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Antileishmanial activity of *Aloe Secundiflora* plant extracts against *Leishmania Major* (2013).

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ABSTRACT
Human leishmaniases are a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*. In this study antileishmanial activity of the methanolic and water leaf extracts from *Aloe secundiflora* plant were analysed by determining the minimum inhibition concentration (MIC), nitric oxide (NO) production stimulation, infection rates (IR) and multiplication index (MI). Cytotoxicity of these plant extracts was also assessed. The MIC levels of water and methanolic plant extracts, amphotericin B and pentostam were 2000 µg/ml, 1000 µg/ml, 125µg/ml and 250 µg/ml respectively against *Leishamnia major* promastigotes. This study revealed that water and methanolic plant extracts significantly inhibited the growth of *Leishmania* parasites ($P \leq 0.05$) as compared to amphotericin B with respect to the parasite infection rates and MIC levels. The IC_{50} for the water and methanolic plant extracts was 279.488 µg/ml and 42.824 µg/ml respectively. The elevated inhibitory activity observed in this study against *Leishmania* major parasites provides evidence and basis for their potential use as therapeutic agents against leishmaniasis.
Key words: *Aloe secundiflora*, Plant extracts, *Leishmania major* and Minimum Inhibition Concentrations (MIC)

1.0 INTRODUCTION

Human leishmaniases are a spectrum of diseases caused by protozoan parasites of the genus *Leishmania*. Leishmaniases are roughly classified into three types of diseases, cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML) and visceral leishmaniasis (VL) according to their clinical manifestations. Visceral leishmaniasis, generally caused by species of the *L. donovani* complex, i.e. *L. donovani*and *L. Infantum* (*chagasi*), is the most severe form, with approximately 500,000 new cases reported annually (www.who.int/leishmaniasis/en/). Active VL is characterized by haematological and hepatosplenic abnormalities, and is generally fatal unless properly treated. Antimicrobials of plant origin have enormous therapeutic potential for various ailments[2, 3]. They are effective in the treatment of infectious diseases and mitigate many of the side-effects that are often associated with synthetic antimicrobials. Natural products are evolutionary shaped molecules with a profound impact on human health[4]. The World Health Organization (WHO) estimates that more than 80% of the world population is dependent (wholly or partially) on plant-based drugs[5]. In East Africa, 90% of the population relies on traditional medicines (TM) and traditional health practitioners (THPs) as the primary source of healthcare. Nature's biosynthetic engine produces innumerable secondary metabolites with distinct biological properties that make them valuable as health products or as structural templates for drug discovery. Aloes are reputed to have been used therapeutically since Roman times[6]. The genus Aloe is common in Kenya, with about 60 taxa recognized[7]. Aloe species have antibacterial, antifungal, anticancer, antiviral and immunomodulatory properties[8]. Herbalists from the Lake Victoria region have traditionally used *Aloe secundiflora* to treat ailments including chest problems, polio, malaria and stomach ache but with no knowledge of the scientific base of their activities[9]. Increasing incidence of deaths due to leishmaniasis in addition to shortfalls of the current existing drugs have led to a renewed quest for novel anti-leishmanial agents.

2.0 METHODS AND MATERIALS

2.1 Source of the plant extracts and extraction Process

The leaves from *A. secundiflora* plant were collected from Ruai area, Nairobi, Kenya. These were air-dried in a shade for 14 days and shred using a laboratory blender. The ground material (100 g) was soaked in absolute methanol for 24 hrs. The extracts were filtered, dried with Na_2SO_4 and the solvent removed under vacuum in a rotary evaporator at 30°C-35°C. For aqueous extraction, 100 g of ground material in 600 ml of water was placed in a water bath and maintained at 60°C for 2 hrs. The filtrate was freeze-dried, weighed and stored at -20°C until required for use [10].

2.2 Experimental animals

Eight week old inbred female BALB/c mice were used to obtain macrophages for antamastigote assay. The BALB/c mice were obtained from the stock maintained at the Kenya Medical Research Institute (KEMRI), Nairobi.

2.3 Cultivation of *Leishmania major* parasites

The *Leishmania major* (Strain IDUB/94=NLB-144) saline aspirate was taken from an infected mouse footpad. The parasites were cultivated in Schneider's *Drosophila* medium supplemented with 20% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and 500 µg/ml 5-fluorocytosine arabinoside as previously described[11,12]. The medium was filter-sterilized and aliquoted into 25cm^2 Costar culture flasks. The amastigote aspirates were inoculated into 5 ml of the medium and incubated at 25^{0}C and grown to stationary phase to generate infective metacyclic forms.

2.4 Preparation of the test drugs

Stock solutions of the extract were prepared in culture media for anti-leishmanial assays and re-sterilized by filtering through 0.22 µm filter flasks in the laminar flow hood. If some extracts were found not to dissolve easily in water or media, they were first dissolved in 1% dimethyl sulfoxide (DMSO) to avoid solvent carry over[13]. All prepared drugs were stored at 4^{0}C and retrieved only during use. Assays for the two test drugs were carried out separately.

2.5 Determination of minimum inhibitory concentration (MIC)

Promastigotes at an initial concentration of 1x10^6 per ml of culture medium were grown in culture medium containing several concentrations of the test extracts ranging from 1µg/ml up to 1mg/ml in a 24 well plate. Cell growth was monitored by assessing their turbidity and consequently the MICs. The lowest concentration of the samples that prevented growth was considered as the MIC. The plant extracts were tested in replicates.

2.6 Anti-promastigote Assay

The parasites were incubated at 25°C for 24 hours and 200 µl of the highest concentration of each of the plant extract were added and serial diluted with dilution factor of 2. The experimental plates were incubated further at 25°C for 48 hours. Ten microlitres of thiazolyl blue tetrazolium bromide (MTT) reagent was added into each plate well and the cells incubated for 2 - 4 hours until a purple precipitate was clearly visible under a microscope. The medium together with MTT was aspirated off from the wells, 100µL of DMSO was added and the plates were shaken for 5 min. Absorbance was measured for each well at 562 nm using a micro-titre plate reader (Multiskan MCC) [15] and the 50% inhibitory concentration (IC_{50}) values generated. Percentage promastigote viability was calculated using the formula below at each concentration [14]

$$\text{Viability (\%)} = \frac{\text{(average absorbance in duplicate drug wells – average blank wells x 100)}}{\text{Average absorbance in control wells}}$$

2.7 Anti-Amastigote Assay

The anti amastigote assay was carried out as previously described[16]. The mice were anaesthetized using 100µl pentabarbitone sodium (Sagatal^R). The peritoneal macrophages were obtained from BALB/c mice upon disinfection of mice body surface with 70% ethanol. The abdominal skin was sheared dorsoventrally to expose the peritoneum. Using a sterile syringe and needle, 10ml of sterile cold phosphate-buffered saline was injected into the peritoneum. After shaking the mouse peritoneal macrophages were harvested by drawing the PBS. The contents were transferred into a sterile 50ml centrifuge tube. The suspension was centrifugally washed at 2000rpm (Hitachi, 05PR-22) for 10minutes and the pellet re-suspended in complete RPMI 1640 medium. Macrophages were adsorbed in 24-well plates and allowed to adhere for 4 hours at 37^{o}C in a 5% CO_2. Non adherent cells were washed with cold PBS and the macrophages incubated overnight in RPMI. Adherent macrophages were infected with parasite: macrophage ratio of 6:1 and incubated at 37^{o}C in the 5% CO_2 for 4 hours. Unattached promastigotes were removed by extensive washing with PBS and the cultures incubated in RPMI for 24 hours. A one time treatment of infected macrophages with the drugs was done. Pentostam and Amphotericin B were used as positive control drugs for comparison of parasite inhibition. The medium and drug were replenished daily for 3 days. After 5 days, the monolayers were washed with PBS at 37^{o}C, fixed in the methanol and stained with 10% Giemsa. The numbers of amastigotes were determined by counting at least 100 macrophages in duplicate cultures. The results were expressed in terms of infection rate (IR) and the multiplication index (MI) as used previously[17]

IR= Number of infected macrophages in 100 macrophages

$$\text{MI} = \frac{\text{Number of amastigotes in experimental culture/100 macrophages x 100}}{\text{Number of amastigotes in 100 control culture/100 macrophages}}$$

The infection rate was used in calculation of the association index (AI). The association indices were determined by multiplying the percentages of the infected cell. Association indices were interpreted as the numbers of parasites per infected cell. Association indices were interpreted as the numbers of parasites that actually infected the macrophages.

2.8 Determination of Nitric oxide production

Nitric Oxide (NO) release in macrophages culture was measured using the Greiss reaction for nitrites[18]. Supernatants (100ul) were collected 48 hours after introducing the test drug into the culture medium. The assay was done in triplicate wells in the 96-well micro-titre plates. To achieve this, 60 ml of Greiss reagent A (1% Sulphonilmide in 1.2 M HCL) was added followed by 60ml of Greiss reagent B (0.35 N[1-naphthy] ethlenediamine). The plates were read at 540nm in the Enzyme Linked Immunosorbent Assay (ELISA) reader. Sodium nitrite in RPMI were used to construct a standard curve for each plate reading.

2.9 Cytotoxicity Assay

African green monkey kidney (Vero) cells were cultured and maintained in minimum essential medium (MEM) supplemented with 10% FBS at 37^{o}C in 5% CO_2 for 24 hrs. They were harvested by trypsinization, pooled in a 50ml vial and 100µl cell suspensions (1x10^{5}cells/ml) added to each well in a 96-well micro-titre plate. Each sample was replicated 2 times. A volume of 150µl of the highest concentration of the extract of the test samples (a serial dilution, prepared in MEM) was added and the experimental plates further incubated at 37^{o}C for 48 hrs. The cells in media without the extract were used as controls. In each well, 10µl 3-(4, 005-dimethylthiazol-2-yl)-2 5-diphenyltetrazolium bromide) (MTT) were added and the cells incubated for 2-4hrs, until a purple precipitate was clearly visible under a microscope. The medium together with MTT was aspirated off the wells. DMSO (100 µl) was added and the plates shaken for 5 min. The absorbance for each well was measured at 562 nm in a micro-titre plate reader and the percentage cell viability calculated manually[19], using the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Average absorbance in duplicate drug wells-average blank wells x 100}}{\text{Average absorbance in control wells}}$$

2.10 Data Analysis

Comparison between the means of water and methanolic plant extracts of *A. secundiflora,* untreated infected negative controls and pentostam, amphotericin B (standard drug controls) was done using the Student's T-test. All the analyses were carried out at 5% level of significance.

3.0 RESULTS

3.1 Minimum inhibition concentration and IC50 of methanolic and water extracts of *Aloe secundiflora*

In this study water plant extract had highest MIC and IC $_{50}$ values as compared to the rest of test samples. The water and methanol extract were active against *L. major* at the lowest concentration of 2000 µg/ml and 1000 µg/ml respectively). Methanollic plant extract showed less ant-leishmanial activity as compared to Pentostam and Amphotericn B with MIC of 1000µg/ml, 250µg/ml and 125µg/ml respectively. Similar observation was seen with IC_{50} values of methanol plant extract, Pentostam and Amphotericin B of 42.824 µg/ml, 14.104 µg/ml and 8.623 µg/ml respectively (Table 1 and Fig. 1).

3.2 Stimulation of Nitric Oxide production activity of methanolic and water plant extracts of *Aloe secundiflora*

The nitric oxide production in supernatants of macrophage culture treated with methanolic, water plant extracts of *A. secundiflora* were determined using a representative standard curve for samples at concentrations between 0 and 1000 µg/ml. No significant nitric oxide levels ($p > 0.05$) were produced compared to the negative control. None of the samples had Optical Density (OD) readings of more than 0.10 indicating that less than 2 µm of nitric Oxide was produced compared to negative controls that produced similar levels.

3.3. Anti amastigote assay of water and methanolic plant extracts of *Aloe secundiflora* to *L. major* Amastigotes

Different concentrations of samples of water and methanolic plant extracts of *Aloe secundiflora* were tested for their efficacy against amastigotes in macrophages. In order to determine the extent of infection, the number of infected macrophages and the number of amastigotes per 100 macrophages was determined. Infection rates were the number of parasites that actually infected the macrophages. The number of *L. major* amastigotes in macrophages treated with methanolic, plant extracts of *Aloe secundiflora* were significantly lower ($p<0.05$) compared to the negative control and Pentostam and Amphotericin B at all concentrations. The P values of methanolic and water plant extracts for infection rates were 0.004 and 0.017 respectively with reference to Amphotericn B as compared with pentostam whose P values were 0.002 and 0.013 respectively using the same standard. The multiplication index of amastigotes of methanolic and water plant extracts in reference to Amphotericin B were 0.107 and 0.017 respectively as opposed to Pentostam of 0.112 and 0.014 respectively.

3.4 Cytotoxicity of Methanolic and Water Extracts of *Aloe secundiflora* on Vero Cells.

The methanolic and aqueous plant extracts of *A. secundiflora* were screened for cytotoxic activity against vero cells using the MTT assay. Methanol and water extracts showed low toxicity against healthy Vero cells (IC_{50} 0.686 and 0.01µg/ml, respectively). The low cytotoxic potential of the extracts in this study is of great significance for their traditional usefulness in the treatment of leishmaniasis (Table 2).

4.0 DISCUSSION

Various experimental studies have shown that several plants in different genera contain compounds that have antileishmanial activity and can be used as alternative therapies, even though their effectiveness differs per group of compound [19]. The differential effects of plant products has been identified as the target site of the *Leishmania* . The advantages of using plant based products have been shown to be as a result of lack of easy development of resistance by parasites [25].

In this study ant-leishmanial activity of the methanolic and water leaf extracts from *Aloe secundiflora* plant was assessed utilizing *in-vitro* tests. It was interesting to note that *Aloe secundiflora* extracts reduced the level of production of nitric oxide in the macrophages, suggesting that the extracts could be acting directly on the parasites rather than stimulating the immune system to counteract them. The water plant extract significantly ($p<0.05$) reduced the infectivity of *L. major* amastigotes in macrophages compared to the standard reference drugs. The methanol plant extract ($P > 0.05$) had no significant difference in infectivity of *L. major* amastigotes in macrophages when compared to standard reference drugs. These findings suggest that water extracts from *Aloe secundiflora* have better active ingredients against leishmania parasites than methanolic extracts (Fig. 1 and 2).

This study describes for the first time investigations into the anti-leishmanial potential of methanolic and water plant extracts from *Aloe secundiflora* by screening for cytotoxic activity against Vero cells. The results showed that water and methanollic plant extracts had lower toxicity against Vero cells.

In this study, the activity of *Aloe secundiflora* extracts against *L. major* showed that the plants contained some pharmacologically active substances. These results suggested that *Aloe secundiflora* had compounds or groups of substances that could prevent growth and proliferation of *L. major* promastigotes. Studies elsewhere have revealed that flavonoids like catechins have antimicrobial properties against fungi, Gram-positive and Gram-negative bacteria[1], [28]. The ant-leishmanial activity observed in this study against *L. major* strains could be due to the ability of flavonoids to form complexes with the parasite cell wall, affecting cell-linked processes thereby inhibiting its growth. Flavonoids are also known to inhibit cell enzyme activities[28]. The antileishmanial activity of *Aloe secundiflora* observed in this study was in agreement with the reports by Chakraborty and Chakraborti who demonstrated that catechins exhibited antibacterial activity by inhibiting the action of the DNA polymerase. The leishmanial inhibitory activity of *Aloe secundiflora* could also be attributed due to the presence of tannins. The tannins could be disrupting the cell membranes of the *L. major*, hence their inhibitory activities. *Aloe secundiflora* extract has been observed to play a role in the control of fowl typhoid, suggesting the possibility of the efficacy of *Aloe secundiflora* in *L. major* infections[8].

Some of the local plants that have been shown to have antileishmanial activities are the garlic onion *Allium sativum* L (Liliaceae) [24]. The active ingredients in this plant are allicin, alliin and dialyl disulphide which are known to stimulate the interferon gamma genes in *L. major* infected macrophages hence promoting the destruction of engulfed amastigotes [24.]

The root bark and leaf extracts of the flowering plant *Plumbago capensis* (Plumbaginaceae) have been shown to inhibit development of *L. major* both in *in vitro* and *in vivo*[20]. The active ingredient has been identified as plumbagin. African moringa ,*Moringa stenopetala* Baker. F Cufodontis (Moringaceae) which grows on an island in Lake Baringo and the local ornamental bottle brush tree *Callistemon citrinus* Curtis (Keels)(Myrtaceae) have also been shown to have antileishmanial activities[26]. The active ingredients in *C. citrinus* are 1,8-Ceole (eucalyptol) and alpha pinene essential oils. Both alpha pinene and 1,8-cineole are non polar terpenes. Since linalool is a monoterpene that has a strong antleishmanial activity[23.] There is a high possibility that alpha pinene may be active antleishmanials agents. *Warbugia Ugandensis* (Canellaceae), the East African greenheart, is one of the most utilized medicinal plant trees in tropical and subtropical Africa Extracts from this plant have been shown to have antileishmanial activities[29]. The active ingredients in this plant are thought to be flavonids, saponins and tannins which have antimicrobial properties [21].

Plant within the genus Aloe such as *Aloe vera* contain flavonoids which like catechins are well known for their antimicrobial properties against fungi, Gram-positive and Gram-negative bacteria [1,28]. Extracts of *A.* secundiflora have been used to treat paludism, ringworm, wounds and pimples[22]. Since the extracts were not toxic to vero cells (fig 5) and macrophages and, did not produce sufficient amounts of nitric oxide (fig 3and 4), the actual mode of action by the extracts is not fully known. Nitric Oxide is known to mediate macrophage cytotoxicity against microbes and tumor cells. The activity of *A. secundiflora* aqueous and methanolic extracts against *L. major* strains could be due to the ability of flavonoids to form complexes with the parasite cell wall and inhibiting the action of DNA polymerase[23]. The plant also contains terpenoids, flavonoids and tannins which are known for disrupting the cell membranes of the *L. major*. This could also account for the inhibitory activitiy of *A. secundiflora* extracts[23].

5.0 CONCLUSION

A.secundiflora methanollic and water extracts produced inhibitory activities against *L. major* promastigotes and amastigotes. They marked higher antileishmanial activities though not of comparative concentrations than most of the reference drugs. The results also showed that plant extracts had lower toxicity against Vero cells as compared to the standard drug amphotericin B. The water and methanollic extracts did not stimulate the macrophages to produce sufficient amounts of nitric oxide, hence the extracts could be acting directly on the parasite rather than stimulating nitric oxide production to kill the parasites. This study could provide some scientific basis on the plant being used as an antileishmanial therapeutic agent, subject. It is therefore possible that if fortified and/or used together with other drugs, the extracts could provide additive or synergistic effects in the control of resistant strains of *Leishmania* parasite

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REFERENCES

Berman J.D., and Lee L.S., Activity of antileishmanial agents against amastigotes in human monocyte derived macrophages and in mouse peritoneal macrophages. *Journal of Parasitology* 1984;70: 220-225.

Calixto, J.B., (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of Medical and Biological Research* **33** (2), 179–190.

Chakraborty D, Chakraborti S. Bioassay-guided isolation and identification of antibacterial and antifungal component from methanolic extract of green tea leaves (Camellia sinensis) *Res J Med Plant* 2010; 4:78–86.

Cohen M.F, Sakihama Y, and Yamasaki H. Roles of plant flavonoids in interactions with microbes: From protection against pathogens to the mediation of mutualism. *Recent Res Dev Plant Physiol2001*; 2:157–73.

Croft, S.L., Sunder, S. and Fairlamb, A.H. (2006). Drug resistance in leishmaniasis. *Clinical Microbiology Review*, **19**(1): 111-126

Delorenzi JC, Attias M, Gattass CR, Andrade M, Rezende C, Da Cuhna Pinto A, Henriques AT, Bou-Habib DC and Saraiva EM. Anti-leishmanial activity of an indole alkaloid from *Peschieraaustralis. Antimicrobial Agents Chemotherapy* 2001;45: 1349-1354.

Dorin D, Le Roch K, Sallicandro P, Alano P, Parzy D, Poullet L, Meijer L and Doerig C. Pfnek-1, a NIMA-related kinase from the human malaria parasite *Plasmodium falciparum*. Biochemical properties and possible involvement in MAPK regulation. *European Journal of Biochemistry* 2001;268: 2600-2608.

Ferreira I.C.P, Lonardoni M.V.C, Machado G.M.C, Leon L.I, Filho L.G, Pinto L.H.B., and de Oliveira A.J.B., Anti-leishmanial activity of alkaloid extract from *Aspidospermaramiflorum. Memorias do InstitutoOswaldo Cruz* 2004;99(3): 325-327.

Hendricks L.D., and Wright N. Diagnosis of Cutaneous leishmaniasis by *in vitro* cultivation of saline aspirates in Schneider's *Drosophila* medium. *American Journal of Tropical Medicine and Hygiene* 1979;28: 962-964.

Holzmuller P, Sereno D, Cavalrero M, Mangot L, Dauloude S, Vincendeau P and Lemesre JL. Nitric oxide-mediated proteasome-development oligonuleosomal DNA fragmentation in *L. amazonensis*amastigotes. *Infection and Immunity* 2002;70: 3727-3735.

Kigondu E.V.M, Rukunga G.M, Keriko J.M, Tonui W.K, Gathitwa J.W, Kirira P.G, Irungu B, Ingonga J.M and Ndiege I.O. Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. *Journal of Ethnopharmacology* 2009;123(3): 504-509.

Kimber C.D, Evans D.A, Robinson B.L and Peters W. Control of yeast contamination with 5-fluorocytosine in *in vitro* cultivation of *Leishmania* spp. *Annals of Tropical Medicine and Parasitology* 1981;75: 453-454.

Kinuthia G.K., Kabiru E.W., Gikonyo N.K., Ingonga J.M., Kigondu E.M., & Anjili C.O., (2012). *In vitro* activity of acqueous and methanollic crude extracts of *callistemon citrinus* against *leishmania major.*

Kishore N, Mishra B.B, Tripathi V, Tiwari V.K. Alkaloids as potential anti-tubercular agents. *Fitoterapia* 2009;80:149–63.

Kuete V, Ngameni B, Simo C.C, Tankeu R.K, Ngadjui B.T, Meyer J.J., Antimicrobial activity of the crude extracts and compounds from *Ficuschlamydocarpa* and *Ficuscordata* (Moraceae).*J Ethnopharmacol*2008; 120:17–24.

Lindsey K.L, Jäger A.K, Viljoen A.M. Cyclooxygenase inhibitory activity of Aloe species. *S Afr J Bot* 2002; 68:47–50.

Makwali J.A, Wanjala F.M, Kaburi J.C, Ingonga J, Byrum W.W, Anjili C.O., (2012) Combination and monotherapy of Leishmania major infection in BALB/c mice using plant extracts and herbicides. *Journal for vector borne diseases*, **49** (3):123-30.
Manisha V, Neha S, Satish S. Antimicrobial Activity of Stem Bark Extracts of Nyctanthesarbortristis Linn. (Oleaceae).*Int J PharmacognPhytochem Res* 2009;1:12–4.
Maobe A.G, Gatebe.E, Gitu L, and Rotich, H.(2013). Preliminary Phytochemical Screening of Eight Selected Medicinal Herbs Used for the treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya *European Journal of Applied Sciences* **5** (1): 01-06,
Mariita M.R, Okemo O.P, Orodho J.A, Kirimuhuzya C, Otieno J.N, Magadula J.J., Efficacy of 13 medicinal plants used by indigenous communities around Lake Victoria, Kenya, against tuberculosis, diarrhoea causing bacteria and Candida albicans. *Int J Pharm Technol* 2010; 771–971.
Mosmann T. Rapid colorimetric assay for cellular growth and survival: application tp proliferation and cytotoxicity assays. *Journal Immunology Methods* 1983;16: 55-63.
Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Mungai, G.M. and Njagi, E.N.M. (2007). Traditional phytotherapy of some remedies used in treatment of malaria in Meru District of Kenya. *South Africa Journal of Botany*, **73**(3): 402-411.
Ngure P.K ., Tonui W.K., IngongaJ., Mutai C., KigonduE., Ng'ang'a Z., Rukunga G., and Kimutai A..(2009) *in vitro* antileishmanial activity of extracts of *Warburgia ugandensis* (Canellaceae), a Kenyan medicinal plant. *Journal of Medicinal Plants Research* Vol. **3**(2), pp. 061-066,
Orwa JA, Jondiko IJ, Minja RJ, Bekunda M. The use of Toddaliaasiatica (L) Lam.(Rutaceae) in traditional medicine practice in East Africa. *J Ethnopharmacol* 2008; 115:257–62.
Polonio T & Effert , T. (2008) Leishmaniasis : drug resistance and natural products *International journal of molecular medicine*, **22**: 277-286
Wabuyele E, Bjora CS, Nordal I, Newton LE. Distribution, diversity and conservation of the genus Aloe in Kenya. *J East Afr Nat Hist* 2006;95:213–25.
Wabwoba B.W., Anjili C.O., Ngeiywa, M.M, Ngure,P.K., Kigondu E.M., Ingonga J.M and Makwali J., (2010) . Experimental chemotherapy with Allium sativum (Liliaceae) methanolic extract in rodents infected with Leishmania major and Leishmania donovani *Journal of Vector Borne Diseases* (4) pg160
Waihenya RK, Mtambo MM, Nkwengulila G, Minga UM. Efficacy of crude extract of *Aloe secundiflora* against Salmonella gallinarum in experimentally infected free-range chickens in Tanzania. *J Ethnopharmacol* 2002;79:317–23.
Wang X, Ge JM, Wang K, Qian J and Zou Y. Evaluation of Emodin-induced cytotoxicity. *Assay Drug Development Technology* 2006; 4: 203-207.

TABLES AND FIGURES

Table 1: MIC Values of Methanollic and Water Plant Extracts.

Drugs	Methanollic extracts	Water extracts	Amphotericin B	Pentostam
MIC (µg/ml)	1000	2000	125	250
IC_{50} (µg/ml)	42.824	279.488	8.623	14.104

Table 2: IC50 for cytotoxicity of methanolic and water plant extracts

Test sample	Methanollic Extracts	Water Extracts	Amphotericin B	Pentostam	DMSO
IC_{50} (µg/ml)	119.86	547.88	68.6	106.8	1.468

FIGURES

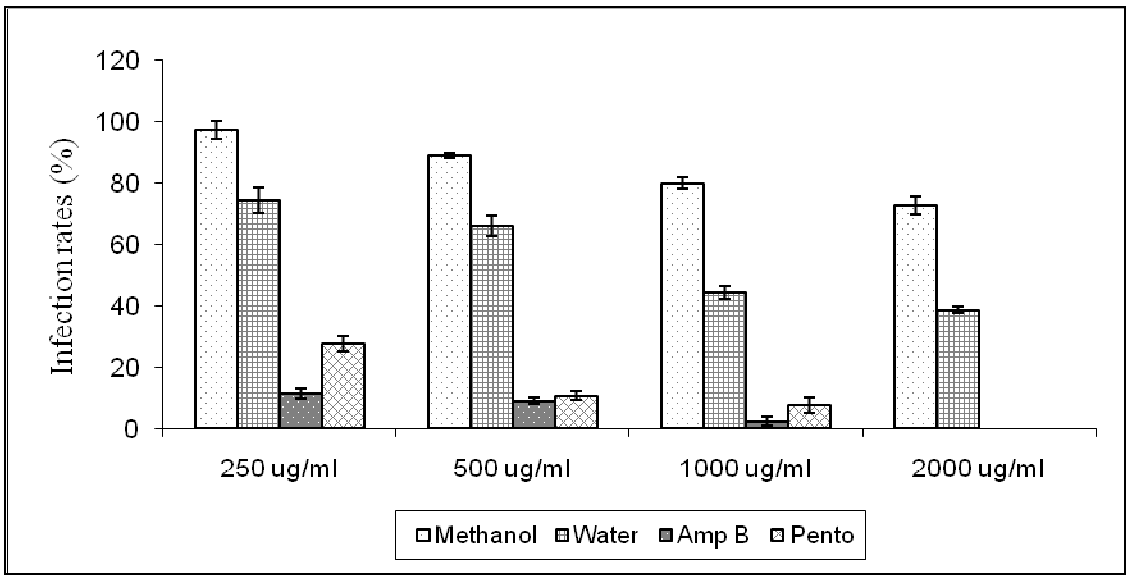

Figure 1: The infection rates on macrophages when treated with methanolic and water plant extracts compared with pentostam and amphotericin B drugs.

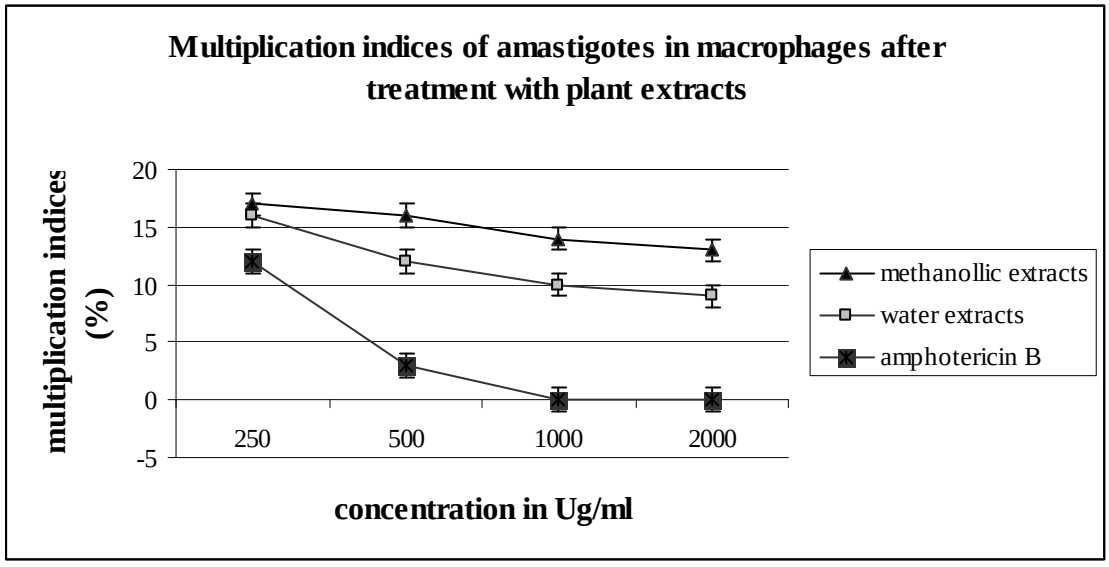

Figure 2: The amastigote growth rate in macrophages treated with methanolic, water extracts of A. secundiflora compared to Amphotericin B drug.

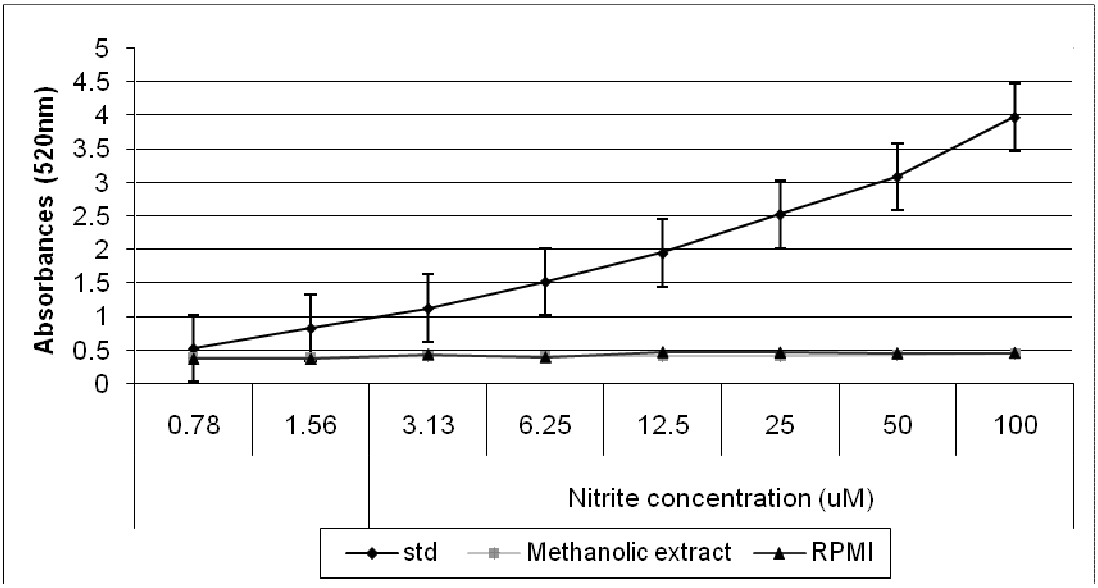

Figure 3: Nitric oxide produced by treated BALB/c mice macrophages with methanolic extracts

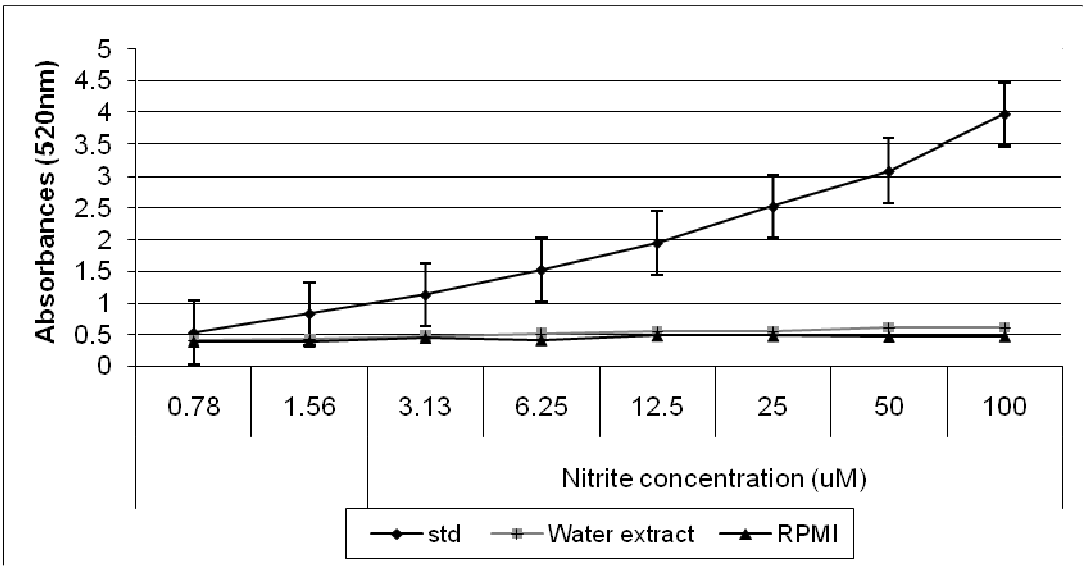

Figure 4: Nitric oxide produced by treated BALB/c mice macrophages with water extracts

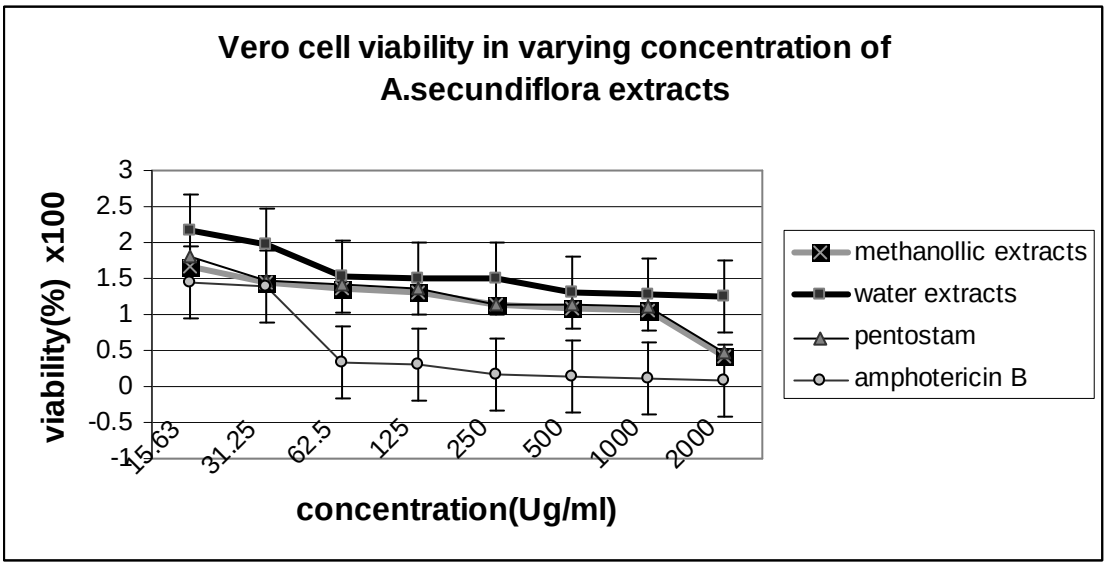

Figure 5: Showing the trend of viability of vero cells against concentrations (serial dilutions) dilution factor 2 of the *Aloe secundiflora* extracts and control drugs amphotericin B and pentostam