Int. J. Med. Arom. Plants, ISSN 2249 – 4340
Vol. 2, No. 1, pp. 185-194, March 2012

RESEARCH ARTICLE

The identification of putative antitrypanosomal compounds in *Tridax procumbens* extracts

A. ABUBAKAR[1*], E.O. OGBADOYI[2], J.I. OKOGUN[3], T.I. GBODI[4], G.F. IBIKUNLE[5]

[1]*Nigerian Institute for Trypanosomiasis Research, P. M. B. 1147, Birnin Kebbi, Kebbi State, Nigeria*

[2]*Global Institute for Bioexploration, Federal University of Technology, Minna/Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria*

[3]*Department of Traditional Medicine and Medicinal Plant Research, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria*

[4]*Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria*

[5]*Chemistry Department, Federal University of Technology, Minna, Niger State, Nigeria*

*****Corresponding Author, Tel: +2348035895490

Article History: Received 29th January 2012, Revised 20th February 2012, Accepted 20th February 2012.

Abstract: The therapeutic potential of *Tridax procumbens* extracts were screened for antitrypanosomal properties in mice. Whole *T. procumbens* was sequentially extracted with hexane, ethyl acetate, methanol and water. The extracts obtained were intraperitoneally administered at doses of 100, 200, 300 and 400mg/kg body weight respectively for 14 consecutive days to test for antitrypanosomal activity in mice infected with *Trypanosoma brucei brucei.* The ethyl acetate and methanol extracts gave a mean survival of 11.7 ± 5.4 and 14.3 ± 10.2 days respectively ($P<0.05$) when compared to untreated control. Phytochemical screening revealed the presence of steroids, saponins, tannins, alkaloids, flavonoids, phenols and carbohydrate in the crude methanol extract and phenols, flavonoids and steroids in the crude ethyl acetate extract. The bioassay-guided fractionation of the crude ethyl acetate and methanol extracts gave 12 and 11 fractions respectively. Fraction 11 of ethyl acetate exhibited better antitrypanosomal activities than fraction 7 of methanol which was significantly different from thier crude extract counterparts and the untreated controls ($P\leq 0.05$). Thin layer chromatographic (TLC) profile of the active ethyl acetate fraction 11 showed 3 spots, with preparative TLC band 2 producing highest antitrypanosomal effects. The TLC profile showed the presence of phenolic compounds. The spectrum of ^{13}C and ^{1}H NMR indicates the presence of sugars, fatty acids and phenolic related compounds namely cathechin, 3, 7 dihydroxyflavone, 3 – hydroxyflavone and quercetin. Although the demonstrated antitrypanosomal activities are insufficient, synthetic and/or chemical modification of detected phenolic compounds may generate effective antitrypanocidal drugs with novel modes of action.

Keywords: *Tridax procumbens*; *Trypanosoma brucei brucei*; *Antitrypanosomal*;^{13}C NMR; ^{1}H NMR; TLC.

Introduction

In tropical Africa, protozoan parasites cause several diseases of social and economic importance. One of the most devastating, trypanosomiasis is caused by infection with trypanosomes, which are transmitted by tsetse flies to people, domestic livestock and wildlife. The disease constrains agricultural development on over a third of the African continent by causing livestock production losses due to poor weight gains, stunted growth, poor milk production, reproductive failure and finally death (ILRAD, 1990). Diseases caused by protozoa are responsible for considerable mortality and morbidity throughout the world. There are an estimated 60 million people are at risk of infection with African trypanosomiasis, with about 300,000 new cases each year (WHO, 1998). It has been predicted that global warming will cause the spread of many tropical diseases (Sharp, 1996).

There are many problems with the currently available drugs for treating protozoal diseases since the development of vaccine against African trypanosomiasis remains elusive due to

*Corresponding author: (E-mail) abukadir65<A.T.>yahoo.com

antigenic variation in trypanosomes. Many of these drugs for treating trpanosomiasis are poorly tolerated because of side effects. Other problems include limited availability, prohibitive cost and increasing drug resistance (Tagboto and Townson, 2001). As a consequence of this, new, cheap, safe and effective drugs are urgently needed.

Tridax procumbens is known for several potential therapeutic activities like antiviral, antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity (Suseela et al 2002). The plants has been extensively used in Ayurvadic system of medicine for various ailments and is shown to possess significant antiinflamatory, hepatoprotective, wound healing and antimicrobial properties (Diwan et al., 1989; Pathak et al., 1991; Saraf et al., 1991; Udupa et al., 1991; Perumal et al., 1999; and Taddei and Rosas 2000). The entire plant is used by indigenous people in Gautemala for the treatment of protozoal infections (malaria, leishmaniasis, vaginitis, dysentry) and gastrointestinal disorders (colic/stomach pains, gastritis/enterocolitis) (Caceres et al., 1998; Berger *et al.*, 1998).

The main aim of these studies is to obtain possible compounds from *T. procumbens* capable of treating African trypanosomiasis which may form the basis for the chemical synthesis of modern pharmaceuticals.

Methodology

Parasites (Trypanosoma brucei brucei)

A stabilate of *Trypanosoma brucei brucei*, a parasite originally isolated from cattle in Lafia, Nasarawa State and kept in liquid nitrogen at the Nigerian Institute for Trypanosomiasis Research Vom, Plateau State was used. It was maintained in rats by serial passaging.

Plant Materials

The *Tridax procumbens* were collected in the months of May and June within and around Kaduna Vom, Plateau state and FUT Minna, Bosso campus, Niger State, Nigeria. The plant was identified with a voucher number, NIPRD/H/6155, and was deposited at the herbarium of National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.

Preparation of Plant Materials

About 1 kg of the *Tridax procumbens* was freshly obtained washed with running tap water and dried at room temperature to a constant weight. The dried plant samples were grinded into powder form using mortar and pestle and the powdered samples were stored in clean polythene bags until required for use.

Phytochemical Analysis

The crude extracts were screened for the presence of alkaloids, saponins, sterols, tannins, flavonoids, phenols, carbohydrate, tarpenes, resins, and anthraquinones using simple chemical tests as variously described by Evans (1989) and Sofowora (1993).

Preparation of Crude Extracts

The extracts were prepared using the method described by Ogbadoyi et al., (2007). In this method, fifty grams (50g) of the dried powdered samples of *Tridax procumbens* were extracted sequentially under reflux with 400ml of hexane, ethyl acetate, methanol and water in that order for 2 hrs in each case. Extracts were filtered hot using muslin cloth and solvent was removed using rotatory evaporator for organic solvents and freeze-drier for water extracts. The extracts, are finally dried in steam bath and transferred into sterile sample bottles for storage at refrigerated temperature until when required for use. The residue was dried after each extraction for the next extraction process.

Initial Screening of the Extracts for Antitrypanosomal Activities

In *Tridax procumbens* the ethyl acetate, methanol and aqueous extracts of the whole plants were subjected to the screening against *T. b. brucei* infected mice. For each extract, there were 7 groups (A - G) of 3 mice each. Groups A

– F were each inoculated with 0.1ml of the inoculum containing about 10^6 trypanosomes and with the appearance of parasitaemia, animals in Groups A – D were each treated intraperitoneally with the extract at 100, 200, 300 and 400 mg/kg body weight respectively for 14 consecutive days. Mice in Group E were treated once intraperitoneally with berenil at 3.5 mg/kg body weight while Group F was untreated and Group G was neither infected nor treated and served as control. Parasitaemia in the blood of infected animals was monitored daily and estimated using the "Rapid Matching Method" described by Herbert and Lumsden (1976).

Bioassay- Guided Fractionation of Tridax procumbens Extracts

In column chromatography, the stationary phase, a solid adsorbent (Silica gel- 60-120 mesh) soaked in hexane, was placed in a vertical glass column and the mobile phase, hexane, was added to the top and allowed to flow down through the column by gravity. Both the methanol and ethyl acetate extracts were separately subjected to column chromatography to obtain various fractions. Consequently, 12 fractions were obtained for ethyl acetate extract and 11 fractions were obtained for methanol extract. Using the RF values of the fractions after visualization of spots with iodine vapour and acid spray, fractions with same values were pooled together as single fractions from which their anti-trypanosomal activities were compared at 200mg/kg body weight for ethyl acetate and 300mg/kg body weight for methanol extract. The fraction with highest activity was further subjected to analytical and preparative thin layer chromatography developed in chloroform and methanol (4: 1). The plates were removed from the tank, air dried and visualised with sulphuric acid spray and ultra violet light at 366nm. The bands obtained from Preparative Thin Layer Chromatography were separately screened for antitrypanosomal activity using five groups (A – E) of three mice each inoculated with *T. b. brucei* and groups A – C were separately treated with bands 1 – 3 respectively. Group D was treated with fraction 11 while group E was infected untreated and group F served as uninfected untreated. All the animals were treated at 20 mg/kg body weight intraperitoneally for 3 consecutive days. The parasitaemia was monitored daily and mean survival and prolongation of life were determined.

Determination of Chemical Composition of Active Fractions

The band obtained from ethyl acetate Fraction 11 was subjected to Nuclear Magnetic Resonance (NMR) analysis in order to elucidate the chemical composition of each band of the active fraction. In addition, the TLC profiles of the 3 bands that constitute the fraction 11 were also determined using normal and reverse phase TLC. The plates were developed in dichloromethane and methanol (4:1) for the normal phase and 50% methanol for reverse phase. The plates were then visualized under UV light at 366 and 254nm and sprayed with 10% vanillin in concentrated sulphuric acid and ferric chloride solution.

Results

Treatment with the *Tridax procumbens* at all the dose levels resulted to lowering of the parasitaemia leading to prolongation of life (Figures 1 to 3). Ethyl acetate and methanol extracts had the best trypanostatic effect resulting to significant prolongation of life by 11.7 and 14.3 days respectively ($P<0.05$). Antitrypanosomal effect of *T. procumbens* of different ethyl acetate fractions (Figures 4-5) showed that fraction 11 gave the highest survival period of 15 days ($P<0.05$) (Table 2) while fraction 7 of methanol gave the highest survival period of 12.3 days (Table 3). The Rf value of 0.4 in ethyl acetate fraction 11 was absent from methanol fraction 7, crude extracts of methanol and ethylacetate (Table 5) . Antitrypanosomal activities of different ethyl acetate bands obtained after PTLC (Figure 8) showed that band 2 gave the highest survival period of 3 days while band 3 recorded the least of 1 day (Table 4). Phytochemical analysis of the crude extracts showed that flavonoid is highly pesent in ethyl acetate than in methanol extract (Table 6).

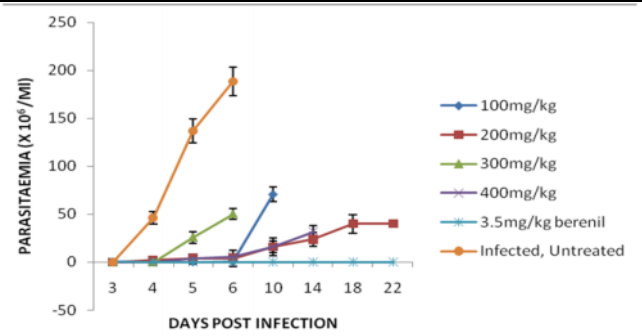

Figure 1: Effect of Different Doses of *Tridax procumbens* Ethyl acetate Extract on *T. b. brucei* Infected Mice

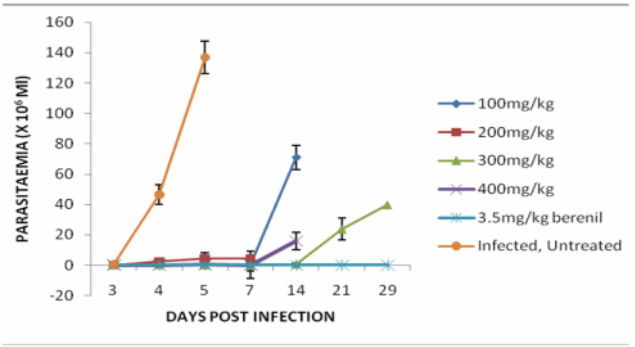

Figure 2: Effect of Different Doses of *Tridax procumbens* Methanolic Extract on *T. b. brucei* Infected Mice.

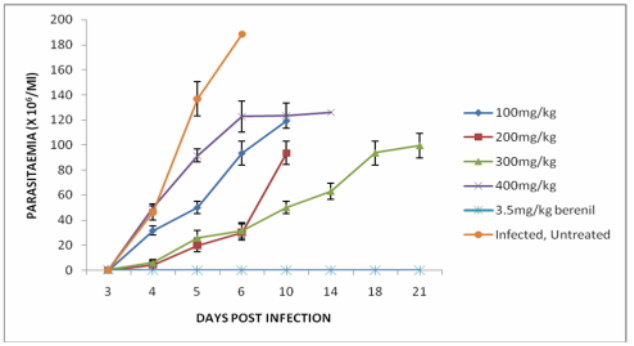

Figure 3: Effect of Different Doses of *Tridax procumbens* Aqueous Extract on *T. b. brucei* Infected Mice.

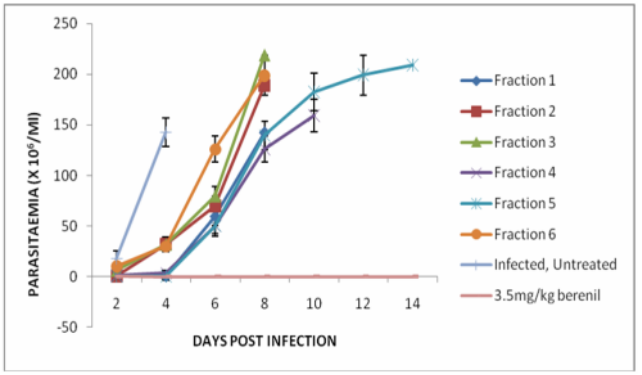

Figure 4: Effect of *Tridax procumbens* Ethyl acetate Fractions (1-6) on *T. b. brucei* Infected Mice.

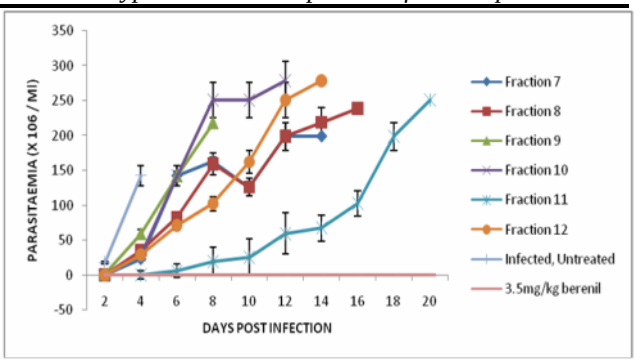

Figure 5: Effect of *Tridax procumbens* Ethyl acetate Fractions (7-12) on *T. b. brucei* Infected Mice.

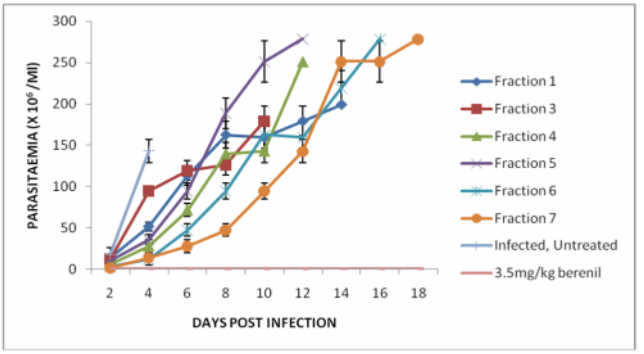

Figure 6: Effect of *Tridax procumbens* Methanol Fractions (1-7) on *T. b. brucei* Infected Mice.

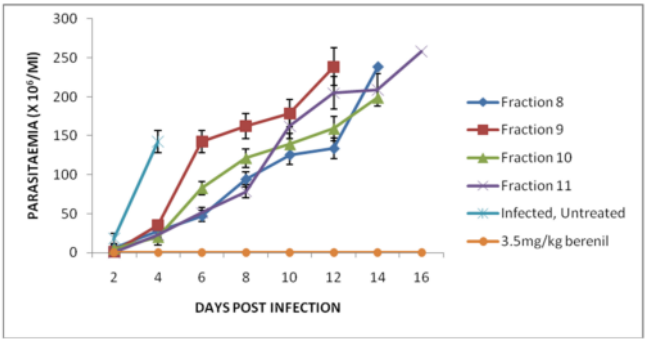

Figure 7: Effect of *Tridax procumbens* Methanol Fractions (8-11) on *T. b. brucei* Infected Mice.

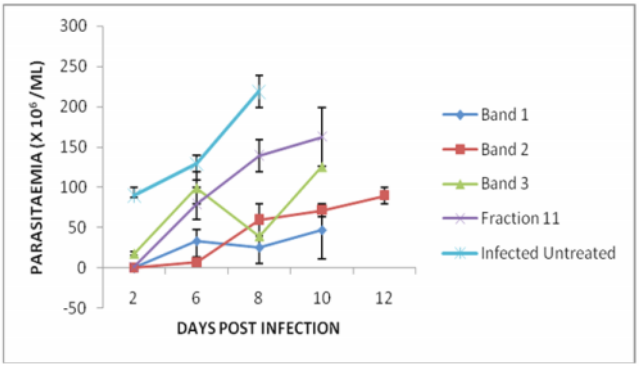

Figure 8: Effect of *Tridax procumbens* Bands (1-3) of Fraction 11 on *T. b. brucei* Infected Mice.

Table 1: Summary for Initial Screening of *Tridax procumbens* Extracts

Extraction Solvent	Dose (mg/Kg)	Survival Range	Survival beyond Control (Days) Min	Max	Means Survival (±SD)
Ethyl acetate	100	8-10	3	4	3.6±0.5
	200	9-22	4	16	11.7±5.4
	300	7-9	2	3	2.7±0.8
	400	6-16	1	10	6.3±3.8
	100	6-17	1	11	7.0±4.3
Methanol	200	6-10	1	4	2.6±1.2
	300	7-30	2	24	14.3±10.2
	400	5-15	0	10	5.6±4.2
Aqueous	100	8-10	3	4	3.7±0.5
	200	7-12	2	6	4.3±1.7
	300	7-21	2	15	8.0±5.4
	400	8-16	3	10	7.3±3.1
	Infected, Untreated	5-6	-	-	-

Table 2: Summary of Screening of *T. procumbens* Ethyl acetate Fractions

Fraction Type	Survival Range (Days)	Survival Beyond Control(Days) Min.	Max.	Means Survival (±SD)
Fraction 1	8	-	3	3.0±0.0
Fraction 2	8	-	3	3.0±0.0
Fraction 3	8	-	3	3.0±0.0
Fraction 4	11	-	6	6.0±0.0
Fraction 5	11-15	7	10	8.2±1.2
Fraction 6	7-8	3	3	2.7±0.5
Fraction 7	9-14	5	9	7.0±1.6
Fraction 8	9-16	5	11	8.3±2.5
Fraction 9	8-9	4	4	3.7±0.5
Fraction 10	8-13	4	8	6.0±1.6
Fraction 11	18-21	14	16	15.0±0.8
Fraction 12	10-15	6	10	8.0±1.6
Crude	11-15	7	10	8.7±1.2

Table 3: Summary of Screening of *T. procumbens* Methanol Fractions

Fraction Type	Survival Range (Days)	Survival Beyond Control (Days) Min.	Max.	Means Survival (±SD)
Fraction 1	9-14	5	9	7.0±1.6
Fraction 2	NIL	-	-	-
Fraction 3	7-11	3	6	4.7±1.2
Fraction 4	10-12	6	7	6.7±0.5
Fraction 5	9-12	5	7	6.0±0.8
Fraction 6	10-16	6	11	8.6±2.1
Fraction 7	14-19	10	14	12.3±1.7
Fraction 8	13-14	9	9	9.0±0.0
Fraction 9	9-13	5	8	6.7±1.2
Fraction 10	9-15	5	10	7.7±2.1
Fraction 11	12-16	8	11	9.7±1.2
Crude	11-13	7	8	7.6±0.5
Positive Control	4-5	-	-	-

Table 4: Summary of Screening of *T. procumbens* Ethyl acetate Bands and Fraction 11

Type	Survival Range (Days)	Survival Beyond Control (Days) Min.	Max.	Means Survival (±SD)
Band 1	10-11	2	2	2.0±0.0
Band 2	11-12	3	3	3.0±0.0
Band 3	8-10	0	1	0.5±0.5
Fraction 11	10-11	2	2	2.0±0.02
Infected, Untreated	8-9	-	-	-

Table 5: Rf Values of *Tridax procumbens* Crude and Fractions Using Choloroform and Methanol (4:1) as Mobile Phase.

Types of Extract	Rf Values	Visualization
Crude Methanol	0.67	Visible
	0.93	Acid Spray
Methanol Fraction	0.93	Visible
Crude Ethyl Acetate	0.67	Acid Spray
	0.84	Acid Spray
	0.93	Visible
Ethyl Acetate Fraction	0.40	UV Light/Acid Spray
	0.67	UV Light/ Acid Spray
	0.84	Acid Spray
	0.93	Visible

Figure 9: TLC plate of Different *T. procumbens* extract (Solvent = Chloroform: Methanol, 4:1) Where MC = Crude Methanol, MF = Methanol Fraction, EC = Crude Ethyl acetate, EF = Ethyl acetate Fraction.

NMR result and TLC profile of the Bands

The chemical compounds present were deduced from the standard NMR correlation charts. From the spectra data obtained (Figures 13-15), the ^{1}H NMR of active band 2 shows olefinic hydrogens between 6.6 and 7.7 delta typical of aromatic hydrogen absorptions as shown by phenolic compounds including flavonoids. The spectrum also shows –CH-O- hydrogen absorptions typical of sugar fragments in the region 3.3 – 3.8 delta. Probable absorptions due to impurity from the Band 1 sample are also present. ^{13}C NMR confirms olefinic / aromatic carbon absorptions at 130.03δ and 115.67δ, -CH-O, CH_2-O carbon absorptions typical of sugar fragments between 102 and 61ppm and polymethylene group absorption at 29.581ppm. There are weak absorptions around 200, 190, 170, 158, 140 and 134ppm some of which are ascribable to aromatic carbonyl compounds including phenols and flavonoid compounds.

Figure 10: Normal Phase TLC Sprayed with Vanillin/Sulphuric Acid.

Table 6: Phytochemical Constituents of *T. procumbens* Crude Methanol and Ethyl acetate Extracts

Phytochemical	Methanol	Ethyl acetate
Carbohydrates	+	-
Steroids	+	++
Saponins	+	-
Flavonoids	++	+++
Tannins	+	-
Alkaloids	+	-
Anthraquinones	-	-
Resins	-	-

Notations: +++ Highly present, ++ Moderately present, + Present, - Absent.

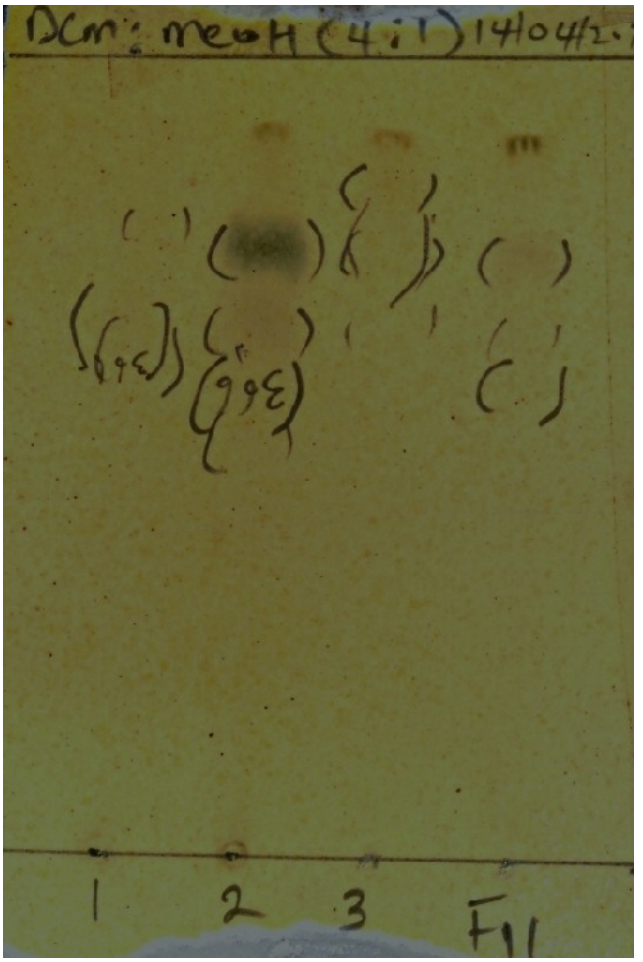

Figure 11: Normal Phase TLC Sprayed with Ferric Chloride Solution

The result of normal phase TLC detected by vanillin in sulphuric acid indicates a prominnent yellow reacting spot at Rf 0.81 common to both the active fraction 11 as well as the PTLC band 2 (Figure 10). Similarly, another normal phase TLC detected by ferric chloride solution indicate a normal blue-black reacting spot (phenol) at Rf 0.81 common to both the fraction 11 and

PTLC band 2 (Figure 11). The prominent colour was yellow in vanillin/ sulphuric acid sprayed while it is blue-black in ferric chloride sprayed. The Rf value is also the same in normal phase TLC plates but was however lower in reverse phase TLC plate (Figure 12). The yellow reacting spot in vanillin/H_2SO_4 is the same as the black-blue reactions of spot in ferric chloride suggesting the spot to be phenolic. The compound present is related to flavonoids eg flavones, 7, 8 Dihydroxyflavone, 3 Hydroxyflavone, Genistein, Quercetin and catechin which all have phenolic groups. Their structures are hereby presented in Figure 16.

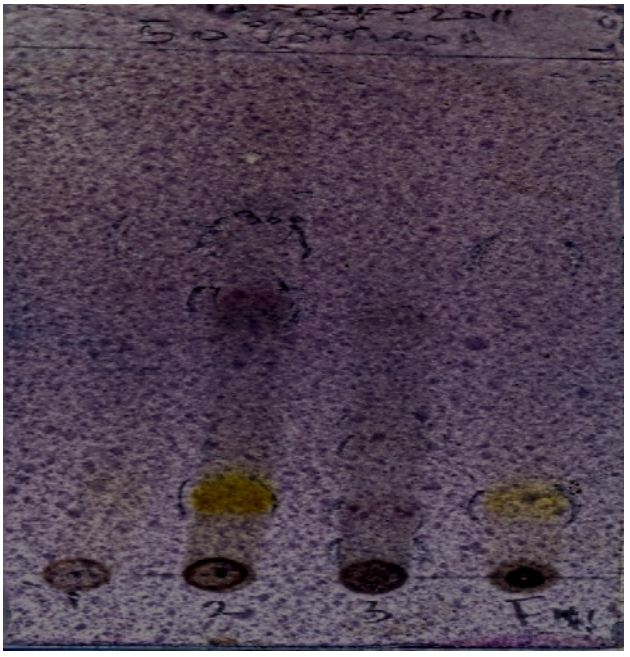

Figure 12: Reverse Phase TLC Sprayed with Vanillin/Sulphuric Acid
KEY: 1= BAND 1; 2= BAND 2; 3= BAND 3; F11= FRACTION 11

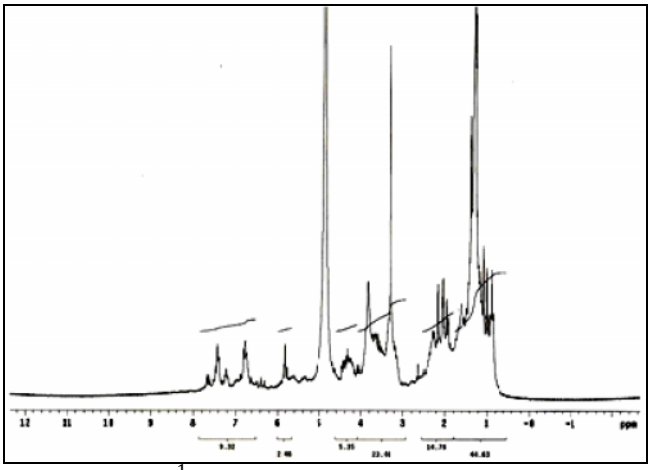

Figure 13: ^{1}H NMR Spectrum in Deuterated Methanol of Band 2

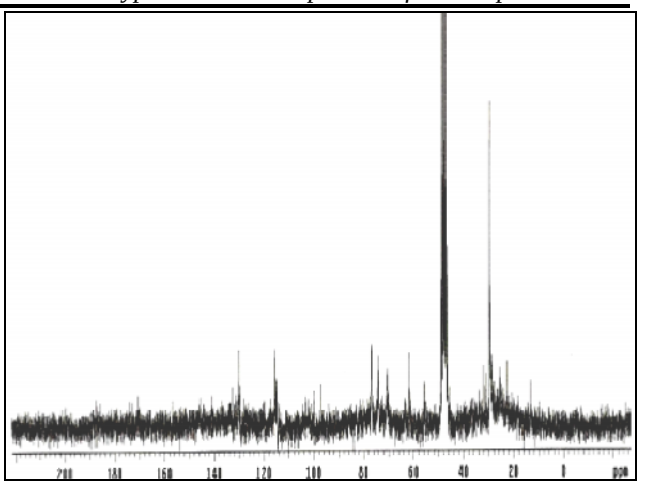

Figure 14: ^{13}C NMR Spectrum in Deuterated Methanol of Band 2

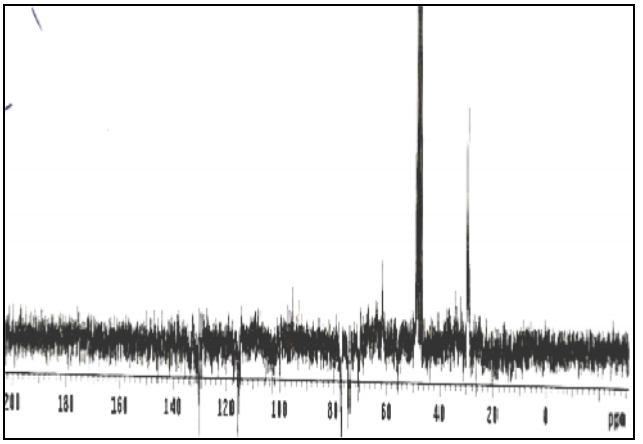

Figure 15: Attached Protein Test ^{13}C NMR in Deuterated Methanol of Band 2

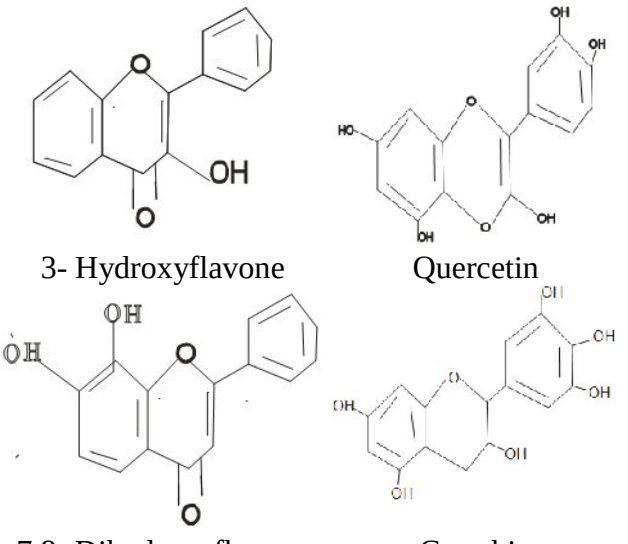

Figure 16: Possible Chemical Compounds in B and 2

Discussion

Extracts of *Tridax procumbens* have shown antitrypanomal potential against *T. b. brucei* infected mice. Both the prolongation of life and suppression of parasitaemia in infected animals is possible probably because *T. procumbens* was earlier reported to have immunomodulatory activity which suggest it's therapeutic usefulness (Tiwari et al., 2004). *T. procumbens* has been demonstrated to stimulate both humoral as well as cell mediated immune system vis-a-vis assists in genesis of improved antibody response against specific clinical antigen (Tiwari et al., 2004). Since trypanosome infection causes immunosuppression, any herbal preparation that is immune boosting may have a significant effect on trypanosomes. Infection with trypanosomes has been known to impair the immune system of the host, cause anaemia, weight loss, reproductive disorders and death of animals if not treated (ILRAD 1991). Consequently in the presence of antigen (parasites) in the blood circulation, the administration of *Tridax procumbens* therefore may have activated the lymphocytes to increase the effectiveness of antigen clearance by phagocytosis or to secrete various immune effector molecules. This may be possible reason for the observed low parasitaemia and prolongation of life beyond the untreated control of extracts of this plant investigated.

Furthermore, the presence of various phytochemicals particularly, flavonoid in this plant could be an additionally responsible for the observation recorded. An important effect of flavonoids is the ability to scavenge oxygen-derived free radicals (Robert, et al. 2001). *In vitro* experimental systems also showed that flavonoids possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties (Middleton, 1998). Therefore any anticarcinogenic plant may serve as a good source of trypanocide since eflornithine currently in use to treat sleeping sickness is known to have some level of anticancer activities (Barrett and Barrett, 2000).

The antitrypanosomal activity was not high in the first few fractions collected where the polarity was low while the activities increased towards the last fractions where the polarity was high. This implies that the active compound must be a highly polar compound. Fraction 7 of methanol that gave the highest survival period of 11.3 days is not as polar as fraction 11 of ethyl acetate. It is possible therefore, that the remaining compound that was not completely extracted by ethyl acetate during the successive extraction was now removed by methanol hence this observation. The results obtained in these studies tend to lay credence to the fact that polar solvents as extracting solvents, have the ability to extract phytochemicals that exhibit strong antitrypanosomal profile, thus making methanol and ethyl acetate good extracting solvents for plants that are reported to have antitrypanosomal activity. This is because all the fractions of the 2 extracts showed antitrypanosomal activity and prolonged the survival of the treated animals. The antitrypanosomal activity exhibited by both the crude and fractions of *T. procumbens* was very obvious because mice in the control group that were infected but not treated presented massive parasitemia culminating in death within one week post infection.

On the basis of prolongation of life, band 2 gave the highest activity of three days while band one and crude gave 2 days each. Consequently, there could be presence of certain compound in this band that has been responsible for the antitrypanosomal activity recorded throughout the screening process. Band 2 contain high amount of phenolic compound (flavonoid) when compared with both the crudes and other bands. This could be reason for the observed highest activity.

The bioassay guided fractionation of crude extract of *T. procumbens* shown that the antitrypanosomal activity was more in fraction 11. The result of both proton and ^{13}C- NMR, generated spectra that indicated the presence of phenols (aromatic compound) which include flavonoids. Phenols give a characteristic blue-black colouration with ferric chloride and yellow with vanillin/H_2SO_4 solution. Thus, this implies that the aromatic compound indicated by NMR suggest it to be phenols. Phenols and flavonoids have been known for a long time to exert diverse biological effects and bioflavonoids in particular to act as antioxidants and preventive agents against cancer (Harborne and Williams, 2000). They are also common constituents of medicinal plants, and the therapeutic effects of many traditional

medicines have been ascribed to these phytochemicals.

The antitrypanosomal activity observed in this study could be as a result of high concentration of the phenolic compounds possibly 7, 8-dihydroxyflavone may be included in the active band 2. This is because, the antitrypanosomal activity produced was similar to the activity obtained when the pure compound was screened for activity (Tasdemir et al, 2006). 7, 8-dihydroxyflavone significantly reduced the level of parasitemia; but the mice relapsed and showed a mean survival of 13 days, which represents an extension of the length of survival of 6 days for the untreated control group (Tasdemir et al, 2006). In this study, the partially purified fraction and band 2 recorded higher mean survival than the crude. Therefore, the prolongation of life observed a higher mean survival could also be due the presence of this compound in the band 2 acting synergistically with other phenolics. However, since metabolism might transform one class of flavonoid into another (Nikolic and van Breemen, 2004) new pharmacological activity or the loss of previous activity may be observed. All these facts may underlie the absence of notable *in vivo* activity for the majority of the polyphenols. Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a potent immunomodulating flavonoid, was shown to directly induce the death of *Trypanosoma brucei gambiense*, the causative agent of HAT, without affecting normal human cell viability (Mamani-Matsuda et al 2004). Quercetin was reported to directly promote *T. b. gambiense* death by apoptosis. These results highlight the potential use of quercetin as an antimicrobial and anti-inflammatory agent for the treatment of African trypanomiasis. However, 7, 8-dihydroxyflavone and quercetin appeared to have some *in vivo* activity and therefore, could serve as pharmacophore models for the rational design of synthetic analogs with higher *in vitro* and *in vivo* activities and more favourable chemical properties.

Conclusion

The result of this study has shown that both the ethyl acetate and methanol extracts of *T. procumbens* exhibited antitrypanosomal activity which was due to the presence of flavonoids. Based on the TLC profile and NMR analysis, the fraction with highest antitrypanosomal activity contains flavonoid compounds.

Acknowledgements: This work was funded in part by a research grant from the Organization for the Prohibition of Chemical Weapons to Emmanuel O. Ogbadoyi

References

Barrett S. V. and Barrett M. P. 2000. Anti-sleeping sickness drug and cancer chemotherapy. *Parasitolology Today* 16 (1), 7-9

Berger, T., Barrientos, A. C., Caceres, A., Hernandez, M., Rastrelli, L., Passreiter, C. M. and Kubelka, W. 1998. Plants used in Gautemala for the treatment of protozoal infections. *Journal of Ethnopharmacology*; 62:107 – 115.

Caceres, A, Lopez, B., Gonzalez, S., Berger, T., Tada, T. and Maki, J. 1998. Plants used in Gautemala for the treatment of protozoal infections. 1. Screening of activity to bacteria, fungi and American trypanosomes of 13 native plants. *Journal of Ethnopharmacology*; 62:195 – 202.

Diwan P. V., Karwande I., Margaret I. and Sattar P. B. 1989. Pharmacology and biochemical evaluation of *Tridax procumbers* on inflammation. *Indian journal of pharmacology* 21. Pp 1 – 7.

Evans W. C. 1989. Trease and Evans Pharmacognosy. 13th Edn., Balliere Tindall, London, pp: 378-689.

Harborne, J. B., and Williams C. A. 2000. Advances in flavonoid research since 1992. *Phytochemistry* 55**:**481–504.

Herbert, W.J. and Lumsden, W.H.R. 1976. *Trypanosoma brucei*: A rapid "matching" ethod for estimating the host's Parasitaemia. *Experimental Parasitology* 40: 427-31.

ILRAD, 1990. July and October report of the International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

ILRAD, 1991. Annual report of the International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

Mamani-Matsuda, M., Rambert, J., Malvy, D., Lejoly-Boisseau, H., Daulouède, S., Thiolat, D., Coves,S., Courtois, P., Vincendeau, P., and Djavad Mossalayi M. 2004. Quercetin Induces Apoptosis of *Trypanosoma brucei gambiense* and Decreases the Proinflammatory Response of Human Macrophages. *Antimicrobial Agents Chemotherapy*. 2004 March; 48(3): 924–929.

Middleton E. J. 1998. Effect of flavonoids on immune and inflammatory cell function. Adv. Exp. Med. Biol., 439 : 175- 182

Nikolic, D., and R. B. van Breemen. 2004. New metabolic pathways forflavanones catalyzed by rat liver microsomes. *Drug Metabolism and Disposal*. **32:**387–397.

Ogbadoyi, E.O., Hafsatu, B., Adamu. Y. K., Chinenye. E. N., Solomon**,** I., Rasheedat, B.A., Emmanuella, I.O., Paul, B.M., Akinsunbo, A.O and Theophilus, Z.A. 2007. Preliminary Studies of Antitrypanosomal Properties of Selected Nigerian Medicinal Plants. *Journal of Research in Bioscience*., **3** (3):38-43.

Pathak AK, Saraf S, Dixit V.K. 1991. Hepatoprotective activity of *Tridax procumbens* - Part I. *Fitoterapia*; 62:307-13.

Perumal, S.R., Ignacimuthu, S. and Raja, D.P. 1999. Preliminary screening of ethnomedicinal plants from India. Journal of Ethnopharmacology, 66; pp. 235-240

Udupa S. L., Udupa A.L. and Kulkarni D.R. 1991. Influence of *Tridax procumbens* on lysyl oxidase activity and wound healing. *Planta Medica* 57: 525-327

Saraf S, Pathak AK, Dixit V.K. 1991 Hair growth promoting activity of *Tridax procumbens* . *Fitoterapia*;62:495-8.

Sharp, D. 1996. Malaria range set to spread in a Warmer World. *The Lancet* 347: 1612.

Sofowora A. 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books, Ibadan, pp: 150.

Suseela, L.; Sarsvathy, A.; Brindha, P. 2002. Pharmacognostic studies on *Tridax procumbens* L. (Asteraceae)". *Journal of Phytological Research* **15** (2): 141-147.

Taddei A. and Rosas R. A. J. 2000. Bioactivity studies of extracts from *Tridax procumbens. phytomedicine* 7: 235 – 238.

Tagboto S. and Townson S. 2001. Antiparasitic properties of Medical Plants and other naturally occurring products. *Advances in Parasitology*, 50: 200-295.

Tasdemir D., Kaiser M., Brun R., Yardley V., Schmidt T. J., Tosun F., and Ruedi P. 2006. Antitrypanosomal and Antileishmanial Activities of Flavonoids and Their Analogues: In Vitro, In Vivo, Structure-Activity Relationship, and Quantitative Structure-Activity Relationship Studies. Antimicrobial Agents and Chemotherapy, p. 1352–1364 Vol. 50, No. 4. *American Society for Microbiology.*

Tiwari U., Rastigi B., Singh P., Saraf D.K. and Vyas S.P. 2004. Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals. *Journal of Ethnopharmacology*, Vol 92, Issue 1,Pages 113-119

Udupa S. L., Udupa A. L. and Kulkarni D. R. 1991. Influence of *Tridax procumbers* on lysyl oxidase activity and wound healing. *Planta Medica* 57:525 – 327.

WHO 1998. Control and surveillance of African trypanosomiasis. Report of a WHO Committee World Health Organization Technical Report Series 881, I-VI, 1-114. Geneva: World health Organization.