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In Vitro Antiprotozoal Activities and Cytotoxicity of Selected Sudanese Medicinal Plants

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Abstract: *Entamoeba histolytica* is ranked third on the list of parasitic protozoan infections leading to death behind malaria and schistosomiasis. It is estimated also up to 200 millions of people are chronically infected with *Giardia lamblia* globally. Metronidazole is the drug currently widely used and recommended in the treatment of amoebiasis and giardiasis. However; sometimes it causes adverse effects such as myoplasia, neuralgia, allergic dermatitis, and others. The *in vitro* antiprotozoal activities of some selected Sudanese medicinal plants (*Acacia nilotica* subsp. *nilotica*, *Adansonia digitata*, *Cyperus rotundus* and *Nigella sativa*) were determined against *Entamoeba histolytica* and *Giardia lamblia* by employing the sub-culture method. The mammalian cytotoxicity of the investigated plants against normal Vero cell line was determined by applying MTT [(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] method. All plants examined 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 95% inhibition at concentration 312.5 µg/ml at the same time against *Entamoeba histolytica* and *Giardia lamblia*. In addition, cytotoxicity (MTT-assay) of these plants against normal Vero cell line which verified the safety of the examined extracts with an IC₅₀ less 100 µg/ml. These studies prove the potent activity of extracts against *E. histolytica* and *G. lamblia* trophozoites *in vitro* with verified safety evidence for use.

Keywords: *Entamoeba histolytica*, *Giardia lamblia*, Medicinal Plants, Metronidazole, Cytotoxicity

1. Introduction

Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against these disabling diseases [1] [2].

Sudan represents one of the largest African countries and

characterized by rich flora described by many botanists. They observed that Sudan herbal medicine represents a unique blend of indigenous cultures with Egyptian, Arabian, west and East African cultures. In an attempt to collect information on biology and phytochemistry of Sudanese medicinal plants it is important to collect information about the plants used by herbalists in different part of Sudan. From the observations of many botanist working in the field of medicinal plants, a lot of work need to be done to identify species that are used by traditional herbalists over years for

curing specific ailments. This can be achieved by encouraging interested botanists and medical doctors in Sudan to collect information from their respective regions by working very close with the established herbalists [3].

The World Health Organization (WHO) estimates that the protozoan *Entamoeba histolytica* is a major cause of morbidity worldwide, causing approximately 50 million cases of dysentery and 100,000 deaths annually [4-5]. Intestinal amoebiasis due to the infection of *E. histolytica* is ranked third on the list of parasitic protozoan infections leading to death behind malaria and schistosomiasis [6]. This infection remains a significant cause of morbidity and mortality world-wide [7]. Amoebiasis is a rare occurrence in developed countries of the world, but only found in travelers, immigrants, homosexuals and institutionalized persons. *E. histolytica*-associated dysentery is a common occurrence in the less developed and developing countries of the world, but is more common in areas of low socioeconomic status, poor sanitation and nutrition especially in the tropics [5].

Giardiasis is the most common cause of parasitic gastrointestinal disease and it is estimated that up to two hundred million people are chronically infected with *Giardia lamblia* globally, and 500,000 new cases reported annually [8]. *G. lamblia* is a major cause of diarrhoea in humans [9]. *Giardia* is a flagellate protozoan with worldwide distribution that causes significant gastrointestinal diseases in a wide variety of vertebrates including cats and human. *G. lamblia* is one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries. *G. lamblia* is considered to be one of the leading causative agents of diarrhoea in both children [10-11-12] and adults [13-14].

Metronidazole is the drug now widely used and recommended in the treatment of amoebiasis and giardiasis [15]. But it is less effective in the tissue than in the gut lumen [16]. In addition, it can eradicate only up to 50% of laminae infections [17]. Metronidazole sometimes causes adverse effects, example, myoplasia, neuralgia, and allergic dermatitis [18]. The present study was conducted to investigate the new anti-amoebic, anti-giardial and cytotoxicity of four Sudanese medicinal plants (*Acacia nilotica* subsp. *nilotica*, *Adansonia digitata*, *Cyperus rotundus* and *Nigella sativa*) were used for treatment of clinical signs associated with Amoebiasis and giardiasis such as venereal diseases were selected to be evaluated for the activity of ethanol crude extracts against *E. histolytica* and *G. lamblia* trophozoites *in vitro*.

2. Materials and Methods

2.1. Plant Materials

The leaves of *Acacia nilotica* subsp. *nilotica* and *Adansonia digitata*, seeds of *Nigella sativa* and whole plants of *Cyperus rotundus* were collected from central Sudan between January 2016 and February 2016. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research

Institute (MAPTMRI). All plant parts were air-dried, under the shadow with good ventilation and then ground finely in a mill until their uses for extracts preparation.

2.2. Preparation of Crude Extracts

Extraction was carried out for the leaves, seeds and whole plant of selected plants by using overnight maceration techniques according to the method described by Harbone [19]. About 50 g were macerated in 250 ml of ethanol for 3 h at room temperature with occasional shaking for 24 h at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration, the solvent was then removed under reduced pressure by rotary evaporator at 55°C. Each residue was weighed and the yield percentage was calculated then stored at 4°C in tightly sealed glass vial ready for use. The remaining extracts which is not soluble by successively extracted by Ethanol using the previous technique. Extracts kept in deep freezer for 48 h, then induced in freeze dryer (Virtis, USA) until completely dried. The residue was weighed and the yield percentage was calculated. The extracts were kept in 4°C until the time of their use.

2.3. Parasite Isolate

Entamoeba histolytica and *Giardia lamblia* used in all experiments were taken from patients of Ibrahim Malik Hospital (Khartoum). All taken samples were examined by wet mount preparation; the positive samples were transported to the laboratory in nutrient broth medium. Trophozoites of *E. histolytica* and *Giardia lamblia* were maintained in RPMI 1640 medium containing 5% bovine serum at 37 ± 1°C. The trophozoites were maintained for the assays and were employed in the log phase of growth.

2.4. Inoculums

E. histolytica and *Giardia lamblia* was inoculated in the RPMI 1640 medium and incubated at 37 ± 1°C for 48 h. parasites were counted under the microscope by haemocytometer chamber.

2.5. In Vitro Susceptibility Assays

In vitro susceptibility assays used the sub-culture method of Cedillo-Rivera *et al.* [20]. which is being described as a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *E. histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis* (Arguello-Garcia *et al.*, 2004) [55]. 5 mg from each extract and compound was dissolved in 50 µl of dimethyl sulfoxide (DMSO) at Eppendorf tube containing 950 µl D. W in order to reach concentration of 5 mg/ml (5000 ppm). The concentrates were stored at -20°C for further analysis. Sterile 96-well microtitre plate was used for different plant extracts, positive control and negative control. Three columns of a microtitre plate wells [8 columns (C) × 12 rows (R)] were chosen for each extract, 40 µl of an extract solution (5 mg/ml) were added to the first column wells C-1: On the

other hand, 20 μ l of complete RPMI medium were added to the other wells of the second column and third column (C-2 and C-3). Serial dilutions of the extract were obtained by taking 20 μ l of extract to the second column wells and taking 20 μ l out of the complete solution in C-2 wells to C-3 wells and discarding 20 μ l from the total solution of C-3 to the remaining 20 μ l serial solutions in the successive columns. 80 μ l of culture medium was complemented with parasite and added to all wells.

The final volume in the wells was 100 μ l. In each test,

$$\text{Mortality of parasites (\%)} = \left(\frac{\text{Negative control} - \text{Tested sample with extract}}{\text{Negative control}} \right) \times 100\%$$

Only 100% inhibition of the parasite considered, when there was no motile parasite observed.

2.6. Cytotoxicity Screening

Micro-culture-tetrazolium MTT-assay was utilized to evaluate the cytotoxicity of plants. This colorimetric assay is based on the capacity of mitochondria-succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) into an insoluble, blue colored-formazan, a product measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells [21].

2.7. Cell Line and Culture Medium

Vero (Normal, African green monkey kidney) cells were

$$(\text{Cells/ml}) N = (\text{Number of cells counted} \times \text{Dilution factor} \times 10^4) / 4$$

*Dilution factor is usually 2 (1:1 dilution with trypan blue), but may need to further dilute (or concentrate) cell suspensions.

2.9. MTT Assay

Serial dilutions of extract were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer walls of the plate were filled with 250 μ l of in-complete culture medium except the last row 6 middle wells (B - G), which were used for the negative control receiving 50 μ l of culture medium and 2 μ l of sterile 0.5% Triton X. 50 μ l/wells complete culture medium (CCM) were added and 30 μ l more were added to second column wells (B - G) that were used as first extract dilution wells. To the first dilution wells in the row, 500 μ g of c suspension extract were added to the 80 μ l. extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 250 μ l to the next well after proper mixing. From the last dilution wells (B-11), 50 μ l were discarded. Each compound was tested in triplicate. Cell suspension in a complete culture medium containing 2.5×10^5 /ml was properly mixed, and 150 μ l of it were transferred into each well of the plate. The plate was covered and placed in 5% CO₂ incubator at 37°C for three-five days (72 hours-120 hours). On the third/fifth day, the supernatant was

Metronidazole (a trichomonocide) pure compound [(1-(2-hydroxyethyl)-2-methyl-5 Nitroimidazole)], a was used as positive control in concentration 312.5 μ g/ml, whereas untreated cells were used as a negative controls (culture medium plus trophozoites). For counting, the samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 0, 24, 48, 72 and 96hs. The mortality% of parasite for each extracts activity was carried out according to

cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37°C. The cells were sub cultured twice a week.

2.8. Cell Counting

Cells were counted using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. was used for calculating the cells:

removed from each well without detaching cells. MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) stock (5 mg/ml) was prepared earlier in 100 ml PBS (phosphate buffer saline). MTT suspension was vortexed and kept on magnetic stirrer until all MTT dissolved. The clear suspension was filter sterilized with 0.2 μ Millipore filter and stored at 4°C or - 20°C until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 μ l of diluted MTT were added. The plate was incubated further at 37°C for 2 to 3 hours in CO₂ incubator. MTT was removed carefully without detaching cells, and 200 μ l of DMSO were added to each well. The plate was agitated at room temperature for 15 minutes then read at 540 nm using micro plate reader. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ cell inhibition} = 100 - \left\{ \frac{\text{Ac-At}}{\text{Ac}} \right\} \times 100$$

Where, At = Absorbance value of test compound; Ac =Absorbance value of control.

2.10. Statistical Analysis

All data were presented as means \pm S. D. Statistical analysis for all the assays results were done using Microsoft

Excel program (2013).

3. Results and Discussion

3.1. Yields (%) of Ethanolic Extracts of Medicinal Plants

The yield% of ethanolic extract of *A. nilotica* subsp.

Table 1. Yields Percentage of ethanolic extracts of medicinal Plants.

No.	Scientific Name of Plants	Family Name	Part Used	Weight of Samples (g)	Yield (%)
1	<i>Acacia nilotica</i>	Mimosaceae	Leaves	500	7.5
2	<i>Adansonia digitate</i>	Malvaceae	Leaves		10.3
3	<i>Cyperus rotundus</i>	Cyperaceae	Whole plant		10.5
4	<i>Nigella sativa</i>	Ranunculaceae	Seeds		8.3

3.2. In Vitro Anti-Protozoal Activities of Medicinal Plants

Protozoal diseases constitute major health problems worldwide, particularly in tropical developing countries. Among the protozoal parasites, *Giardia lamblia* and *Entamoeba histolytica* have the highest incidence of diarrheal diseases in developing countries. Chemotherapy is the first choice for the treatment of protozoal diseases; however it has proven side effects. Therefore there is need for safe and effective treatment alternatives including plants as one of the readily available alternatives for control and prevention of amoebiasis and giardiasis.

Amoebiasis is an increasingly important parasitic disease among patients with HIV infection regardless of whether they have AIDS. Although HIV/AIDS patients are not especially prone to infection with *Entamoeba histolytica*, it has been suggested that they are more susceptible to an invasive form of the disease than are normal patients [22-23-23]. Infection with *E. histolytica* has also been reported to be an important cause of acute and chronic diarrhoea in HIV patients [24-25-26].

Giardia lamblia is an important cause of acute and chronic gastrointestinal disease throughout the world and has been identified as the etiologic agent in numerous waterborne outbreaks of diarrheal disease. Although *G. lamblia* is among the most prevalent enteric protozoal infections in humans, it is relatively recently that improvements in the *in vitro* cultivation of this organism have allowed reliable, reproducible tests to assess the *in vitro* activity of therapeutic agents against *G. lamblia* [27]. Despite the previous comprehensive screening of Sudanese medicinal plants for their antiprotozoal activity [28-29-30-31-32].

All plants examined against *Entameba hitolytica* and *Giardia lamblia* have shown varying trophozoiticidal activities *in vitro*.

The antiamebic potential of the ethanolic extract of *A. nilotica* subsp. nilotica and *A. digitata* (leaves), *Cyperus rotundus* (whole plants) and *Nigella sativa* (seeds) at different concentrations (500, 250 and 125 ppm) and Metronidazole (the reference control) with concentration (312.5 µg/ml) was investigated against *E. histolytica* and *G. lamblia* trophozoites *in vitro*.

The antiamebic and anti giardial activities of the ethanolic

nilotica and *A. digitata* (leaves), *Cyperus rotundus* (whole plant) and *Nigella sativa* (seeds) were 7.5%, 10.3%, 10.5% and 8.3% respectively Tables (1). The ethanolic extracts investigated antiamebic and anti giardial activity *in vitro* against *E. histolytica* and *G. lamblia* (trophozoites), and cytotoxicity (MTT assay).

extract of *A. nilotica* 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 95% inhibition at concentration 312.5 µg/ml at the same time against *Entamoeba histolytica* and *Giardia lamblia* (Figure 1 and 2). Ethanolic leaves of *A. nilotica* which was screened for antiamebic and anti giardial activities against (*E. histolytica* and *Giardia lamblia*) trophozoites *in vitro* showed antiamebic and anti giardial activities with an inhibition concentrations (IC₅₀) more than 34.5 µg/ml and 44.2 µg/ml and increasing successively during the day three of the screening respectively Tables (2 and 3). This result similar to Antitrichomonal activity in Sudan [33].

These results agree with traditional uses of *A. nilotica* in Sudan which indicate the plant claimed to be of anti-parasitic properties. The plant extract was found to exhibit anti diarrhoeal, antibacterial, antimalarial and inhibition of lipid peroxidation [34-35-36]. Moreover, the results obtained in this study are similar to studies carried out for antiplasmodial activity in mice by Alli *et al.* [37]. anti-malarial activity against CQ sensitive (3D7) and CQ-resistant (Dd2 and INDO) strains of *P. falciparum* in culture using the fluorescence-based SYBR. *A. nilotica* was reported with significant inhibiting activity and IC₅₀ was 13µg/mL [38]. anti-Diarrhea activity [39-40-41-28].

El Shanawny [54] used *A. nilotica* fruit for treatment of sore throat, cold, bronchitis, pneumonia, ophthalmia, diarrhea, dysentery, leprosy and venereal diseases. Some of these diseases such as, dysentery and venereal diseases (this study did not determine which type of venereal disease) are protozoal diseases and may possibly confirm the antiprotozoal activity of the plant fruit. Indeed, it could explain the antiprotozoal activity of *A. nilotica* on different classes of protozoa such as *Trichomonas vaginalis* and *Entamoeba hystolytica*. Also Fatima *et al.*, (2005) [42]. found that the crude methanolic extract of *A. nilotica* possess *in vitro* anti leishmanial activity against *Leishmania major* promastigotes. The promising activity of the plant in this study may be due to the chemical constituents. Bioactive compounds like Steroids, Alkaloids, Tannins, Reducing sugars, contains tannin (12-20%), terpenoids, saponins and glycosides, Phlobetannin, gallic acid, protocatechuic acid pyrocatechol, (+) – catechin, (-) epigallocatechin-5,7-

digallate [43].

The antiameobic and anti giardial activities of the ethanolic extract of *A. digitata* 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 95% inhibition at concentration 312.5 µg/ml at the same time against *Entamoeba histolytica* (Figure 1 and 2). Ethanolic leaves of *A. digitata* which was screened for antiameobic and anti giardial activities against (*E. histolytica* and *Giardia lamblia*) trophozoites *in vitro* showed antiameobic and anti giardial activities with an inhibition concentrations (IC₅₀) more than 48.7 µg/ml and 58.7 µg/ml and increasing successively during the day three of the screening respectively Tables (2 and 3).

The antiameobic and anti giardial activities of the ethanolic extract of *C. rotundus* 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 95% inhibition at concentration 312.5 µg/ml at the same time against *Entamoeba histolytica* (Figure 1 and 2). Ethanolic whole plants of *C. rotundus* which was screened for antiameobic and anti giardial activities against (*E. histolytica* and *Giardia lamblia*) trophozoites *in vitro* showed antiameobic and anti giardial activities with an inhibition concentrations (IC₅₀) more than 36.9 µg/ml and 46.3 µg/ml and increasing successively during the day three

of the screening respectively Tables (2 and 3). Moreover, these results obtained in this study are similar to studies carried out for antimalarial activity by Thebtaranonth and Thebtaranont (1995) [53]. The promising activity of the plant in this study may be explained for the chemical constituents- Cyprotene, cypera-2, 4-diene, a-copaene, cyperene, aselinene, rotundene, valencene, ylanga-2, 4- diene, g-gurjunene, trans-calamenene, d-cadinene, g-calacorene, epi-aselinene, a-murolene, g murolene, cadalene, nootkatene which the plant proved to contain [44]. in addition to mustakone, cyperol [45], isocyperol [46], cyperotundone [47], and acyprone [48-49].

The antiameobic and anti giardial activities of the ethanolic extract of *N. sativa* 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 95% inhibition at concentration 312.5 µg/ml at the same time against *Entamoeba histolytica* (Figure 1 and 2). Ethanolic seeds of *N. sativa* which was screened for antiameobic and anti giardial activities against (*E. histolytica* and *Giardia lamblia*) trophozoites *in vitro* showed antiameobic and anti giardial activities with an inhibition concentrations (IC₅₀) more than 49.0 µg/ml and 58.8 µg/ml and increasing successively during the day three of the screening respectively Tables (2 and 3).

Table 2. Inhibition concentration (IC) of medicinal plants against *E. histolytica*.

No.	Name of Samples	Part Used	IC (µg/ml)			
			IC ₅₀	IC ₉₀	IC ₉₅	IC ₉₉
1.	<i>A. nilotica</i>	Leaves	34.5	305.3	400.8	498.4
2.	<i>A. digitata</i>		48.7	327.5	415.7	483.0
3.	<i>C. rotundus</i>	Whole plant	36.9	309.2	403.4	498.9
4.	<i>N. sativa</i>	Seeds	49.0	327.2	414.9	491.7

Key: IC₅₀ Inhibition concentration 50%, IC₉₀ Inhibition concentration 90%, IC₉₅ Inhibition concentration 95%, IC₉₉ Inhibition concentration 99%.

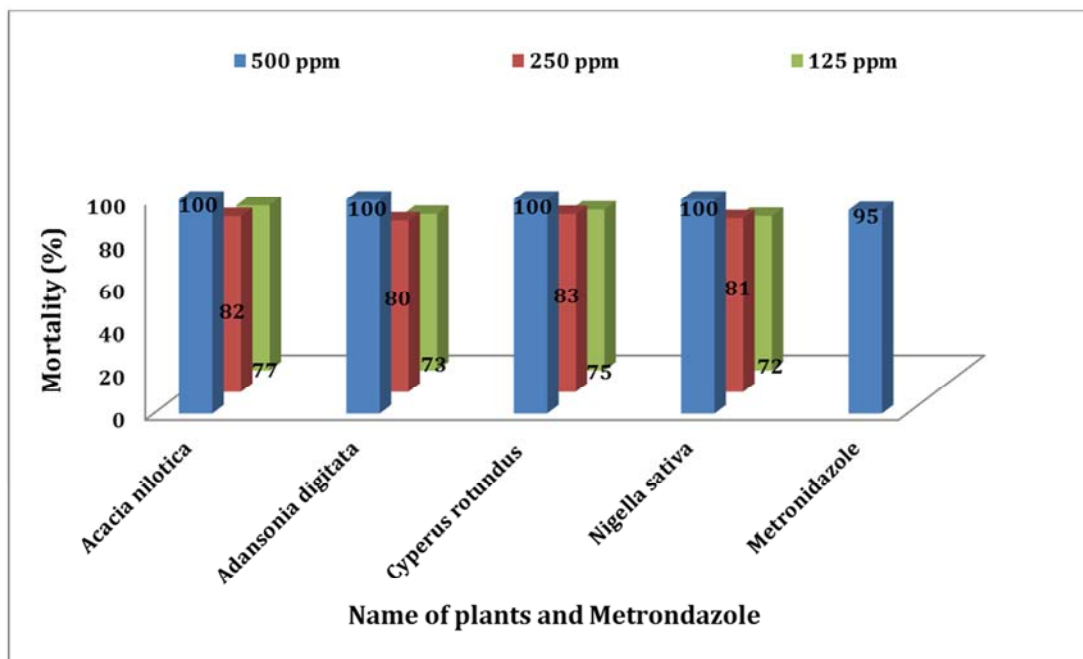


Figure 1. In vitro activity of extracts from medicinal plant against *E. histolytica*.

Table 3. Inhibition concentration (IC) of medicinal plants against *Giardia lamblia*.

No.	Name of Samples	Part Used	IC (µg/ml)			
			IC ₅₀	IC ₉₀	IC ₉₅	IC ₉₉
1.	<i>A. nilotica</i>	Leaves	44.2	320.2	410.2	499.0
2.	<i>A. digitate</i>	Leaves	58.7	339.9	423.3	495.6
3.	<i>C. rotundus</i>	Whole plants	46.3	324.1	413.3	490.1
4.	<i>N. sativa</i>	Seeds	58.8	339.9	423.2	494.4

Key: IC₅₀ Inhibition concentration 50%, IC₉₀ Inhibition concentration 90%, IC₉₅ Inhibition concentration 95%, IC₉₉ Inhibition concentration 99%.

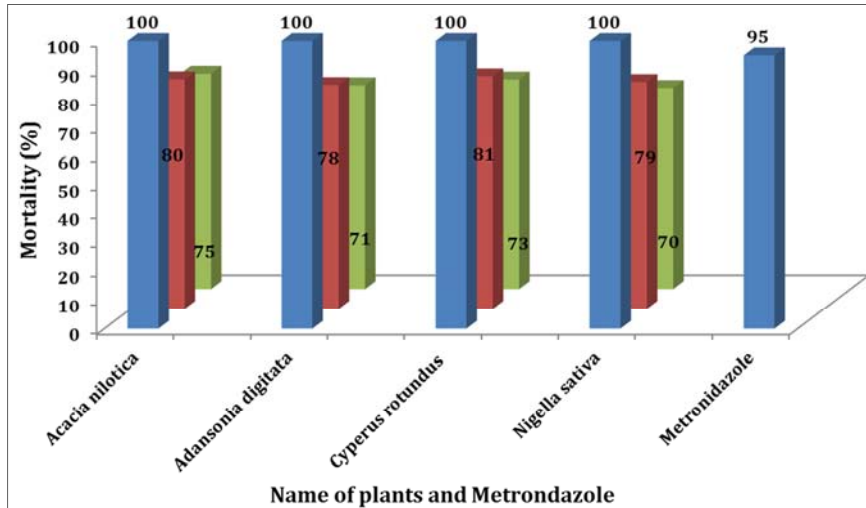


Figure 2. *In vitro* activity of extracts from medicinal plant against *G. lamblia*.

3.3. Cytotoxicity Activity of Medicinal Plants

The cytotoxicity using 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT assay) with different concentrations (500, 250 and 125 ppm) and compare triton-x100 (the reference control).

All tested plants showed no cytotoxic activity. They have IC₅₀ > 100 µg/ml compared to the control “Triton-x” which was highly toxic with IC₅₀ < 30 µg/ml.

Interestingly, the cytotoxicity assays were conducted in this study to evaluate the cytotoxicity effects of ethanolic extracts of *A. nilotica* subsp. *nilotica* and *A. digitata* (leaves), *C. rotundus* (whole plant) and *Nigella sativa* (seeds) by using MTT-assay in Vero cell line. Table (4) indicated the growth

inhibition percentage (%) of Vero cell line *in vitro* by ethanolic extract of *A. nilotica* (leaves) at different concentrations from 125 to 500 µg/ml and showed an IC₅₀>100 (µg/ml) verifying the plant safety. This result was similar to that produced by Riaz *et al.* (2011) [50], using the Human brain microvascular endothelial cells (HBMEC). Solomon and Shittu (2010) [51]. has investigated *in vitro* antimicrobial activity of the crude ethanolic leaf extract of *Acacia nilotica* linn against *Campylobacter coli* isolated from goats. The highest zone of inhibition was observed with the 70 mg/ml concentration. *A. nilotica* has been reported to be very useful in treating diarrhea and cough in human [38].

Table 4. Cytotoxicity of plants extracts on normal cell lines (Vero cell line) as measured by the MTT-assay.

No.	Name of Samples	Part Used	Concentration (µg/ml)			IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	The degree of toxicity
			500	250	125			
			Inhibition (%) ± SD					
1.	<i>A. nilotica</i>	Leaves	50.9 ± 0.05	47.5 ± 0.03	29.1 ± 0.02	377.4	>100	Non-toxic
2.	<i>A. digitate</i>		17.9 ± 0.05	10.8 ± 0.03	5.5 ± 0.02	1816		
3.	<i>C. rotundus</i>	Whole plant	40.39 ± 0.07	30.80 ± 0.09	16.84 ± 0.01	831.8		
4.	<i>N. sativa</i>	Seeds	19.3 ± 0.05	1.8 ± 0.03	-17.5 ± 0.02	1569		
5.	*Control	Drug	95.3 ± 0.01			< 30		Highly toxic

Key: IC₅₀ < 30 µg/ml: high toxic, > 100 µg/ml: no toxic *Control = Triton-x100 was used as the control positive at 0.2 µg/mL.

The cytotoxicity assays were conducted in this study to evaluate the ethanolic extract of *A. digitata* (leaves) their cytotoxicity effects by using MTT-assay include (Vero cell line). The result of MTT-assay verified the safety of the examined extract.

The inhibition percentage (%) of Vero cell line growth *in*

vitro by ethanolic extract of *C. rotundus* (whole plant) for different concentrations 125 to 500 µg/ml and showed an IC₅₀ > 100 (µg/ml) which is verifying the plant safety. This result was similar to that produced by Ahmed *et al.* (2012) [52], who found that the plant extract gave similar result using the Brine Shrimp Bioassay.

The cytotoxicity assays were conducted in this study to evaluate the ethanolic extract of *Nigella sativa* (seeds) their cytotoxicity effects by using MTT-assay include (Vero cell line). The result of MTT-assay verified the safety of the examined extract.

4. Conclusion

This result enhances the ethno botanical uses of plants as anti-amoebic and anti-giardial in cases associated with Amoebiasis and Giardiasis in Sudan. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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