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TRYPANOCIDAL PROPERTIES OF *TERMINALIA IVORENSIS* A. Chev. (COMBRETACEAE)

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Abstract

Human African Trypanosomiasis (sleeping sickness) cause considerable mortality and morbidity throughout the world with Trypansoma *brucei*, *T. gambiense* and *T. brucei rhodesiense* as the major causative agents. The *Terminalia ivorensis* gradient extracts were tested against *T. brucei rhodesiense* parasites and the $E.D_{50}$ values of 11.11 and 12.32 µg/mL were obtained for the ethyl acetate and ethanolic gradient extracts respectively. The column fractions of the ethyl acetate fraction gave $E.D_{50}$ value between 1.98 and 5.46 µg/mL for the very active fractions indicating prospect for the development of potential trypanocidal agent from the plant.

Key words: *Terminalis ivorensis, Trypanosoma brucei rhodesiensis*, trypanocidal

Introduction

It is estimated that 60 million people are at the risk of infection of Human African Trypanosomiasis (HAT) with about 300,000 new cases reported each year (WHO, 1995). The disease is found uniquely in eastern sub-African and is of major public health importance to the majority of these countries. The prevalence rate is in excess of 70% in some villages in the Democratic Republic of Congo according to WHO 2000. In some of the worst affected countries, the disease has become the leading cause of mortality exceeding deaths due to AIDS (WHO, 1998). Sleeping sickness is very difficult to treat particularly at the late stage. The current drugs that are used for the treatment of

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trypansomiasis are toxic, requiring courses of parental administration and have variable efficacy (Croft, 1997).

Suramine and Pentamidine are effective against the early haemolymphatic stages of the disease. The polyamide biosynthesis inhibitor; eflornithine which was previously used to manage advanced stages of the disease is no longer available in the market. The current drug used for the treatment of the late stage of sleeping sickness is the arsenical drug, melarsoprol. In 1999, reports showed that 5% of the patients treated with melarsoprol die as a result of the side effects of the drug (WHO, 1999). In recent years the rate of relapse after treatment with melarsoprol has been recorded and the cause was attributed to the emergence of drug resistant *T. brucei* strain (Barret and Fairlamb, 1999). These limitations in the chemotherapy of sleeping sickness are a cause for major concern because the disease is 100% fatal.

In our earlier work on the screening of some medicinal Nigerian plants for trypanocidal properties Adewunmi et al (2001) it was discovered that *Terminalia ivorensis* showed great promise as a trypanocidal agent Other biological activities associated with the plant include anti-inflammatory and anti-arthritis (Iwu and Anyanwu, 1982) also antibacterial activity (Malcom and Sofowora ,1969). In ethnomedicine, *T. ivorensis* forms part of the treatment for syphilis among the Jukuns. The pulverized leaves are used as poultice to treat burns and bruises. Similarly the decoction of the bark is used as lotion for sores. The ashes of the leaf gall (common on the tree) is mixed with the roasted bulb of *Crinum*, made into an ointment with fresh cow butter is rub on rheumatic and other swollen joints for relief (Hutchinson and Dalziel, 1936). Ekong and Idemudia (1967) reported the presence of terminolic, ellagic glycirrhetic acids and quercetin in the plant. Iwu and Anyanwu (1982) also reported the presence of sericic acid and lonchoterpene (28-hydroxy-18α-glycirrhetinic acid) along with those earlier mentioned in the plant.

In view of our earlier work on the trypanocidal activity on this plant, there was the need to subject *Terminalia ivorensis* to bioassay – guided study in order to determine if purification will enhance bioactivity, which might lead to the development of affordable effective and safe alternative drugs to combat trypanosomiasis.

Materials and Methods

Plant material

Stem bark of *Terminalia ivorensis* was collected at the Ogun state re-forestation project reserve (Area J 4) Ijebu, Nigeria in November, 2002 by Mr. A.T. Oladele and authenticated by Mr. B. O. Daramola of the Department of Botany, Obafemi Awolowo University, Ile-Ife. Voucher specimen deposited at the Obafemi Awolowo University herbarium (numbered IFE 15445).The stem bark was oven-dried at 50^0 C and milled into a powder using the Christy Grinding machine. The powder was stored in an amber coloured bottle until needed.

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Extraction

The powdered stem bark of *Terminalia ivorensis* (1kg) was subjected to Soxhlet extraction successively with hexane, ethyl acetate and methanol as solvents. The extracts were concentrated to dryness in vacuo and yielded 2.36%, 6.4% and 12.0% respectively.

In vitro assay
Parasites

Trypanosoma brucei rhodensiense STIB 900 blood stream form (bsf) trypomastigotes were maintained in HMI -18 medium (Hirumi and Hirumi, 1989) with 15% heat inactivate foetal calf serum (HIFCS) (Harlan – Seralab, UK) at 37 ^{0}C, 5% CO_2 / air mixture as described by Asres et al (2001).

In a 96- well microtitre plate, each sample was diluted in a three fold dilution series to obtain six concentrations with three replicates for each concentration. To each well containing 100 µL of diluted sample was added 2 x 10^4/ mL *Trypanosoma brucei rhodensiense* tryptomastigotes **(**100 µL) **.**Top concentration for the test compound was 60 µg/ml. Pentamidine was used as control. The $E.D_{50}$ for pentamidine is usually between 1.0 and 0.1 ρg/mL. The plates were incubated for 72h at 37 ^{0}C, 5% CO_2 – air mixture. At 72h, the viable parasites were counted on a Neubauer haemocytometer and compared with untreated controls. The plates were assessed microscopically before Alaman Blue was added (Raz et al, 1977). Plates were read after 5-6h on a Gemini fluorescent plate reader (Somifax Pro 3.1.1 molecular Devices, U.K) at EX/ EM 530 585nm with a filter cut-off at 550nm. $E.D_{50}$ values were calculated MSx/ fit (IDBS, U.K.). The samples were tested twice on separate occasions and the standard deviation determined for each of the samples.

Bioactivity guided fractionation

The activities of the gradient i.e. n- hexane (NH), ethylacetate (EA) and ethanolic (EH) extracts were tested against *T. brucei rhodesense* parasites as described above with pentamidine as control. Their $E.D_{50}$ values in µg/mL were determined.The ethyl acetate extract (9.0g) was column chromatographed using silica gel (60-120) mesh and eluted with gradient solvents of n-hexane / chloroform, 100%chloroform and gradient of chloroform / methanol as solvent systems. 39 fractions of 50mL each eluates collected were monitored on TLC using Pet. ether / $CHCl_3$ (3:2) for fractions 1 -21 and $CHCl_3$ / MeOH (9:1) for fractions 21 -39 and bulked into 15 bulked fractions coded as TV/A –TV/P successively. These fractions were subsequently tested against *T. b. rhodesiense* parasite as described above and their median effective concentration $E.D_{50}$ determined.

The most active bulked fraction TV/N3 (190mg) was further purified using PTLC, $CHCl_3$ / MeOH (4:1) as solvent system and silica gel G_{254} as adsorbent . Six bands TV/N1 to TV/N6 were obtained and eluted with $CHCl_3$. TV/N3 (18mg) was obtained as single spot R_f 0.60 in $CHCl_3$: MeOH 17:3 and gave purple colour with Anisaldehyde spray

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reagent. Further in vitro studies will be required to determine the effectiveness of Compound TV/N3 whose structure was yet to be determined.

Results and Discussion

The results are presented in Table 1. Natural products isolated from higher plants have provided novel clinically active drugs which are one of the keys to the discovering of naturally occurring therapeutic agents through bioassay- guided fractionation and purification procedures. When purification enhances activity, it often leads to the discovery of new active molecules.

Table 1: Activities of crude extracts and column fractions of Ethyl acetate extract of *Terminalia ivorensis* stem bark against *T. brucei rhodesiense*

Extract/ Fractions/ Compound	Weight (mg)	Median Inhibitory conc. $E.D_{50}$ µg/mL
n- Hexane	23.6×10^3	>60
Ethyl acetate	64.0×10^3	11.11± 2.05
Ethanol	120.0×10^3	12.32± 1.32
TV/A	5.0	>60
TV/B	17.0	>60
TV/C	12.0	>60
TV/D	12.0	>60
TV/E	20.0	>60
TV/F	8.0	11.26 ± 0.28
TV/G	6.0	>60
TV/H	870.0	>60
TV/I	34.0	5.46 ± 0.17
TV/J	10.0	2.95 ± 0.32
TV/K	40.0	3.37 ± 0.08
TV/L	1.0	13.89 ± 1.12
TV/M	160.0	60.0 ± 0.67
TV/N	190.0	1.98 ± 0.46
TV/P	110.0	33.07± 1.34
Pentamidine		0.0004 ± 0.0001

On the other hand if it leads to reduction in activity, the constituents are synergistic, as such drugs are to be formulated at the stage of highest activity. In this study *T. ivorensis* was subjected to bioassay – guided study the bioactive constituents were found to be in the moderately polar and polar fractions. The ethyl acetate and ethanolic extracts gave $E.D_{50}$

values of 11.11 and 12.32 µg/mL respectively while the n- hexane extract with an $E.D_{50}$ value › 60 µg/mL was inactive . Purification of the ethyl acetate gradient extract using column chromatography led to bulked fractions TV/NA –TV/NP and when tested against same organism gave $E.D_{50}$ values in the range of 1.98 and 33.07µg/mL. The most active fractions TV/I-TV/K and TV/N gave $E.D_{50}$ values of 5.46, 2.95, 3.37 and 1.98µg/mL respectively which were far better than that given by the ethyl acetate extract. Pentamidine the reference drug gave an $E.D_{50}$ value of 4.0 ρg/mL. This report represents an exciting advance in the search for novel trypanocide from natural source.

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