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Antiamoebic and Cytotoxicity of Ethanolic Fruit Extract of *Kigelia africana*

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Abstract

Kigelia africana (family Bignoniaceae), is a common component of the pharmacopeia's of multiple African groupings which inhabit the areas in which it grows. Amongst these groups there is a myriad of medicinal uses in the treatment of a wide variety of bacterial, fungal and protozoal infections, as well as in the treatment of cancers. 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. This study was carried out to evaluate antiamoebic activites (*E. histolyica*) and cytotoxicity (MTT assay) of ethanol extract of *K. africana* (fruits). The extract of *K. africana* (fruits), with different concentrations (500, 250 and 125 ppm) and Metronidazole concentration (312.5 µg/ml) to be investigated *in vitro* against *E. histolyica* trophozoites, and cytotoxicity (MTT) assay with test concentrations and Triton-100 (the reference control) was studied. The ethanolic fruit extract exhibited 100% mortality within 96 h, at a concentration 500 ppm, whereas, metronidazole gave 96% inhibition at concentration 312.5 µg/ml. MTT assay verified the safety of the examined extract. The studies conducted for *K. africana* fruit extract was proved to have potent activities against *E. histolyica* trophozoites *in vitro*.

Keywords

Antiamoebic
Entamoeba histolyica
Fruit extract
Cytotoxicity
Kigelia africana

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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 (Amaral et al., 2006; Koko et al., 2008).

Kigelia africana (family Bignoniaceae), commonly known as sausage tree due to the shape of its fruit, is an African plant with a wide geographical range of usage, ranging from Southern Africa, through Central Africa, to Western Africa. Whilst the fruit is most often cited as having therapeutic properties, multiple parts of the *K. africana* tree have been used in traditional healing systems in the treatment of a variety of medical conditions and complaints. The powdered mature fruit is used to treat wounds, abscesses, and ulcers, whilst the green fruit is used to treat syphilis and rheumatism. An infusion made from the ground bark and fruit is used to treat stomach problems in children. Roots and bark are used to treat pneumonia. In West Africa, leaves and twigs are used to treat wounds, dysentery, stomach and kidney disorders, snakebite, and rheumatism. The fruit is used to treat constipation, gynaecological disorders, haemorrhoids, lumbago and dysentery. Slices of mature baked fruits are used to ferment and flavor traditional African beer (Arkhipov et al., 2014). Due to its range of medicinal uses, *K. africana* may provide a source of useful phytochemicals with therapeutic properties which could be used as alternatives to currently used medicines.

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 (WHO, 1997; Ravdin and Stauffer 2005). 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 (Farthing et al., 1996).

Amoebiasis is the infection of human gastrointestinal tract by *E. histolytica*, a protozoan parasite capable of invading the intestinal mucosa and that may spread to other organs, mainly the liver which usually leads to amoebic liver abscess. This infection remains a significant cause of morbidity and mortality world-wide (Stanley and Reed, 2001). 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 socio-economic status, poor sanitation and nutrition especially in the tropics (Ravdin and Stauffer, 2005). Thus the majority of *E. histolytica* infections, morbidity and mortality occur in Africa, Central and South America and the Indian sub-continent (Haque et al., 2000).

Metronidazole is the drug now widely used and recommended in the treatment of amoebiasis (Townso net al., 1994). But it is less effective in the tissue than in the gut lumen (Bhopale et al., 1995). In addition, it can eradicate only up to 50% of laminae infections (Tierney et al., 1999). Metronidazole sometimes causes adverse effects, example, myoplasia, neuralgia, and allergic dermatitis (Upcrof et al., 2006). The present study was conducted to investigate the antiamoebic activity and cytotoxicity of *K. africana* fruits in Sudan.

Materials and methods

Plant materials

The *K. africana* (fruits) was collected from central Sudan between January 2008 and February 2008. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants Research Institute (MAPRI). Fruits were air-dried, under shadow with good ventilation and then ground finely in a mill until their uses for extracts preparation. Table 1 indicates the scientific name, family, parts used, yield% of ethanol extract and traditional uses of *K. africana* (fruits).

Table 1. Extract yield and traditional medicinal uses of *K. africana* (fruits).

Name of Plant	Family name	Part Used	Yield %	Traditional medicine
K. africana	Bignoniaceae	Fruits	11.5	Fever, cold, respiratory tract infections, Anti-parasitic, antimicrobial, anticancer.

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Preparation of crude extracts

Extraction was carried out for the fruits of *K. africana* plant by using overnight maceration techniques according to the method described by Harbone (1984). About 50 g was 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.

Antimoebic activity of *K. africana* (fruits) extract

Parasite isolate: E. histolytica used in all experiments were taken from patients of 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 *E. histolytica* 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.

Inoculums: E. histolytica 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

For *in vitro* susceptibility assays, the sub-culture method of Cedillo-Rivera et al. (2002) was used This method is being described as a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *E. histolytica*, *Giardia intestinalis* and *Trichomonas vaginalis* (Arguello-Garcia et al., 2004). Five mg from each extract and compound was dissolved in 50 µl of dimethyl sulfoxzide (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-hydroxyethl)-2-methyl-5 Nitro-imidazole], 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 three 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:

$$\text{Cell mortality (\%)} = \frac{\text{(Control negative – Tested sample with extract)}}{\text{Control negative}} \times 100$$

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

Cytotoxicity screening

Microculture tetrazolium MTT (Methyl Thiazolyl Tetrazolium) assay was utilized to evaluate the cytotoxicity of the studied *K. africana* (fruits).

MTT assay

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

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activity is a measure of the viability of the cells (Patel et al., 2009).

Preparation of extracts and solutions: Using a sensitive balance, 5 mg of each extracts were weighed and put in Eppendorf tubes. DMSO (50 µl) was 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 subcultured 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:

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

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 min. separating the cells from the supernatant that flicked out. One 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. Three duplicated concentrations for each extracts i.e., 6 wells for each extract. 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 was pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts was 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 h.

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 h 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 min. then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

$$\%\ \text{cell inhibition} = 100 - \{(Ac - At)/Ac\} \times 100$$

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 assays results were done using Microsoft Excel program (2007).

Results and discussion

The The fruits of *K. africana* (family Bignoniaceae) were screened for antiamoebic activity against (*E. histolytica*) trophozoites *in vitro* and cytotoxicity studies using 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) with Vero cell line.

Antiamoebic activity of *K. africana* (fruits) extract

The antiamoebic potential of the ethanolic extract of *K. africana* (fruits) was extracted by ethanol, with different concentrations (500, 250 and 125 ppm) and

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Mertronidazole (the reference control) with concentration (312.5 µg/ml) to be investigated against *E. histolytica* trophozoites *in vitro*. Ethanol extracts of *K. africana* (fruits) showed 100% inhibition at a concentration of 500 µg/ml after 96 h; this was compared with Metronidazole which gave 96% inhibition at 312.5 µg/ml against *E. histolytica* (Fig. 1).

Fig. 1: *In vitro* antiamoebic activity of *K. africana* (fruits) ethanolic extract against *E. histolytica.*

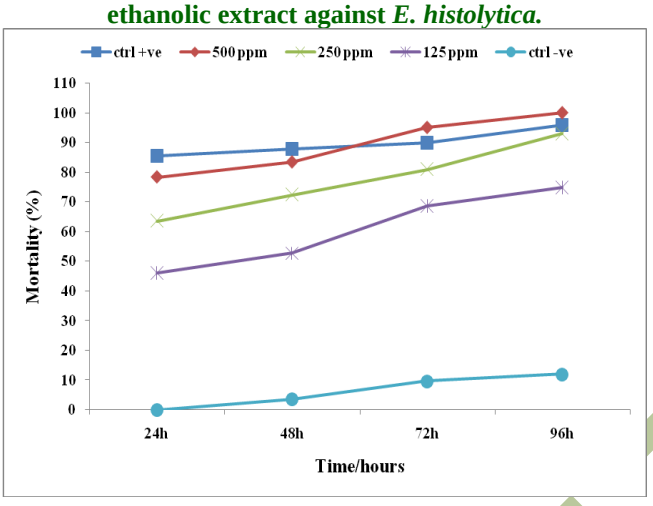

Table 2. Cytotoxicity of *K. africana* extracts on normal cell lines (Vero cell line) as measured by the MTT assay.

Name of plant (part)	Conc. (µg/ml)	Absorbance	Inhibition (%) ± SD	IC_{50} (µg/ml)
K. africana (fruits)	500	2.31	21.1 ± 0.05	> 100
	250	2.55	12.8 ± 0.03	
	125	3.23	-10.6 ± 0.02	
*Control		0.09	95.96 ± 0.01	

*Control = Triton-x100 was used as the control positive at 0.2 µg/ml. Conc. = Concentration.

Cytotoxicity assay of *K. africana* (fruits) extract

The maximum concentration used was 500 µg/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 *K. africana* (fruits). MTT colorimetric assay was used. Reading in triplicate for different concentrations 125-500 µg/ml.

Amoebiasis is caused by *E. histolytica*, a protozoan parasite of humans and the causative agent of intestinal amoebiasis. This disease is a major health problem in developing countries (Stanley, 2003). Although it is asymptomatic in 90% of cases, about 50 million people are estimated to suffer from symptoms associated with amoebiasis, such as hemorrhagic colitis and amoebic liver abscess (Ravdin, 1995). Several means of transmitting *E. histolytica* are known, ingestion of the infective cyst occur in food and water and it may also be transferred via homosexual men (Tanyuksel and Petri, 2003). Ethanol extracts of *K. africana* (fruits) showed 100% inhibition at a concentration 500 µg/ml after 96 h, whereas Metronidazole gave 96% inhibition at 312.5 µg/ml concentration against *E. histolytica* (Fig. 1). The cytotoxicity assays was conducted in this study to evaluate the ethanolic extract of *K. africana* (fruits) their cytotoxicity effects by using MTT-assay with Vero cell line. The result of MTT assay verified the safety of the examined extract. Cytotoxicity assays were conducted in this study to evaluate the ethanolic extract of *K. africana* (fruits) their cytoxtoxicty effects by using MTT-assay include (Vero cell line). The result of MTT assay verified the safety of the examined extract (Table 2).

Conclusion

This result enhances the ethno botanical uses of *K. africana* (fruits) as antiamoebic in cases associated with amoebiasis 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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References

Amaral, F.M.M., Ribeiro, M.N.S., Barbosa-Filho, J.M., Reis, A.S., Nascimento, F.R.F., Macedo, R.O., 2006. Plants and chemical constituents with giardicidal activity. Braz. J. Pharmacog. 16, 696-720.

Arguello-Garcia, R., Cruz-suto, M., Romero-Montoya, L., Ortega-Pierres, G., 2004. Variability and variation in drug susceptibility among *Giardia*

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duodenalis isolates and clones exposed to 5-nitromidazoles and benzimidazoles *in vitro*. J. Antimicrob. Chemother. 54, 711-721.

Arkhipov, A., Sirdaarta, J., Rayan, P., McDonnell, P.A., Cock, I.E., 2014. An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacog. Commun. 4(3), 62-76.

Bhopale, K., Pradhan, K., Masani, K., Karl, C., 1995. Additive effect of diloxanidefuorate and Metronidazole (Entamizole R) in mouse caecalamoebiasis. Indian J. Exp. Biol. 33, 73-74.

Cedillo-Rivera, R., Chave, B., Gonzalez-Robles, A., Tapia-Contreras, A., Yepez-Mulia, L., 2002. *In vitro* effect of nitazoxanide against *Entamoba histolytica*, *Gairdia lamblia* and *Trichomonas vaginalis* trophozoites. J. Eukaryotic Microbiol. 49, 201-208.

Farthing, M.S., Cavellos, A.M., Kelly, P., Cook, G.C., 1996. Intestinal Protozoa. In: Manson's Tropical Disease. 20th Edn. W.B. Saunder Company, London. pp.1255-1267.

Haque, R., Mollah, N.U., Ali, I.K., Alam, K., Eubanks, A., Lyerly, D., Petri, Jr. W.A., 2000. Diagnosis of amebic liver abscess and intestinal infection with the TechLab *Entamoeba histolytica* II antigen detection and antibody tests. J. Clin. Microbiol. 38(9), 3235–3239.

Harbone, B., 1984. Phytochemical Methods. 2nd Edn. Champan Hall, New York. pp.4-7.

Koko, W.S., Mesaik, M.A., Yousaf, S., Galal, M., Choudhary, M.I., 2008. *In vitro* immunomodulating properties of selected Sudanese medicinal plants. J. Ethnopharmacol. 118, 26-34.

Patel, S., Gheewala, N., Suthar, A., Shah A., 2009. *In-vitro* cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and Vero cell line. Int. J. Pharm. Pharmaceut. Sci. 1(1), 38-46.

Ravdin, J.I., 1995. State of the art clinical article. Clin. Infect. Dis. 20, 1453-1466.

Ravdin, J.I., Stauffer, M.M., 2005. *Entamoeba histolytica* (Amoebiasis). In: Principles and Practice of Infectious Diseases (Eds.: Mendell, G. L., Benneth, J. E., Dolin, R.). 6th Edn. P. A. Churchill Livingstone, Philadelphia.

Stanley, Jr., Reed, S.L., 2001. Microbes and microbial toxin: paradigms for microbial- mucosal. Interactions VI. *Entamoeba histolytica*: parasite-host interactions. Am. J. Physiol. Gastrointest. Liver Physiol. 280(6), 1049-1054.

Stanley, S.L., 2003. Amoebiasis. Lancet 361, 1025-1034.

Tanyuksel, M., Petri, W.A., 2003. Laboratory diagnosis of amoebiasis. Clin. Microbiol. Rev. 16, 713-729.

Tierney, L.M., Mcphee, J.S., Papadaksis, M.A. (Eds.), 1999. Current Medical Diagnosis and Treatment. Appleton and Lange, Stanford, CT.

Townson, S.M., Boreham, P.F., Upcrof, T.P., Upcroft, J.A., 1994. Resistance to the nitroheterocyclic drugs. Acta Trop. 56, 173-194.

Upcrof, J.A., Dunn, L.A., Wrigh, J.M., Benakli, K., Upcrof, P., Vanelle, P., 2006. 5-nitroimidazole drugs effective against Metronidazole-resistant *Trichomonas vaginalis* and *Giardia dodenalis* spp. Animicrob. Chemother. 50, 344-347.

World Health Organization, 1997. Weekly Epidemiological Records. 72, 97-100.

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