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Phytochemical Screening and *In Vitro* Antitrypanosomal Activity of Aqueous and Methanol Leaf Extract of *Verbascum sinaiticum* (Scrophulariaceae) against *Trypanosoma congolense* Field Isolate

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Abstract

Aqueous and methanol leaf extracts of *V. sinaiticum* were investigated for the presence of secondary metabolites and their *in vitro* activity against *Trypanosoma congolence*, the main causative agent of African animal trypanosomosis in Sub-Saharan Africa and Ethiopia. The *in vitro* assay was carried out by monitoring test concentrations of 4, 2, 1, 0.4 and 0.2 mg/ml for cessation or reduction in motility of trypanosomes followed by monitoring for loss of infectivity to mice. Phytochemical screening revealed presence of alkaloids, flavonoids, glycosides, phenolic compounds, saponins, steroids and tannins. An appreciable *in vitro* activity was attained by the methanol extract of *V. sinaiticum* at 4 mg/ml concentration. In general, the results obtained suggest ethno-pharmacological usefulness of the plant and necessitate further studies to be carried on isolated active substances from the plant.

Keywords: Trypanosomosis; *Trypanosoma congolense*; *Verbascum sinaiticum*

Introduction

Trypanosomosis is a potentially fatal disease of humans and domestic animals in tropical Africa and South America [1]. The disease has undergone a dramatic and devastating resurgence in recent years especially in Sub-saharan Africa [2]. Some 50 million people in 36 African countries are at the risk of acquiring the infection. Recently, it was estimated that 300,000 to 500,000, people are currently infected and more than 100 deaths are caused each year by the disease [3].

Human African trypanosomiasis (HAT) is caused by the haemoflagellate, *Trypanosoma brucei gambiense* in West and Central Africa, and *Trypanosoma brucei rhodisiense* in Eastern Africa, while African animal trypanosomosis (AAT) is caused by *Trypanosoma brucei brucei*, *T. vivax* and *T. congolense*., which affect health of cattle and other livestock [4]. Thus, the significance of trypanosomiasis to human health, nutrition and economy is enormous.

The fight against the vector (tsetse fly) has not been very successful, and the chemicals used as part of the control measures pollute the environment. Immunization against trypanosomosis has not been possible because of the problem of antigenic variation. Therefore, chemotherapy continues to play a major role in the management and control of trypanosomosis. This is essential because without treatment, the outcome of African trypanosomosis is almost always fatal [5].

Trypanocides are used for the control of AAT in the 37 African countries where animal trypanosomosis is endemic. Three compounds: isometamidium chloride, homidium salts (homidium bromide (Ethidium®) and homidium chloride (Novidium®) and diminazene aceturate (Bernil®, Veriben®) are used in treatment of AAT [6].

The search for vaccination against African trypanosomiasis remains elusive and effective treatment is beset with problems of drug resistance and toxicity [7-9]. In addition existing treatment for trypanosomiasis are old, toxic and/or expensive [10]. Besides, there are problems associated with chemotherapy including drug availability, especially in rural areas, distribution and pharmacological properties of drugs, differences in the epidemiology of the disease response to therapy, and relapses [1,7-10].

One of the major problems that severely limit trypanosomosis chemotherapy is the unwillingness of pharmaceutical companies to invest in development of drugs against trypanosomosis for lack of financial incentives because the disease affects largely the rural poor in Africa. Currently the treatment of animals with trypanocidal drugs still remains the most frequently applied measure to control trypanosomosis. Treatment is mainly carried out by the livestock owners themselves without any supervision by veterinary personnel. It has been observed that under-dosing occurs very frequently, which is an important risk factor for the development of drug resistance [11]. In Ethiopia, presences of moderate to high prevalence of trypanosomes resistant to drugs were reported in different sites [12,13]. Therefore, the need to search for cheaper, more effective, easily available and less toxic drugs cannot be over-emphasized.

In the immediate past, the possibility of sourcing for new generations of trypanocidal agent from medicinal plants has been receiving some consideration [14-25].

Freiburghaus et al., [26,27] evaluated several medicinal plants of Tanzanian and Ugandan origin for their in vitro trypanocidal activity. Their results revealed that plants could indeed be a good source of trypanocidal drugs.

Several reports on the evaluation of different chemicals/drugs for trypanocidal activity have appeared [28,29] just as are interesting reports on the antitrypanosomal effects of plant extracts and plant derivatives [26,27,30-32]. Some of these reports have indeed shown that, at least under in vitro conditions, various medicinal plants possess trypanocidal activity. The anititrypanosomal activity of medicinal plants is believed to be due to the various phytochemicals present in the plants. It is known from the literature that secondary plant metabolites exhibit antitrypanosomal activity [29,33-35].

Recently, Fulas [36], Teklehaymanot [37] reported plants claimed to be useful in the treatment of African trypanosomosis in Ethiopia. As a follow up to these works, we present in this publication, report on in vitro activity of aqueous and methanol extracts of *Verbascum sinaiticum* for their trypanocidal activity using *T. congolense* as test organism.

Verbascum sinaiticum **(Scrophulariaceae)**

The family Scrophulariaceae is a cosmopolitic family with 300 genera and about 5400-5500 species, mainly found in the tropical mountains. Verbascum is a genus having about 360 species. *Verbascum sinaiticum* known by the Amharic name 'qetetina' is a biennial plant, 60-150 cm tall [38].

Traditional uses of *V. sinaiticum* include: for wound treatment, stomachache [39]; viral infection, cancer [37]; sun stroke fever, abdominal colic, diarrhea, hemorrhage, anthrax [40]; hepatitis [41]. Moreover, powder of the leaves of *V. sinaiticum* mixed with water is given orally [36,37] or the filtrate is instilled into left ear and nose [40] for treatment of animal trypanosomosis. Investigation of the leaves of *V. sinaiticum* has afforded two flavonolignans, hydrocarpin and the novel sinaiticin, as well as two flavones, chrysoeriol and luteolin [42]. Tadeg et al., [43] have shown in vitro broad spectrum antimicrobial activity of the methanol extract of *V. sinaiticum* leaves against Gram (+) *Staphylococcus aureus* and Gram (-) *Pseudomonas aeruginosa* bacteria.

Material and Methods

Reference Drug: Diminazine aceturate (Veriben® containing 1.05 gm diminazene aceturate+2.36 gm antipyrine, (Ceva Santé Animale, France; batch number-719A1) a commercial trypanocidal drug was used.

Test organism: The test organism *T. congolense* was isolated from infected cattle in Sebategna kebele, Ilu-Aba-Bora-Zone, Bedele town, Dabo Hana woreda, 480 km. from Addis Ababa in South west direction. The presence of *T. congolense* in the screened cattle was detected from blood samples collected from the peripheral ear of the animals by then an animal with peak parasiaemia of (~108 trypanosomes/ml) [44] was selected and blood was collected to the ethylene diamine tetra acetic acid (EDTA) coated tube from the jugular vein of the animal and diluted with PBS. Then 0.2 ml of blood containing (~104 trypanosomes/ml) was inoculated to laboratory mice for subsequent use of the infected blood the invitro test.

Experimental animals: For the blood incubation infectivity test, healthy swiss albino mice (weighing 20-30 gm. and age of 8-12 weeks) were obtained from the animal house of Ethiopian Health and Nutrition Research Institute (EHNRI) and School of Pharmacy, Addis Ababa University. Animals were housed in polypropylene cages (6-10 animals per cage), maintained fewer than 12 h light and 12 h dark cycle and allowed free access to pellet diet and clean water ad libitum. All procedures complied with the guide for the care and use of laboratory animals [45,46].

Collection of Plant specimens

The leaves of *V. sinaiticum* were collected in the month of March 2013, from Entoto-mountain about 10 km north of the center of Addis Ababa, Ethiopia. The fresh leaves were wrapped by plastic sheets during transportation. Taxonomic identification was done and a voucher specimen was deposited (Collection EM/002) at the National Herbarium, College of Natural sciences, Addis Ababa University. The leaves of the plant materials were thoroughly washed with distilled water to remove dirt, soil and any other foreign materials and left to drain off. The leaves were then spread on laboratory bench and dried under shade. The dried leaves were pulverized using mortar and pestle at medicinal plants laboratory of ALIPB.

Plant Extraction

Preparation of aqueous extract: 200 g of dried leaf powder of *V. sinaiticum* was separately macerated with 1000 ml of distilled water and methanol for 48 hours with frequent agitation in orbital shaker and the resulting liquid was filtered using Whatman No. 3 filter paper (Whatman Ltd., England). Extraction was repeated three times and the filtrates of all portions were pooled in one vessel. The aqueous extract was placed in a Petridish and lyophilized for one week to yield a solid residue, while the methanol extract was concentrated using Rota vapor (BÜCHI Rota-vapor, Switzerland) at no more than 40°C in order to obtain dry extract. The resulting dried mass was then powdered, weighed and packed into a glass vial and stored in a desiccator over silica gel until use.

The percentage yield was calculated as:

Percentage yield = (Amount of extracted obtained / Amount of initial sample) X 100

Phytochemical Screening

Aqueous and methanol extracts of *V. sinaiticum* were screened for the presence of active principles such as alkaloids, anthraquinones, flavonoids, glycosides, phenolic compounds, saponins, steroids, tannins and terpenes.

Test for alkaloids: This was carried out as described by Rafauf [47] and Sofowora [48].

a. Dragendorff's test: 1 ml of Hydrochloric acid (HCl) and 3 drops of Dragendorff's reagent were added to the extract solution. The formation of orange precipitates indicated the presence of alkaloids.

b. Wagner's test: 1 ml of HCl and 3 drops of Wagner's reagent were added to the extract solution. The formation of a brown precipitate indicated the presence of alkaloids.

Test for anthraquinones: This was carried out as described by Tyler et al. [49]:

a. Free Anthraquinones: 5 gm of each plant extract was shaken with 10 ml of benzene and filtered. A 10% ammonium hydroxide solution

(5 ml) was added to the filtrate, and the mixture was shaken. The presence of a pink, red or violet color in the ammonia phase was taken as an indication of the presence of anthraquinones.

b. Combined Anthraquinones: 5 gm of plant extract was boiled with 10 ml of 1% HCl and filtered while hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was removed and 10% ammonium hydroxide (equal to half the volume of benzene) was added to it. A pink, red or violet color in the ammonia phase indicated the presence of anthraquinone derivatives.

Test for flavonoids: This was carried out as described by Dermarderosian and Liberti [50]:

a. Ferric chloride test: Few drops of ferric chloride were added to the extract test solution. Formation of blackish red color indicated the presence of flavonoids.

b. Alkaline reagent test: 3 ml of 10% sodium hydroxide (NaOH) was added to the extract test solution followed by 3 ml of 10% HCl. The formation of a yellow color on addition of NaOH, which disappeared on addition of the HCl, indicated the presence of flavonoids.

c. Lead acetate solution test: formation of yellow precipitate after addition of few drops of lead acetate (10%) solution to the extract solution indicated the presence of flavonoids.

Test for glycosides: This was carried out as described by Evans [51]:

a. Keller Killiani test: the extract test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Formation of lower reddish brown layer and upper acetic acid layer which turns bluish green was taken as an indication for presence of glycosides.

b. Bromine water test: the extract test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Test for saponins

a. Foam test: To 1 ml of each extract, 3 ml of water was added and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result (Evans) [51].

Test for steroids and terpenes

a. Liebermann Burchard test: Extract solution was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer indicated the presence of steroids while the formation of deep red color in the lower layer indicated a positive test for terpenes [52].

Test for tannins

a. Gelatin test: To 1 ml of the extract solution, 5 ml of 1% gelatin containing sodium chloride (NaCl) was added. Formation of a yellow precipitate denoted the presence of tannins [51].

Test for phenolic compounds

To test for presence phenolic compounds, few drops of ferric sulfate were added to each extract solution. Formation of dark-violet color indicated the presence of phenolic compounds [48].

In Vitro Activity Test

The *in vitro* test was performed in triplicates in 96 well micro-titter plates (Flow laboratories Inc.). Infected blood obtained by cardiac puncture of mice at peak parasitaemia (~108 trypanosomes/ml) [4] was put into EDTA tube. Stock solutions of the aqueous and methanol leaf extracts of *V. sinaiticum* were first prepared in 2% tween 80 in phosphate buffered saline glucose (PBSG) as used by Endeshaw et al. [53], Ene et al. [54], Johnson et al. [55] and Feyera et al. [19]. Aliquot of 50 µl of crude extracts solution of 20.0 mg/ml, 10.0 mg/ml, 5 mg/ml, 2.0 mg/ml, and 1 mg/ml were mixed with 200µl of blood containing about 20-25 trypanosomes/field (~108 trypanosomes/ml) in micro-titter plates to produce effective test concentrations of 4 mg/ml, 2 mg/ml, 1 mg/ml, 0.4 mg/ml, and 0.2 mg/ml respectively.

To ensure that the effect monitored was that of the extract alone, negative and positive controls were included which contained the parasite (200 µl of infected blood) suspended in 50 µl of 2% tween 80 in PBSG and similar effective test concentrations of diminazene aceurate respectively [15,54,56,57].

After 5 min incubation in covered micro-titter plates maintained at 37°C, a drop of the test mixture was placed on separate microscope slide covered with cover slip and the motility of the trypanosomes was observed under the microscope (400X) at 10 min interval for 2 hours. The procedure was carried out separately for the aqueous and methanol extracts of each plant in triplicates.

Cessation or drop in motility of the trypanosomes in extract-treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of antitrypannosomal activity. Time (minute) after which motility ceased or reduced drastically was recorded for comparison. The movement of the parasite are grouped as; actively motile (motile parasite in ≤ 5 microscopic fields), drastically reduced motility (motile parasite in the range of 10-20 microscopic fields), ceased (no motile parasite in 10-20 microscopic fields). The shorter the time of cessation of motility of the parasite, the more active the extract was considered to be. Under this *in vitro* system, parasites survived for about 4 h when no extract was present [19,58].

Blood Incubation Infectivity Test

For the validation of the *in vitro* antitrypanosomal activity, similar concentrations of extracts as used in the *in vitro* test were assessed for blood incubation infectivity test. Parasite suspension was incubated in the presence of the aqueous/methanol leaf extract of *V. sinaiticum*, as described in the *in vitro* study then contents of the *in vitro* mixtures in the micro-titter plates were injected intraperitoneally into five healthy mice and the level of parasiteamia was assessed every other day by collecting blood from tail of each mouse and checked for the presence of trypanosomes using the wet blood film by Microhaematocrit Buffy Coat Technique (MHBCT) [59]. The loss of infectivity of the trypanosomes to mice was concluded if no trypanosome was detectable within 21 days as described in the works of Maikai [60], Atawodi et al. [15], Wurochekke and Nok [58] and Abu et al. [61]. In addition effect of the extracts in prolongation of establishment of infection was monitored by comparing with the negative control.

Results

Yield for plant extraction

As shown in Table 1, among the solvents used for extraction methanol provided maximum percentage yield as compared to the aqueous solvent.

Phytochemical screening

Phytochemical screening of the aqueous and methanol leaf extracts of *V. sinaiticum* had revealed the presence of different secondary metabolites (Table 2).

Plant species	Part extracted	Solvent	Percentage yield (%w/w)
V. sinaiticum	Leaf	Distilled water	13.09%
		Methanol	18.13%

Table 1: Percentage yields of aqueous and methanol leaf extracts of *Verbascum sinaiticum*

Constituents	*V. sinaiticum*	
	Aqueous extract	Methanol extract
Alkaloids	+	+
Anthraquinones	-	-
Flavonoids	-	+
Glycoside	-	+
Saponins	+	-
Steroids	-	+
Phenolic compounds	-	+
Tannins	-	+
Terpenes	-	-
+ = present, - = absent		

Table 2: Phytochemical screening results for the aqueous and methanol leaf extracts of *Verbascum sinaiticum*

In vitro antitrypanosomal activity

As shown in Table 3, the methanol extracts of *V. sinaiticum* had ceased motility of the trypanosomes within 50 and 80 min at 4 and 2 mg/ml concentration, respectively, while the aqueous extract shown similar effect within 60 min only at the 4 mg/ml concentration. Drastic reduction in motility of trypanosomes was observed after 70 min at 2 mg/ml aqueous extract of *V. sinaiticum*. However the positive control diminazine aceturate immobilized motility of trypanosomes within 20, 30 and 60 min at 4, 2, and 1 mg/ml test concentrations, respectively, whereas the negative control 2% tween 80 and lower test concentrations (1, 0.4 and 0.2 mg/ml) of aqueous and methanol extracts of *V. sinaiticum* neither immobilized nor reduced motility of trypanosomes.

Treatment	Time (min.) of cessation or drastic reduction in motility					
	Test concentrations					
	Extract	4 mg/ml	2 mg/ml	1 mg/ml	0.4 mg/ml	0.2 mg/ml
V. sinaiticum	Aqueous	60*	70**	NE	NE	NE
	Methanol	50*	80*	NE	NE	NE
Positive control	Diminazine aceturate	20*	30*	60*	NE	NE
Negative control	2% Tween 80 in PBSG	NE				
*=ceased motility; **drastically reduced motility, NE=no noticeable effect on motility						

Table 3: *In vitro* antitrypanosomal effect of the aqueous and methanol leaf extracts of Verbascum sinaiticum on motility of *Trypanosoma congolense.*

Blood incubation infectivity test

The mice which received the test concentrations containing 4 mg/ml diminazine aceturate were found to be aparasitaemic after 21 days monitoring period, while the mice which received the test concentrations containing 4 and 2 mg/ml methanol extract of *V. sinaiticum* and 1 and 2 mg/ml diminazine aceturate lost infectivity to some of the animals and had prolonged establishment of infection (pre patent period) as compared to the negative control (Table 4).

Discussion

Despite the enormity of the health and economic implication of African trypanosomosis, current chemotherapeutic options are very limited and far from ideal for both human and livestock [5,62]. Therefore, the need for safer, cheaper and readily available sources of medicaments cannot be over-emphasized.

Literature surveys and field studies have shown that plants are used in traditional medicine in Africa to treat trypanosomes in humans and animals [63]. In this regard the objective of this study was to screen and evaluate the *in vitro* antitrypanosomal activity of aqueous and methanol extracts of *V. sinaiticum* leaves which have traditional use in the treatment of infectious diseases including trypanosomosis [36,37].

The observed antitrypanosomal activity of the plant extracts confirm earlier in vivo and *in vitro* studies which suggest that plant extracts could contain potent trypanocidal constituents [15,21,22,24,26,27,30]. However, it is not possible to compare many of our results with those of earlier reports because the plant investigated here was not previously studied for trypanocidal activity, although the use of the plant in the traditional management of trypanosomiasis have recently been reported (Fulas, [36]; Teklehaymanot, [37] and Weldegerima et al. [40]).

Plant	Extract	Test Concentration	Number of mice which developed infection	Infection interval in days (Mean±SEM)
V. sinaiticum	Methanol	4 mg/ml	2/5	16.00 + 0.00
		2 mg/ml	4/5	14.50 + 0.50
		1 mg/ml	5/5	13.60 + 0.40
		0.4 mg/ml	5/5	13.20 + 0.48
		0.2 mg/ml	5/5	12.80 + 0.48
	Aqueous	4 mg/ml	5/5	13.20 + 0.48
		2 mg/ml	5/5	12.80 + 0.48
		1 mg/ml	5/5	12.40 + 0.40
		0.4 mg/ml	5/5	12.40 + 0.40
		0.2 mg/ml	5/5	12.00 + 0.00
Positive control	Diminazine aceturate	4 mg/ml	0/5	Ni
		2 mg/ml	3/5	18.66 + 0.66
		1 mgml	4/5	16.50 + 0.50
		0.4mg/ml	5/5	15.60 + 0.40
		0.2mg/ml	5/5	14.80 + 0.48
Negative control	2% Tween 80 in PBSG		5/5	11.80 + 0.37
Values are Mean ± SEM; N= 5; Ni=No infection				

Table 4: The effect of aqueous and methanol leaf extract of *Verbascum sinaiticum* on blood incubation infectivity test

The aqueous extract of the plant was prepared by macerating the dried leaves in distilled water in order to simulate the way it is traditionally used [36,37]. With the assumption that some of the active ingredients responsible for the claimed antitrypanosomal activity might not be soluble in water adequately; the methanol leaf extract of the plant was also included in the study. The choice of absolute methanol was based on the review on extraction methods of plants with antitrypanosomal activity by Mbaya and Ibrahim [63] who stated that, in most situations, where air dried materials were powdered into small particles, and extraction was most productive with 100% methanol or ethanol. Extraction of *V. sinaiticum* with water and methanol yielded 13.09% and 18.13%, respectively. The yield obtained from the methanol extracts of the plant was found to be higher as compared to the aqueous extracts which could be an indication of the extracting power of the solvent which was also noticed in the phytochemical screening. This yield, if the extracts are found to be active and promising for further development, can add advantage to the commercial production of the plant.

Recognition of the biological properties of myriad natural products has fuelled the current focus of this field, namely, the search for new drugs against trypanosomosis. One of the objective for evaluating plants for biological activity is to isolate one or more biologically active compounds that may be potentially useful in treating certain disease conditions or serve as a structural analogue (template) from which better synthetic modifications can be derived. Therefore, the leaves of *V. sinaiticum* were screened for the presence of different phytochemicals of therapeutic interest using chemical method with the objective of finding out the possible class of compounds present in the plant (Table 2). According to the results of the phytochemical screening study, the extracts were found to show a positive test for the presence of saponins, steroids, phenols, alkaloids, glycosides, flavonoids and tannins, while methanol extract of *V. sinaiticum* showed positive test for the presence of steroids, phenols, glycosides, flavonoids and tannins, the aqueous extract showed a negative result for the same test.

Numerous *in vitro* and in vivo studies conducted on the antitrypanosomal activities of the class of compounds listed above reported the potential of each class of compounds in killing or inhibiting the growth of wide ranges of trypanosomes. The results of phytochemical screening had shown that methanol extract *V. sinaiticum* contain phenolic compounds. Phenolics and polyphenols have been reported in the literature to have antitrypanosomal potential by inhibiting the trypanosome alternative oxidase (TAO) [64].

The presence of flavonoids in methanol extract of *V. sinaiticun* is in agreement with the report Afifi et al. [42], who reported the occurrence of two flavonolignans, hydrocarpin and sinaiticin, as well as two flavones, chrysoeriol and luteolin in the same plant. And it has

been known that flavonoids and flavonoid-derived plant natural products are effective antitrypanosomal substances against different trypanosome species [29,65].

In addition, the finding of alkaloids in aqueous extract of *V. sinaiticum* concurs with the findings of Samia et al. [66], who reported the presence of alkaloids in Verbascum species. Also, saponins (mullinsaponins) were isolated from the same plant by Tatli et al. [67]. The positive test for glycosides in the methanol extract of *V. sinaiticum* in this study coincide with the report of Elgindi et al. [68], who reported the occurrence of phenylethanoid glycoside, verbascoside (acteoside) in *V. sinaiticum*.

Therefore, the observed *in vitro* antitrypanosomal activity of *V. sinaiticum* might be attributed to either the individual class of compounds present in each herb, or to the synergistic effect that each class of compounds exert to give the observed biological activity. Hence, further in-depth investigations should be carried out to resolve this issue.

Parasites motility constitutes a relatively reliable indicator of viability of most trypanosomes [69] and a complete elimination or reduction in motility of trypanosomes when compared to the control could be taken as index of trypanocidal activity [58]. *V. sinaiticum* crude leaf extracts had shown considerable *in vitro* antitrypanosomal activity, with the methanol extracts exhibiting the highest activity than the aqueous extract. The *in vitro* antitrypanosomal activity of the aqueous and methanol extract of *V. sinaiticum* at 4 mg/ml concentration which ceased motility of parasites within 50 and 60 min is in agreement with *in vitro* antitrypanosomal activity of *Pseudocedrella kotschi* which similarly immobilized motility of *T. congolense* at 4 mg/ml concentration within 55 min of incubation [15].

The mechanism by which the extracts immobilized or reduced motility of the trypanosomes is not known at this stage of the work. However, accumulated evidences suggested that many natural products exhibited their antitrypanosomal 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 [15,70,71].

Respiration of trypanosomes is obligatory for their motility as well as for managing the energy reserve required for the synthesis of the variable surface glycoproteins. The inhibition of cellular and mitochondrial respiration by any chemotherapeutic agent will obviously compromise all the energy dependent processes. This was confirmed by the microscopy of the trypanosomes, which showed a cessation or reduction in motility after incubation with different concentrations of extracts [72].

It is not known why 2 mg/ml aqueous extract *V. sinaiticum* reduced trypanosome motility (within 70 min) but could not completely eliminate motility. However, it appears reasonable to speculate that these extracts may belong to the group that acts by static action affecting growth and multiplication of trypanosomes rather than eliminating them altogether. The positive control diminazine aceturate immobilized trypanosomes within 20, 30 and 60 min at test concentrations of 4, 2, 1 mg/ml, respectively. This finding is not in agreement with Maikai [25], in which even lower concentration 0.1 mg/ml of diminazine aceturate ceased motility of the trypanosomes within 20 min. The difference might be due to the *T. congolense* isolate that could have developed resistance to the drug as reported by Chaka and Abebe [73]. Comparison analysis revealed that the standard drug exhibited superior *in vitro* antitrypanosomal activity even at lower concentration (2 mg/ml) when compared to the extracts. This is consistent with several reports made on other medicinal plant extracts [16,29,54,61,74].

The *in vitro* antitrypanosomal activities of both extacts were not confirmed by blood incubation infectivity. Yusuf et al. [75] suggested that complete immobility of the parasites *in vitro* may not necessarily indicate that the parasites were dead, but rather the parasites may have lost their infectivity. This may be due to the respective concentration might have only immobilized, but not killed the parasite by causing unfavorable conditions. The parasites might have recovered and become infective in contact with suitable physiological conditions. Prolongation of the pre patent period of animals inoculated with the *in vitro* mixtures of *V. sinaiticum* (2 and 4 mg/ml) for more than 16 days is in agreement with the findings of Feyera et al. [19] and Yusuf et al. [75].

It may appear to contemplate that the highest (2 and 4 mg/ml) concentration level either killed the parasites or caused them to lose their infectivity coupled to cease in motility of the trypanosomes in vitro. Loss of infectivity may be by abrogating some vital metabolic processes in the parasites or could be due to some morphological changes on the parasites induced by the extract at this concentration that render them more susceptible to the mice immune defense systems.

Conclusion

This study gave indications of *in vitro* antitrypanosomal activity of methanol crude leaf extracts of *V. sinaiticum* against *T. congolence* field isolate. The aqueous extracts of the plant exhibited lower *in vitro* antitrypanosomal activity, while the methanol extracts have shown better activity at higher concentration. The higher concentration (4 mg/ml) of the aqueous and methanol extract of *V. sinaiticum* showed superior *in vitro* activity by immobilizing trypanosome motility within 50 and 60 min, respectively and prolonging the prepatent period of trypanosomes infectivity to mice which remained aparasitaemic for more than 16 days after the inoculation of the *in vitro* mixtures. Generally, the current study established that leaves of *V. sinaiticum* could have potential antitrypanosomal activity which can be considered as a potential source for the search of new drugs against Africal animal trypanosomosis.

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