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Antigiaridial Activity and Cytotoxicity of Ethanolic Bark Extract of *Acacia nilotica* (L.)

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Abstract:

Background: *Acacia nilotica* (L.) was used to treat different ailments for instance cold, congestion, fever, gallbladder, hemorrhage, hemorrhoids, leucorrhoea, ophthalmic, sclerosis, small pox, intestinal pains and acute diarrhea. Other preparations are used for gargle, toothache, ophthalmic and syphilitic ulcers. Human parasitic infections still represent a challenging public health problem worldwide, especially in tropical and subtropical regions. Giardiasis is the most common cause of parasitic gastro-intestinal disease and up to two hundred million people are chronically infected with *Giardia lamblia* globally with 500,000 new cases reported annually.
Objectives: The purpose of the paper is to investigate the *in-vitro* antigiardial activity and cytotoxicity (MTT assay) of ethanol extract of *A. nilotica* subsp. *nilotica* (bark).

Method: The ethanolic extract of *A. nilotica* (bark), with different concentrations (500, 250 and 125 ppm) were investigated together with Metronidazole as a reference control at 312.5 µg/ml against *Giardia lamblia* trophozoites. Cytotoxicity (MTT assay) was also performed with different concentrations (500, 250 and 125 ppm) and compared to the reference control Triton-x100.

Result: *A. nilotica* bark ethanolic extract exhibited 100% mortality within 96 h, at a concentration of 500 ppm; this was compared with Metronidazole which gave 96% inhibition at the concentration of 312.5 µg/ml at the same time. In addition cytotoxicity (MTT-assay) verified the safety of the examined extract with an IC_{50} less than 100 µg/ml.

Conclusion: These studies prove the potent activity of *A. nilotica* against *Giardia lamblia* trophozoites *in vitro* with verified safety evidence for use.

Keywords: *In vitro*, Antigiardial activity (*Giardia lamblia*), Metronidazole, Cytotoxicity (MTT-assay), *Acacia nilotica*.

Introduction

Medicinal plants are invaluable, safe, less toxic, cheap, available and reliable natural sources 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 the disabling diseases [1, 2].

Acacia nilotica Subsp. nilotica belongs to the family of Mimosaceae. The tree has yellow mimosa-like flowers and long grey pods constricted between seeds. The bark and branches bear spikes about 2cm long. The five leaves are densely hairy with 3-6 pairs of pinnate consisting of 10-20 pairs of leaflets that

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narrow with parallel margins rounded at the apex and with a central midrib closely crowded.

The inflorescence consist of bright yellow flowers in auxiliary head on stalk half way up. The flowering period of the plant is between November and March [3]. The native distribution of *Acacia nilotica* includes much of Africa and the Indian subcontinent [4]. From the Germplasm Resources Information Network: GRIN database [5], the native distribution includes: Africa (Algeria, Angola, Botswana, Egypt, Ethiopia, Gambia, Ghana, Guinea-Bissau, Kenya, Libya, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe) and Asia (Iran, Iraq, Israel, Oman, Saudi Arabia, Syria, Yemen, India, Nepal, Pakistan)[6]. The bark tinges of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns. The bark is used in the treatment of hemorrhages, cold, diarrhea, tuberculosis, leprosy colds, bronchitis, diarrhea, bleeding piles and leucoderma [7, 8]. Decoction of the bark is largely used as an astringent douche in gonorrhea, cystitis, vaginitis, leucorrhoea, prolapse of the uterus and piles [9].

The protozoan *Giardia lamblia* (Figure 1) is the most frequently isolated intestinal protozoan parasite around the world and it is the causal agent of the disease known as giardiasis [10]. *Giardia lamblia* is a unicellular, flagellated intestinal protozoan parasite isolated worldwide and is ranked among the top 10 human parasites [11, 12]. The morphology of *Giardia* is encountered in two forms: trophozoite and cyst. The trophozoite stage is approximately 12-15 microns by 6-8 microns [13]. The cyst of *Giardia lamblia* is elliptically shaped, range in size from 6 to 10 microns and contains two to four nuclei [14].

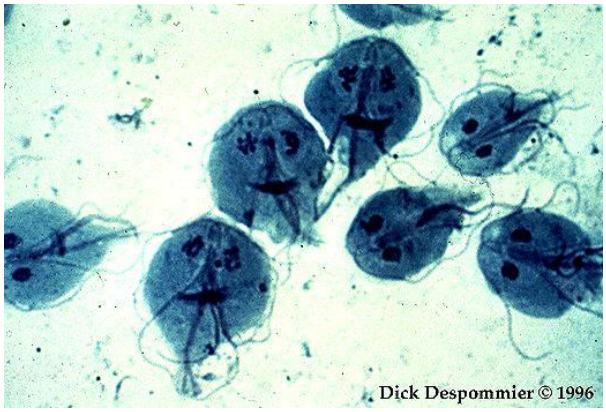

Figure 1. *Giardia lamblia* in Gram staining

Epidemiological studies suggest that the parasite is responsible for about 5% of acute diarrhea and 20% of chronic diarrheal illness in the world [15]. Although symptomatic infection causes a broad spectrum of clinical manifestations, Giardia results in asymptomatic carrier state in a majority of cases. The asymptomatic infections are most common in children and people with prior exposure to a source of infection [13].Typical patient has symptoms for at least one week, including foul-smelling diarrhea, nausea, abdominal cramps, flatulence, and intense fatigue [16].

The transmission of *Giardia* to humans depends on the ingestion of cysts excreted in the faces of infected persons or animals. The principal mode of transmission to humans appears to be person-to-person, although indirect transmission from contaminated water and food such as infected animals has been described [17,23].

The treatment of giardiasis consists of the use of one or more drugs, with metronidazole being the first choice. Other nitroimidazolic derivatives (secnidazole, tinidazole, and ornidazole), benzim idazoles (albendazole, mebendazole), furazolin, quinacrine and paromomycin have also been employed in therapeutic regimens. However, these drugs have adverse effects including gastrointestinal disturbances, nausea, headache, leucopoenia, myopia, neuralgia, allergic dermatitis and an unpleasant taste in the mouth. Furthermore, they can lead to neurotoxic effects, ataxia, convulsions and vertigo, bringing to the interruption of treatment. In addition, mutagenic and carcinogenic effects have been described in laboratory animals [24, 28].

Metronidazole is widely used and recommended in the treatment of giardiasis but it is less effective in the tissue than in the gut lumen [29,30]. In addition, it can eradicate only up to 50% of laminae infections [31]. Metronidazole sometimes causes adverse effects like myoplasia, neuralgia, and allergic dermatitis [32].

The need of alternative drugs to reduce the use of synthetic drugs especially after the growing resistance to Metronidazole [33,34] is urgent and new antigiardial drugs are probably required. The present study was conducted to investigate the antigiardial activity and cytotoxicity of *A. nilotica* bark in Sudan wich maybe a potential natural alternative.

Materials and Methods

Plant materials

Acacia nilotica bark was collected from (Khartoum) Central Sudan during the period of January to February 2014. The plant was identified and authenticated by the researcher **Dr**. **Haider Abdelgadir** from Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) in Khartoum, Sudan. Bark of *A. nilotica* (Figure 2) was air-dried, under the shade, pulverized and stored prior to extraction. Shade with good ventilation and then ground finely in a mill and kept in the herbarium until extract preparation.

Figure 2. *Acacia. nilotica* (bark)

Preparation of crude extract

Extraction was carried out for the bark of *A. nilotica* by overnight maceration techniques according to the method described by Harbone [35]. About 50 g of the dried powder from *Acacia nilotica* barks was macerated in 250 ml of ethanol for 3 h at room temperature. Occasional shaking for 24 h was performed and, the supernatant was decanted into a clean, dry container. After this, the supernatant was filtered using Clevenger's apparatus and kept in freeze dryer for 48 h, (Virtis, USA) until completely dried. The extracts were kept and stored at 4°C until required for analysis.

In vitro testing of extract for antigiardial activity

Parasite isolation:

Giardia lamblia used in the experiments were taken from patient with positive giardiasis from Ibrahim Malik Hospital (Khartoum). Samples were transported from the hospital to the laboratory of research institute in RPMI 1640 medium. Culturing of the *G. lamblia* trophozoites was performed at 37 ± 1°C in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS). The cultured trophozoites were used for the assay in the log phase of growth (Figure 3).

A **B**

Figure 3. Culture of *G. lamblia* in RPMI 1640 medium. A: Laboratory hood B: Culture flask containing medium and protozoan

In vitro susceptibility assays

In vitro susceptibility assays were performed following the sub-culture method of Cedillo-Rivera *et al*. [36], which is a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *E. histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis* [37]. 5 mg from both plant extract and compound was

dissolved in 50 µl of dimethylsulfoxzide (DMSO) in an Eppendorf tube containing 950 µl od DMSO in order to reach the concentration of 5 mg/ml (5000 ppm). The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution. The concentrates were stored at -20°C for further analysis. Sterile 96 multi-well plate [8 columns (C) × 12rows (R)] was used to test the plant extract, the positive and negative control with three columns used for each extract. 40 µl of the plant 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 from C1 to the second column The mortality % of parasite for each extract was calculated according to the following formula:

wells and mixing then, 20 µl were taking out from the solution in C-2 wells to C-3 wells and discarding 20 µl from the total solution of C-3. 80 µl of culture medium was complemented with parasite (1 $X10^3$ cell/ml) and added to all wells. The final volume in the wells was 100 µl. Metronidazole (a trichomonocide) pure compound [(1-(2-hydroxyethl)-2-methyl-5 Nitroimidazole], was used as positive control at a concentration of 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 three times after 24, 48, 72 and 96 h from the assay.

Mortality of cells % = (n° of cells in negative Control -n° of cells in tested sample with extract)×100 n° of cells negative Control

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

Cytotoxicity screening

Micro-culture-tetrazolium MTT-assay was utilized to evaluate the cytotoxicity of the *A. nilotica*. 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 [38].

Cell Line and Culture 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 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. The following formula was used for calculating the cells:

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

*Dilution factor is usually 2 (1:1 dilution with trypan blue), but further dilution may be needed for highly concentrated cell suspensions.

MTT assay

Serial dilutions of extract were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer walls of the plate (A and H) were filled with 300 µl of in-complete culture medium in addition to 2µl of sterile 0.5% Triton X and were used for the negative control. The 6 rows in the middle (B – G) received 250 µl of in-complete culture medium and 50 µl/wells of complete culture medium (CCM), B column wells were used as first extract dilution with 500 µg of suspension extract added. Extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 150 µl to the next well after proper mixing. From the last dilution wells (B-11), 150 µl were discarded. Each compound was tested in triplicate. Cell suspension in a complete culture medium containing 2.5 X 10^5 /ml was properly mixed, and 150 µl 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 removed from each well leaving detaching cells. MTT ((3- (4,5-Dimethyl-

thiazol-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 was 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 µl of diluted MTT were added. The plate was incubated further at 37° C for 2 to 3 hours in CO_2 incubator. MTT was removed carefully without detaching cells, and 200 µl 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 (Figure 4). The percentage growth inhibition was calculated using the formula below:

% cell inhibition = 100-{(Ac-At)/Ac} × 100

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

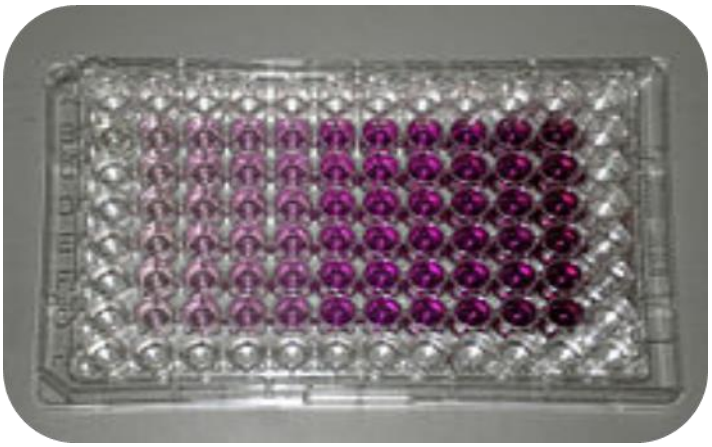

Figure 4. A microtiter plate after MTT assay.

Statistical analysis

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

Results and Discussion

The bark of *A. nilotica* family (Mimosaceae) was screened for antigiardial activity against (*Giardia lamblia*) trophozoites *in vitro* and tested for cytotoxicity using 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) in Vero cell line. The yield % of *A. nilotica* bark ethanol extract was 9.5%.

The antigiardial potential of the ethanolic extract of *A. nilotica* (bark), with different concentrations (500, 250 and 125 ppm) and Mertronidazole (the reference control) with concentration of 312.5 µg/ml was investigated against *G. lamblia* trophozoites *in vitro*. The ethanol extract of *A. nilotica* (bark) showed 100% inhibition at a concentration of 500 µg/ml after 96 h; which was compared with Metronidazole giving 96% inhibition at the concentration of 312.5 µg/ml at the same time against *G. lamblia* (Figure 5).

The bark of *A. nilotica* screened for antigiardial activity against (*G. lamblia*) trophozoites *in vitro* showed antigiardial activity with an inhibition concentrations (IC) more than 1.40µg/ml and increasing successively during the days of screening (Table 1). This results are similar to the antitrichomonal activity in Sudan **[**39**]**.

These results agree with traditional uses of *A. nilotica* in Sudan which claimed that the plant has anti-parasitic properties. The plant extract were found to exhibit antidiarrhoeal, antibacterial, antimalarial and inhibition of lipid peroxidation **[**40- 43**]**.

Moreover, the results obtained in this study are similar to the studies carried out for antiplasmodial activity in mice by Alli et al. [44], 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 IC_{50} was 13µg/mL [40] anti-Diarrhea activity [45a 46-48] and antiprotozoal activity in Sudan [49-51].

El Shanawny, [52] 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*, [53] 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 (12-20%), reducing sugars, terpenoids, saponins, glycosides, phlobetannin, gallic acid, protocatechuic acid pyrocatechol, (+) – catechin, (-) epigallocatechin-5,7-digallate [54, 60**].**

Interestingly, the cytotoxicity assays were conducted in this study to evaluate the cytotoxicity effects of ethanolic extract of *A. nilotica* (bark) by using MTT-assay in Vero cell line. Table 2 indicates the growth inhibition percentage (%) of Vero cell line *in vitro* by ethanolic extract of *A. nilotica* (bark) at different concentrations from125 to 500 µg/ml and showed an IC_{50}>100 (µg/ml) verifying the plant safety. This result was similar to that produced by Riaz et al. [61], using the Human brain microvascular endothelial cells (HBMEC).
Solomon *et al*, [62] 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.

Acacia nilotica has been reported to be very useful in treating diarrhea and cough in human Guinko [63].

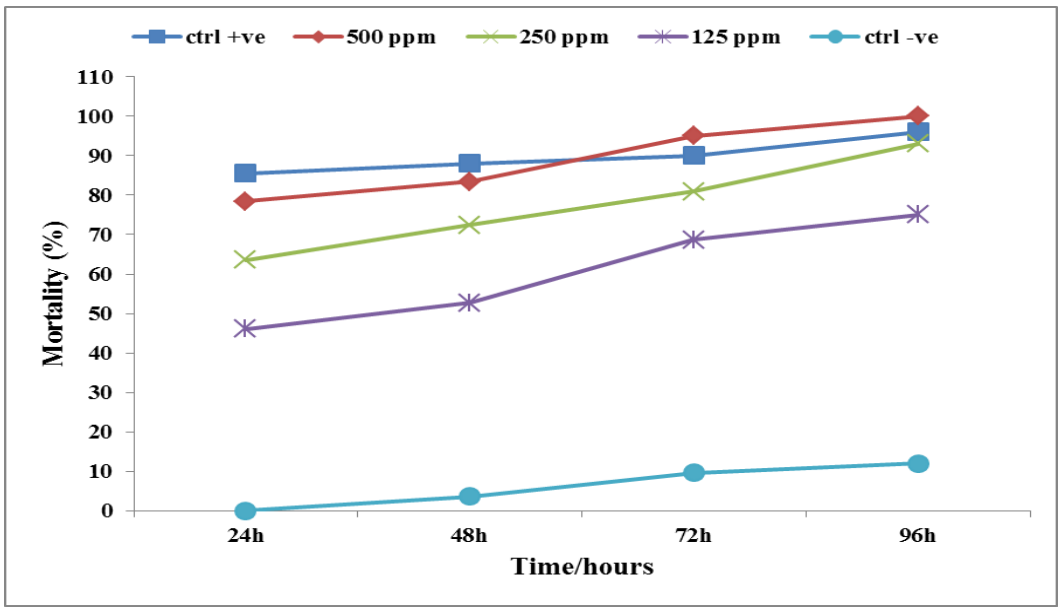

Figure 5. In vitro activity of A. nilotica ethanol extract against G. lamblia.

Table 1. Inhibition concentration (IC) of *A. nilotica* (bark) ethanol extract against *G.lamblia.*

IC (µg/ml)	**Time/days**			
	After one day	After two days	After three days	After four days
IC_{50}	1.40	1.60	1.80	4.20
IC_{90}	920.30	440.15	270.50	210.12
IC_{95}	2090.18	980.15	510.20	330.20
IC_{99}	4010.30	1990.12	850.30	470.10

Key: IC_{50} Inhibition concentration 50%, IC_{90} Inhibition concentration 90%, IC_{95} Inhibition concentration95%, IC_{99} Inhibition concentration 99%.

Table 2. Cytotoxicity of *A. nilotica* extract on normal cell lines (Vero cell line) as measured by the MTT assay.

No.	**Name of** sample	**Concentration (µg/ml)**	**Absorbance**	**Inhibition (%) ± SD**	**IC_{50} (µg/ml)**
1	*A. nilotica* (bark)	500	2.31	21.1 ± 0.05	> 100
		250	2.55	12.8 ± 0.03	
		125	3.23	10.6 ± 0.02	
2	*Control		0.09	95.96 ± 0.01	< 30

Conclusion

Our results revealed a good pharmacological activity of *A. nilotica* extract against *G. lamblia* suggesting that the extract has the potential of being used in parasitic infection. The results presented here provide motivation for further exploration in plant active compounds for the development of new anti-parasitic agents.

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References

1. Amaral FMM, Ribeiro MNS, Barbosa-Filho JM, Reis AS, Nascimento FRF And Macedo RO, (2006). Plants and chemical constituents with giardicidal activity. Braz J Pharmacogn 16: 696-720.
2. Koko WS, Mesaik MA, Yousaf S, Galal M. and Choudhary MI. (2008). *In vitro* immunomodulatingproperties of selected Sudanese medicinal plants. J Ethnopharmacol 118: 26-34.
3. Mann A, Gbate M and Umar A (2003). Medicinal and Economic Plants. Jube Evans Books and Publication, Bide, Nigeria.10-30.
4. Cox ML (1997). Homichloda barker (Jacoby) (Coleoptera: Chrysomelidae: Alticinae) a candidate agent for the biocontrol of prickly acacia, *Acacia nilotica* (Mimosaceae) in Australia. Journal of Natural History 31: 935-964.
5. USDA, ARS, National Genetic Resources Program. (2001). Germplasm Resources Information Network (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available:http://www.ars-grin.gov/cgibin/npgs/html/.
6. Bargali K and Bargali S (2009). *Acacia nilotica*: a multipurpose leguminous plant. Nature and Science. 7(4): 11-19.
7. Malviya S, Rawat S, Anil Kharia A and MeenaVermaM (2011). Medicinal attributes of *Acacia nilotica* Linn. - A comprehensive review on ethnopharmacological claims. International Journal of Pharmacy & Life Sciences. 2(6): 830-837.
8. Del WE (2009). *In vitro* evaluation of peroxyl radical scavenging capacity of water extract of *Acacia nilotica*(L) Afr.J.Biotechnol., 8(7): 1270-1272.
9. Nadkarni KM (2005). The Indian Plants and Drugs. New Delhi: Shrishti Book Distributors, 4, 5.
10. Campanati L, Monteiro-Leal LH 2002. The effects of the antiprotozoal drugs metronidazole and furazolidone on trophozoites of Giardia lamblia (P1 strain). ParasitolRes 88: 80-85.
11. Harris JC, Plummer S, Turner MP, Lloyd D (2000). The microaerophilic flagellate Giardia intestinalis: Allium sativum (garlic) is an effective antigiardial. Microbiology146: 3119-3127.
12. Harris JC, Plummer S, Lloyd D (2001). Antigiardial drugs. Appl Microbiol Biotechnol, 57: 614-619.
13. Morgan UM, Reynoldson JA, Thompson RCA 1993. Activities of several benzimidazoles and tubulin inhibitors against Giardia spp. in vitro. Antimicrobial Agents Chemistry, 37: 328-331.
14. Petri- Jr WA (2003). Therapy of intestinal protozoa. Trends Parasitology, 19: 523-526.
15. Upcrof JA, Dunn LA, Wrigh JM, Benakli K, Upcrof P and Vanelle P (2006). 5-nitro-imidazole drugs effective against Metronidazole-resistant *Trichomonasvaginalis* and *Giardia dodenalis*spp. Animicrob agent Chemother, 50: 344-347.
16. Townson SM, Boreham PF, Upcroft P, Upcroft JA, (1994). Resistance to the nitroheterocyclic drugs. Acta Trop, 56: 173-194.
17. Bhopale K, Pradhan K, Masani K and Karl C (1995). Additive effect of diloxanidefuorate and Metronidazole (Entamizole R) in mouse caecalamoebiasis. Indian J ExpBiol, 33: 73-74.
18. Tierney LM, Mcphee JS andPapadaksis MA, (1999). Current medical diagnosis and treatment Appleton and Lange (Ed.), Stanford, Conn.
19. Iran, M, Kezaeian Mal Izaddoost .M. (2006). In vitro antitrichomonas activity of Allium ehrtifolium(Persian shallot) in comparison with metrondizole. Iranian Journal of Rubi Health 35: 92 -94.
20. Pratibha Tiwari, Divya Singhand Man Mohan Singh. (2008) Anti-Trichomonas activity of Sapindus saponins, a candidate for development as microbicidal contraceptive, Journal of Antimicrobial Chemotherapy 62(3): 526-534.
21. Smith, H. V.; Paget, T., in: Foodborne Diseases: Infectious Dis-eases, Simjee, S., Ed., Humana Press, Inc., Totowa, N.J., 2007, 303-336.
22. Wolfe, M.S. (1992). Giardiasis. Clin. Microbiol. Rev., 5: 93-100.
23. Farthing, M. (1997). The molecular pathogenesis of giardiasis. J. Pediatr. Gastroenterol. Nutr., 24: 79-88.
24. Heresi, G., and Cleary, T. G. (1997). *Giardia*. Ped. in Rev., 18(7): 243-247.
25. Ortega, Y., and Adam, R. (1997). *Giardia*: overview and update. Clin. Infect. Dis., 25(3): 545-549.

26. Thompson, R. C. A., Reynoldsin, J. A., and Mendis, A. H. W. (1993). Goardoa and *Giardia*sis. Advances in Parasitol., 32:71-160.
27. Petri, W.A. (2005). Treatment of Giardiasis. Curr Treat Options Gastroenterol, 8:13-17.
28. Mildran, D., Gells, A. M., and William, D. (1977). Venereal transmission of enteric pathogens in male homosexuals--two case reports. J. A. M. A., 238:1387-1389.
29. Akin, E.W., and Jakubowski W. (1986). Drinking water transmission of giardiasis in the United States. Wat. Sci. Tech., 10:219-26.
30. Payment, P. (1999). Poor efficacy of residual chlorine disinfectant in drinking water to inactivate waterborne pathogens in distribution systems. Can. J. Microbiol., 45(8):709-715.
31. American Academy of Pediatrics, (2000). Red Book, Report of the Committee on Infectious Diseases, pp 252-253.
32. Quick, R., Paugh, K., Addiss, D., Kobayashi, J., and Baron, R. (1992). Restaurant-associated outbreak of giardiasis. J. Inf., 166:673-976.
33. Mintz, E. D., Hudson-Wragg, M., Mshar, P., Cartter, M. L. and Hadler, J. L. (1993). Foodborne giardiasis in a corporate office setting. J. Inf., 167:250-253.
34. Rose, J. B., and Slifko T. R. (1999). *Giardia, Cryptosporidium*, and *Cyclospora* and their impact on foods: a review. J. Food Protection, 62(9):1059-1070.
35. Harbone B. (1984). Phytochemical methods. 2nd. New York, Champan Hall, 4, 4-7.
36. Cedillo-Rivera R, Chave B, Gonzalez-Robles A, Tapia-Contreras A and Yepez-Mulia L (2002). *In vitro* effect of nitazoxanide against *Entamobahistolytica, Gairdialamblia*and *Trichomonasvaginalis*trophozoites. J Eukaryotic Microbiol, 49:201-208.
37. 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 AntimicrobChemother, 54:711-721.
38. Patel S, Gheewala N, Suthar A and Shah A (2009). *In-vitro* Cytotoxicity Activity of *SolanumNigrum* Extract against HelaCell Line and VeroCell Line. International Journal of Pharmacy and Pharmaceutical Sciences, 1(1):38-46.
39. Dahab. M. M., Koko. W. S., Osman. E. A. (2010). *In vitro* Antitrichomonal Activity of *Acacia nilotica* L different extracts. International journal of natural product and pharmaceutical sciences. 1(1): 10-14.
40. Tahir A.E. Satti G.M.H. and Khalid S.A (1999). Antiplasmodia l Activity of Se lected Sudanese Medicinal Plants with Emphasis on Acacia nilotica. Phytother. Res., 13: 474–478.
41. Saleem, A., Ahotupa, M., Pihlaja, K. (2001) Total phenolics concentration and antioxidant potential of extracts of medicinal plants of Pakistan. Zeitschrift fur Naturforschung (C) 56, 973-978.
42. Rani, P., and Khullar, N. (2004) Antimicrobial evaluation of some medicinal plants for their antienteric potential against multi-drug resistant *Salmonella typhi*. Phytotherapy Research 18, 670-673.
43. Agunu, A., Yusuf, S., Andrew, G., Umar Zezi, A., Aburahman, E. (2005) Evalutation of five medicinal plants used in diarrhoea treatment in Nigeria. Journal of Ethnopharmacology 101, 27-30.
44. Alli LA, Adesokan AA, Salawu OA, Akanji MA, Tijani AY. Anti-plasmodial activity of aqueous root extract of Acacia nilotica, African Journal of Biochemistry Research 2011; 5(7):214-219
45. Guinko S (1991) Etude Surle role des Acacia dans Le development rural au Burkin Faso et au Niger. Ouagaduogu (Mimeo).,1: 6-10.45.
46. Gill L.S. (1992) Ethanomedical uses of plants in Nigeria. Un iversity of Benin Press, Benin City Nigeria . 10-30. 46.
47. Patel J.D. Patel D.K. and Ku mar V (2008) Screening of plant extracts used in traditional antidiarrhoeal medic ines against pathogenic Escherichia coli.Scientific World. 6(6): 63-67.
48. Sanni S. Th ilza I.B. Talle M. (2010). The effect of Acacia nilotica pod Ethyl Acetate fraction on induced diarrhea in alb ino rats. New York Science Journal. 3(8): 16-20.
49. Samia HA, ElmalikKH, KhalidHS, AshamatAM and KhojaliSME (2004). Iochemical changes in rates experimentally infected with *Trypanosomaevansi*. Journal of animal and veterinary advances. 3(7):483- 486.
50. Hiba A. Ko¨nigGM, KhalidSA, WrightAD and KaminskyR (2002). Evaluation of selected Sudanese medicinal plants for their *in vitro* activity gainsthemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory, and for cytotoxicity. Journal of Ethnopharmacology 83: 219-228.
51. Koko SW (2005). Antimalarial Activity of Xanthium brasilicumVell*. *In vitro, In vivo* and Toxicological Approaches. Recent progress in medicinal plants, 15:1-10.
52. El-Shanawany, M. A. (1996) Medicinal Plants Used in Saudi Traditional Medicine, King Abdel Aziz City for Science and Technology, Riyadh.144.
53. Fatima, A. K., Nazar, M., Husam Eldin, O., Mohamed., M, Mubark M., Magzoub, M., Siddig, A. (2005) *In vitro* Assessment of Anti - Cutaneous Leishmaniasis Activity of Some Sudanese Plants. Türkiye Parazitoloji Dergisi, 29, 3-6.
54. Seigle r D.S. (2003). Phytochemistry of Acacia-sensu lato.Biochem. Syst. Ecology., 31(8): 845–873.

55. Singh Ra jbir, Singh Bikra m and Singh Sukhpreeet (2008) Anti-free radical activities of kae mpferol isolated from Acacia nilotica(L.) Willd. Ex. De l.Toxicology in Vitro., 22(8): 1965–1970.25.
56. Banso A (2009) Phytochemical and antibacterial investigation of bark e xtracts of Acacia nilotica. J. Med. Plants Res., 3(2): 082-085.26.
57. Pande M.B. (1981) Note on the nutritive value of babul (Acacia niloticaL.)seeds(e xtracted). Indian J. Anim. Sci., 1981, 51(1): 107-108.
58. Wassel G.M . (1990) Study of phenolic constituents and tannins isolated from Acacia nilotica L. Willd and Acacia farnesiana L. Willd growing in Egypt". Herba Hungarica., 29(1,2):43-49.
59. Chaubal R, Ta mbe A (2006) Isolation of new straight chain compound from Acacia nilotica. Ind. J. Chem., 45(B):1231-1233.
60. Bushra S, Fa rooq A, Ro man PAntioxidant activity of phenolic components presentin barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica, and Eugenia jambolanaLam. T rees. Food Chemistry. 2007, 104 (3): 1106-1114.
61. Riaz S., Faisal M., Hasnainand S., Ahmed K. N. (2011). Antibacterial and Cytotoxic Activities of Acacia niloticaLam (Mimosaceae) Methanol Extracts Against Extended Spectrum Beta-Lactamase Producing Escherichia coli and Klebsiella Species. Tropical Journal of Pharmaceutical Research, 10 (6): 785-791.
62. Solomon G.O. and Shittu G.A. (2010) In vitro antimicrobial and phytochemical activities of Acacia nilotica leaf extract. J. Med. Plants. Res., 4(12):1232-1234.
63. Guinko S (1991) Etude Surle role des Acacia dans Le development rural au Burkin Faso et au Niger. Ouagaduogu (Mimeo).,1: 6- 10.