

Efficacy of a Combination of *Plumbago Capensis* and *Aloe Secundiflora* Aqueous and Methanolic Plant Extracts in the Treatment of *Leishmania Major* in Balb/C MicePaul K Ndungu¹, Johnstone M Ingonga¹, Michael Gicheru² and Christopher O Anjili¹¹Centre for Biotechnology Research and Development, Kenya Medical Research Institute, Kenya²Department of Zoological Sciences, Kenyatta University, Kenya

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Abstract

Background & Objectives: Monotherapy and combination therapy studies were to determine antileishmanial activities of the methanolic and water leaf extracts from *Aloe secundiflora* and *Plumbago capensis*.

Methods: The plants were analyzed 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.

Results: The MIC levels of water and methanolic plant extracts, amphotericin B and Pentostam were 2000µg/ml, 1000µg/ml, 125µg/ml and 250µg/ml respectively against *Leishmania major* promastigotes. 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 279.488µg/ml and 42.824µg/ml respectively. A similar extraction procedure was done for *P. capensis*. When administered orally, a combination of *P. capensis* and *A. secundiflora* proved to be more effective than that of the methanolic extract with LDU units of 4,800 and 15,300 respectively. Water extracts of *P. capensis* when used alone gave LDU of 5,200 units, methanolic extract gave 11,000 units. *Aloe secundiflora* water and methanolic extracts gave the same LDU of 10,700. Methanolic extract combinations of the two plants were the least effective, yielding the highest LDU of 21,000 units.

Interpretation & Conclusion: The results suggest that a combination oral therapy of extracts of the two plants and monotherapy using *P. capensis* can be effective methods of partially treating leishmaniasis.

Introduction

Leishmaniasis is a deadly vector borne disease that causes significant morbidity and mortality in Africa, Asia, Latin America and Mediterranean regions. Worldwide, there are 2 million new cases each year and one out of ten of the world's population is at risk of infection. There are three clinical forms of leishmaniasis: Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL) and Visceral Leishmaniasis (VL). Cutaneous leishmaniasis is caused by infection with *L. tropica* and *L. braziliensis*. Cutaneous leishmaniasis caused by *L. tropica* is common along the shores of Mediterranean, throughout Middle East, Africa and parts of India, while that caused by *L. braziliensis* is mainly confined to Central America and South America. In Kenya, CL is endemic in Rift Valley, Central and Western regions [1]. Currently, the only available drugs for the treatment of the leishmaniasis are pentavalent antimonials such as sodium stibogluconate (Pentostam), aminosidine, amphotericin B as well as paromomycin and sitamaquine [2]. Drugs for leishmaniasis are highly expensive, toxic and are associated with high resistance and relapse rates [3]. Antimicrobials of plant origin have enormous therapeutic potential for various ailments [4,5]. 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 [6]. The World Health Organization (WHO) estimates that more than 80% of the world population is dependent (wholly or partially) on plant-based drugs [7,8]. 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 [7,8]. The objective of this study was to evaluate the efficacy of a combination of *P. capensis* and *A. secundiflora* extracts in the treatment of *L. major* in BALB/c mice.

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Materials and Methods

The study was carried out at the Centre for Biotechnology Research and Development (CBRD), Kenya Medical Research Institute (KEMRI). *Plumbago capensis* leaves were collected from Upper Hill area, Nairobi County whereas *A.secundiflora* leaves were collected from Ruai, Kiambu County and transported to the University of Nairobi, Botany Department Herbarium, for identification and specimen voucher preparation. Extraction of the dried plant materials was done at the Center of Traditional Medicine and Drug Research (CTMDR) at KEMRI. The *A.secundiflora* was processed according to the method of Kigundu et al. (2009). Briefly, they were air-dried in shade for 14 days after which they were shredded using laboratory blender. The ground materials (100g) were soaked in absolute methanol for 24 hours. The extract was filtered, dried with Na_2SO_4 and the solvent removed under vacuum in a rotary evaporator at 30–35°C. For aqueous extraction, 100g of ground material in 600ml of water was placed in a water bath and maintained at 60°C for 2 hours. The filtrate was freeze-dried, weighed and stored at -20°C.

Leishmania Parasites

Leishmania major (Strain IDUB/KE/94=NLB-144) saline aspirates were taken from the foot of an infected mouse. These parasites were cultivated in Schneider's Insect Medium (SIM) supplemented with 20% heat-inactivated Foetal Bovine Serum (FBS), 100µg/ml penicillin and 50µg/ml streptomycin [9] and 250µg/ml 5-fluorocytosine arabinoside [10]. Promastigotes were incubated at 25°C and grown to stationary phase to generate infective metacyclic forms.

Preparation of the Test Drugs

Stock solutions of the fractions were made in culture media for antileishmanial assays and re-sterilized by filtering through 0.22µm filter flasks in a laminar flow hood. All the extracts dissolved easily in water, Schneider's Insect Medium (SIM) and Phosphate Buffered Saline (PBS) thus there was no any kind of solvent carry-over. All prepared drugs were stored at 4°C.

In-Vitro Evaluation against *Leishmania Major* Promastigotes

Stationary phase promastigotes harvested as described above were counted and re-suspended in SIM. The tests were performed as previously described [11]. The tests were performed in 96-well plates maintained at 26°C under 5% CO_2 environment. Two hundred microliters of SIM were placed in the wells containing the maximum concentrations of the test compounds and 100µl in the next wells (2 to 12) and controls; 2µl of compound solutions of 20 mg/ml in distilled water were added to wells number 1 and serial dilutions (Ranging from 1000µg/ml to 31.25µg/ml) in the wells were performed. Hundred microliters of culture medium containing 2.0×10^6 stationary phase *Leishmania* parasites/ml were added to each test compound concentration. Parasite observation and counting was done using a haemocytometer. The results were expressed as the concentration inhibiting parasite growth by 50% after 72 hours incubation.

In-Vivo Evaluation against *Leishmania Major*

Eight week old inbred BALB/c mice of the same sex were obtained from the KEMRI animal house. Mice hind footpads were

measured using direct reading vernier caliper prior to infection. The mice were divided into 16 groups of 5 mice each. Each mouse was inoculated with 1×10^6 metacyclic promastigotes / 40µl PBS in the left hind footpad. Infected mice were left for 4 weeks for disease development. Treatment was done orally in six groups using a canula, one group with *P.capensis* water extract, another with *A.secundiflora* water extract, another with *P.capensis* methanolic extract, another with *A.secundiflora* methanolic extract, another with combined water extract from the two plants and the other with a combined methanolic extract from the two plants. The intraperitoneal dose was given to another six groups, one group with *P.capensis* water extract, another with *A.secundiflora* water extract, another with *P.capensis* methanol extract, another with combined water extract from the two plants and the other with a combined methanolic extract from the two plants. The control group was given intramuscularly 20mg/kg/day of Pentostam® and Amphotericin B. The positive control group was administered with PBS orally and through intramuscular injection. Selection of drug doses was based on earlier studies using *P.capensis* [12] and *A.secundiflora* [13].

Evaluation of Minimum Inhibitory Concentration (Mic)

Leishmania major promastigotes (1×10^6 parasites/ml) were grown and incubated in culture media containing different concentrations of the test extracts ranging from 1µg/ml to 1mg/ml. Cell growth was monitored by assessing their turbidity and consequently the MICs [14]. The lowest concentration of samples that prevented growth of *L. major in vitro* was considered as the MIC.

Anti-Promastigote Assay

Leishmania major promastigotes were cultured at a concentration of 1×10^6 . They were incubated in 24-well plates for five days at 25°C after which they were transferred into 96-well micro-titre plates. They were incubated further at 26°C for 24 hours and 200 µl of the highest concentration of the test samples added before serial dilution was carried out. The experimental plates were incubated further at 26°C for 48 hours. The controls contained culture media only. The Medium Together With Thiazolyl Tetrazolium Bromide (MTT) 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 shaken for 5 minutes. Absorbance was measured for each well at 562 nm using a micro-titre plate reader [14] and the 50% inhibitory concentration (IC_{50}) values generated. Percentage promastigotes viability was calculated using the formula by Mossman (1983) for each concentration:

$$\text{Viability(\%)} = \frac{(\text{Absorbance in duplicate drug wells} - \text{Absorbance in blank wells})}{\text{Absorbance in control wells}} \times 100$$

Anti-Amastigote Assay

The *L.major* anti-amastigote assay was carried out as described by Delorenzi et al. (2001). Peritoneal macrophages were obtained from BALB/c mice. The mice were anaesthetized using 100µl pentobarbitone sodium (Sagatal®). The body surface was disinfected with 70% ethanol. The torso skin was torn dorsoventrally to expose the peritoneum. Using a sterile syringe and needle, 10ml of

sterile cold Phosphate-Buffered Saline (PBS) was injected into the peritoneum. After which the peritoneal macrophages were harvested by withdrawing the PBS. The contents were transferred into a sterile 50ml centrifuge tube. The suspension was centrifugally washed at 2,000rpm for 10 minutes 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°C in 5% CO₂. Non-adherent cells were washed with cold PBS and macrophages incubated overnight in RPMI. Adherent macrophages were infected with a parasite/macrophage ratio of 6:1 and further incubated at 37°C in 5% CO₂ for 4 hours. Free promastigotes were removed by extensive washing with PBS and the cultures incubated in RPMI for 24 hours. After 5 days, the monolayers were washed with PBS at 37°C, fixed in methanol and stained with Giemsa. The number of amastigotes was determined by counting at least 100 macrophages in duplicate cultures, and the results expressed as Infection Rate (IR) and Multiplication Index (MI) [15] as follows:

$$IR = \text{No. of infected macrophages in 100 macrophages}$$

$$MI = \frac{\text{No. of amastigotes in experimental culture} / 100 \text{ macrophages}}{\text{No. of amastigotes in 100 control cultures} / 100 \text{ macrophages}} \times 100$$

The infection rate was used to calculate the Association Index (AI). The association indices were determined by multiplying the percentages of infected macrophages. Association indices were interpreted as the number of parasites that actually infected the macrophages.

Nitric Oxide Production Assay

Nitric oxide release in macrophage cultures was measured using the Greiss reaction for nitrites [16]. Briefly, 100µl of the supernatants were collected 48 hours after introducing the test drug into the culture medium. The assay was done in triplicate wells in 96-well microtiter plates. To this, 60 µl of Greiss Reagent-A (1% sulphanilamide in 1.2M HCL) was added followed by 60µl of Greiss Reagent B (0.3% N (1-naphthyl) ethylenediamine). The plates were read at 540 nm in Enzyme-Linked Immunosorbent Assay (ELISA) reader. Sodium nitrite in RPMI was used to construct a standard curve for each plate reading. The representative nitrite standard reference curve in RPMI medium was prepared as described in the Greiss reagent system instructions manual (Promega® USA).

Cytotoxicity Assay

The cytotoxicity of the extracts for mammalian cells was measured using vero cells. The vero cell line was obtained from KEMRI, Center for Virus Research (CVR) where it has been maintained through cryo-preservation and passaging weekly. The 100µl of media containing vero cells were cultured and maintained in Minimum Essential Medium (MEM) supplemented with 10% FBS. The cells were incubated at 37°C in 5% CO₂ for 24 hours by trypsinization, pooled in 50ml centrifuge tubes and in 100µl cells suspension (1x10⁶cells/ml) put into 2 wells of rows A-H in a 96 well micro-titer plate for one sample to attach. The medium were aspirated and 150µl of the highest at 562nm concentration of each of the test samples serially diluted. The experimental plates with the cells were further

incubated at 37°C for 48 hours. The cells in media without the extract were used as controls. Ten microliters (10µl) of MTT were added into each well and cells incubated for 2 hours until a purple precipitate was visible under a microscope. The medium together with the MTT were aspirated off the wells after which 100µl of DMSO was added and plates shaken for 5 minutes. The absorbance was measured for each well plate using a micro titer plate reader [17] and the percentage cell viability was calculated manually at each concentration using the following formula:

$$\text{Viability(\%)} = \frac{(\text{Absorbance in duplicate drug wells} - \text{Absorbance in blank wells})}{\text{Absorbance control wells}} \times 100$$

The IC₅₀ values of the test extracts and the control leishmaniasis drugs were determined automatically by inserting the absorbance values in the chemosen software program designed to determine IC₅₀ for test drugs.

Monitoring of Body Weights

To assess the effect of *L.major* infection in mice as well as drug efficacy in treated experimental mice, body weights were taken before infection, shortly before treatment commenced and at the end of the experiment. Weights for the treated group were compared with those from the control group to make evaluations on effect of *L.major* infection or the effectiveness of the test drugs.

Monitoring of Lesion Development

At the start of the study, the size of the right and left hind footpad of the mice were taken using a direct reading vernier caliper. The size of the infected footpad was taken weekly during the treatment period. Lesion progression was monitored by comparing the differences in thickness of infected footpad against the thickness of the uninfected contra-lateral footpad [7].

Determination of Parasite Load

At the end of the treatment period, all the mice were inoculated with 100 µl sagatal, the liver and spleen removed and weighed. Portions of the liver, spleen and the site of infection were cultured in Schneider's insect medium to detect parasites and to determine visceralization. Impression smears were made from the spleen, they were fixed in methanol and stained with Giemsa. They were used to calculate the parasite load using the method by Bradley and Kirkley (1997). At the end of the experiment, all the mice were disposed-off by incineration as per the regulations set up by KEMRI's Animal Care and Use Committee (ACUC).

Data Analysis

The mean values of different data sets of mice groups were compared using one way Analysis of Variance (ANOVA). Linear regression analysis and statistical graphs were used to compare the effectiveness of water and methanolic plant extracts either separately or in combination against change in mice weight and lesion sizes over the treatment period, where *p*-values of 0.05 (95% confidence) or less were considered significant. The IC₅₀ were determined using Chemosen Software program. Descriptive statistics were used where applicable. Data were organized into tables, bar graphs and line graphs.

Table 1: MIC and IC₅₀ of methanolic and water plant extracts.

Drug	MIC (µg/ml)	IC ₅₀ (µg/ml)
<i>Plumbago capensis</i> (H ₂ O extract)	1000	855.93
<i>Plumbago capensis</i> (Methanol extract)	2000	148.28
<i>Aloe Secundiflora</i> (H ₂ O extract)	500	643.08
<i>Aloe Secundiflora</i> (Methanol extract)	1000	717.58
<i>Plumbago capensis</i> and <i>Aloe Secundiflora</i> (H ₂ O extract)	500	118.57
<i>Plumbago capensis</i> and <i>Aloe Secundiflora</i> (Methanol extract)	1000	190.34
Pentostam	250	171.29
Amphotericin B	125	29.58

Results

Minimum Inhibition Concentration (Mic) and IC₅₀ Values

Plumbago capensis water and methanolic extract inhibited *L. major* parasite growth at IC₅₀ of 855.93µg/ml and 148.28 µg/ml respectively. *Aloe secundiflora* water and methanolic extracts inhibited parasite growth at IC₅₀ of 643.08µg/ml and 717.58µg/ml respectively. A combination of *P. capensis* and *A. secundiflora* water extracts exhibited synergistic/additive effect and inhibited parasite growth at IC₅₀ of 118.57µg/ml which was better than a combination of *P. capensis* and *A. secundiflora* methanolic extract at IC₅₀ of 190.34µg/ml, almost similar to Pentostam whose IC₅₀ was 171.58µg/ml. Amphotericin B inhibited parasite growth at IC₅₀ of 29.58µg/ml (Table 1).

Nitric Oxide

BALB/c mice peritoneal macrophages were incubated in RPMI medium. Using the Greiss Reagent system, the *A. secundiflora* water extract had Optical Density (OD) readings ranging from 0.051 to 0.066 at a concentration ranging from 0 to 100µm. *Plumbago capensis* had OD readings ranging from 0.032 to 0.045 at a concentration ranging from 0 to 100µg/ml. The aqueous extracts of *A. secundiflora* and *P. capensis* did not produce significant NO levels (p>0.05). None of the samples had Optical Density (OD) readings of more than 0.10 (Tables 2 and 3).

In-Vitro Activity of Methanolic and Water Crude Extracts

At a concentration of 250µg/ml, *A. secundiflora* water extract was able to reduce the number of amastigotes from 150 to 78. As the concentration increased to 500µg/ml, 1000µg/ml, 2000µg/ml, the number of amastigotes reduced to 68, 46 and 38 respectively. At a

Table 2: Absorbance (OD) units ±SD of the standard nitrite (positive control), RPMI (negative control) and water extract from the study plants.

Concentration (µg/ml)	Standard Nitrite Curve (+ve control)	RPMI (-ve control)	<i>Aloe secundiflora</i>	<i>Plumbago capensis</i>
0	0.054±0.011	0.021	0.051	0.032
20	0.153±0.013	0.030	0.053	0.035
40	0.178±0.001	0.038	0.061	0.036
60	0.331±0.000	0.048	0.051	0.040
80	0.559±0.004	0.055	0.059	0.042
100	0.983±0.010	0.079	0.066	0.045

Table 3: Absorbance (OD) units ±SD of the standard nitrite (positive control), RPMI (negative control) and methanolic extract from the study plants.

Concentration (µg/ml)	Standard Nitrite Curve (+ve control)	RPMI (-ve control)	<i>Aloe secundiflora</i>	<i>Plumbago capensis</i>
0	0.061±0.012	0.062	0.064	0.064
20	0.075±0.037	0.064	0.063	0.071
40	0.097±0.067	0.054	0.061	0.064
60	0.145±0.0135	0.055	0.062	0.066
80	0.225±0.246	0.056	0.062	0.063
100	0.298±0.359	0.055	0.063	0.069

concentration of 250µg/ml, 500µg/ml, 1000µg/ml and 2000µg/ml, *A. secundiflora* methanolic extract showed the following number of amastigotes 100, 88, 81 and 76 respectively (Table 4.3). *Plumbago capensis* water extract at a concentration of 250µg/ml, 500µg/ml, 1000µg/ml, 2000µg/ml, the number of amastigotes reduced from 150 to 110, 96, 54 and 42 respectively while *P. capensis* methanolic extracts showed almost similar activity reducing the amastigotes number from 150 to 115, 100, 66 and 51 at a concentration of 250µg/ml, 500µg/ml, 1000µg/ml and 2000µg/ml respectively. A combination of *A. secundiflora* and *P. capensis* water extract reduced the number of amastigotes from 150 to 70, 42, 28 and 12 at a concentration of 250 µg/ml, 500µg/ml, 1000µg/ml and 2000µg/ml, while a combination of *A. secundiflora* and *P. capensis* methanolic extract reduced the number of amastigotes from 150 to 78, 55, 42 and 18 at a concentration of 250µg/ml, 500µg/ml, 1000µg/ml and 2000µg/ml respectively. Amphotericin B reduced the number of amastigotes from 28 at a concentration of 250µg/ml to 11, 5 and 0 at a concentration of 500µg/ml, 1000µg/ml and 2000µg/ml respectively while Pentostam reduced the number of amastigotes from 13 at the minimum concentration of 250µg/ml to 10, 4 and 0 at a concentration of 500µg/ml, 1000µg/ml and 2000µg/ml respectively (Table 4).

Weight Measurements

Mice administered with *A. secundiflora* water extract initial weight was 22.6g, after 4 weeks of treatment, the weight reduced to 22.2g for the orally administered group and maintained the same weight of 22.6g for those administered the drugs intraperitoneally while *A. secundiflora* methanolic extract initial weight was 23.0g which increased to 23.2g for the mice which were administered the drugs orally and maintained the same weight of 23.0g for those mice administered intraperitoneally. After 4 weeks of treatment, the group administered with *P. capensis* water extract reduced the weight from 25.4g to 23.4g for those mice administered orally but mice administered intraperitoneally maintained the weight at 25.4g while the group administered orally with *P. capensis* methanolic extracts increased in weight from the initial weight of 23.5g to 24.0g while those administered intraperitoneally maintained the same weight of 23.5g. Oral administration of mice with a combination of *A. secundiflora* and *P. capensis* water extract reduced the mice weight from 25.6g to 25.2g, after 4 weeks of treatment, but maintained the same weight of 25.6g when administered intraperitoneally. After 4 weeks of experimental drug administration of a combination of *A. secundiflora* and *P. capensis* methanolic extract, the mice maintained the initial weight of 26.0g when administered both orally and intraperitoneally while

Table 4: Infectivity of *L.major amastigotes* in macrophages after treatment with various test drugs.

Conc.	Extract							
	A.s (H ₂ O)	A.s (Methanol)	P.c (H ₂ O)	P.c (Methanol)	A.s and P.c (H ₂ O)	A.s and P.c (Methanol)	Amph B	Pento
250	78	100	110	115	70	78	28	13
500	68	88	96	100	42	55	11	10
1000	46	81	54	66	28	42	5	4
2000	38	76	42	51	12	18	0	0

P.c: *Plumbago capensis*; *A.s:* *Aloe secundiflora*; *P.c & A.s:* Combination of *P. capensis* and *A. secundiflora*; AmphB: Amphotericin B, Pento-Pentostam; Conc.: Concentration

those administered with Amphotericin B and Pentostam maintained the average weight of 23.0g and 22.6g respectively. Mice administered with PBS orally reduced in weight from 24.75g to 19.2g while those administered intraperitoneally weight reduced from 24.75g to 18.9g after the 4 weeks of treatment (Table 5).

Lesion Sizes

The mice administered with *A.sekundiflora* water extract lesion size reduced from 3.0 mm to 2.01 mm and 1.89 mm when administered orally and intraperitoneally respectively, while those administered with *A.sekundiflora* methanolic extract lesion sizes reduced from 3.0mm to 1.70mm and 2.09mm when administered orally and intraperitoneally respectively. *Plumbago capensis* water extract when administered orally, lesion size reduced from 3.3mm to 2.30mm but when administered intraperitoneally lesion size reduced from 3.3mm to 2.60mm while oral admission of *P.capensis* methanolic extract reduced lesion size from 3.3mm to 2.17mm while intraperitoneal administration reduced lesion size from 3.3mm to 2.72mm. A combination of *A.sekundiflora* and *P.capensis* water extract oral and intraperitoneal admission reduced lesion sizes from 3.4mm to 2.31mm and 2.70mm respectively, while a combination of *A.sekundiflora* and *P.capensis* methanolic extract reduced lesion sizes from 3.0 mm to 2.10mm when administered orally and 2.24mm when administered intraperitoneally. When Amphotericin B and Pentostam were administered intraperitoneally, the lesion sizes reduced from 3.4mm and 3.6mm to 1.94mm and 1.96mm respectively while those mice administered with PBS orally, the lesion size reduced from 3.1mm to 2.79mm while those mice administered with PBS intraperitoneally, the lesion sizes increased from 3.1mm to 3.13mm (Table 6).

Table 5: Mean weight (g) change of mice against the route of Drug Administration.

Plant Extract/Mice Group	Initial weight (g)	Route of drug Administration	
		Oral	IP
<i>Aloe secundiflora</i> (H ₂ O)	22.6	22.2	22.6
<i>Aloe secundiflora</i> (Methanol)	23	23.2	23
<i>Plumbago capensis</i> (H ₂ O)	25.4	23.4	25.4
<i>Plumbago capensis</i> (Methanol)	23.5	24	23.5
<i>Aloe secundiflora</i> and <i>Plumbago capensis</i> (H ₂ O)	25.6	25.2	25.6
<i>Aloe secundiflora</i> and <i>Plumbago capensis</i> (Methanol)	26	26	26
Amphotericin B	23		23
Pentostam	22.6		22.6
PBS	24.75	19.2	18.9

Table 6: Mean lesion size (mm) of mice against the route of Drug Administration.

Plant Extract	Initial Lesion size (mm)	Route of Administration	
		Oral	IP
<i>A.sekundiflora</i> (H ₂ O)	3	2.01	1.89
<i>A.sekundiflora</i> (Methanol)	3	1.7	2.09
<i>P.capensis</i> (H ₂ O)	3.3	2.3	2.6
<i>P.capensis</i> (Methanol)	3.3	2.17	2.72
<i>A.sekundiflora</i> and <i>P.capensis</i> (H ₂ O)	3.4	2.31	2.7
<i>A.sekundiflora</i> and <i>P.capensis</i> (Methanol)	3	2.1	2.24
Amphotericin B	3.4		1.94
Pentostam	3.6		1.96
PBS	3.1	2.79	3.13

Leishman Donovan Units as Per the Route of Drug Administration

A combination of *A.sekundiflora* and *P.capensis* water extract had LDU of 4092 when administered orally and LDU of 19712 when administered intraperitoneally. *Plumbago capensis* water extract had LDU of 6580 and 25860 when administered orally and intraperitoneally respectively. The oral and intraperitoneal administration of *P.capensis* methanolic extract had LDU of 11316 and 14058 respectively. *Aloe secundiflora* water extract had LDU of 13788 and 19418 when administered orally and intraperitoneally respectively. A methanolic extract of *A.sekundiflora* when administered through the oral route had LDU of 14040 and when administered through the intraperitoneal route had LDU of

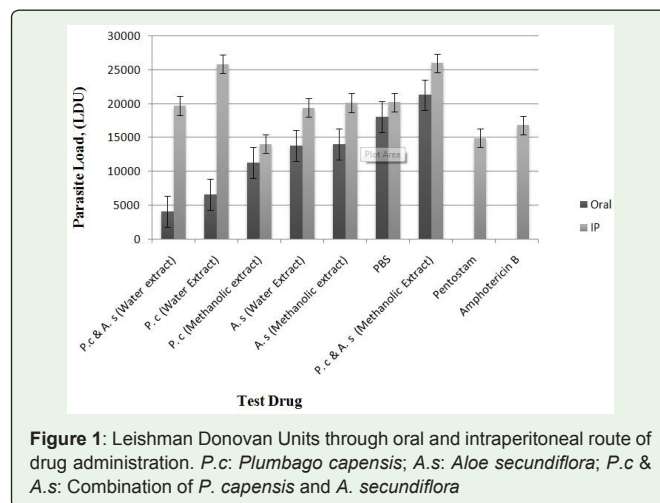


Figure 1: Leishman Donovan Units through oral and intraperitoneal route of drug administration. *P.c:* *Plumbago capensis*; *A.s:* *Aloe secundiflora*; *P.c & A.s:* Combination of *P. capensis* and *A. secundiflora*

20130. A combination of *P.capensis* and *A.secundiflora* methanolic extracts had LDU of 21280 and 25980 when administered orally and intraperitoneally respectively. The control group administered with PBS only had LDU of 18018 and 20202 when administered orally and intraperitoneally respectively. Pentostam had LDU of 14924 while Amphotericin B had LDU of 16800 (Figure 1).

Discussion

In this study, antileishmanial activity of the methanolic and water extracts from *A.secundiflora* and *P.capensis* was assessed using *in-vitro* tests [18,19]. It was noted that the plant extracts reduced the NO production 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 reduced the infectivity of *L.major* amastigotes in macrophages compared to the standard reference drugs. The methanolic plant extract 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 *A.secundiflora* have better active ingredients against *Leishmania* parasites than methanolic extracts. In this study, the activity of *A.secundiflora* against *L.major* showed that the plant contained some pharmacologically active substances. These results suggest that *A.secundiflora* had compounds or group 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 [3,20]. As observed in the current study, the test extracts had IC₅₀ levels of 118.57 µg/ml and 190.34 µg/ml for the water and methanolic extract respectively, while Amphotericin B and Pentostam showed IC₅₀ levels of 29.58 µg/ml and 171.29 µg/ml respectively. Although the standard drugs were effective against *Leishmania* promastigotes, they have been reported to be highly toxic to patients and they require a protracted administration [21,22].

Conclusions

The water and methanolic extracts of *A.secundiflora* and *P.capensis* have inhibitory activities against *L.major* parasites both *in-vitro* and *in-vivo*. The water and methanolic extracts of *A.secundiflora* and *P.capensis* can be used either separately or in combination as antileishmanial therapeutic agents.

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