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Anti-trypanosoma activities of stem-back methanolic extract of *ficus sycomorous* against *trypanosoma congolense*: A preclinical study for aquatic *trypanosoma* infections

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Abstract
This study is aimed at investigating the *Trypanosoma* potential of stem-back methanolic extract of *Ficus sycomorous* against *Trypanosoma congolense*. This investigation is aimed at establishing a study base for subsequent screening of the plant against aquatic *trypanosomes*. Firstly, the fresh plant of *Ficus sycomorous* was obtained from Sokoto central market, which was properly identified at Department of Biological sciences, Botany unit, processes and extracted with methanol at Biochemistry department, Usmanu Danfodiyo University Sokoto, Nigeria, the experimental parasite was obtained from department of biochemistry Ahmadu Bellow University Zaria, Nigeria, and the study was conducted at the department laboratory of veterinary helminthology, faculty of veterinary medicine, Ahmadu Bellow University Zaria, Nigeria. Assessment of invitro Trypanosoma activity was performed in triplicate in 96 well microtiter plates (Flow laboratory inc., Mclean Virginia 22101, USA). The concentration of the extracts used are 1mg/ml, 2mg/ml, 4mg/ml, 8mg/ml and 16mg/ml against 20µl of blood containing about 20-23 parasites per field, each concentration of the extract was incubated for 60 minute and the mobile parasite after every 10 minutes of incubation was recorded in mean±SD. To ensure that the effects monitored was that of the extract alone, a set of control was included which contained the parasite suspended in 10% DMSO only as negative control and diminazen aceturate as the positive control. The result shows that the stem-back methanolic extract of *Ficus sycomorus* dramatically inhibit the parasite growth at all concentration, the number of mobile parasites decreases as the concentration of the extract increases with respect to time. It was found out that, the extract at these concentrations shows No significant potentiality when compare with the positive control (Diminazen aceturate). Furthermore, the lowest concentration that can kill half of the parasite was further extrapolated graphically as IC_{50} = 561.3 µl/ml. The research therefore suggests that, this plant could be a promising anti-trypanosome which demand for further trials like in-vivo evaluations, molecular investigations, toxicology study, isolation and characterization of the active components.

Keywords: Trypanosoma, Infection, Parasite, Inhibition, Aquatic, diseases

1. Introduction
Both human and animal *trypanosomiasis* are acknowledged as one of the major health challenges of human and animal public health restrictions worldwide (Venturelli *et al*., 2022) [33]. The number of wildlife-livestock-human diseases is rising due to increased human activity and the strain on land resources (Ranjan *et al*., 2025) [31], the closer human settlements and grazing areas are to game parks and wildlife reserves, the greater the zoonotic risk (Jingyi *et al*., 2019) [15]. For instance, *trypanosomiasis* is a zoonotic disease that affect both animals and humans, it can be transmitted from animals to human and human to animals (Ohaeri and Eluwa, 2011) [29]. *Trypanosomiasis* epizootics have been documented in freshwater cultured Nile tilapia (*Oreochromis niloticus*) and cultured marine fish (*Late Calcarifer, Epipenephelus fuscoguttatus*, and *Cromileptes altivelis*) (Chen *et al*., 2022) [5]. According to multiple studies, this parasite poses a major threat to aquatic creatures' health. For example, in one study involving the evaluation of hematological parameters of infected fishes, the result showed a variety of changes, such as nuclear anomalies (micronuclei), eccentric nuclei, and shattered

nuclei. Serum total protein and globulin levels were lower in infected fish, but phagocytic activity, lysozyme activity, which is an indication of anemia, and nitric oxide production were significantly higher in infected fish than in non-infected fish (Alam *et al*., 2023) [3]. In another research, *Clarias* which is one of the most popular farm fishes in Africa were revealed to have more susceptibility to *trypanosome* infection than gift tilapia (Harutyunyan, 2023) [8]. Furthermore, according to a different study performed in Egypt, to look at *trypanosome* infections in *Clarias* and *Oreochromis niloticus*, it also showed that, irrespective of the species of fish collected, *Trypanosoma mukasai* was the most common pathogen found during the investigations. Additionally, the study found a positive correlation between *trypanosome* infection and water quality parameters. This indicates that *trypanosome* infection affects the parameters of water quality as well (Hanaa *et al*., 2024) [7]. Another study used clinical examination on evaluating histological parameters, serum biochemistry, and hemograms to assess the impact of *trypanosomes* on cultured largemouth bass (*Micropterus salmoides*). Lethargy, anorexia, and histological lesions in the liver, head, kidney, and spleen were among the tissue damage that were noticed in diseased fish, the infected fishes also had significantly greater levels of alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) and significantly lower levels of glucose, triglycerides, and low-density lipoprotein in their serum (Jiang *et al*., 2019) [14]. All these assessments have demonstrated the lower chances of survival of the fishes that are infected by *Trypanosoma* parasite, which in turn may lead to serious economic lost. However, many synthetic drugs are used for the treatment of these diseases in the past (Agrawal *et al*., 2025) [2], but the problems of synthetic drugs include environmental pollution, side effects issues, cost, lack of efficacy and health implications on consumers (Abdullahi *et al*., 2025) [1]. Therefore, due to the above issues, there is a pressing need for the development of substantial and alternative drugs other than the synthetic drugs for the treatment this life-threatening diseases. Thus, this study focusses on evaluating the antiparasitic properties of the *Ficus sycomorous* plant, particularly with regard to *trypanosomes*. The study was aimed at evaluating the anti-*Trypanocide* potential of *Ficus sycomorous* plant against *Trypanosoma Congolense*, this study is a preclinical examination on the potentiality of the plant against, subsequent studies shall look at the potential of the experimental plant on *trypanosome* isolated from fish species, below are some of the isolated *trypanosome* species isolated from freshwater and marine ecosystem.

Table 1: Some aquatic *trypanosomes* and the aquatic habitat they were identified is summarized below

S/no	*Trypanosome* species	Species of Fish	Habitat	Reference
1	*Trypanosoma carassii*	Tilapia	Freshwater	Zhang *et al*., 2023 [35]
2	*T. danilewskyi*	Tilapia	Freshwater	Zhang *et al*., 2023 [35]
3	*T. Mukasai*	Many fishes	Freshwater	Nico *et al.*, 2020 [27]
4	*T. hypostome*	Catfish	Freshwater	Lemos *et al*., 2015 [21]
5	*T. chagasi*	Catfish	Freshwater	Lemos *et al*., 2015 [21]
6	*T. lopesi*	Catfish	Saltwater	Lemos *et al*., 2015 [21]
7	*T. nudigobii*	Leeches	Marine	Hayes *et al*., 2014 [9]
8	*T. murmanensis*	Gadiform fish	Marine	Khan, 1977 [18]
9	*T. larimichthysi*	*L. crocea*	Marine	Yang *et al*., 2025 [34]
10	*T. nudigobii*	Klipfish	Marine	Le Roux *et al*., 2025 [20]

Pathogenesis and Pathophysiology of *Trypanosomes* infection in aquatic Habitat

In aquatic habitat, *trypanosome* pathogenesis involves a host-parasite-vector cycle that alter the normal healthiness of the fish, in aquatic ecosystem, leeches are the primary vector of the parasite. The parasites enter the bloodstream through the bite wound when an infected leech affixes itself to a fish host (Hayes *et al*., 2014) [9]. *Trypanosomes* grow outside the cell in the blood and tissues of the fish species, producing parasitemia and causing immunological suppression, anemia, sluggishness, and reduced oxygen transport. Particularly in stressed or farmed fish populations, severe cases can result in tissue damage and death (Magez *et al*., 2020) [22], figure below summarizes the process. When uninfected leeches consume parasitemia fish, they absorb *trypanosomes*, which grow within the leech before spreading to new victims, thus perpetuating the cycle of transmission. This cycle maintains resuscitation of *trypanosomes* infections in aquatic environments and contributes to consistent outbreaks of disease in fish habitat (Kua and Leaw, 2024) [19].

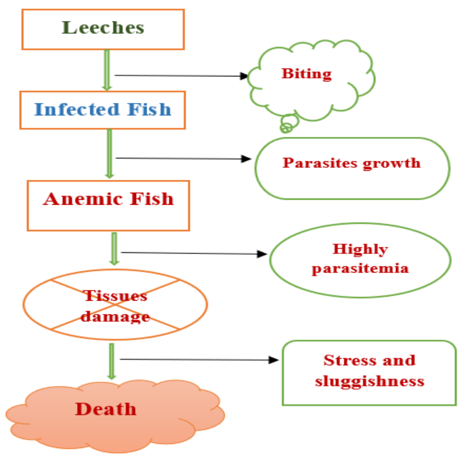


Fig 1: A simple illustration, involving the stages of *trypanosome* pathogenesis and pathophysiology in aquatic organisms

***Ficus sycomorous* plant**

Ficus sycomorus, is a huge deciduous tree that is indigenous to sections of the Middle East, the Mediterranean, and Africa's tropical and subtropical areas (Falistocco, 2024) [6]. Along with other noteworthy species like the common fig (*Ficus carica*) and the rubber plant (*Ficus elastica*), it is a member of the *Moraceae* family (Falistocco *et al*., 2024) [6]. The species has great therapeutic and practical value in addition to its enormous historical and cultural significance for the communities that use it in the history. It also plays a crucial ecological role (Charles and Patrick, 2024).

Fig 1: Picture of a fig plant

Botanical description of the experimental plant

Ficus sycomorus tree can reach a height of 20 meters and has a wide canopy that frequently reaches more than 15 meters in circumference. The tree's grayish bark, which becomes more and more broken as it matures, covers its rough, fluted trunk. Because of its extensive root system, it may thrive in a variety of soil types, including poor and desert soils. (Orwa *et al*., 2009) [30]. The tree has a dark, huge and elliptical leaves that grows up to 30 cm in length. They have a characteristic glossy upper surface with a lighter, hairy underside and are oriented alternately. The syconium, a distinctive structure with many tiny flowers, is one of the most remarkable characteristics of the sycamore fig (Keller, 2014) [17]. The fruit turns crimson or yellowish when mature, though depends on the type (Nahon, 2012) [25]. Even though other fruit of the fig plants are sweater, the fruit is eaten either dried of fresh and can also be used to make jams and jellies (Hussain *et al*., 2021) [12].

***Ficus sycomorous* medicinal insight**

Ficus sycomorus Traditional medicine has long known for their medicinal uses, various part of the plant has been used for treatment of many diseases. The fruit is highly reach in fiber, vitamins (particularly vitamin C), and minerals, which are consider eaten as a dietary supplement (Josias *et al*., 2017) [16]. The tree's latex has antibacterial potential; hence it has been used locally for the treatment of wound and infections. Respiratory and gastrointestinal issues are also being treated locally using the leaves of the plant as well as treatment of fever (Breijeh and Karaman, 2024) [4]. Thanks to its phytochemical composition, which reveals several bioactive compositions responsible for its therapeutic qualities. The plants phytochemical composition was said to serve as support for antibacterial, antidiabetic, anti-inflammatory, and antioxidant properties of the tree (Nawaz *et al*., 2020) [26].

Methodology and Materials

Phytochemical screening of Ficus sycomorous

Table 2: The table below shows the result phytochemical screening of *Ficus sycomorous* obtained from Sokoto metropolitan area

S/No	Phytochemicals	Test	Reference
1	Alkaloid	Mayer test Wagner test	Nagaraju *et al*., 2019 [24] Mohammed *et al*., 2014 [23]
2	Saponin	Frothing test	Sharma *et al*., 2023 [32]
3	Balsams	Ferric chloride	Nagaraju *et al*., 2019 [24]
4	Anthraquines	10% Ammonia Test	Mohammed *et al*., 2014 [23]
5	Tannins	Ferric chloride	Nigussie *et al*., 2021 [28]
6	Cardiac glycosides	Keller-Killiani	Nagaraju *et al*., 2019 [24]
7	Volatile oils	HCl	Mohammed *et al*., 2014 [23]
8	Flavonoids	NaOH	Hossain *et al*., 2013
9	Saponin glycoside	Fehling	Nagaraju *et al*., 2019 [24]

Parasite collection, identification and inoculation

A highly parasitized mice infected with *Trypanosoma congolense* was obtained from Department of Biochemistry, Ahmadu Bellow University Zaria, Nigeria. The parasite was maintained in Helminthology laboratory Department, Faculty of Veterinary, Ahmadu Bellow University Zaria, Nigeria, by continuous passage of infected blood from the infected mice to the uninfected mice. The passage was considered necessary when parasitemia was in the range of 16-32parasites per field (Usually 3-5 days post infection). In passaging, $1X10^3$ parasite were introduced intraperitonially and intramuscularly into mice in 0.1- 0.2 ml/Phosphate buffer saline solution. For several passages, approximately 80% blood solution (v/v) was obtained by cardiac puncture into 1ml syringe containing 0.2ml Ethylene diamine tetra acetone (EDTA 1% w/v). About 0.1- 0.2 of the blood was collected as described above was injected into other mice and was acclimatized for one week.

Determination of parasitemia

Parasitemia in infected mice was monitored from the blood obtained from the picking the tail of the infected mice, the number of parasite count was determined using microscope at X400 magnification using rapid matching method of Herbert and Lumsden, (1976). This involved microscopic counting of parasite per field in either pure blood or diluted blood with phosphate buffer (PBS, P^H 7.2).

Logarithm values of these counts obtained by matching method of Herbert and Lumsden, (1976) is converted to antilog so as to provide absolute number of trypanosomes per ml of blood.

Invitro test Determination of Trypanocidal activity

Assessment of invitro *Trypanosoma* activity was performed in triplicate in 96 well microtiter plates (Flow laboratory inc., Mclean Virginia 22101, USA). The concentration of the extracts used are 100µl, 200µl, 400µl, 800µl and 1600µl against 20µl of blood containing about 20-23 parasites per field obtained as described under "determination of parasitemia". Serial dilution was further performed containing 5µl of parasitized blood with extracts concentrations; 8mg/ml, 0.008mg/ml, 0.08mg/ml and 0.8mg/ml to determine IC_{50}. To ensure that the effects monitored was that of the extract alone, a set of control was included which contained the parasite suspended in 10% DSMO only. For reference purposes test were also performed with the same concentrations of Dimina1R (445mg diminazen aceturate+ 555mg phenazone/g Eagle chemical company LTD, Ikeja, Nigeria)- a commercial trypanocide drug.

Results and Discussion

Table 3: Phytochemical screening of *Ficus sycomorous*. The table below summarizes the phytochemical screening result of the *Ficus sycomorous* obtained from Sokoto metropolitan area

S/No	Phytochemicals	Test	Result
1	Alkaloid	Mayer test Wagner test	++ ++
2	Saponin	Frothing test	ND
3	Balsams	Ferric chloride	+++
4	Anthraquines	10% Ammonia Test	+++
5	Tannins	Ferric chloride	+++
6	Cardiac glycosides	Keller-Killiani	+++
7	Volatile oils	HCl	++
8	Flavonoids	NaOH	+
9	Saponin glycoside	Fehling	+
10	Glycoside	Fehling	+++
11	Steroids	Chloroform	+++

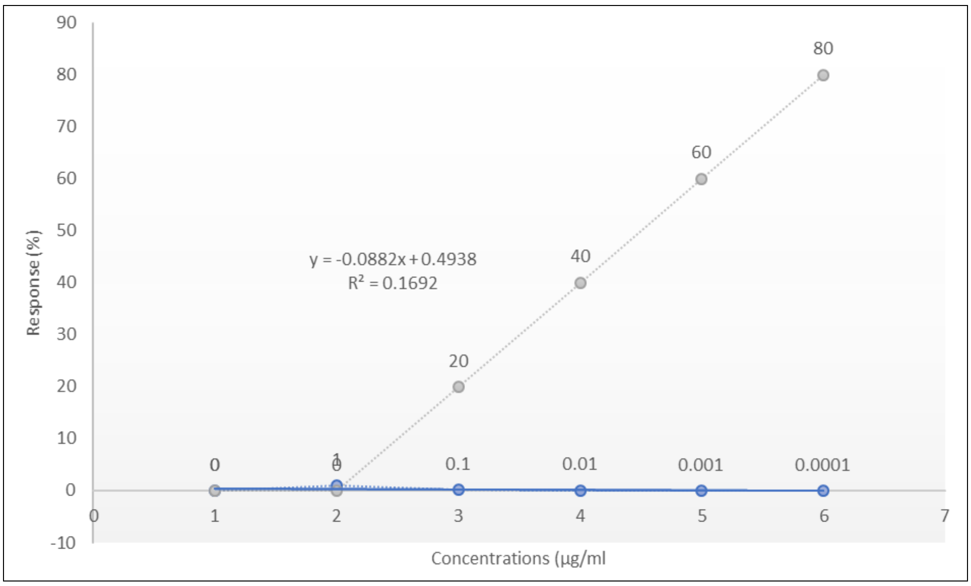
Table 4: Number of parasites counts with respects to time of incubations

Time (Min)	0	10	20	30	40	50	60
DM	0.0±00	11.3±3.36	18.7±4.32	23±4.70	23±4.70	23±4.70	23±4.70
T1mg/ml	0.0±00	0.0±00	0.0±00	0.0±00	0.0±00	0.0±00	0.0±00
T2mg/ml	0.0±00	0.0±00	1.7±1.30	2.0±1.41	2.7±1.64	3.0±173	3.7±1.92
T4mg/ml	0.0±00	0.0±00	2.3±1.15	2.7±1.64	4.3±2.07	4.7±2.17	7.3±2.70
T8mg/ml	0.0±00	0.0±00	3.7±1.92	6.7±2.59	14.7±3.83	17.0±4.12	20.0±4.47
T16mg/ml	0.0±00	0.3±0.12	6.0±2.45	11.0±3.32	16.3±4.03	20.0±4.47	22.7±4.76
DMSO	0.0±00	0.0±00	1.3±1.14	2.0±1.41	2.3±1.51	3.0±1.73	4.0±2.00

Abbreviations: DM: diminazen aceturate, T: test extract, DMSO: Dimethyl Sulphur-oxide

The table above is the parasite count from 0 minute of incubation to 60minutes of parasitic blood/extract incubations. The table shows the potential of methanolic extract of *Ficus sycomorous* against *Trypanosoma congolense*. As the concentration of the extract increases, the mobile parasite decreases, i.e., the dead parasite is proportional to extracts concentrations. For example, All the parasites were mobile and intact at T1 mg/ml from 0 minutes to 60 minutes, but as the concentration increases at T16 mg/ml at 0 minute to 60 minutes the number of parasites count decreases to mean ± SD = 22.7±4.76, that means out of 23 parasite per field that was counted, about 22.7±4.76 were killed by the extract. Nonetheless, the methanolic extracts of *Ficus sycomorous* shows no significant activities when compare to diminazen aceturate commercial medicine whose concentrations at 60minutes kill all the parasite. While, the extract at highest concentration in µl/ml kill 98.7% of the parasite and the commercial drug clear the parasite 100%. The normal trend seen in the table suggested that, the higher the concentration of the extract, the more the number of the parasite killed. Although, the impact of cellular toxicity cannot be predicted, whether the extract have additional threat on the normal functions of the cells or the organelles. Furthermore, 8mg/ml of the methanolic extract was further diluted serially i.e., and incubated for another 30 minutes the number of non-motile parasite were recorded and are plotted against concentrations as seen below;

Determination of IC_{50}



To calculate the IC_{50} (the concentration at which the response is reduced by 50%), when the response Y is 50%.

$Y = 0.0882x + 0.4938$

Set ($Y = 50$) and solve for (x):

$50 = 0.0882x + 0.4938$

$IC_{50} = 561.3$ µg/ml

Conclusion

The study involving the invitro anti-*Trypanosoma* activities of *Ficus sycomorous* was performed with the aim of establishing if the plant has anti-*Trypanosoma* potential. However, under the favorable laboratory condition, the result shows that, the plant have a promising active compound that can inhibit the growth of the parasite, the test result also shows no significant differences between the commercial drug that was used. The research further recommends the *in vivo* analysis, followed by the isolation, purification and characterization of the plant active metabolites, toxicological study and lastly the main clinical trial.

List of Abbreviations

- **DMSO:** dimethyl Sulphur-oxide
- **PBS:** Phosphor buffer saline glucose
- **AST:** Aspartate amino transferase
- **ALT:** Alanine amino transferase
- **LDH:** Lactate dehydrogenase
- **EDTA:** Ethylene diamine tetra-acetone
- **DM:** Diminazen aceturate

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