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https://dx.doi.org/10.4314/nvj.v40i1.3 **ORIGINAL ARTICLE**

# Aqueous Extract Of Fruit Pulp Of *Adansonia digitata* (Linn): Phytochemical Screening And *In Vitro* Antitrypanosomal Effect

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## SUMMARY

Chemotherapy is the most widely used means of controlling Trypanosomosis, a major health problem to man and his livestock over much of Tropical Africa. However, effectiveness of the drugs available is limited by a number of factors which include increasing parasite resistance, treatment failures and unacceptable toxicity. This study investigated the phytoconstituents of aqueous extract of fruit pulp of *Adansonia digitata* and its *in vitro* anti-trypanosomal effects on Federe strain of *Trypansoma brucei brucei*. Qualitative phytochemical analysis of the extract was carried out using standard technique. While in the *in vitro* study, about $3 \times 10^5$ *T. brucei brucei* in 0.3mls of blood suspended in 0.4mls Ringer's solution were each dispensed into tubes (A-D) containing 0.3mls of the aqueous extract at concentrations of 0.02mg/ml, 0.2mg/ml, 2mg/ml and 20mg/ml respectively. The fifth tube (E) was an untreated control (Ringer's solution and parasite). The tubes were incubated at $37^0$C and examined for the presence and motility of trypanosomes at 15 minutes intervals for 2hours. After the incubation and motility assessment, 0.2ml of the contents of each tube was inoculated intraperitoneally into group of 3 rats, 3 other rats served as uninfected controls. The inoculated animals were then examined daily for the presence of trypanosomes for a period of 60 days. The phytochemical analysis showed the presence of tannins, saponin, phenol, terpenoid, cardiac active glycoside, anthraquinone, reducing sugar, alkaloids, flavonoids and steroids. The extract demonstrated a concentration and time dependent inhibitory effect on trypanosomal motility. Highest effect was observed at concentration of 20mg/ml, with total ceassation of trypanosome motility from 75 minutes of exposure all through the 120 minutes of the incubation. Also rats inoculated with content of the tubes containing the 20mg/ml of the extract did not show parasitaemia and survived the 60 days infectivity test period. However, all rats inoculated with trypanosomes exposed to lower concentrations of the extract showed high parasitaemia with 100% mortality within 5 days post inoculation.

**Keywords:** *Adansonia digitata*, Trypanosomiasis, Phytochemistry, *Trypanosoma brucei brucei*.

## INTRODUCTION

African Animal trypanosomiasis (AAT) remains a major constraint to health and productivity of cattle and other domestic animals in tsetse infested areas of tropical Africa (Essan *et al.*, 2009). AAT is a major factor retarding the growth of the livestock industry in Africa. The disease has undergone a dramatic and devastating resurgence in recent years especially in sub-Saharan Africa where it causes serious economic losses in the livestock industry (Welburn *et al*., 2001). Trypanosomosis hinders profitable livestock farming in significant part of arable land mass of Africa (Molyneux, 1997), causing death of well over 3 million cattle annually with an estimated cost potential of about 6-12 billion US dollars (Mortlmans, 1986; ILRAD, 1994).

Despite the economic and public health importance of African Trypanosomosis, it still remains a disease with unsatisfactory medical control. Chemotherapy, the major control strategy for trypanosomosis is confronted with problems of drugs resistance, unacceptable toxicity and long treatment protocols (Ogbadoyi *et al*.,2007), promoting the need for the search of cheap, less toxic and readily available ethno botanical treatment of the disease. Plants have been used for centuries in ethno pharmacological treatment of different types of diseases and still offer the potentials for discovery of novel chemotherapeutic agents (Tagboto and Townson, 2001). Plants possess variety of bioactive compounds with activities against viruses, cancer and parasites (Ahmed *et al.,* 2001). These plants contain compounds mainly secondary metabolites such as alkaloids, glycosides, flavonoids, terpenes and coumarins (Rates, 2001). They have been reported to provide better and cheaper alternatives to synthetic chemotherapeutics (Freiburghans *et al.,* 1996; Nok *et al.,* 1996, Adewummi *et al.,* 2001, Nok, 2005). In Nigeria, several ethnobotanical studies of Nigerian plants used in the traditional management of trypanosomosis indicated both significant *in vitro* or *in vivo* antitrypansomal activity.

*Adansonia digitata* also known as Baobab is a large iconic tree indigenous to Africa where it is found in many countries. It is emblematic, culturally important and physically majestic sub-tropical tree. In the past decade, it has attracted the interest of several pharmaceutical companies and researchers due to its various traditional uses such as medicinal, nutritional, and cosmetic (Addy, 2009). Various parts of the plant (e.g. leaves, bark, fruit pulp), have traditionally been used as immune-stimulant, anti-inflamatory, analgesic, insect repellant and pesticide properties, in the treatment of diarrhea and dysentery in many African countries, and have been evaluated as a substitute for imported western drugs (El-Rawy *et al*.,1997). As a result of its high natural vitamin C content, Baobab fruit pulp has a well-documented antioxidant capability (Brady, 2011) and could therefore be very useful in the prevention and treatment of oxidative stress related disease (Blomhoff, 2010).

Our interest in *A. digitata* in the search for new trypanocides stems from the various claims for its use in alternative medicine, and findings on its potential anticancer properties. This was hinged on the fact that antitumour drugs have been screened for trypanocidal action (Williamson and Scott-Finnigan 1978) and trypanocidal drugs have also been screened for anticancer activity (Barret and Barret 2000, Ivan *et al.,* 2014). This is perhaps due to the fact that protozoan parasites, such as those of malaria, trypanosomiasis and leishmaniasis, have a number of features in common with the proliferating cells of cancer and some forms of heart disease (Hide 1989). In this regards, Manfredini (2002) have tested the anti-trypanosomal potentials of the vegetative parts of *A. digitata*, but, to the best of our knowledge

there is no available report on the effect of the fruit pulp of this plant against trypanosomes. The aim of this study therefore, is to determine the phytoconstituents and explore the anti-trypanosomal potential of aqueous extract of fruit pulp of *A. digitata.*

## MATERIALS AND METHODS

### Plant material

Dry fruits of *Adansonia digitata* were collected around the environs of the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The fruits were cracked open using a hammer, and the fruit pulp was manually harvested and allowed to further dry under room temperature on the laboratory bench and stored until use.

### Preparation of Aqueous Extract of *Adansonia digitata*

The fruit pulp was detached from the seed using mortar and pestle. The pulp was then separated from the seeds and fiber by sieving. A total of 280 mg of the dried fruit pulp was weighed and placed in a conical flask containing 7 litres of distilled water and allow to stand for 72 hours in a refrigerator at +4°C with periodic agitation to ensure even mixture of the pulp with water. The mixture was then filtered using 850nm and 150nm sieves in succession. The third stage of filtration was done using Whatman Filter Paper No.1. Cotton wool was placed on the filter paper to facilitate the filtration processes. It was then frozen and dried using freeze- drying machine (ILSHIN freeze dryer with concentrator, Ilshin Lab. Co. Ltd, Netherlands).

### Phytochemical screening of the extract

Phytochemical analysis of the aqueous extract of the fruit pulp of *Adansonia digitata* was carried out according to the methods described by Sofowora (1993) and Evans (1998).

### Parasite

Stabblate of *Federe* strain of *Trypanosoma brucei brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna and was maintained in the laboratory by passaged into albino rats until when used.

### *In vitro* Antitrypanosomal Activity of Aqueous Extract of the Fruit Pulp of *Adansonia digitata*.

A donor albino rat obtained from the animal house of the Nigerian Institute for Trypanosomiasis Research (NITR) was inoculated with *T. brucei brucei* (Federe strain) and at massive parasitaemia, the rat was sacrificed and blood was collected into a beaker containing about 10 mg of Ethylene Diamine Tetra Acetic Acid. A suspension of trypanosomes was prepared by the addition of normal saline into the beaker. The concentration of the suspension was adjusted to about $1x10^6$ organisms per ml, by careful addition of the normal saline.

A total of 0.4 ml of the suspension of Ringer's solution (Sodium chloride 8.60 g/L, Potassium chloride 0.30 g/L, Calcium chloride dehydrate 0.33 g/L) and 0.3ml of the suspension of trypanosomes (containing about $3 \times 10^5$ trypanosomes) were each dispensed into 4 tubes containing 0.3 ml each of the extract at concentrations of 0.02 mg/ml, 0.2 mg/ml, 2 mg/ml and 20 mg/ml respectively for the first 4 tubes (A-D). The fifth tube (E) was an untreated control (Ringer's solution and parasite). The tubes were then incubated at $37^0$C after which the contents of the tubes were each examined at time 0 minutes and subsequently at interval of 15 minutes for 2 hours by aspirating a drop using a Pasteur pipette onto clean slides, covered with cover slips and examined for the presence and

motility of the parasites under the microscope at x 40 objective lens (Ene *et al.*, 2014).

**Infectivity Test**

After the 2 hours of incubation and motility assessment, 0.2ml of the contents of each tube was inoculated intraperitoneally into 3 rats for each group. 3 other rats served as uninfected controls. Daily examination of blood of the inoculated rats was done to detect the possible presence of trypanosomes over a period of 60 days.

**Ethical Statement**

The animals were maintained and used at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, following the guidelines of NITR Ethical Committee. The study was carried out in accordance with the principles of Laboratory Animal Care (National Institute of Health Publication No. 86.23, revised 1985).

**RESULTS**

**Phytochemical Screening**

The result of the qualitative phytochemical screening of aqueous extract of the fruit pulp of *Adansonia digitata* is shown in table I. The qualitative analysis showed the presence of tannins, saponin, phenol, terpenoid, cardiac-active glycoside, anthraquinone, reducing sugar, alkaloids, flavonoids and steroids.

**TABLE I:** Qualitative phytochemical constituent of aqueous extract of fruit pulp of *Adansonia digitata*.

| S/NO | Phytochemical constituents | Inference |
|---|---|---|
| 1 | Tannins | + |
| 2 | Saponin | + |
| 3 | Phenol | + |
| 4 | Terpenoid | + |
| 5 | Cardiac-active glycoside | + |
| 6 | Anthraquinone | + |
| 7 | Reducing sugar | + |
| 8 | Alkaloids | + |
| 9 | Flavonoid | + |
| 10 | Steroids | + |

Note: + Detected.

### *In vitro* Antitrypanosomal Activity

The result of the *in vitro* anti-trypanosomal activity of aqueous extract of fruit pulp of *Adansonia digitata* on *Trypanosoma brucei brucei* is shown in Table II. The control sample and samples exposed to 0.02mg/ml and 0.2 mg/ml revealed actively motile trypanosomes through the 120 minutes of observation. However, samples exposed to 2mg/ml of the extract revealed very active, active and sluggish trypanosomes at 0 minute, 15 minutes and 30 – 60 minutes respectively, after which the parasite became very sluggish from minutes 75 to 120minutes of observation. Samples exposed to 20mg/ml of the extract showed very sluggish trypanosome between 15 to 75 minutes after which they remained immotile from 90 to 120 minutes of observation.

**TABLE II:** *Invitro* trypanocidal activity of aqueous extract of fruit pulp of *Adansonia digitata*.

| Concentration of Extract (mg/ml) | Time of exposure (minutes) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
| 20 | ++++ | + | + | + | + | + | - | - | - |
| 2 | ++++ | +++ | ++ | ++ | ++ | + | + | + | + |
| 0.2 | ++++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| 0.02 | ++++ | ++++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Control | ++++ | ++++ | ++++ | ++++ | +++ | +++ | +++ | +++ | +++ |

Key: ++++ Very active; +++ Active; ++ Sluggish; +Very Sluggish; -Full inhibition of motility.

### Effect of Aqueous Extract of Fruit Pulp of *Adansonia digitata* on the Infectivity of *Trypanosoma brucei brucei*.

The infectivity test (Table III) showed that all the animals inoculated with contents of the tubes containing the highest concentration of the extract (20mg/ml) did not show parasitaemia and survived the 60 days infectivity observation period. For the lower concentrations 2 mg/ml, 0.2 mg/ml and 0.02 mg/ml none of the animals survived the observation period with the animals expressing parasitaemia by the second day of the test and died at an average of 4.3, 3.7 and 2.6 days post inoculation respectively.

**TABLE III:** Effect of aqueous extract of fruit pulp of *Adansonia digitata* on the infectivity of *Trypanosoma brucei brucei* after incubation period.

| Extract Concentration(mg/ml) | No of rats inoculated | Infection/parasitaemia | Survival of rats |
|---|---|---|---|
| 20 | 3 | - | S |
| 2 | 3 | + | NOS* |
| 0.2 | 3 | + | NOS* |
| 0.02 | 3 | + | NOS** |
| Control | 3 | + | NOS** |

Key: +Parasitaemia positive. -Parasitaemia negative. S=All rats survived the 60days infectivity observation period.NOS=None of the rats survived the 60days infectivity observation period.NOS*=Rats died 3-5days after inoculation.NOS**=Rats died 1-3days after inoculation.

## DISCUSSION

This work has revealed that the fruit pulp of *Adansoina digitata* contains phtyochemicals, such as saponins, tannins, flavonoids, cardiac glycosides, steroids and terpenes which concur with the work of Anani *et al.* (2009). In addition, several authors have identified the presence of flavonoids, saponins, tannins, cardiac glycosides in plants that showed trypanocidal activities (Nok, 2002; Nok, 2005; Atawodi *et al*., 2011, Nwodo *et al.,* 2015) which could be responsible for the in vitro anti-trypanosomal activity observed in this study.

In *in vitro* studies, cessation or decrease in the parasite motility is taken as a measure of the anti-trypanosomal effect. The concentration of 20 mg/ml had the highest inhibition of motility of trypanosomes, which is an indication that at this concentration the fruit pulp of *A. digitata* possess anti-trypanosomal activity. This is because parasite motility constitutes a relatively reliable indicator of viability of most zooflagellate parasites. Cessation in motility of trypanosomes therefore served as a measure of anti trypanosomal potential of the aqueous extract when compared to the control that appeared very active throughout the 120 minutes of observation. This *in vitro* results also corroborates the findings of Manfredini (2002) who reported that extracts of baobab roots eliminate the motility in *T. congolense* within 60 minutes and drastically reduce motility in *T. brucei brucei.*

Although the mechanism of the inhibition of the parasite motility in this study is not known, the direct contact of the trypanosomes with the extract and possibly the disruption of the functions of some important organelles in the trypanosome might be responsible. Natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase which is very sensitive to alterations in redox balance. (Bala *et al*, 2009). Furthermore, (Atawodi *et al.*, 2003) 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, and natural products may also act by binding with the kinetoplast DNA of the parasites. The anti-trypanosomal effect of *A.digitata* from the results obtained is concentration dependent. Highest concentration of the extract used in the present study (20 mg/ml) completely immobilized and possibly killed all the parasites after 120 minutes of exposure evident by the loss of infectivity of the parasites in albino rats.

## CONCLUSION

This study has shown that the aqueous extract of *Adansonia digitata* contains phytoconstituents such as tannins, saponin, phenol, terpenoid, anthraquinone, alkaloids, flavonoids and steroids that were previously detected in plants with anti-trypanosomal effects. The extract demonstrated a concentration and time dependent *in vitro* inhibitory effect on trypanosomal motility and infectivity with a better effect associated with the highest concentration of 20 mg/ml used in this study. To discover the full potential of the fruit pulp of *A. digitata* as source of possible novel anti-trypanosomal agent, there is need for further work on the *in vivo* ant-trypanosomal effect of this extract and to isolate the constituents having the anti-trypanosomal activity as well as their mechanism of action.

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