



Original Article

Antigiardial Activity and Cytotoxicity of *Adansonia digitata*

Ahmed Saeed Kabbashi* and Amel Mahmoud Abdrabo

Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan

ARTICLE INFO

Received 26 Sep. 2015
Received in revised form 10 Oct. 2015
Accepted 24 Oct. 2015

Keywords:

In vitro,
Antigiardial,
Giardia lamblia,
Metronidazole,
Cytotoxicity (MTT-assay),
A. digitata (leaves).

Corresponding author: Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan

E-mail address: ahmedsak88@gmail.com

ABSTRACT

Background: *Adansonia digitata* L. (Family: Malvaceae) is a majestic tree revered in Africa for its medicinal and nutritional value. The plant parts are used to treat various ailments such as diarrhoea, malaria and microbial infections. It is reported that it is an excellent anti-oxidant due to the vitamin C content which is seven to ten times higher than the vitamin C content of oranges. *A. digitata* has numerous biological properties including antimicrobial, antiviral, anti-oxidant and anti-inflammatory activities amongst others. Giardiasis is the most common cause of parasitic gastro-intestinal 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.

Objectives: The purpose of the paper was to investigate the *in-vitro* anti-giardial activity and cytotoxicity (MTT assay) of ethanol extract of *A. digitata* L. (leaves).

Method: The ethanol extract of *A. digitata* (leaves), with different concentration (500 ppm, 250 ppm and 125 ppm) and metronidazole concentration (312.5 µg/ml) to be investigated *in vitro* against *giardia lamblia* trophozoites. And cytotoxicity (MTT assay) with different concentration (500 ppm, 250 ppm and 125 ppm) and compare triton-100 (the reference control).

Result: The result was obtained from *A. digitata* leaves ethanol extract which exhibited 100% mortality within 96 h, at a concentration 500 ppm; this was compared with Metronidazole which gave 96% inhibition at concentration 312.5 µg/ml at the same time. And MTT assay verified the safety of the examined extract.

Conclusion: These studies conducted for both *A. digitata* leaves was proved to have potent activities against *giardia lamblia* trophozoites *in vitro*. MTT assay verified the safety of the examined extract.



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}.

The leaves of *Adansonia digitata* are important protein sources in complementing the amino acid profile and thereby improving the protein quality of the diet. Young leaves are commonly used as a vegetable in soups or cooked and eaten as spinach. Dried green leaves are used throughout the year, mostly in soups served with the staple dish of millet. Flowers can be eaten raw or used as flavour in drinks. In the folk medicine *A. digitata* is used in the treatment of fevers, diarrhea, malaria, haemoptysis and scorbutic complaints (vitamin C deficiency) and dysentery. Pulp extract is applied as eye-drops in cases of measles. In many medicinal uses, stem bark is used. When prepared it is made into a decoction for internal use and functions due to its soluble and insoluble tannin, and gummy and albuminous constituents beta-sitosterol has been studied and this occurs in the bark and also the seed oil. Root bark is also used in traditional medicine. This contains beta-sitosterol and two glycosides. The leaves form a component or herbal remedies and a mash prepared from the dried powdered roots is given to malarial patients as a tonic. A semi-fluid gum obtained from *A. digitata* bark is used to treat sores³.

Adansonia digitata products (e.g. fruits, seeds, leaves, bark) contribute to the livelihood of many populations in Africa as it is a source of food, fibre and medicine⁴⁻⁸. More than three hundred traditional uses have collectively been documented in Benin, Mali, Zimbabwe, Cameroon, the Central

African Republic, Kenya, Malawi, South Africa and Senegal⁹. Various plant parts (e.g. leaves, bark, fruit pulp), have traditionally been used for immunostimulant, anti-inflammatory, analgesic, insect repellent and pesticidal properties, in the treatment of diarrhoea and dysentery in many African countries, and have been evaluated as a substitute for imported western drugs^{10,11}.

Giardiasis is the most common cause of parasitic gastro-intestinal 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 annually¹². *Giardia lamblia* is a major cause of diarrhoea in humans¹³. *Giardia* is a flagellate protozoan with worldwide distribution that causes significant gastrointestinal diseases in a wide variety of vertebrates including cats and human. Giardiasis is one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries. *Giardia lamblia* is considered to be one of the leading causative agents of diarrhoea in both children^{14,15,16} and adults^{17,18}.

Metronidazole is the drug now widely used and recommended in the treatment of giardiasis¹⁹. But it is less effective in the tissue than in the gut lumen²⁰. In addition, it can eradicate only up to 50% of laminae infections²¹. Metronidazole sometimes causes adverse effects, example, myoplasia, neuralgia, and allergic dermatitis²². The present study was conducted to investigate the anti-giardial activity and cytotoxicity of *A. digitata* (leaves) in Sudan.

Materials and Methods

Plant materials

The *A. digitata* (leaves) were collected from central Sudan between

January 2015 and February 2015. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

Preparation of crude extracts

Extraction was carried out for the leaves of *A. digitata* plant by using overnight maceration techniques according to the method described by Harbone²³. About 50 g round material was macerated in 250 ml of ethanol for 3 h at room temperature. Occasional shaking for 24 h at room temperature was performed and, the supernatant was decanted. Thereafter, the supernatant was filtered under reduced pressure by rotary evaporatorion at 55°C. Each residue was weighed and the yield percentage was calculated and then stored at 4°C in tightly sealed glass vial ready for use. The remaining extracts which were not soluble were successively extracted using ethanol with the described technique. The extracts were kept in freez dryer for 48 h, (Virtis, USA) until they were completely dried. The residue was weighed and the yield percentage was calculated. The extracts were kept and stored at 4°C until required.

In vitro testing of extract for anti giardial activity

Parasite isolate

G. lamblia used in all experiments were taken from patients from Ibrahim Malik Hospital (Khartoum). All taken samples were examined by wet amount preparation; the positive samples were transported to the laboratory in nutrient broth medium. Trophozoites of *G. 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.

Inoculum

G. 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.

In vitro susceptibility assays

In vitro susceptibility assays used the sub-culture method by Cedillo-Rivera *et al*²⁴, 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*²⁵. 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 µl 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 µl of extract to the second column wells and taking 20 µl out of the complete solution in C-2 wells to C-3 wells and discarding 20 µl from the total solution of C-3 to the remaining 20 µl serial solutions in the successive columns. 80 µl of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 100 µl.

In each test, Metronidazole (a trichomonocide) pure compound [(1-(2-hydroxyethyl)-2-methyl-5 Nitroimidazole], a was used as positive control in concentration 312.5 µg/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 four times for counting after 24, 48, 72 and 96 h. The mortality % of parasite for each extracts activity was carried out according to the following formula:

$$\text{Mortality of parasites (\%)} = \frac{\text{Control negative} - \text{tested sample with extract}}{\text{Control negative}} \times 100\%$$

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

Cytotoxicity Screening

Microculture tetrazolium MTT assay was utilized to evaluate the cytotoxicity of the *A. digitata*.

Microculture Tetrazolium (MTT) Assay

Principle

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-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is 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 cells²⁶.

Preparation of *A. digitata* extract

Using a sensitive balance 5 mg of each extracts were weighed and put in eppendorf tubes. 50 µl of DMSO were

added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

Cell Line and Culturing Medium

Vero (Normal, African green monkey kidney) cells were 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.

Cell line used

Vero cells (Normal, African green monkey kidney).

Cell counting

Cell counts were done 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. The following formula was used for calculating cells:

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

Procedure

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. 1 ml complete medium was added to the cells and all the cell suspension was contained in

a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 µl taken from row B were pipetted and mixed well in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated three concentrations 500, 250, 125 µg/ml for each extract. Then the plate was covered and incubated at 37°C for 96 hours.

On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96-well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37°C. MTT was removed carefully without detaching cells, and 100 µl of DMSO were added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

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

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

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assay results were done using Microsoft Excel program (2007).

Results

The leaves of *A. digitata* (family: Malvaceae) was screened for anti-giardial activity against (*Giardia lamblia*) trophozoites *in vitro*, and screened for cytotoxicity using 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) Vero cell line.

The leaves of *A. digitata* was screened for anti-giardial activity against (*Giardia lamblia*) trophozoites *in vitro*, and screened for cytotoxicity using 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) Vero cell line. And the yield percentage (%) of ethanol leaves extract of *A. digitata* investigated for anti-giardial activity and cytotoxicity was found to be 9.3% (**Table 1**).

Anti-giardial activity of *A. digitata* (leaves) extract

The anti-giardial potential of the ethanolic extract of *A. digitata* (leaves) was extracted by ethanol, with different concentrations (500, 250 and 125 ppm) and Mertronidazole (the reference control) with concentration (312.5 µg/ml) to be investigated against *Giardia lamblia* trophozoites *in vitro*. Ethanolic extract of *A. digitata* (leaves) showed 100% inhibition at a concentration 500 µg/ml after 96 h; this was compared with Metronidazole which gave 96% inhibition at concentration 312.5 µg/ml at the same time against *Giardia lamblia* (**Figure 1**).

The leaves of *A. digitata* was screened for anti-giardial activity against (*Giardia lamblia*) trophozoites *in vitro*. Showed anti-giardial activity with an inhibition concentrations (IC) more than

25.74 $\mu\text{g/ml}$ after day tow and gave an IC_{50} , IC_{90} , IC_{95} and IC_{99} after one day, after tow day, after three day and after four day respectively (Table 2).

Cytotoxicity assay of *A. digitata* (leaves) extract

The maximum concentration used was 500 $\mu\text{g/mL}$. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.

This table indicates the % inhibition of Vero cell line growth *in vitro* by ethanolic extract of *A. digitata* (leaves). MTT colorimetric assay was used. Reading in triplicate for different concentrations 125-500 $\mu\text{g/mL}$.

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

Discussion

The leaves of *A. digitata* (family: Malvaceae) was screened for anti-giardial activity against (*Giardia lamblia*) trophozoites *in vitro*, and screened for cytotoxicity using 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) Vero cell line.

Antigiardial activity of *A. digitata* (leaves) extract

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* (Boreham *et al*; 1984). Despite the previous comprehensive screening of Sudanese medicinal plants for their antiprotozoal activity^{27, 28, 29}.

Ethanolic extract of *A. digitata* (leaves) inhibited 100% inhibition concentration 500 $\mu\text{g/ml}$ after 96 h; this was compared with Metronidazole which gave 96% inhibition at concentration 312.5 $\mu\text{g/ml}$ at the same time against *G. lamblia* (Figure 1).

Interestingly, the cytotoxicity assays was 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 leaves of *A. digitata* was screened for anti-giardial activity against (*G. lamblia*) trophozoites *in vitro*. Showed anti-giardial activity with an inhibition concentrations (IC) more than 25.74 $\mu\text{g/ml}$ after day tow and gave an IC_{50} , IC_{90} , IC_{95} and IC_{99} after one day, after tow day, after three day and after four day respectively (Table 2).

Cytotoxicity assay of *A. digitata* (leaves) extract

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 (Table 3).

Conclusion

This result enhances the ethno botanical uses of *A. digitata* (leaves) as anti-giardial in cases associated with 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.

Acknowledgements

The authors are grateful to **Mr. Mohammed Ismail Garbi**, Department of Microbiology, Faculty of Medical Laboratory Sciences, International University of Africa. Khartoum, Sudan.

References

1. Amaral F. M. M., Ribeiro M. N. S., Barbosa-Filho J. M., Reis A. S., Nascimento F. R. F. and Macedo R. O. (2006). Plants and chemical constituents with giardicidal activity. *Braz. J. Pharmacogn.*, 16:696-720.
2. Koko W. S., MESAİK M. A., Yousaf S., Galal M. and Choudhary M. I. (2008). *In vitro* immunomodulating properties of selected Sudanese medicinal plants. *J. Ethnopharmacol.*, 118:26-34.
3. Yusha'u M., Hamza M. M. and Abdullahi N. (2010). Antibacterial activity of *Adansonia digitata* stem bark extracts on some clinical bacterial isolates. *Int. J. Biomed Health Sci.*, 6(3):129-135.
4. Wickens, G. E. (1982). The baobab, Africa's upside-down tree. *Kew. Bulletin.*, 37, 171–202.
5. Codjia, J. T. C., Fonton, B. K., Assogbadjo, A. E. and Ekue, M. R. M. (2001). Le Baobab (*Adansonia digitata*). Une Espèce à Usage Multiple au Bénin. Cecodi, CBDD, Veco, SNV, FSA.
6. Sidibe M. and Williams, J. T. (2002). Baobab. *Adansonia digitata*. Fruits for the Future, 4. International Centre for Underutilised Crops, Southampton, UK, pp. 100.
7. Chadare, F. J., Linnemann, A. R., Hounhouigan, J. D., Nout, M. J. R. and Van Boekel, M. A. J. S. (2009). Baobab food products: a review on their composition and nutritional value. *Critical Reviews in Food Science and Nutrition*, 49:254–274.
8. De Caluwé, E., Halamová, K. and Van Damme, P. (2009). Baobab (*Adansonia digitata* L.): a review of traditional uses, phytochemistry and pharmacology. In: Rodolfo, H., Simon, J.E., Ho, C.-T. (Eds.), African natural plant products: new discoveries and challenges in chemistry and quality. *Oxford University Press*, USA, pp. 51–84.
9. Buchmann, C., Prehsler, S., Hartl, A. and Vogl, C. R. (2010). The importance of baobab (*Adansonia digitata* L.) in rural West African subsistence—suggestion of a cautionary approach to international market export of baobab fruits. *Ecology of Food and Nutrition*, 49, 145–172.
10. El-Rawy, E. M., Gergis, S. M., Bazaid, S. and El-Mougy, S. A. (1997). The immunostimulant effect of *Adansonia digitata* on the immune response of chicken vaccinated with avian cholera vaccine. *Journal of Egyptian Veterinary Medical Association*, 57, 959–970.
11. Ramadan, A., Harraz, F. M. and El-Mougy, S. A., (1994). Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of *Adansonia digitata*. *Fitoterapia*. LXV, 418–422.
12. World Health Organization. (1998). Control of tropical disease. WHO, Geneva.
13. Lauwaet, T., Y. Andersen, *et al.* (2010). "Rapid detachment of *Giardia lamblia* trophozoites as a mechanism of antimicrobial action of the isoflavone formononetin." *J. Antimicrob Chemother*, 65(3):531-534.
14. Noor Azian M. Y., San Y. M., Gan C. C., Yusri M. Y., Nurulsyamzawaty Y., Zuhazam A. H., Maslawaty M. N., Norparina I. and Vythilingam I. (2007). Prevalence of intestinal protozoa in an aborigine community in Pahang, Malaysia. *Trop. Biomed.*, 24:55-62.
15. Dib H. H., Lu S. Q. and Wen S. F. (2008). Prevalence of *Giardia lamblia* with or without diarrhea in South East, South East Asia and the Far East. *Parasitol. Res.*, 103(2):239-251.
16. Addy S., Antepim G. and Frimpong E. H. (2004). Prevalence of pathogenic *Escherichia coli* and parasites in infants with diarrhoea in Kumasi, Ghana. *E. Afr. Med. J.*, 81(7):353-357.

17. Ayeh-Kumi P. F., Quarcoo S., Kwakye-Nuako G., Kretchy J. P., Osafo-Kantanka A. and Mortu S. (2009): Prevalence of Intestinal Parasitic Infections among Food Vendors in Accra, Ghana. *J. Trop. Med. Parasitol.*, 32(1):1.
18. Nyarango R. M, PA A., EW K. and BO N. (2008). The risk of pathogenic intestinal parasite infections in Kisii Municipality, Kenya. *BMC Public Health*, 8:237.
19. Townson S. M., Boreham P. F., Upcroft P. and Upcroft J. A. (1994). Resistance to the nitroheterocyclic drugs. *Acta. Trop.*, 56:173-194.
20. Bhopale K., Pradhan K., Masani K. and Karl C., (1995). Additive effect of diloxanidefurate and Metronidazole (Entamizole R) in mouse caecal amoebiasis. *Indian J. Exp. Biol.*, 33:73-74.
21. Tierney L. M., Mcphee J. S. and Papadakis M. A. (1999). Current medical diagnosis and treatment Appleton and Lange (Ed.), Stanford, Conn.
22. Upcrof J. A., Dunn L. A., Wrigh J. M., Benakli K., Upcrof P. and Vanelle P. (2006). 5-nitroimidazole drugs effective against Metronidazole-resistant *Trichomonas vaginalis* and *Giardia dodenalis* spp. *Animicrob. Agent. Chemother.*, 50:344-347.
23. Harbone , B. (1984). Phytochemical methods. 2nd. New York, Champan Hall, 4:4-7.
24. Cedillo-Rivera R., Chave B., Gonzalez-Robles A., Tapia-Contreras A. and Yopez-Mulia L. (2002). *In vitro* effect of nitazoxanide against *Entamoeba histolytica*, *Gairdia lamblia* and *Trichomonas vaginalis* trophozoites. *J. Eukaryotic Microbiol.*, 49:201-208.
25. Arguello-Garcia R., Cruz-suto M., Romero-Montoya L. and Ortega-Pierres G. (2004). Variability and variation in drug susceptibility among *Giardia duodenalis* isolates and clones exposed to 5-nitromidazoles and benzimidazoles *in vitro*. *J. Antimicrob. Chemother.*, 54:711-721.
26. Patel S., Gheewala N., Suthar A., and Shah A. (2009). *In vitro* Cytotoxicity Activity of *Solanum Nigrum* Extract Against Hela Cell Line And Vero Cell Line. *International Journal of Pharmacy and Pharmaceutical Sciences*, 1(1):38-46
27. Samia H. A., Elmalik K. H., Khalid H. S., Ashamat A. M. and Khojali S. M. E. (2004). Biochemical changes in rats experimentally infected with *Trypanosoma evansi*. *Journal of animal and veterinary advances*, 3(7):483-486.
28. Hiba Ali., Ko'nig G. M., Khalid S. A., Wright A. D. and Kaminsky R. (2002). Evaluation of selected Sudanese medicinal plants for their *in vitro* activity against hemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory, and for cytotoxicity. *Journal of Ethnopharmacology*, 83:219-228.
29. El Tahir, A., Satti, G. and Khalid, S. (1999). Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Acacia nilotica*. *Phytotherapy Research*, 13, 474-478.

Table 1: Preliminary quantitative data on the amount of *A. digitata* leaves used in the anti-giardial activity and cytotoxicity study

Scientific Name of Plant	Family name	Part Used	Yield %
<i>A. digitata</i>	Malvaceae	Leaves	9.3

Table 2: Inhibition concentration (IC) *A. digitata* (leaves) ethanol extract against *G. lamblia*

IC (µg/ml)	IC ₅₀	IC ₉₀	IC ₉₅	IC ₉₉
After day one	164.68	1454.30	1909.45	2374.16
After day tow	70.17	1085.90	1529.30	2011.21
After day three	42.18	393.25	519.84	649.87
After day four	25.74	287.81	389.18	495.45

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

Table 3: Cytotoxicity of *A. digitata* extract on normal cell lines (Vero cell line) as measured by the MTT assay

S. No.	Name of plant (part)	Concentrations (µg/ml)	Absorbance	Inhibition (%) ± SD	IC ₅₀ (µg/ml)
1	<i>A. digitata</i> (leaves)	500	2.42	17.9 ± 0.05	> 100
		250	2.87	10.8 ± 0.03	
		125	3.42	5.5 ± 0.02	
2	*Control		0.14	95.3 ± 0.01	

Key: *Control = Triton-x100 was used as the control positive at 0.2 µg/mL.

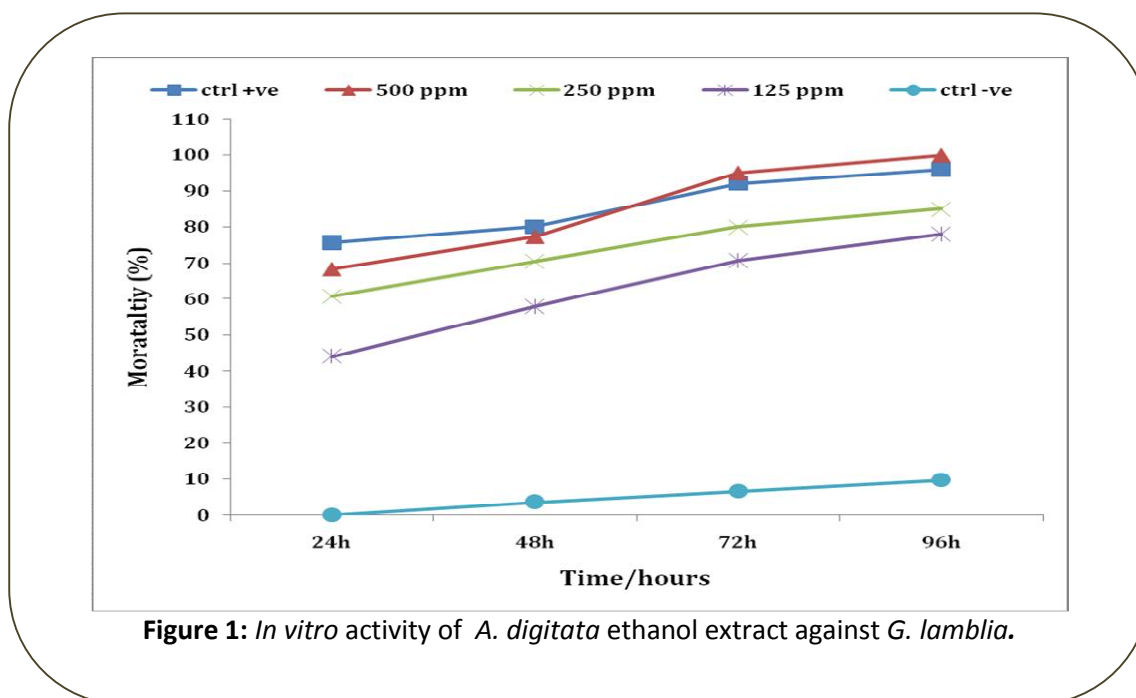


Figure 1: *In vitro* activity of *A. digitata* ethanol extract against *G. lamblia*.