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EVALUATION OF ANTITRYPANOSOMAL AND ANTI INFLAMMATORY ACTIVITIES OF SELECTED NIGERIAN MEDICINAL PLANTS IN MICE

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Abstract

The extracts of nine selected Nigerian medicinal plants were investigated on *Trypanosoma brucei brucei* infected mice. The anti-inflammatory properties of hexane fraction of the most promising *U. chamae* extract was assessed by acute oedema of the mice paw model while the modulatory effect of the extract on Delayed-Type Hypersensitivity (DTH) response on *in vivo* leucocytes mobilization was evaluated. 'Dose- probing acute toxicity tests' established an oral and intraperitoneal LD_{50} for *T. ivorensis* stem bark as >1600 < 5000 mg/kg and 100 mg/kg respectively, while the oral LD_{50} of *Uvaria. chamae* was >5000 mg/kg. Extracts of *Khaya senegalensis, Harungana madagascariensis, Terminalia ivorensis, Curcuma longa, Ocimum gratissimum* and *Alcornea cordifolia* showed weak anti-trypanosomal effect and did not exhibit significant clearance in parasitemia at the test dose administered compared with the positive control (Diminal®). However, the leaf extract of *U. chamae* and its hexane fraction demonstrated a significant response ($P < 0.01$). The fraction at 1000 mg/kg inhibited oedema by 107%. *Uvaria. chamae* demonstrated both anti-trypanosomal and anti-inflammatory properties by increasing the survival time of infected mice due to reduction in parasitemia caused by *T. brucei brucei*.

Keywords: antitrypanosomiasis, anti-inflammatory, acute toxicity, screening, medicinal plants

Introduction

Trypanosomosis is a fatal disease of human and domestic animals in tropical Africa and South America spread by the bite of an infected tsetse fly (*Glossina* Genus) (Fairlamb, 1982). It has undergone a dramatic and devastating resurgence within the last two decades (Smith et al., 1998), especially in Sub-Saharan Africa (Welburn et al., 2001). The significance of trypanosomosis on human health, nutrition and economy is enormous, thereby necessitating continuous research for better ways of eradicating the disease (Atawodi, 2005). Unfortunately, the high cost of chemical drugs, with the high incidence of their side effects and the emergence of resistance strains has rendered existing chemotherapy inadequate (Atouguia and Costa, 1999). Therefore, there is need to explore other agents, especially of plant origin for new generations of anti-trypanocidal agents that are more effective, less toxic, and readily available.

There have been several reports that a vast majority of the population, particularly those living in rural settings, largely depend on herbal medicines (Gupta, 1994). Natural products (plants and animals) are important sources of new drugs because their derivatives are extremely useful as lead structures for synthetic modification and optimization of bioactivity (Sülsen et al., 2007). In furtherance of the efforts on sourcing for novel molecules from natural sources in combating African animal trypanosomosis, previous research on *in vivo* antitrypanosomal activity is very limited compared to the in *vitro* experiments, and in the few *in vivo* studies found in literature, no complete cure without relapses was recorded for plants such as *Alstonia boonei* bark, *Annona senegalensis* root, *Morinda lucida* leaves and *Picralima nitida* (Wosu and Ibe, 1989). Hence, this work was undertaken to screen selected Nigerian medicinal plants for *in vivo* antitrypanosomal activity. The studied plants include *Khaya senegalensis*, A. Juss, *Harungana madagascariensis* (Choisy) Poir, *Terminalia ivorensis* A. Chev., *Curcuma longa* L., *Ocimum gratissimum* L., *Aloe schweinfurthii* Bak., *Achyranthes aspera* Hook F., *Uvaria chamae* P. Beauv., and leaf and stem of *Alcornea cordifolia* Schumach & Mill Arg (Table 1). *U. chamae* being the most active plant extract was partitioned using solvents of increasing polarity. Since infection models in mice have indicated that African trypanosomosis trigger anti-inflammatory responses (Namangala et al., 2001), the most active partitioned fraction was therefore evaluated for its anti-inflammatory response(s) in mice.

Materials and Methods

Plant collection and authentication

The fresh leaves and stem of *A. cordifolia* Schumach & Mill Arg (Euphorbiaceae), the stem bark of *T. ivorensis* A. Chev. (Combretaceae) and *U. chamae* (R.Br.) Pichon, (Apocynaceae) were collected from Obafemi Awolowo University (OAU) campus, Ile-Ife, Osun State. The plants were identified and authenticated by Mr. Bernard Omomoh, Herbarium Unit, Department of Botany, OAU Ile-Ife. Voucher specimens IFE HERBARIUM, 16790, 16398 and 16791 were deposited for *A. cordifolia, T. ivorensis and U. chamae* respectively. Other plant extracts of *Khaya senegalensis* (A. Juss) stem barks, *Harungana madagascariensis* (Choisy) Poir, the rhizome of *Curcuma longa* L., the stem of *Ocimum gratissimum* L., the leaves of *Aloe schweinfurthii* Bak., and *Achyranthes aspera* (Hook F.) leaf, were obtained from the extract store of the Drug Research and Production Unit (DRPU), OAU, Ile-Ife.

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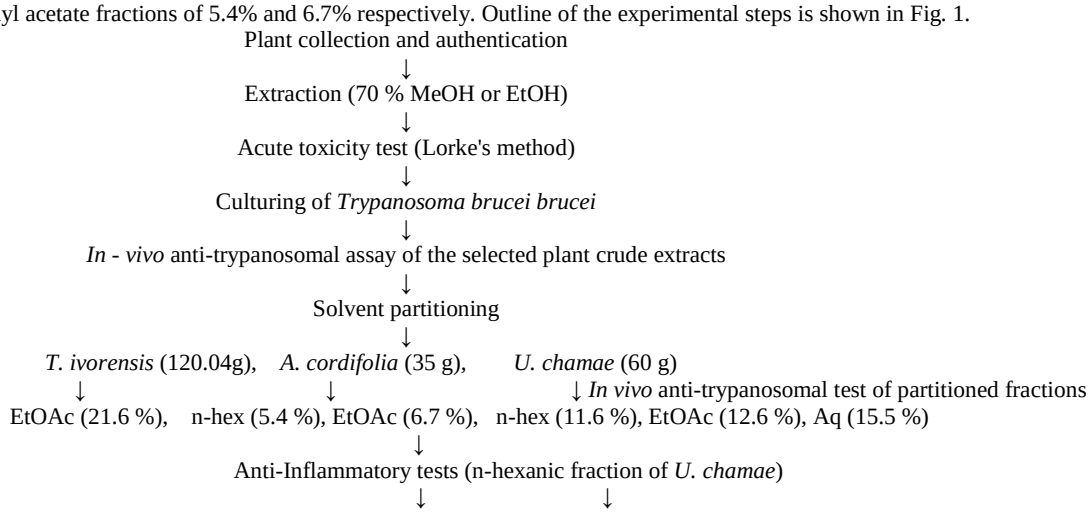
Table 1: The selected medicinal plants belong to different families and were evaluated for their potential as antitrypanosomosis agent.

Studied plant	Family	Local name(s)	Plant part used	Ethnomedicinal uses	References
Khaya senegalensis	Meliaceae	Ogonwo (Yoruba, Nigeria)	stem bark	Healing agent of burns skin ailments and wounds.	Makut et al., 2008
Harungana madagascariensis	Hypericaceae	Ayin (Yoruba), Marike (Hausa), Atara (Ibo).	stem bark	Sap used in the treatment of scabies and as an anthelmintic (tapeworms), leaves used as a remedy for hemorrhages, diarrhoea, gonorrhoea, sore throats, headaches and fevers.	Keay 1989, Dalziel,1956, Kokwaro, 2009.
Terminalia ivorensis	Combretaceae	Idigbo (Yoruba, Nigeria)	stem bark	Stem bark is boiled in salted water and used in treating chronic respiratory infections.	Katende et al., 1995. Ofukwu et al., 2008.
Curcuma longa	Zingiberaceae	Atale pupa (Yoruba) Gangamau (Hausa)	Rhizome	Stomach ache, skin problems muscular problems and arthritis.	Royal Botanical Gardens, 2012.
Ocimum gratissimum	Lamiaceae	Efinrin (Yoruba), Daidoya (Hausa), Nchonwu (Ibo).	Stem	Treatment of diarrhoea, dysentery and other gastrointestinal infections; upper respiratory tract infections associated with coughing, pneumonia, asthma and bronchitis; urogenital infections including sexually trans-mitted diseases, skin infections (dermatitis, eczema, scabies), wounds and ulcers.	Huxley**,** 1992. Adjanahoun et al., 1991, Prabhu et al., 2009, El-said et al., 1969 and Chukwuka et al., 2011.
Aloe schweinfurthii	Aloaceae	Gbadu (Nigeria)	Leaf	Healing agent of burns, skin ailments and wounds.	Burkill, 1985.
Uvaria chamae	Annonaceae	Akisan (Yoruba, Nigeria)	Leaf	Leaf sap is used to treat wounds and sores, leaf infusions is used as eye wash and leaf decoction is used as febrifuge.	Ainsilie, 1937
Alcornea cordifolia	Euphorbiaceae	Ewe ipa (Yoruba)	leaf and stem	Treatment of toothaches, snake bites, sore etc. diuretic etc.	Cesario, 1993.

Plant extraction and solvent partitioning

Terminalia ivorensis stem bark was oven dried at 60ºC, while the leaf and stem of *A. cordifolia* and the leaf of *U. chamae* were oven dried at 40ºC. Dried plant materials were powdered prior to extraction. *T. ivorensis* and *A. cordifolia* (1Kg each) were extracted in 70% ethanol separately, while *U. chamae* (1Kg) was extracted in 70% methanol. The samples were allowed to extract in the cold for 72 h with constant shaking using an electronic laboratory shaker. The filtrates were evaporated *in-vacuo* at 40ºC to a minimum volume using a rotary evaporator and lyophilized to complete dryness to give corresponding extract yields. *T. ivorensis* (14%), *U. chamae* (7.3 %) and *A. cordifolia* leaf and stem were 15.4 and 8.1 % respectively. *T. ivorensis* (120.04 g) was further partitioned into water and ethyl acetate (1:1v/v) to afford ethyl acetate fraction (21.6 %) yield. *U. chamae* (60g) gave the respective partitioned fractions; n-hexane (11.6 %), ethyl acetate (12.6 %) and aqueous (15.5 %), while *A. cordifolia* extract (35g) afforded n-hexane and ethyl acetate fractions of 5.4% and 6.7% respectively. Outline of the experimental steps is shown in Fig. 1.



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SAOMP test DHTR test

Figure 1: Outline of the procedures; SAOMP - Systemic Acute Oedema of the mice paw; DHTR - Delayed Type Hypersensitivity Reaction

Test organism

T. brucei brucei strain was obtained from Federal University of Technology, Minna, Niger State, Nigeria. The parasite was maintained in the laboratory in rats by continuous passage.

Determination of parasitaemia

Parasitemia was monitored in blood obtained from the mice tail, pre-sterilized with ethanol. The number of parasites was determined microscopically at X400 magnification using the "Rapid Matching" method (Herbert and Lumsden, 1976).

Experimental animals

Healthy mice weighing between 19-25g were purchased from the animal house of Faculty of Pharmacy, OAU, Ile-Ife and kept in well ventilated cages. They were exposed to 12h light and dark cycles and were fed with pelletized growers mash and water *ad libitum*. The animals were allowed a minimum of 7 days acclimatization before use. The animals were treated according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (National Academy Press, 1996).

Test for toxicity

Acute toxicity of the extract of *T. ivorensis* (oral and intraperitoneal routes) and *U. chamae* (oral route) were evaluated using established method (Lorke, 1983) with 4 groups (n=3) of animals. The control group (I) received 10 % Tween-20, the test groups (II, III, IV) received geometric doses of 10, 100 and 1000 mg/kg of the extracts respectively. The experimental animals were fasted for 12 h prior to administration of the extracts. The result obtained from phase 1 test determined the dose selection of 1600, 2900 and 5000 mg/kg for the phase 2 tests. The animals were closely observed for any signs of toxicity during the first 5 hours, and the number of deaths was recorded at 24, 48 and 72 h.

***In - vivo* antitrypanosomal assay**

For each plant extract, *in-vivo* antitrypanosomal screening was conducted using 25 mice inoculated with 10^5 *T. brucei brucei* parasites in 0.2ml of infected blood via the intraperitoneal route. The parasitaemia count was determined using the method of Herbert and Lumsden (1976). The animals were divided into 5 groups of 5 animals each. Group 1 mice received 10% Tween-20, groups 2, 3 and 4 mice received different individual doses of extracts ; (125, 250 and 500) mg/kg ethanolic crude extract and ethyl acetate fraction of *T. ivorensis* respectively, (3000, 1500 and 750) mg/kg of *C. longa*, (2500, 1250 and 625) mg/kg of *O. gratissimum*, (500, 250 and 125) mg/kg of *A. schweinfurthii*, (2000, 1000 and 5000) mg/kg of *H. madagascariensis*, (1000, 500 and 250) mg/kg of *A. cordifolia*, (150, 75 and 35) mg/kg of *K. senegalensis*, (1000, 500 and 250) mg/kg of *U. chamae* and (600, 300 and 150) mg/kg of *A. aspera*. Mice in group 5 served as the positive control and were treated with Diminazene aceturate® at 10 mg/kg. Administered doses of *T. ivorensis* and *U. chamae* were based on the experimental LD_{50} as determined in this study, while the LD_{50} of other extracts were used as reported in literature. All treatment commenced when the average parasitaemia was one parasite per field and treatment continued daily for 6 days. Wet blood film was carried out at every other day to estimate parasitaemia changes and mortality recorded.

Anti-inflammatory tests
Systemic acute Oedema of the mice paw

The systemic acute oedema of the mice paw was investigated using the method of Winter et al. (1962). In this experiment, animals (n=5/group) received 250, 500 or 1000 mg/kg of *U. chamae* n- hexane (the most active) fraction orally. Oedema was induced one hour later by injecting 0.1ml of egg albumin into the sub-planter region of the right hind paw of the mice. The volume of distilled water displaced by the treated paw was measured at 0, 1, 2, 3, 4 and 5 h after induction of oedema. The negative control group received 10 % Tween-20, while the positive control group received 100mg/kg indomethacin. Inflammation was assessed as follows:

$V_t - V_{0,}$ where V_0 = volume of the treated paw at 0 hr, and V_t = volume at various time.

Delayed type hypersensitivity reaction (DTHR) test

Swiss albino mice (20-25g) of either sex were divided into 5 groups. Each group (n=5) received 250, 500 or 1000 mg/kg of n-hexane fractions orally. Control animals received either Levamisole (2.5 mg/kg) or 10 % Tween-20. On day zero, one hour after the administration of the fractions, the mice were sensitised by injecting fresh sheep red blood cells (SRBCs) (0.1 ml of 7.3×10^6 cells/ml) intradermally into the right hind foot paw. Extract administration was continued daily for 7 days. On day 7, the sensitised animals were challenged by intradermal injection of SRBCs (0.1ml) into the paw of the left hind foot. The paw size was determined by the volume of distilled water displaced by the paw before and after 24h and 48h. Oedema formation was quantified as the difference in volume of the inflamed paw (Doherty, 1981).

Statistical Analysis

Data were analysed by employing the paired t-test (Snedecor and Cochran, 1967).

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Results

Acute Toxicity Tests

The result of acute toxicity of *T. ivorensis* and *U. chamae* showed that *T. ivorensis* (stem bark) extract had low toxicity with LD_{50} greater than 1600 mg/kg but lesser than 5000 mg/kg for the oral route, while the LD_{50} via the intraperitoneal route was 100 mg/kg. *U. chamae* leaf extract showed lower toxicity with LD_{50} of 5000 mg/kg (orally) with no mortality recorded in any test dose up to 5000 mg/kg. The LD_{50} values of *K. senegalensis* (stem bark), *H. madagascariensis* (bark), *C. longa* (rhizome), *A. cordifolia* (leaf), *A. aspera* (leaf) and *O. gratissimum* (stem) as obtained from literature (Table 2) were used for the dose selection for the anti-trypanosomal activity of the plants.

Table 2: Literature LD_{50} data of *K. senegalensis, H. madagascariensis, C. longa, A. cordifolia, A. aspera* and *O. gratissimum*

Plant	Plant part	Route of administration	LD_{50} values	Reference
K. senegalensis	Stem bark	Oral	>2000mg/kg	Ishaku et al., 2012
H. madagascariensis	Bark	Oral	6.25g/kg	Prosper et al., 2012
C. longa				
C. longa	Rhizome	Oral	10g/kg	Sittisomwong et al.,1990
	Rhizome	Intraperitoneal and subcutaneous	> 15g/kg	Sittisomwong et al.,1990
A. cordifolia				
A. aspera	Leaf	Oral	> 32g/kg	Donatien et al., 2010
O. gratissimum	Leaf	Oral	>2000mg/kg	Barua et al., 2012
	Stem	Oral	> 300mg/kg	Nema et al., 2011

In vivo Antitrypanosomal Experiments

In the *in vivo* antitrypanosomal experiments, parasites were first detected in all the infected groups on day 2 post-infection (Table 3). Parasite counts thereafter increased sharply afterwards except for Diminazene aceturate® treated control group with a complete clearance of parasite on day 3 post inoculation without relapse. In the test groups, extract and fractions of *U. chamae* showed a complete clearance of the parasite on day 5. However, relapse was observed on the day 7 post infection.

Anti-trypanosomal activity of the partitioned extracts of *U. chamae*

Animals treated with n-hexane and ethylacetate fractions of *U. chamae* had a longer survival period up to day 11 (Table 4). However, the initial clearance of parasitaemia was followed by a relapse and then death of the animals due to high parasitaemia level.

Table 3: *In vivo* Antitrypanosomal effects of crude extracts, ethyl acetate fraction of *Terminalia ivorensis*, Diminazene aceturate® and 10% Tween-20

Test Sample	Dose (mg/kg)	Death/ survival	Mean Trypanosome counts at day 0, 3, 5 and 7			
			0[a]	3	5	7
Control (untreated)	-	5/0	0	2.13±0.13	15.67±1.43	39.80±0.23
Diminal®	10	5/5	0	0.00±0.00**	0.00±0.00**	0.00±0.00**
TISE	500	5/0	0	1.07±0.29*	9.13±0.88	35.07±1.17*
	250	5/0	0	1.07±0.22*	6.20±0.77	38.07±0.71
	125	5/0	0	1.13±0.17*	7.87±1.01	37.20±1.82
TISEt	500	5/0	0	0.73±0.19**	16.07±1.93	31.13±1.64*
	250	5/0	0	0.87±0.25*	16.07±1.44	35.00±1.69
	125	5/0	0	0.47±0.13**	14.53±1.30	33.53±0.81**
UCL	1000	5/0	0	1.27±0.036*	0.00±0.00**	12.53±0.85**
	500	5/0	0	1.07±0.25	0.00±0.00**	21.93±0.87**
	250	5/0	0	1.27±0.13*	0.00±0.00**	26.60±0.34**
OGS	100	5/0	0	3.00±0.67	14.2±0.65	33.87±2.24*
	50	5/0	0	0.73±1.19*	12.47±0.77	34.67±1.23*
	25	5/0	0	1.40±0.50	13.13±1.47	32.87±0.40**
ASL	500	5/0	0	0.93±0.19**	11.67±0.84	26.60±1.90**
	250	5/0	0	1.47±0.20	11.67±1.07	32.20±0.87**
	150	5/0	0	1.00±0.24*	12.40±0.73	36.67±1.77
AAL	500	5/0	0	1.33±0.18*	12.13±0.77	34.60±0.86**

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	250	5/0	0	1.37±0.20	10.27±1.38	29.07±1.46**
	125	5/0	0	1.33±0.24	9.67±0.46*	27.60±3.70*
ACL	1000	5/0	0	1.20±0.20*	8.53±1.31**	31.00±1.29
	500	5/0	0	1.60±0.32	10.40±1.61**	30.80±1.91*
	250	5/0	0	1.20±0.27*	10.00±0.97	35.13±2.39
ACS	1000	5/0	0	1.20±0.23*	5.87±0.43**	28.33±1.70**
	500	5/0	0	1.07±0.13**	8.47±1.30**	34.00±1.28*
	250	5/0	0	1.67±0.11*	12.67±1.12	34.87±1.80
ACSh	30	5/0	0	1.31±0.22	11.21±1.50	29.51±1.31
	15	5/0	0	1.63±0.13	11.22±1.51	31.33±2.10
	7.5	5/0	0	1.41±0.51	10.81±2.51	33.24±1.04
HMS	2000	5/0	0	1.27±0.19*	13.87±1.56	35.67±1.51
	100	5/0	0	1.07±0.22**	13.80±0.69	38.00±1.23
	500	5/0	0	1.40±0.13*	14.13±0.89	34.40±1.88
CLR	3000	5/0	0	2.53±0.77	18.00±1.38	36.87±0.70*
	1500	5/0	0	1.47±0.17*	12.60±0.87	33.47±1.71*
	750	5/0	0	1.47±0.29	11.33±0.89	32.93±1.12**

Legend: *Values are Mean ± Standard Error of the Mean.* [a]*Day of infection,* **Significant as compared to untreated control group (P < 0.05),* ****Significant as compared to untreated control group (P < 0.01). TIS - Terminalia ivorensis (stem bark, crude ethanolic extract), TISEt - Terminalia ivorensis (stem bark, ethylacetate fraction), UCL - Uvaria chamae (leaf extract), OGS - Ocimum gratissimum (stem extract), ASL - Aloe schweinfurthii (leaf extract), AAL - Achyranthes aspera (leaf extract), ACL - Alcornea cordifolia (leaf extract), ACS - Alcornea cordifolia (stem extract), HRM - Harungana madagascariensis (stem bark extract), CLR - Curcuma longa (rhizome)*

Anti-Inflammatory activities of *Uvaria chamae*

Anti-Inflammatory activities of *U. chamae* **using systemic Acute Oedema of the mice paw**

The inhibitory effect of the hexanic fraction of *U. chamae* was expressed in all the doses administered (Table 5). Administration of the fraction profoundly suppressed the development of acute oedema of the mice paw. Generally, it was found to evoke an overall non-dose related effect with the lowest dose (250 mg/kg) exhibiting the highest inhibition at the 5th hour which was better than that of indomethacin (100 mg/kg). In comparison with the standard agent, Indomethacin, *U. chamae* n-hexane fraction at 1000 mg/kg inhibited inflammation all through the experimental time with the exception of the 5th hour as shown in Table 6.

Delayed Type Hypersensitivity Reaction (DHTR) test

In the Delayed Type Hypersensitivity Reaction (DHTR) anti-inflammatory test (Table 7), extract of *U. chamae* profoundly inhibited DTHR induced by SRBCs in a no-dose related manner. The effect of the lowest dose (250 mg/kg) on day 7(a) shows the left paw volume before challenge; day 7(b) and day 8 show the paw volumes at the 24th and 48th hours after challenge with SRBCs. The paw volume was most reduced by *U. chamae* at 1000mg/kg on day 8 (1.08 ± 0.08).

Table 4: Antitrypanosomal activities of partitioned fractions of *U. chamae*

Test fractions / administered doses	**Mean parasitemia counts at day 3, 5, 7, 9 and 11.**				
	3	**5**	**7**	**9**	**11**
Control (Untreated, 10% tween-20)	1.07±0.27*	9.33±0.49*	17.93±0.87*	33.00±0.73*	------
n-Hexane (1000 mg/kg)	0.00±0.00*	0.00±0.00**	1.13±0.23**	9.53±0.56**	13.73±8.41
Ethylacetate (1000mg/kg)	0.00±0.00*	0.27±0.19**	6.67±0.89**	22.13±1.93*	25.73±3.15
Aqueous (1000 mg/kg)	0.00±0.00*	9.3±0.19**	9.80±0.75**	25.53±1.53*	------
Diminal® (10 mg/kg)	0.00±0.00**	0.00±0.00**	0.00±0.00**	0.00±0.00**	0.00±0.00

Legend: Values are Mean ± Standard Error of the Mean, *Significant as compared to untreated control group (P < 0.05), **Significant as compared to untreated control group (P < 0.01), ----- 100% mortality)

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Table 5: Measured paw volume of the mice oedema by n-hexane fraction of *U. chamae*

	Paw Volume (cm) / dose (mg/kg)				
Hours	**0[a]**	**250**	**500**	**1000**	**Indomethacin**
0	1.13 ± 0.0250	1.22 ± 0.0200	1.24 ± 0.0245	1.28 ± 0.0250	1.24 ± 0.0245*
1	1.90 ± 0.0408	1.94 ± 0.0510	1.66 ± 0.0678	1.38 ± 0.0250*	1.60 ± 0.0632*
2	1.78 ± 0.0479	1.54 ± 0.0678	1.50 ± 0.0000*	1.23 ± 0.0250*	1.44 ± 0.0600*
3	1.83 ± 0.0854	1.38 ± 0.0583*	1.38 ± 0.0735*	1.40 ± 0.0707*	1.42 ± 0.0663*
4	1.78 ± 0.0250	1.32 ± 0.0735*	1.48 ± 0.0200	1.43 ± 0.0750*	1.54 ± 0.0510*
5	1.78 ± 0.0000	1.29 ± 0.0583*	1.32 ± 0.0583*	1.53 ± 0.0854*	1.48 ± 0.0490*

Legend: Values are Mean ± Standard Error of the Mean, [a]Untreated control group, *Significant as compared to untreated control group ($P < 0.05$)

Table 6: Percentage inhibition of the systemic acute oedema of the mice paw by the hexane fraction of *U. chamae*

Time (Hours)	Inhibition of systemic acute oedema of the mice paw (%)				
	Doses (mg/kg)	250 mg/kg	500mg/kg	1000mg/kg	Indomethacin
0	100	100	100	100	100
1	0	6	45	87	53
2	0	51	60	107	69
3	0	77	80	83	74
4	0	85	63	77	54
5	0	90	88	63	64

Table 7: Delayed Type Hypersensitivity Reaction (DTHR) test of the hexanic fraction of *U. chamae*

	Paw Volume (cm) / dose (mg/kg)				
Days	**Negative control [a]**	**250**	**500**	**1000**	**Levamisole**
0	0.88 ± 0.0490	0.96 ± 0.0400	1.03 ± 0.1453	0.90 ± 0.0577	0.95 ± 0.0500
7(a)	1.58 ± 0.0860	1.43 ± 0.0250	1.57 ± 0.0667	1.38 ± 0.0479**	1.38 ± 0.0629
7(b)	1.52 ± 0.0583	1.15 ± 0.0250*	1.40 ± 0.0577	1.22 ± 0.0800	1.20 ± 0.0707
8	1.26 ± 0.0510	1.10 ± 0.0408*	1.23 ± 0.0333	1.08 ± 0.0800	1.18 ± 0.0629

Legend: Values are Mean ± Standard Error of the Mean, 0 = paw volume of the left hind paw of mice before SRBC injection, 7(a) = paw volume of the right hind paw of mice before SRBC injection, 7(b) = paw volume of the right hind paw of mice after 24h of injection, 8 = paw volume of the right hind paw of mice after 48h of injection, *= significant as compared to untreated control group ($P < 0.05$), **= significant as compared to untreated control group ($P < 0.01$)

Table 8: Percentage inhibition of oedema in DTHR test of the hexanic fraction

	Inhibition of oedema (%)				
Days	**250**	**500**	**1000**	**Levamisole (2.5)**	**Negative control**
0	-	-	-	-	-
1	33	23	31	39	0
2	70	42	50	61	0
3	63	47	53	77	0

Legend: % inhibition of the hexanic fraction at different concentrations was significant ($P< 0.05$) compared with the negative control over the period of assessment. However, they were not significant compared with the reference drug levamisole.

Discussion

The LD_{50} values of *T. ivorensis* stem bark ethanolic extract was found to be 100 mg/kg intraperitoneally, but its oral toxicity was less than 5000 mg/kg. However, the LD_{50} of *U. chamae* by the same route of administration was found greater than 5000 mg/kg, and no mortality was observed in all the test groups. The result indicated the relative safety of both extracts to experimental mice. However, the differences observed in the LD_{50} values of *T. ivorensis* via the oral and intraperitoneal routes despite the fact that same extract and doses were administered could be due to different uptake mechanisms, absorptions, distributions and excretion of the extracts as earlier indicated (Patricia et al., 2005). In previous studies, the *in vitro*

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antitrypanocidal effect of *T. ivorensis* ethanolic stem bark extract was evaluated on calf endothelia cells with an LC_{50} value of 55.71µg/ml (Adewunmi et al., 2001). In another study, the ethylacetate and ethanolic crude extracts of this plant on *Trypanosoma brucei in vitro* showed ED_{50} of 11.11µg/ml and 12.32µg/ml respectively. However, in this current *in vivo* study, the previous results were not reproduced. The observed difference in activities might likely be due to inactivation of the plant's active molecules by metabolic transformation which inhibited the trypanocidal activity under *in-vivo* condition as observed with other antitrypanosomal agents (Balber et al., 1985).

The result for the *Ocimum gratissimum* screening in this work corresponds with earlier reports that no parasitemia clearance was observed *in-vivo* (Adamu et al., 2009). Therefore, it does not have trypanocidal effect. Other plants tested in this study did not exhibit significant clearance in parasitemia at the test doses administered. However, *U. chamae* extract treated groups demonstrated significant effect with a clearance of parasitemia at day 5 post inoculation (Table 4). Though a relapse was observed afterwards with some opportunistic manifestations like facial oedema and gradual loss of condition, the activity of the crude extract necessitated a successive partitioning into n-hexane (11.6%), ethyl acetate (12.6%), and aqueous (15.5%). The non-polar n-hexanic fraction showed a significant activity with about 50% reduction in parasitemia (13.73 ± 8.41) on day 11 of infection compared with the ethyl acetate having 25.73 ± 3.15 while the aqueous fractions recorded 100% mortality arising from very high level of infection in the animals. This result showed that the antitrypanosomal activity exhibited by *U. chamae* lies in the non-polar hexanic fraction.

This work shows the effect of *U. chamae* in acute and delayed type anti-inflammatory tests where it inhibited mice paw oedema induced by egg albumin (Tables 5 - 8). The anti-inflammatory effect of this plant was more pronounced in the acute test where hypersensitivity reaction was inhibited by 107% at the dose of 1000 mg/Kg (Table 6). This was further supported by the fact that immunosuppression has been linked with increased susceptibility of trypanosome hosts to secondary infections and has been further studied in this light (Doherty, 1981). This is a relevant phenomenon, as cattle infected in the field often die of opportunistic infections. Disease-associated suppression also affects host immune response to trypanosomes.

The presence of alkaloids, saponin, terpenoids, tannin and phenols has been found in the root of the plant (Okwu and Iroabuchi, 2009). Scientific evidence suggested that these classes of natural constituents exhibit antitrypanosomal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defences against oxidative stress (Sepulveda–Boza and Cassels, 1996). 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. Furthermore, a novel monobenzylated monoterpene (Chamanen) had been isolated from the root bark of *U. chamae* (William and Charles, 1977). Further studies could disclose the presence of a common moiety for possible interactions with a putative target receptor and a potential drug-likeness found in the leaf extract.

Conclusion

The n-hexanic fraction of *U. chamae* exhibited significant *in-vivo* antitrypanosomal and anti-inflammatory activities. Its anti-inflammatory responses in the experimental animals could be a probable mechanism for the antitrypanosomal activity demonstrated by the plant. Further purification of this fraction could indicate a better *in-vivo* antitrypanosomal activity and this is ongoing in our laboratory.

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