**Efficacy of crude methanolic extracts of Allium sativum L. and Moringa stenopetala (Baker f.) Cufod. against Leishmania major**

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**Abstract:** Leishmania major is a protozoan parasite responsible for cutaneous leishmaniasis (CL) in humans. CL is transmitted via a bite by infected female phlebotomine sand fly. Research on herbal therapy for leishmaniasis is increasing globally because conventional drugs are costly, toxic and require a prolonged administration. In vitro and in vivo antileishmanial activities of dried Allium sativum (garlic) and Moringa stenopetala methanolic extracts against L. major were studied. Minimum inhibitory concentrations (MICs) of methanolic extracts of A. sativum (A) and M. stenopetala (M) against L. major were 3 and 5 mg/ml and IC₅₀ of 863.12 and 1752.92 µg/ml respectively. The blend AM (1:1) had IC₅₀ of 372.1 µg/ml and promastigotes’ viability of 71.03% compared to IC₅₀ of 0.26 and 0.82 µg/ml for Pentostam and Liposomal amphotericin B respectively. Multiplication indices (MIs) of L. major amastigotes ranged from 43.67% to 45.93% after treatment with extracts A or M or blend AM at 125 µg/ml and were significantly different (P < 0.05) from Liposomal amphotericin B at 12.5 µg/ml. Oral extract A reduced significantly (P > 0.05) L. major caused foot pad lesions in BALB/c mice while oral extract M did not. Blend AM (ip) reduced the lesion sizes and its efficacy was close to Pentostam and Liposomal amphotericin B. Oral extract A had a high parasite reduction rate of 60.70% and average LDU of 0.22±0.15 compared to Pentostam at 66.40% and LDU of 0.18±0.08. In conclusion, methanolic extract of A. sativum showed anti-leishmanial activity both in vitro and in vivo and it decreased L. major caused foot pad lesions in BALB/c mice. A blend of garlic and moringa methanolic extracts (AM at 1:1) were active against L. major. The active ingredients in crude methanolic extracts of garlic and moringa plants should be established and tested against L. major when blended.

**Keywords:** A. sativum; M. stenopetala; methanolic extracts; blend; antileishmanial; Leishmania major.

**Introduction**

Cutaneous leishmaniasis (CL) is one of the neglected tropical protozoan diseases and it is transmitted by a bite of an infected female phlebotomine sand fly (Diptera: Psychodidae). The protozoan responsible for this disease belongs to the genus Leishmania (Kinetoplastida: Trypanosomatida) and it is endemic in 98 countries worldwide (WHO, 2010). An estimated yearly incidence of about 1.2 million cases of CL occur each year worldwide (Alvar et al., 2012). The main clinical manifestations of CL are painful skin lesions that can elicit social stigmatization and significant morbidity in the sufferers (Bailey, 2013).
According to Nilforoushzadeh et al. (2007), there is no effective and safe treatment for leishmanias. The CL drugs which include Pentavalent antimonials, Amphotericin B and Pentamidine among others are toxic, expensive and are often associated with several health complications. Drug resistances by Leishmania parasites and therapeutic failure have also been observed in leishmaniasis patients. Leishmania major (Kinetoplastida: Trypanosomatidae) is one of the species that cause CL in Kenya and it is endemic in Baringo County, in the great Rift Valley.

Herbal products are potential sources of anti-leishmanial compounds since they have a wide chemistry, remarkable diversity and a high accessibility in nature (Monzote, 2009). Increased research on plant derived anti-leishmanial compounds has identified herbal products that are effective against Leishmania parasites. Allium sativum L. (garlic) is a perennial plant that belongs to the family Amaryllidaceae and it has been used as food, spice and medicine for thousands of years in different parts of the world (Singh & Singh, 2008). The medicinal properties of A. sativum range from antimicrobial, hypolipidemic, antithrombotic to antitumour activities (Augusti, 1996). Previous studies have indicated that garlic extracts or their fractions augment parasite engulfment and destruction of intracellular Leishmania amastigotes by BALB/c mice peritoneal macrophages (Ghazanfari et al., 2006). Similarly, McClure et al. (1996) reported that Allicin, the active antimicrobial compound in A. sativum, demonstrates a significant inhibitory effect on leishmanial cell growth.

Moringa stenopetala (Baker f.) Cufod. is a smooth barked, deciduous flowering plant widely distributed in southern parts of Ethiopia and northern Kenya (Mekonnen et al., 1999) particularly in Lake Baringo islands. M. stenopetala belongs to the monogeneric family Moringaceae (Order Capparales). In Ethiopia, the leaves and fruits of M. stenopetala are eaten as vegetables which are rich in proteins, calcium, phosphorous, iron plus vitamins A and C. The leaves, roots, fruits and the barks of the M. stenopetala are used to treat different ailments including stomach problems, malaria, hypertension, diabetes, asthma and expelling retained placenta (Mekonnen et al., 1999). Fresh root wood ethanol extracts and dried leaves acetone extracts of M. stenopetala have been reported to possess antitrypanosomal property (Mekonnen et al., 1999). In our previous study, dried leaves crude aqueous extract of M. stenopetala was demonstrated to possess in vitro inhibitory effect against L. major promastigotes at a concentration of 3mg/ml (Kinuthia et al., 2013).

It is against this background that the present study was designed to investigate the in vitro and in vivo activity of crude methanolic extracts of A. sativum L. and M. stenopetala (Baker f.) Cufod. against L. major. The crude methanolic extracts were studied singly or as blends.

**Materials and methods**

**Plant materials**

Bulbs of A. sativum were purchased from Nakumat super market in Nairobi, Kenya while young leaves of M. stenopetala were picked from trees growing on the slopes of Lake Baringo islands in Kenya. The study plants were authenticated at University of Nairobi Herbarium, in the department of Botany, Chiromo Campus. The young leaves of M. stenopetala and thin slices of A. sativum cloves were dried at room temperature at Kenya Medical Research Institute (KEMRI), Leishmania unit, until they attained a constant weight. The dried plant materials were labeled appropriately and then transferred to the Center of Traditional Medicine & Drug Research (CTMDR) at KEMRI, where they were ground into a powder using an electric mill and then stored at minus 20°C until required for extraction.

**Crude Extracts**

The crude methanolic extracts were prepared as described by Cock (2008). Briefly, a 100g of ground plant material was soaked in 500 ml of analytical grade methanol for 72 hours at room temperature with gentle shaking, then filtered and concentrated using a rotary evaporator to obtain the crude methanolic extracts. The dry extracts were weighed and stored at 4°C until required for the bioassays. The methanolic crude extracts were stored at minus 20°C until required for testing.

**Purification and screening of the extracts**

The crude methanolic extracts were prepared and screened as described by Cock (2008). Briefly, a 100g of ground plant material was soaked in 500 ml of analytical grade methanol for 72 hours at room temperature with gentle shaking, then filtered and concentrated using a rotary evaporator to obtain the crude methanolic extracts. The dry extracts were weighed and stored at 4°C until required for the bioassays. The methanolic crude extracts were stored at minus 20°C until required for testing.

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extracts of *A. sativum* was coded as extract A while that of *M. stenopetala* was coded as extract M. With an initial weight of 100g ground plant material, the methanolic crude extracts of *A. sativum* (A) was 5.698g (5.70%) while that of *M. stenopetala* (M) was 9.217g (9.22%).

**Leishmania parasites**

The *Leishmania major* strain (IDUB/KE/94=NLB-144) used in this study was acquired from Institute of Primate Research (IPR) Kenya, where it had been maintained by cryopreservation in liquid nitrogen. The parasites were grown to stationary phase at 25°C in Schneider’s insect medium supplemented with 20% heat inactivated fetal bovine serum, 100 U/ml penicillin and 500 g/ml streptomycin (Hendricks & Wright, 1979), and 250 g/ml of 5-fluorocytosine arabinoside (Kimber et al., 1981). The stationary-phase metacyclic stage promastigotes were then harvested by centrifugation at 1500 rpm for 15 minutes at 4°C. The metacyclic promastigotes were then used for the in vitro and in vivo assays.

**Experimental animals**

Eight week old male inbred BALB/c mice were used for in vivo macrophage assay for the plant extracts. The inbred BALB/c mice were obtained from International Livestock Research Institute (ILRI), Kenya. They were then housed at the KEMRI animal house at 23°C to 25°C and were fed on standard commercial diet in the form of mice pencils and were given tap water ad libitum. The mice were handled in accordance with the regulations that have been set by KEMRI’s Animal Care and Use Committee (ACUC).

**Evaluation of minimum inhibitory concentration (MIC)**

The MIC was determined as described by Wabwoba et al. (2010). Promastigotes at a concentration of $1 \times 10^6$ metacyclic promastigotes per ml of culture medium were exposed to several concentrations of the test individual plant extracts A and M. The lowest concentration of the test plant extracts that inhibited promastigotes growth was taken to be the MIC.

**In vitro anti-promastigote assay**

This was evaluated as described by Wabwoba et al. (2010). The metacyclic promastigotes at a concentration of $1 \times 10^6$ promastigotes per ml were incubated in culture medium in 24-well plates in presence of different concentrations of plant extracts for five days at 25°C. The aliquots of parasites were then transferred into 96-well micro-titer plates, and incubated at 27°C for 24 hours. Two hundred micro liters of test extracts A and M samples were then added to the parasite cultures at concentrations ranging from 5 mg/ml to 0.5 mg/ml of extracts. The plates were then incubated further at 27°C for 48 hours. The control wells contained culture alone. Ten micro liters of 2, 5-diphenyltetrazolium bromide (MTT) reagent was added into all the wells and incubated for 4 minutes. The medium together with MTT were aspirated off, followed by addition of 100 l of dimethyl sulfoxide (DMSO) per well and shaken for 5 minutes. Absorbance was measured for each well at 562 nm using a micro-titer reader. The absorbance readings were used to generate the 50% inhibitory concentration (IC$_{50}$) values for extracts A and M. Percentage promastigotes’ viability (%) was determined using the formula described by Mosmann (1983), in which, viable promastigotes (%) = (at – ab) / (ac) × 100, where at was the absorbance of treated samples and ab was the absorbance of the blank wells and ac was the absorbance of the control wells.

**In vitro anti-amastigote assay**

This was carried out as described by Delorenzi et al. (2001). Peritoneal macrophages were obtained from clean BALB/c mice. Ten milliliters of sterile cold phosphate-buffered saline (PBS) was injected into the peritoneum of anaesthetized BALB/c mice whose body surface had been disinfected with 70% ethanol. The PBS containing the macrophages was washed through centrifugation at 2,000 rpm for 10 minutes and the macrophages were adsorbed in sterile 24-well plates for 4 hours at 37°C in 5%
CO₂. Adherent macrophages were infected with promastigotes and further incubated at 37°C in 5% CO₂ for 4 hours and then washed with sterile PBS to remove the free promastigotes. This was followed by further incubation of the infected macrophages for 24 hours in RPMI 1640 culture medium. The infected macrophages were then treated with extracts A and M. Pentostam and Liposomal amphotericin B were used as positive control drugs. The medium, test extracts and control drugs were replenished daily for 3 days. After 5 days, the macrophages were washed with PBS at 37°C, fixed in methanol and stained with 10% Giemsa. The number of amastigotes was determined by counting at least 100 macrophages in duplicate cultures, and the results was expressed as infection rate (IR) and multiplication index (MI) as described by Berman & Lee (1984).

Infection and treatment of BALB/c mice

The BALB/c mice were placed into 10 groups caged separately, where each group was comprised of at least 5 mice. All the mice in each group were infected with *L. major* promastigotes in the foot pads as described by Wabwoba et al., 2010. Briefly, the thickness of the hind legs footpads was measured using a reading vernier caliper prior to infection. The left hind footpads of the mice were then subcutaneously inoculated with 1×10⁶ stationary phase infective metacyclic promastigotes of *L. major* in 40 μl sterile PBS. Lesion development was monitored for four weeks after which treatment commenced. A four week treatment was given to the infected mice that had developed footpad lesions. Groups of mice were treated with individual methanolic extracts A, extracts M, blend of A and M in a ratio of 1:1, and with positive and negative controls. Treatment was administered orally daily using a cannula for each extract or intra-peritoneally using a fine 1ml 30 gauge insulin needles (BD Micro-Fine Plus®, USA) at a dose of 20 mg/kg daily for each extract. The positive control groups of mice were treated intra-peritoneally with Pentostam and Liposomal amphotericin B leishmaniasis drugs at a dose of 20 mg/kg per day. Two groups of negative control mice were treated with phosphate buffered saline (PBS), one group orally and the other intraperitoneally. The progression of the foot pads lesions sizes was monitored weekly using a vernier caliper to measure the thickness of the infected left hind foot pad and comparing it with that of non infected right hind foot pad as described by Nolan & Farrel (1987).

Post treatment parasite burden in BALB/c mice spleens

After a four week treatment period, the mice were sacrificed using 100 l pentobarbitone sodium (Sagatal®). At necropsy, the spleens were weighed and spleen impression smears were made as described by Chulay & Bryceson (1983). The impression smears were fixed in methanol and stained with Giemsa. The smears were examined under a microscope to enumerate the number of amastigotes per 1000 nucleated spleen cells. The relative and total numbers of parasites in the spleen were estimated by calculating the spleen index (%), Leishman-Donovani Units (LDUs) and total Leishman-Donovani Units (total LDUs) as described by Bradley & Kirkley (1977). Spleen index (%) was determined using the formula: Spleen weight (g) divided by body weight (g) × 100%. LDU was the number of amastigote per 1000 nucleated splenocytes while the total LDU was calculated using the formula: LDU × spleen weight (g) × (2 × 10⁵).

Data analysis

Data was analyzed using SPSS version 17.0 for windows at 5% level of significance. One way ANOVA (F test) was used to compare promastigotes viabilities (%) after different treatments. Other variables compared using F test were infection rates (IRs) and multiplication indices (MIs) of amastigotes in peritoneal macrophages and also the lesion sizes in different groups of BALB/c mice under different treatments. Cases in which homogeneity test of variance (Levene’s test) were significant, robust tests of equality of means that included Brown-forsythe and Welch tests were carried out as alternative versions of the F-statistics. Multiple comparisons of the individual treatments were
Results

MIC, IC_{50} and viability of L. major promastigotes

The MICs of crude methanolic extracts A and M against L. major were 3 and 5 mg/ml respectively compared to 1.25 \times 10^{-2} and 6.25 \times 10^{-3} mg/ml for Pentostam and Liposomal amphotericin B respectively. The IC_{50} of extracts A and M were 863.12 and 1752.92 µg/ml respectively compared to 0.26 and 0.82 µg/ml for Pentostam and Liposomal amphotericin B respectively. The viabilities (%) of L. major promastigotes after treatment with crude extracts A and M at concentrations that ranged from 5 to 0.5 mg/ml were 67.80% and 80.84% respectively while the promastigotes viability in Pentostam and Liposomal amphotericin B were 18.41% and 12.22% respectively at an initial concentration of 0.1mg/ml which was serially diluted by a factor of 2. A blend of crude methanolic extracts of A. sativum (A) and M. stenopetala (M) at fixed ratios (AM) and at the MIC based concentrations, exhibited additive interaction at ratios 1:1, 2:8, and 1:9 with corresponding promastigotes’ viability of 76.56%, 59.79% and 34.59% respectively. The IC_{50} of blend AM at ratios ranging from 9:1 to 1:9 was 372.1µg/ml with overall promastigotes viability of 71.03%.

Table 1: The in vitro infection rates (IR) and multiplication indices (MI) of L. major amastigotes in peritoneal macrophages following treatment with methanolic extracts and control drugs.

<table>
<thead>
<tr>
<th>Test extracts &amp; controls</th>
<th>Concentration (µg/ml)</th>
<th>IR (%)</th>
<th>Amastigotes per 100 cells</th>
<th>MI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single extracts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>125.00</td>
<td>46</td>
<td>193</td>
<td>43.67</td>
</tr>
<tr>
<td></td>
<td>62.50</td>
<td>49</td>
<td>201</td>
<td>45.48</td>
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<td></td>
<td>31.25</td>
<td>51</td>
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<td></td>
<td>15.63</td>
<td>70</td>
<td>230</td>
<td>52.04</td>
</tr>
<tr>
<td>M</td>
<td>125.00</td>
<td>70</td>
<td>203</td>
<td>45.93</td>
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<td></td>
<td>62.50</td>
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<td>210</td>
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<td></td>
<td>31.25</td>
<td>83</td>
<td>316</td>
<td>71.49</td>
</tr>
<tr>
<td></td>
<td>15.63</td>
<td>87</td>
<td>353</td>
<td>79.86</td>
</tr>
<tr>
<td>Blend (1:1)</td>
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<td>73</td>
<td>193</td>
<td>43.67</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPMI * (3 replicas)</td>
<td>1st</td>
<td>89</td>
<td>451</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>79</td>
<td>403</td>
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<tr>
<td></td>
<td>3rd</td>
<td>86</td>
<td>472</td>
<td>n/a</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>84.67±2.96</td>
<td>442</td>
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</tr>
<tr>
<td>Pentostam</td>
<td>50.00</td>
<td>14</td>
<td>51</td>
<td>11.54</td>
</tr>
<tr>
<td></td>
<td>25.00</td>
<td>38</td>
<td>123</td>
<td>27.83</td>
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<tr>
<td></td>
<td>12.50</td>
<td>67</td>
<td>210</td>
<td>47.51</td>
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<td></td>
<td>6.25</td>
<td>81</td>
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</tr>
<tr>
<td>Lip ampho B</td>
<td>50.00</td>
<td>6</td>
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<td>6.25</td>
<td>23</td>
<td>62</td>
<td>14.03</td>
</tr>
</tbody>
</table>

RPMI *: Negative control in which the amastigotes multiplied in RPMI 1640 culture medium; n/a = not applicable; A = A. sativum while M = M. stenopetala; Lip ampho B = Liposomal amphotericin B.

Extracts in vitro effect on L. major amastigotes

RPMI 1640 medium supported the growth of L. major amastigotes in peritoneal macrophages more effectively as was indicated by a high infection rate (IR) of 84.67± 2.96 %. Liposomal amphotericin B and Pentostam inhibited the survival of L. major amastigotes peritoneal macrophages as was indicated by low IRs of 6% and 14% respectively at a concentration of 50µg/ml. A similar trend was observed in multiplication...
indices (MIs) as shown in Table 1. Effects of extracts A and M was dose dependent, where high extracts concentration led to low IRs and MIs. Methanolic extracts of garlic (A) were more effective in inhibiting the survival of amastigotes in peritoneal macrophages in vitro (Table 1). Blend AM at a concentration of 125 µg/ml was associated with 43.67% multiplication index for L. major amastigotes in peritoneal macrophages, and this activity was close to that of pentostam (47.51%) at 12.50 µg/ml (Table 1). Tukey’s post hoc test showed that the difference between the IRs and MIs of extracts A, M and blend AM and those of Liposomal amphotericin B were statistically significant (P < 0.05).

The orally administered methanolic extract A (A. sativum) into infected BALB/c mice led to a decrease of their foot pad lesions. The difference between the mean lesion sizes of mice treated with extract A and that of mice treated with drugs Pentostam and Liposomal amphotericin B (Figure 1) was not significant (P = 0.986 and 0.898 respectively). Orally administered methanolic extract M (M. stenopetala) did not reduce the footpad lesions in the infected BALB/c mice and this compared closely with that of mice treated with PBS (Figure 1). Tukey’s post hoc test showed that orally administered extracts M had an efficacy that was significantly different from that of orally administered extract A (P = 0.0001). Extracts A, administered intra-peritoneally (ip) had a significant reduction of the footpad lesions in BALB/c mice while extracts M were not very active in reducing foot pad lesions when compared to intra peritoneally administered PBS (P = 0.853).

![Figure 1](http://www.openaccessscience.com)
Pentostam indicated that the difference was not significant (F (2,28) = 0.946, P = 0.479). Blend AM administered intra-peritoneally into infected BALB/c mice, reduced the lesion sizes steadily from the second week (Figure 2) and its efficacy was significantly different from that of Pentostam and Liposomal amphotericin B (P < 0.05).

**Figure 2:** The foot pad lesion sizes in *L. major*-infected BALB/c mice after treatment with intra peritoneally administered blend AM (1:1) comprising of methanolic extracts of *A. sativum* (A) and *M. stenopetala* (M) compared to intra peritoneally administered Pentostam (Pento), Liposomal amphotericin B (Lip amph B), and phosphate buffered saline (PBS).

**Table 2:** The average spleen index ± SE, LDU ± SE and total LDU ± SE for groups of *L. major* infected BALB/c mice that were treated with methanolic extracts, and Pentostam, Liposomal amphotericin B and PBS controls.

<table>
<thead>
<tr>
<th>Extracts &amp; controls</th>
<th>Route</th>
<th>Ave spleen index (%)</th>
<th>Ave LDU</th>
<th>Ave total LDU (×1000)</th>
<th>% parasite reduction&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (Garlic)</td>
<td>oral</td>
<td>0.60±0.05</td>
<td>0.22±0.15</td>
<td>4.83±3.20</td>
<td>60.70</td>
</tr>
<tr>
<td></td>
<td>ip&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55±0.05</td>
<td>0.52±0.04</td>
<td>11.31±0.21</td>
<td>07.97</td>
</tr>
<tr>
<td>M (Moringa)</td>
<td>oral</td>
<td>1.31±0.13</td>
<td>0.36±0.07</td>
<td>15.00±1.53</td>
<td>-22.05</td>
</tr>
<tr>
<td></td>
<td>ip</td>
<td>1.31±0.31</td>
<td>0.16±0.02</td>
<td>6.36±2.90</td>
<td>48.25</td>
</tr>
<tr>
<td>Blend (1:1)</td>
<td>oral</td>
<td>0.54±0.11</td>
<td>0.53±0.18</td>
<td>12.14±6.53</td>
<td>01.22</td>
</tr>
<tr>
<td></td>
<td>ip</td>
<td>0.59±0.11</td>
<td>0.44±0.17</td>
<td>10.48±4.19</td>
<td>14.73</td>
</tr>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pentostam</td>
<td>ip</td>
<td>0.73±0.19</td>
<td>0.18±0.08</td>
<td>4.13±1.10</td>
<td>66.40</td>
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<tr>
<td>Lip amph B</td>
<td>ip</td>
<td>0.61±0.02</td>
<td>0.24±0.02</td>
<td>4.84±0.38</td>
<td>60.62</td>
</tr>
<tr>
<td>PBS</td>
<td>ip</td>
<td>0.54±0.04</td>
<td>0.38±0.21</td>
<td>8.74±5.30</td>
<td>28.89</td>
</tr>
<tr>
<td>PBS</td>
<td>oral</td>
<td>0.56±0.06</td>
<td>0.61±0.22</td>
<td>12.29±4.49</td>
<td>00.00</td>
</tr>
</tbody>
</table>

<sup>a</sup> means that the % was calculated in reference to total LDU for PBS oral which was taken to represent 100% parasite burden; <sup>b</sup> = intra-peritoneal; A = *Allium sativum*; M = *Moringa stenopetala*; Lip amph B = Liposomal amphotericin B; PBS = phosphate buffered saline.
Effects of extracts on spleen parasite burden in mice

Orally administered extract A (A. sativum) had the highest parasite load reduction of 60.70% while orally administered extract M (M. stenopetala) had the lowest at -22.05% compared to 66.40% for Pentostam (Table 2). The average values of LDU±SE for intra peritoneally administered extracts A and M were 0.52±0.04 and 0.16±0.02 respectively compared to 0.18±0.08, 0.24±0.02, and 0.38±0.21 for intra peritoneally administered Pentostam, Liposomal amphotericin B and PBS respectively (Table 2). The route of extracts’ administration had a significant influence on the total LDU values obtained as was the case for extract M whose mean total LDU for oral route and that for intra peritoneal route differed significantly (P = 0.016). Blend AM (1:1) reduced the spleen parasite burden by 14.73% when administered intra peritoneally compared to 60.62% and 28.89% for Liposomal amphotericin B and PBS respectively.

Discussion

Among the new strategies of controlling leishmaniasis is the use of herbal materials, which are regarded as safer, cheap and less likely to be associated with drug resistance. The medicinal properties of A. sativum L (garlic) includes antibacterial, antifungal, antiviral and antiparasitic (Goncagul & Ayaz, 2010). The medicinal property of garlic has been attributed to its organosulfur compounds which include alliin, allicin, diallyl sulfide, diallyl disulfide, ajoene among others (Islam et al., 2011). Previous studies have reported on the antileishmanial property of garlic extracts in vitro and in vivo (Ahmadi-Renani, et al., 2002; Gamboa-Leon et al., 2007; and Wabwoba, et al., 2010). According to McClure et al. (1996), allicin (diallyl thiosulfinate) inhibits leishmanial cell growth significantly and it is less inhibitory to the growth of mammalian cell lines. The present study concurs with the previous studies because it demonstrates that methanolic extract of dried garlic have a low toxicity and inhibits the survival of L. major promastigotes and amastigotes in vitro, as well as reducing the size of L. major induced foot pad lesions in BALB/c mice. In addition, this study shows that garlic lowers the amastigotes burden in the spleens of infected mice. According to Ahmadi-Renani et al. (2002), aqueous extract of garlic reduced the diameter of L. major induced lesions within 30 days of treatment and the healing correlated with nitric oxide (NO) release by the host macrophages. According to Gamboa-Leon (2007), garlic shows immune modulatory property that enhances the release of nitric oxide (NO) by the host macrophages and kills Leishmania parasites in experimental BALB/c mice.

The medicinal and nutritional values of Moringa stenopetala (Baker f.) Cufod. have been widely investigated by Mekonnen and her research collaborators (Mekonnen et al., 1999). The current study indicates that intra peritoneally administered methanolic extract of M. stenopetala into L. major infected BALB/c mice was active in reducing the amastigotes burden by 48.25% in their spleens at a dose of 20mg/kg/day for four weeks, but it seemed not to be active in reducing the L. major caused foot pad lesions in the infected mice at the same dose and treatment period. Plants in the order Capparales, including M. stenopetala are rich in glucosinolates which are phytochemicals that are precursors of a wide range of bioactive anti biotic compounds (Fahay, 2005; Bellostas et al., 2010). This could explain the plant’s in vivo inhibitory activity against L. major amastigotes in BALB/c mice, observed in the current study. In our previous study, aqueous extracts of M. stenopetala supported an in vitro L. major amastigotes infection rate of 58% in BALB/c mice peritoneal macrophages (Kinuthia, et al., 2013).

Recent literature reports have established the effectiveness of natural products as potentially rich sources of new and selective agents for the treatment of tropical diseases caused by protozoan parasites (Mishra et al., 2011). In most community based herbal therapies, different herbal materials are used in combination to treat a specific medical condition. The blending of herbal materials or drugs could be advantageous when synergistic or additive interactions ensue. Antagonistic interactions are often disadvantageous (Tahany, 2010).
In the present study, blends of methanolic extracts of A. sativum and M. stenopetala at 1:1, 2:8 and 1:9 ratios interacted additively. This could have explained the activity of the blend (ratio 1:1) in reducing the spleen parasite burden by 14.73% when administered intra-peritoneally in BALB/c mice and also in significantly reducing the L. major caused foot pad lesion sizes in the mice. Since garlic is rich in organosulfur compounds while moringa is rich in glucosinolates, therefore a blend of the two may have contained the two compounds thus explaining the antileishmanial activity. Previous studies have shown that blends of aqueous extracts of A. sativum and M. stenopetala that interacted synergistically or additively showed in vitro and in vivo antileishmanial activity (Kinuthia, et al., 2013).

Conclusion

The methanolic crude extracts of Allium sativum L (garlic) are less toxic compared to leishmaniasis standard drugs Pentostam and Liposomal amphotericin B, and in addition the crude extracts are active against L. major both in vitro and in vivo. Intra-peritoneally administered M. stenopetala methanolic crude extracts into L. major infected BALB/c mice lowered the amastigotes burden in their spleens. Similarly, a blend of A. sativum and M. stenopetala methanolic crude extracts at a ratio 1:1 was relatively less toxic and it reduced the size of L. major caused foot pad lesions in BALB/c mice significantly.

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References


