemical classes have been identified (Hopp *et al.*, 1976; Oliver–Bever, 1986; Sepulveda-Boza and Cassels, 1996). This investigation did not involve structure elucidation. It may be difficult to extrapolate this *in vitro* result to mean efficacy *in vivo* because discrepancies between *in vitro* and *in vivo* correlations due to metabolic processes which occur in multicellular organisms are well known (Fans worth and Moris, 1976). Nevertheless, and for practical purposes bioactive screening *in vitro* remains a useful method for preselection of plant for anti-trypanosomal activity (Freiburghaus *et al.*, 1996). Therefore, plants found to be active in this report must be tasted *in vivo* before a definite statement can be made on their trypanocidal potentials.

Also previous workers (Freiburghaus *et al.*, 1997) have shown that the mean MIC value of common trypanocidal drugs is 10.7 mg/ml and that agent with MIC value between 5 – 20 mg/ml could be regarded as very active. In this study, methanolic leaves extract of *Lannea schimperi* was found to be active at 3, 6, 12 and 24 mg/ml. The extract inhibited the motility of the trypanosomes in a dose dependent manner, with the highest concentration (24 mg/kg) inhibited within 30 minutes and the lowest concentration (3 mg/kg) at 330 minutes.

So also, the infectivity to mice of all trypanosomes incubated at various extract concentrations were negative four days post inoculation, but the infectivity of the control incubated for 360 min was positive with parasitic load of  $2.5 \times 10^6$  four days post inoculation, this further revealed the trypanocidal potential of the plant extract. This result is in line with earlier findings by Kaminsky *et al.*, (1990) which demonstrated that Berenil- or Samorin-resistant trypanosomes can be distinguished from drug-sensitive parasites by the drug incubation infectivity test.

It is difficult to speculate the mechanism by which these extracts exhibit their trypanocidal action. However, accumulated evidence (Supulveda–Boza and Cassels, 1996) suggest that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite. The trypanocidal principles of the plants tested in this study is unknown, until further studies are carried out

## Conclusion

The findings of this research revealed that the methanolic leaves extract of *Lannea schimperi* is moderately toxic and posses trypanocidal principles that require further scientific elucidations.

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