

Full Length Research Paper

In-vitro* and *-vivo* anti-*Trypanosoma evansi* activities of extracts from different parts of *Khaya senegalensis

I. A. Umar*, M. A. Ibrahim, N. A. Fari, S. Isah and D. A. Balogun

Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Accepted 19 April, 2010.

The *in vitro* activities of the aqueous and ethanolic extracts of the leaves, root bark and stem bark of *Khaya senegalensis* on *Trypanosoma evansi* were evaluated. The ethanolic extract of the stem bark was found to possess the highest *in vitro* activity among the six extracts tested; as it eliminated the parasites within 5 min post incubation at concentrations of 0.5 and 1 mg/ml. This extract was therefore used to treat rats experimentally infected with *T. evansi* at concentrations of 20, 40 and 80 mg/kg body weight, beginning 7 days post infection (p.i). At the termination of the experiment on day 13 p.i, the stem bark ethanolic extract significantly ($P < 0.05$) kept the parasitemia lower than was observed in the untreated infected rats, whereas the parasites were eliminated from the bloodstream of Diminal-treated rats at day 9 p.i. All the infected animals developed anaemia whose severity could not be ameliorated by the extract treatment. It was therefore concluded that the stem bark ethanolic extract of *K. senegalensis* possessed both *in-vitro* and *-vivo* anti-*T. evansi* activity but could not prevent the disease-induced anaemia.

Key words: *Khaya senegalensis*, *Trypanosoma evansi*, anti – trypanosomal.

INTRODUCTION

Animal trypanosomiasis is still 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 (Welburn et al., 2001); and thus an important priority for biomedical and public agencies, agricultural sector and the scientific community (Aksoy, 2003). One of the important pathogenic trypanosomes in animals is *Trypanosoma evansi*; the causative agent of *Surra* that is highly fatal to a number of domesticated mammals such as camels, horses and water buffaloes among others (Vanhollebeke et al., 2006). Since the adaptation of the parasite to mechanical transmission by blood sucking insects (tabanids), the disease has spread beyond its original distribution in sub-Saharan Africa and is now also present in South America, North Africa and large parts of Asia (Vanhollebeke et al., 2006).

The existing treatments of trypanosomiasis are challenged with problems comprising drug resistance, toxicity and expensive/limited drugs (Gutteridge, 1985, Atawodi et al., 2002). Therefore, there is a need to search for cheaper, more effective, easily available and less toxic chemotherapeutic agents for combating trypanosomiasis. The use of herbal preparations for the treatment of the disease still holds a strong potential in that some ethnomedicinal plants have been demonstrated to contain potent trypanocides (Igweh and Onabanjo, 1989; Owolabi et al., 1990; Nok et al., 1993; Atawodi, 2005).

The indigenous use of *Khaya senegalensis* (Juss), a dry zone mahogany belonging to the family *Meliaceae*, in the treatment of trypanosomiasis has been reported (Atawodi et al., 2002) and *in vitro* anti-trypanosomal activity of the plant against *Trypanosoma brucei* has been demonstrated (Wurochekke and Nok, 2004; Atawodi, 2005). More recently, the *in vivo* action of the stem bark aqueous extract of the plant against *T. brucei* (Ibrahim et al., 2008) has been reported, but information on the *in vitro* and/or *in vivo* action of the plant against *T. evansi*, with broadest host and geographic range among the pathogenic animal trypanosomes is still lacking.

Hence this work was designed to evaluate the *in vitro*

*Corresponding author. E-mail: smaumar@yahoo.com. Tel: +2348023586896.

and *in vivo* anti-trypanosomal activities of extracts from various parts of *K. senegalensis* against *T. evansi*.

MATERIALS AND METHODS

Sample collection

The stem bark, root bark and leaves of mature *K. senegalensis* were collected from the botanical garden of Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria; and were identified at the herbarium of the same Department with a voucher number of 90081 which was deposited. The parts of the plant were thoroughly washed and shade-dried for a week to a constant weight. The dried parts were pounded to fine powder with mortar and pestle and then stored in dry containers until needed.

Experimental animals

A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations of the World Health Organization (WHO). The principles of laboratory animal care were also duly followed in this study. Apparently healthy white albino rats of both sexes weighing between 90-172g were used for the work and were obtained from Pharmacology Department, Ahmadu Bello University, Zaria, Nigeria. The animals were kept in well ventilated laboratory cages with 12 hours day/night cycles. The rats were maintained on a commercial poultry feed (ECWA Feeds, Jos-Nigeria) and drinking water *ad libitum*.

Extracts preparation

One hundred grams of the fine powdered plant parts were soaked in 300 ml of either distilled water or ethanol and sequentially extracted by shaking for 6 h on wrist action shaker. The preparations were left to stand for a further 24 h. After filtration through Whatmann's filter paper, samples were concentrated to dryness on a water bath at 40°C, packaged in water-proof polythene bags and stored in the refrigerator at 4°C until required (Atawodi, 2005).

Trypanosome

Trypanosome evansi (Sokoto strain) was obtained from an infected mouse in the Parasitology Department, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Parasites harvested from a donor rat at peak parasitaemia were used for both the *in vitro* studies and infection of experimental animals.

In vitro screening

Exactly 1 mg of the different extracts were weighed and dissolved in 1 ml of phosphate buffered saline (PBS). Serial dilution of this stock solution was done using PBS to obtain concentrations ranging from 0.25 to 1 mg/ml. Assessment of the *in vitro* anti-trypanosomal activity was performed in triplicates in 96 well microtitre plates (Flow laboratories Inc., Mclean, Virginia, USA). In wells of the microtitre plates, 20 µl of each extract was incubated at 37°C, with 40 µl of the infected blood (obtained from a donor rat with about 10⁹ *T. evansi* per ml of blood), achieving effective extract concentrations of 0.083 to 0.332 mg/ml in the reaction mixtures. For control, the 20 µl of extract was replaced with PBS. Parasite count was then monitored on a glass slide (covered with a covering slip) and observed under a microscope at ×400 magnification. The number of motile parasites was counted at 5 min intervals for 1 h.

In vivo activity of the stem bark ethanolic extract

35 rats were divided into seven groups of five rats each. The rats in five groups were intraperitoneally infected with 10⁶ *T. evansi* per 100 g body weight (b.w) and the level of parasitaemia monitored daily by the Herbert and Lumsden (1976) method for the 14 days period of the experiment. Exactly, 20, 40 and 80 mg/kg b.w. of the extract (Inf +EDI, Inf + EDII and Inf + ED III respectively) were administered orally and 0.5 ml/100 g b.w. of diminal[®] (a standard drug for African Trypanosomiasis that contains 445 mg diminazene aceturate + 555 mg phenazone/g. Eagle Chemical Company LTD, Ikeja, Nigeria) given intraperitoneally to four different groups of the infected rats, starting a day after the parasites were first detected in the bloodstream (Day 7 post infection); the remaining infected group was left untreated (infected control). One group of the uninfected rats was orally administered with 40 mg/kg b. w. of the extract (extract control) whereas the other group was maintained as uninfected untreated (normal) control. The pre-infection and terminal packed cell volumes of all the rats were determined by the microhaematocrit method.

Analysis of data

Paired means were compared using students' t-test.

RESULTS

Both the aqueous and ethanolic extracts of the various parts of *K. senegalensis* showed *in vitro* anti-trypanosomal activity in a dose-dependent fashion (Figures 1 and 2) with the ethanolic extracts seemingly exhibiting a higher activity against the *T. evansi* parasites. However, the stem bark ethanolic extract possessed the highest activity against the parasite and thus was used for the *in vivo* studies.

The parasitaemias of both the infected control and the infected treated groups are presented in Figure 3. While a progressive increase in parasitaemia was observed in the infected controls; treatments with the extract significantly ($P < 0.05$) lowered the level of parasitaemia when compared to the infected untreated rats, whereas the parasites totally disappeared from the bloodstream of diminal[®]-treated infected group on day 9, p.i.

The *T. evansi* infections in this work caused significant fall in PCV of infected rats; indicative of anaemia. However, while administration of Diminal[®] to infected animals significantly reduced the magnitude of decline in PCV, administration of the extract at the various doses had no effect on the disease-induced anaemia. The PCVs of the two groups of uninfected animals remained relatively constant.

DISCUSSION AND CONCLUSIONS

Some plant extracts have been demonstrated to contain potent trypanocidal constituents (Igweh and Onabanjo, 1989; Owolabi et al., 1990; Atawodi, 2005). So far, only aqueous and methanolic extracts of stem bark of *K. senegalensis* have been shown to possess *in vitro*

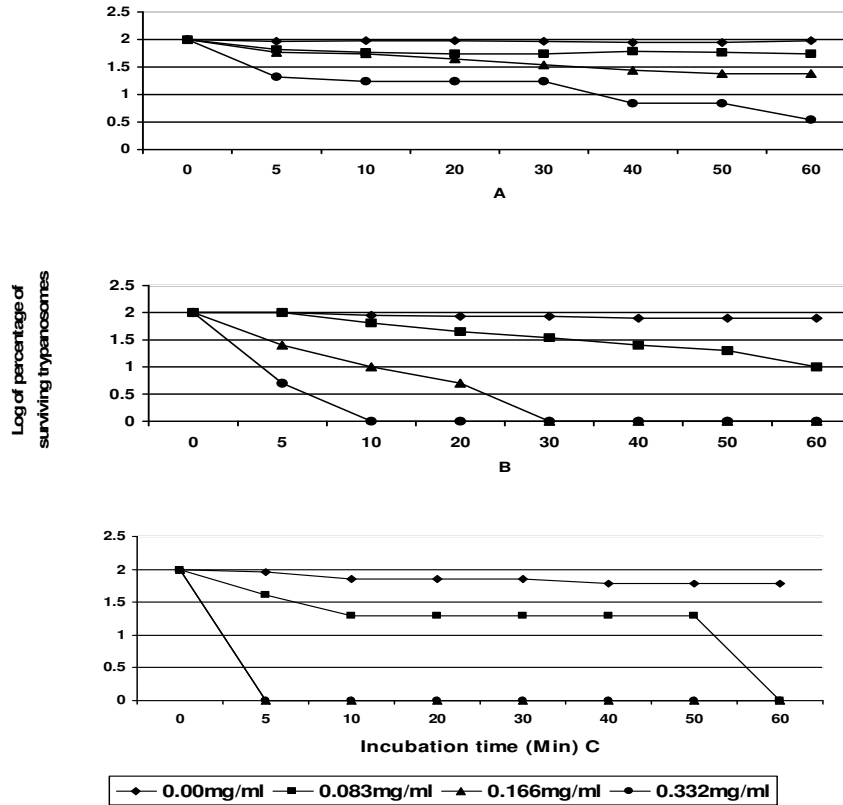


Figure 1. Profiles of log percentage surviving trypanosomes after incubation with various concentrations of ethanolic extracts of the leaves (A) root bark (B) and stem bark (C) of *Khaya senegalensis*.

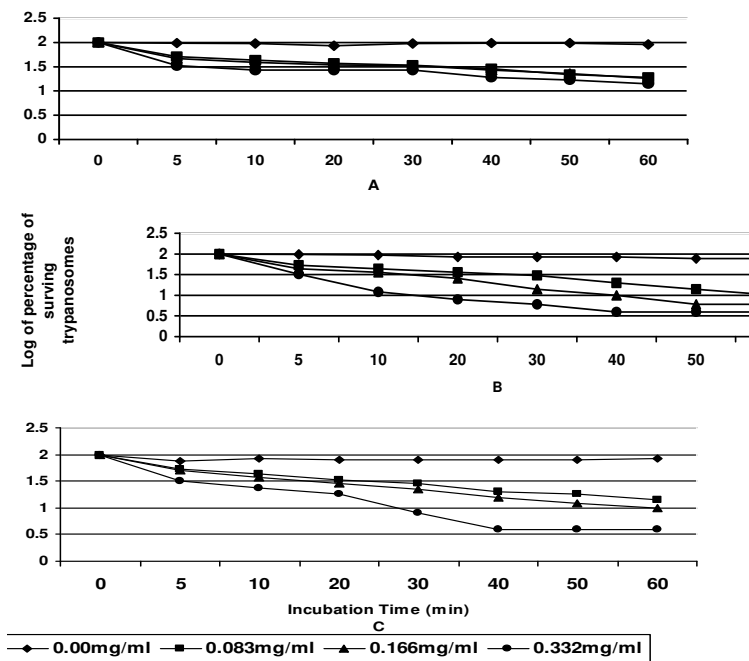


Figure 2. Profiles of log percentage surviving trypanosomes after incubation with various concentrations of aqueous extracts of the leaves (A) root bark (B) stem bark (C) of *K. senegalensis*.

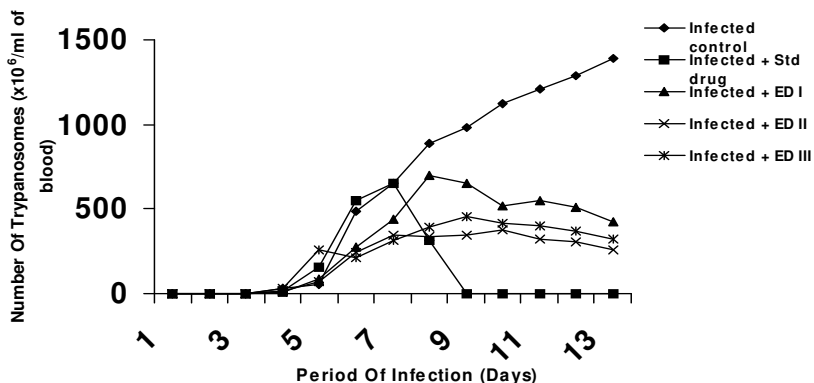


Figure 3. Effect of ethanolic extract of stem bark of *K. senegalensis* on *T. evansi* infected rats.

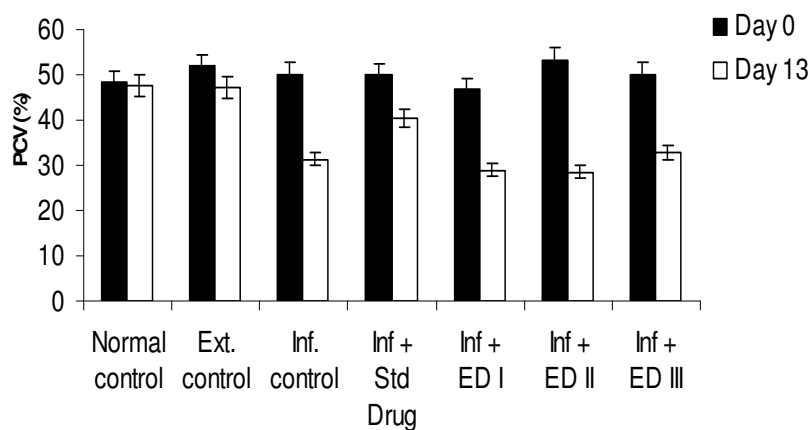


Figure 4. Effect of ethanolic extract of stem bark of *K. senegalensis* on the PCV level of *T. evansi* infected rats.

activity against *T. brucei*. (Wurochekke and Nok, 2004, Atawodi, 2005). This report reveals that the leaves, stem bark and root bark of *K. senegalensis* also contain some water and ethanol-extractable phytochemicals that possess *in vitro* activities against *T. evansi*.

Parasite motility constitutes a relatively reliable indicator of viability of most zooflagellate 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 anti-trypanosomal activities among the plant parts could be attributed to the variation(s) in concentration and composition of phytochemicals in the different parts since distinct function(s) is performed by all the parts and hence tend to produce slightly different chemical constituents. Since a plant with high *in vitro* anti-trypanosomal activity may have no *in vivo* activity and vice versa, because of peculiarities in the metabolic disposition of the plant's chemical constituents, we tested

the most active extract (ethanolic extract of the stem bark) for *in vivo* anti-trypanosomal activity so that a definite statement can be made on the anti-*T. evansi* activity of the plant.

The observed *in vivo* anti-*T. evansi* activity of the stem bark ethanolic extract of this plant support earlier reports that some plant extracts possess *in vivo* activities against trypanosomes (Asuzu and Chineme, 1990; Nok et al., 1993; Ibrahim et al., 2008). This could also provide the scientific basis for the traditional use of *K. senegalensis* in the management of trypanosomiasis (Atawodi et al., 2002).

The exert mechanism for the *in vivo* action of this extract is unknown since the active ingredient(s) were not isolated. However, previous reports attributed the trypanocidal activity of a number of tropical plants to the flavonoids (azaanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine (Hopp et al., 1976, Nok, 2001). Furthermore, Sepulveda-Boza and Cassels (1996) suggested that many natural products

exhibit their trypanocidal activity through interference with 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 (Atawodi et al., 2003). Since the stem bark ethanolic extract of *K. senegalensis* has also been shown to contain flavonoids (Makut et al., 2008), it is thus possible that this extract acted through one or more of these mechanisms.

Anaemia is a constant feature of trypanosome infections whose severity is linked to the level of parasitemia (Umar et al., 2000). The extract did not affect the severity of anaemia in infected animals probably because parasitaemia, albeit low grade, was persistent in the animals and the aetiological factors involved in the haemolysis have been established before the extract treatment. The Diminal[®]-treated infected rats showed significant improvement in PCV from infection levels perhaps because the drug was able to eliminate parasites from the blood to levels undetectable by microscopic examination. We therefore concluded that the stem bark ethanolic extract of *K. senegalensis* possessed both *in-vitro* and *-vivo* anti-*T. evansi* activity. However, the antitrypanosomal activity of this plant reported herein appears to be relatively lower than the previously reported activity against *T. brucei* (Ibrahim et al., 2008) but further work on the toxicology, isolation and identification of the bioactive components would certainly reveal whether this plant could be exploited for the development of new generation of trypanocides. Furthermore, information reported in this study could be useful in assessing the overall antitrypanosomal activity of this plant.

REFERENCES

- Aksoy S (2003). Control of tsetse flies and trypanosomes using molecular genetics. *Vet. Parasitol.*, 115(2):125-145.
- Asuzu IU, Chineme CN (1990). Effects of *Morinda lucida* leaf extract on *T. brucei brucei* infection in mice. *J. Ethnopharmacol.*, 30: 307-313.
- Atawodi SE (2005). Comparative *in vitro* trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigeria savannah plants. *Afri. J. Biotechnol.*, 4(2): 177-182.
- Atawodi SE, Ameh DA, Ibrahim S, Andrew JN, Nzelibe HC, Onyike E, Anigo KM, Abu EA, James BD, Njoku GC, Sallau AB (2002). Indigenous knowledge system for treatment of trypanosomiasis in Kaduna State of Nigeria. *J. Ethnopharmacol.*, 79: 279-282.
- Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M, (2003). *In vitro* trypanocidal effects of methanolic extracts of some Nigeria savannah plants. *Afr. J. Biotechnol.*, 2(9): 317-321.
- Gutteridge WE (1985). Existing Chemotherapy and its limitations. *Brit. Med. Bull.*, 41: 162-168.
- Hopp KH, Cunningham IV, Bromel MC, Schermester LJ, Wahba KSK (1976). *In vitro* antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi*. *Lloydia*, 39(5): 375-377.
- Ibrahim MA, Njoku GC, Sallau AB (2008). *In vivo* activity of stem bark aqueous extract of *Khaya senegalensis* against *Trypanosoma brucei*. *Afri. J. Biotechnol.* 7(5): 661-663.
- Igweh AG, Onabanjo AO (1989). Chemotherapeutic effects of *Annona senegalensis* in *T. brucei brucei* infection in mice. *J. Ethnopharmacol.*, 30: 307-313.
- Kaminsky F, Nkuna MHN, Brun R (1996). Evaluation of African medicinal plants for their *in vitro* trypanocidal activity. *J. Ethnopharmacol.*, 55: 1-11.
- Makut MD, Gyar SD, Pennap GRI, Anthony P (2008). Phytochemical screening and antimicrobial activity of the ethanolic and methanolic extracts of leaf and Bark of *Khaya senegalensis*. *Afri. J. Biotechnol.* 7(9): 1216-1219.
- Nok AJ (2001). Azaanthraquinone inhibits respiration and *in vitro* growth of long slender blood stream forms of *T. congolense*. *Cell Biochem. Func.*, 20: 205-212.
- Nok AJ, Esiebo KAN, Hondjet I, Arowosafe S, Onyenekwe PC, Gimba CE, Kagbu JA (1993). Trypanocidal potential of *Azadirachta indica*: *In vivo* activity of leaf extract against *T. brucei brucei*. *J. Clin. Biochem. Nutr.*, 15: 113-118.
- Owolabi OA, Makanga B, Thomas EW, Molyneux DH, Oliver RW (1990). Trypanocidal potentials of Africa woody plants. *In vitro* trials of *Khaya grandifolia* seed extracts. *J. Ethnopharmacol.*, 30: 227-231.
- Sepulveda-Boza S, Cassels BK (1996). Plants metabolites active against *Trypanosoma cruzi*. *Planta Medica*, 62: 98-105.
- Umar IA, Toh ZA, Igbalajobi FI, Igbokwe IO, Gidado A (2000). The effect of orally administered vitamin C and E on severity of anaemia in *Trypanosoma brucei* – infected rats. *Trop. Veterinarian*, 18: 71-77.
- Vanhollebeke B, Truc P, Poelvoorde P, Pays A, Joshi PP, Katti R, Jannin JG, Pays E (2006). Human *Trypanosoma evansi* infection linked to a lack of Apolipoprotein 1. *New Engl. J. Med.*, 355: 2752-2756.
- Welburn SC, Coleman PG, Fevre E, Mandlin I (2001). Sleeping sickness- a tale of two diseases. *Trends Parasitol.*, 17: 19-24.
- Wurochekke AU, Nok AJ (2004). *In vitro* antitrypanosomal activity of some medicinal plants used in the treatment of trypanosomiasis in Northern Nigeria. *Afri. J. Biotechnol.* 3(9): 481-483.