In Vitro Leishmanicidal Activity of Momordica foetida against Leishmania Major

Chepkemei J K¹, Makwali J¹, Ngeiywa M¹, Anjili C.²

¹Department of Biological Sciences, University of Eldoret - Kenya
²Kenya Medical Research Institute (KEMRI), Nairobi - Kenya

Abstract: Cutaneous leishmaniasis is caused by different species of leishmania and produces a skin ulcer that heals spontaneously in most cases, leaving scar. The antileishmanial activity of extracts of M. foetida (family Cucubitaceae), a known traditional therapy and one of the commonly used medicinal plants in Kenya was evaluated. Methanolic and aqueous extracts of this plant were tested for possible antileishmanial activity in vitro. Different concentration of methanol and aqueous extracts of M. foetida were tested against Leishmania major promastigotes and amastigotes. The inhibitory concentration (IC₅₀) on promastigotes, percentage rates of macrophages infected by amastigotes and cytotoxicological concentration (CC₅₀) effects on vero cells were determined. Data were analyzed using Stat graphic, for antileishmanial activities within and between all groups; the t-test and ANOVA analysis were used respectively. The results showed that M. foetida extracts significantly (P < 0.05) produced inhibition activities against L. major promastigotes and amastigotes compared to controls. The Minimum inhibition concentration (MIC) for aqueous extracts (125µg/ml) significantly demonstrated higher inhibitory factor than that of methanolic extracts (250µg/ml) by 125 units. Antileishmanial activities significantly (P < 0.05) increase with the increase in concentrations of M. foetida extracts. The extracts had no significant toxicity (P > 0.05) against vero cells compared to standard reference drugs and did not stimulate the macrophages to produce sufficient amount of nitric oxide, hence the extract could be having active compounds that act directly on parasites, therefore, supports its traditional use as antileishmanial remedy and it should also be tested against other species of the parasite such as L. donovani, L. tropica and L. aethiopica.

Keywords: Antileishmanicidal, Momordica foetida, Leishmania major, leishmaniasis, promastigote, amastigote and In vitro.

1. Introduction

The leishmaniases are diseases caused by obligate intracellular, kinetoplastid protozoa of the genus Leishmania (Singh, 2006). Although it is not a household name like malaria, the diseases caused by infection with Leishmania continue to have a major impact on much of the world’s population and are currently considered to be an emerging illness with high morbidity and mortality in the tropics and subtropics (Handman, 2001). Leishmaniases are endemic in 88 countries of the world in which 350 million people who are considered at risk of infection live; there are 2 million new cases of leishmaniasis annually and 14 million infected people worldwide (WHO, 2007). An increase in the incidence of leishmaniasis can be associated with urban development, destruction of forests, environmental changes, migrations of people to areas where the disease is endemic and wars which contributes to its spread due to displacement of people (Kolaczinski et al., 2007). Proven therapies against human leishmaniases include pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), amphotericin B, pentamidine, miltefosine and paromomycin (Berman; 1997). These drugs are unsatisfactory because of their limited efficacy, frequent side effects and increasing drug resistance, therefore, new, safer and more efficacious drugs are urgently required (Croft et al., 2005). Moreover, there is no effective vaccine against leishmaniasis (Handman, 2001). In this regard, medicinal plants offer prospects for discovering new compounds with therapeutic properties. M. foetida, (Cucubitaceae) is one of the most highly utilized medicinal plants in tropical and subtropical Africa. (Afolayan&Sunmonu, 2010). The ripe fruits of M. foetida is commonly eaten by children in the regions the plant grow and the leaves are chewed and swallowed as a remedy for stomach ache, constipation, toothache, venereal diseases, fever, muscle pains, weak joints and general body pains. The leaves are boiled in water and used to baths as cure for skin diseases and drunk to treat malaria and diabetes. M. foetida which is known as “Chepterenderet/Cheptenderet” in nandi/keiyo tribes is used by traditional healers to treat visceral leishmanias is (VL) in west pokot orally in boiled water and topical application to cutaneous leishmaniasis (CL) lesions. Previous studies on M. foetida have shown good antimicrobial, antiplasmodial and antidiabetic effects (Froelich, et al., 2007). Aqueous and methanolic extracts compounds of M. foetida showed activity against L. major promastigotes and amastigotes, however, the effect on Leishmania parasites has not been documented. The objective of the present study was to determine the effect of extracts of M. foetida in vitro on the growth and viability of L. major promastigotes in cell-free culture and amastigotes in macrophages extracted from BALB/c mice.

2. Materials and Methods

Collection of M. foetida: M. foetida aerial parts were collected from Sugoi village in Turbo constituency, Uasin Gishu County, in the Rift Valley Province in Kenya. Botanical identification was done by University of Eldoret botanists. Voucher specimens were kept in the herbarium of University of Eldoret.

Preparation of samples and extraction of M. foetida: The aerial parts were cut into small pieces and air-dried for three weeks under a shed. The dried specimens were shred using
an electrical mill in readiness for extraction. The sample preparation and extraction procedures were carried out as described by Harbone (1994). Cold sequential extraction was carried out on plant material with analar grade methanol and distilled water. 600ml of methanol for methanolic extracts and water for aqueous extracts was added to 300g of the shred specimen and flasks placed on a shaker and soaked for 48 hrs. The residue was filtered using a Buchner funnel under vacuum until the sample dried. The filtrate was then concentrated under vacuum by rotary evaporation at 30 - 35°C. The concentrate was transferred to a sample bottle and dried under vacuum; the weight of the dry extract was recorded and stored at -4°C until required for bioassay.

Bioassays for antileishmanial activity of M. foetida: Leishmania parasites: Metacyclic promastigotes of L. major strain (NLB-144) was used. Parasites were maintained as previously described (Titus et al., 1984) and metacyclics were isolated from stationary phase cultures by negative selection using peanut agglutinin (Tonui et al., 2004). Briefly, promastigotes were cultured in Schneider’s Drosophila medium supplemented with 20% foetal bovine serum (FBS), penicillin G (100 U/ml), and streptomycin (100µg/ml). Stationary-phase promastigotes were obtained from 7 day-old cultures.

Preparation of test compounds: Stock solutions of the fractions were made in culture media for anti-leishmanial assay and re-sterilized by passing through 0.22 µm microfilters under sterile conditions in a laminar flow hood. The extracts that were insoluble in water or media were first dissolved in 1% DMSO to avoid solvent carry over, (Dorinet et al., 2001). All the prepared compounds were stored at -4°C and retrieved only during use.

**Anti-amastigote assay:** The anti-amastigote assay was carried out as described by Delorenzi et al. (2001). Briefly, peritoneal macrophages were obtained from two BALB/c mice. The body surface was disinfected, the skin torn dorso-ventrally to expose the peritoneum and a sterile syringe used to inject 10 ml of phosphate buffered saline (PBS) into the peritoneum. Mouse peritoneal macrophages were harvested by withdrawing the fluid into sterile centrifuge tubes. The cell suspension was centrifuged at 2000rpm at 4°C for 10 minutes and the pellet re-suspended in 5 ml of complete RPMI 1640 medium.

Macrophages were absorbed in 24-well plates and allowed to adhere for 4 hours at 37°C in 5% CO2. Non-adherent cells were washed with phosphate buffered saline (PBS), and the macrophages incubated overnight in RPMI 1640 media.

Evaluation of minimum inhibitory concentration (MIC): Leishmania promastigotes (10^5 parasites/ml) were maintained in culture or in the presence of several concentrations (1µg/ml to 10µg/ml) of test compounds. Cell growth was evaluated daily by assessment of visibility turbidity in order to evaluate MIC. The lowest concentration of the samples that prevented the growth of Leishmania parasites in vitro was considered as the MIC.

Anti-promastigote assay: L. major promastigotes were cultured in NNN media overlaid with 2 ml of Schneider’s Drosophila insect medium (SIM-F) supplemented with 20% foetal bovine serum, 100 (g/ml streptomycin and 100 U/ml penicillin-G, and 5-fluorocytosine. Promastigotes were incubated in 24-well plates. After five days of cultivation, aliquots of parasites were transferred to 96-well micro-titer plate. The parasites were incubated at 27°C for 24 h and 200µl of the highest concentration of each of the test samples was added and serial dilution carried out. The experimental plates were incubated further at 27°C for 48 h. The controls used were promastigotes with no test compounds and medium alone. Ten microlitres of thiazoly blue tetrazolium bromide (MTT) reagent was added into each well and the cells incubated for 2 - 4 hrs until a purple precipitate was clearly visible under a microscope. The medium together with MTT was aspirated off from the wells, a hundred microlitres of DMSO added and the plates shaken for 5 min. Absorbance was measured for each well at 540nm using a micro-titer plate reader (Mosmann, 1983) and the 50% inhibitory concentration (IC50) values generated. Percentage promastigotes viability was calculated using the formula:

\[
\text{Promastigote viability} = \frac{\text{Average absorbance in duplicate drug wells - average blank wells}}{\text{Average absorbance control wells}} \times 100
\]

**Nitric oxide production determination:** Nitric oxide release in supernatants of macrophage culture was measured

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

The infection rate was used in the calculation of the association index (AI). The AIs were determined by multiplying the percentage of infected macrophages by the number of parasites per infected cell. Association indices were the number of parasites that actually infected the macrophages.
by the Griess reaction for nitrates (Holzmuller et al., 2002). 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 a 96-well micro-titer plate. To this, 60μl of Griess reagent A (1% sulphanilamide in 1.2 M HCl) was added, followed by 60μl of Griess reagent B (0.3% N-[1-naphthyl]ethylenediamine). The plates were read at 540nm in an ELISA plate reader. Sodium nitrite in RPMI was used to construct a standard curve for each plate reading.

Cytotoxicity assay: Vero cells were cultured and maintained in Minimum Essential Medium supplemented with 10% FBS. The cells were cultured at 37°C in 5% CO2, harvested by trypsinization, pooled in a 50 ml vial and 100μl cell suspension (1 x 10^4 cell/ml) put into 2 wells of rows A-H in a 96-well micro-titer plate for one sample. The cells were incubated at 37°C in 5% CO2 for 24 h to attach, the medium aspirated off and 150μl of the highest concentration of each of the test samples serial diluted. The experimental plates were incubated further at 37°C for 48 h. The controls used were cells with no drugs, and medium alone. MTT reagent (10μl) was added into each well and the cells incubated for 2 - 4 h until a purple precipitate was clearly visible under a microscope. The medium together with MTT was aspirated off from the wells, after which 100μl of DMSO was added and the plates shaken for 5minutes. The absorbance was measured for each well at 540nm using a micro-titer plate reader (Wang et al., 2006). Cell viability (%) was calculated using the formula:

\[
\text{Cell Viability} (%) = \frac{\text{Ave absorbance in duplicate drug wells} - \text{Average blank wells}}{\text{Average absorbance control wells}} \times 100
\]

Statistical analysis: All experiments were done in triplicate. The mean and standard deviation of at least three experiments were determined. The data was analyzed using Stat-graphic software. Statistical analysis of the differences between mean values obtained for the experimental groups were done by analysis of variance (ANOVA) and student’s t test. P values of less than 0.05 were considered significant.

### 3. Results

#### Activity of M. foetida Extracts on Promastigotes (10^6) and Minimum Inhibition Concentration (MIC)

The antipromastigotes effects of *M. foetida* are shown in Table 1 and Figure 1. The results observed indicate that *M. foetida* extracts and standard reference drugs significantly (P ≤ 0.05) inhibited promastigotes of *Leishmania* parasite *in vitro* after 72 hours of incubation compared to RPMI which did not inhibit promastigotes. Methanolic extracts showed the highest inhibition concentration value (IC_{50}) of 23.5μg/ml followed by aqueous extracts, Amphothericin B and pentostam with 15.6μg/ml, 17.8μg/ml and 11.7μg/ml respectively that inhibited 50% of about 10^6 promastigotes. On the other hand, the study showed that *M. foetida* methanol extracts also showed the highest (MIC) value of 250 ± 0.003μg/ml which was significantly difference (P ≤ 0.05) from the other tested compounds to kill 10^6 promastigotes. The MIC values for water extracts, Pentostam and Amphothericin B were 125 ± 0.001μg/ml, 62.5 ± 0.002μg/ml and 31.3 ± 0.001μg/ml and respectively. The inhibition effects of *M. foetida* extracts and standard reference drugs significantly (P ≤ 0.05) increase with increase in test compound concentrations compared to RPMI. The small the IC_{50} and MIC values, the higher antipromastigote activity of the test compounds.

![Figure 1](https://example.com/figure1.png)

**Figure 1:** The trend showing percentage of promastigote inhibited by *M. foetida* extracts and control treatments

#### Activity of extracts of *M. foetida* against L. major infected macrophages from BALB/c mice

There was no significant difference (p>0.05) in the number of *L. major* amastigotes in macrophages treated with the *M. foetida* extracts compared to Pentostam and Amphothericin B at concentrations of 50 to 200μg/ml as shown in Figure 2. The results showed that the slopes of the trends significantly (P ≤ 0.05) dropped with increase of concentrations in *M. foetida* extracts and standard reference drugs from 50μg/ml to 100μg/ml to 200μg/ml reducing number of infected macrophages compared to RPMI which showed 90% infected macrophages at all concentrations. Methanolic extract showed the highest Macrophages infection rates by *M. foetida* extracts and control treatments.

### Table 1: Table showing Mean of IC_{50} and MIC% inhibition of parasites by extracts

<table>
<thead>
<tr>
<th>Test samples</th>
<th>RPMI</th>
<th>H2O-extract</th>
<th>Met-extract</th>
<th>Pentostam</th>
<th>AmB</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC_{50} (μg/ml)</td>
<td>0 ± 0.01</td>
<td>15.6 ± 0.054</td>
<td>23.4 ± 0.053</td>
<td>11.7 ± 0.054</td>
<td>7.8 ± 0.053</td>
</tr>
<tr>
<td>MIC (μg/ml)</td>
<td>0 ± 0.09</td>
<td>125 ± 0.001</td>
<td>250 ± 0.03</td>
<td>62.5 ± 0.002</td>
<td>31.3 ± 0.001</td>
</tr>
</tbody>
</table>
200 µg/ml. Pentostam killed 64% parasites infected macrophages at 50 µg/ml, 75% at 100 µg/ml and 82% at 200 µg/ml. Amphotericin B showed the higher antileishmanial activity at all concentrations hence slope almost flat by killing 85% parasites infected macrophages at concentration of 50µg/ml, 90% at 100µg/ml and 93% at 200µg/ml showing significance different (P ≥ 0.05) in antiamastigotes compared to M. foetida extracts.

**In vitro activity of M. foetida extracts to L. major amastigotes**

Different concentrations of samples of aqueous and methanol M. foetida extracts were tested for their efficacy are shown in Figure 3. The percentages of amastigotes and infected macrophages from tested compounds data were calculated then transformed into natural logarithms because coefficient of variation covered a wide range of values due to effectiveness of M. foetida extracts and standard reference drugs compared to RPMI and these are based on constant e (2.72). Results showed that M. foetida extracts and standard drug significantly (P ≤ 0.05) kill L. major amastigotes reducing macrophages infection compared with RPMI which showed the highest value of infection of LN 6.6 and was the same for the concentration of 50µg/ml, 100µg/ml and 200µg/ml followed by methanolic extract, LN of 6.5 at 50ug/ml, 6.2 at 100ug/ml and 6.0 at 200ug/ml and aqueous extract with LN 5.8 at 50ug/ml, 5.4 at 100ug/ml and 50ug/ml. The reduction rates of parasites of M. foetida extracts showed no significance different (P ≥ 0.05) compared with standard reference drugs. Pentostam reduced to LN 4.6 at 50ug/ml, 4.4 at 100ug/ml and 4.2ug/ml while Amphotericin B reduced the highest number of parasites to LN 3.5 at 50ug/ml, 3.2 at 100ug/ml and 3.0ug/ml. The results also showed no significant difference (P ≥ 0.05) between macrophage infections of M. foetida methanolic and aqueous extracts. The increase in M. foetida extracts and standard reference drugs concentrations significantly (P ≤ 0.05) kill amastigotes reducing infections of macrophages compared to RPMI.

**Multiplication Index (MI)**

The results of MI of parasites in macrophages infections under different test compounds treatments are shown in figure 4. The results showed that increase in M. foetida extracts and standard reference drugs concentrations significantly (P ≤ 0.05) reduced macrophages infections by amastigotes compared to steady parasite load in negative control (RPMI). Methanol extracts reduce the number of parasites multiplication from 100% to 80%, 55% and 30%, Aqueous from 100% to 45%, 30% and 17% at concentration of 50µg/ml, 100µg/ml and 200µg/ml respectively while pentostam and Amphotericin B reduces from 100% to 15%, 13% and 12% and 100% to 10%, 8% and 7% respectively compared with RPMI whose MI of parasites is high >90%. The M. foetida extracts and standard drugs showed significant reduction of amastigotes infections in macrophages in comparison with RPMI showed 90% macrophages infections at all the concentrations. The significance difference (P ≤ 0.05) was observed between M. foetida and standard reference drugs at concentration of 50µg/ml and 100µg/ml.

**Stimulation of nitric oxide production by M. foetida extracts in cell free cultures**

The nitric oxide production in supernatants from macrophage culture treated with and test samples were determined compared to sodium nitrite standard curve for test samples at concentrations between 0 and 1000µg/ml as shown in Figure 5. None of the samples had Optical Density (OD) readings of more than 0.10 indicating that less than 2µm of NO was produced compared to negative controls that produced similar levels. Production of Nitric oxide...
(NO) by different test compounds was shown to be concentrations dependent. RPMI showed increase of NO production from 75% at 50µg/ml to 90% at 200µg/ml. Methanolic and aqueous extracts showed small amount of NO production from 56% at 50µg/ml to 68% at 200µg/ml respectively. Amphotericin B and Pentostam showed decrease in nitric oxide productions in macrophages infected with L. major from 73% at 50µg/ml to 54% at 200µg/ml and 70% at 50µg/ml to and 57% at 200µg/ml respectively meaning they didn’t excite macropgages to enhance L. major amastigotes killing.

Figure 5: Nitric oxide productions by L. major infected macrophages after treatment with M. foetida extracts and controls

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CC50 µg/ml</th>
<th>%PI</th>
<th>IC50</th>
<th>a/b</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI</td>
<td>0.000</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
<td>0.000</td>
</tr>
<tr>
<td>Methanol extracts</td>
<td>104 ± 0.1</td>
<td>72</td>
<td>0.220 ± 5</td>
<td>-0.87 ± 0.4</td>
<td>0.010</td>
</tr>
<tr>
<td>Aqueous extracts</td>
<td>122.5 ± 0.07</td>
<td>80.0 ± 0.02</td>
<td>0.26 ± 0.01</td>
<td>-0.64 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Pentostam</td>
<td>76.2 ± 0.12</td>
<td>80.6 ± 5</td>
<td>0.46 ± 0.14</td>
<td>-0.49 ± 1</td>
<td>0.074</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>88.6 ± 0.19</td>
<td>88.0 ± 9.5</td>
<td>0.68 ± 11</td>
<td>-0.28 ± 0.9</td>
<td>0.062</td>
</tr>
</tbody>
</table>

*CC50 = Cytoxic concentration (CC < 2µg/ml = toxic, CC > 2µg/ml and < 99µg/ml = Moderate toxic and CC > 99µg/ml = non toxic), Pl = Parasites inhibition, IC50 = Inhibition concentration, a/b = Slope/interval and P value = Probability value.

4. Discussion

In the study, the in vitro activity of M. foetida extracts against L. major also showed that the plant may contain some pharmacologically active substances that could prevent growth and proliferation of L. major promastigotes and amastigotes. The study showed that promastigotes and amastigotes were very susceptible to M. foetida extracts giving herbs some advantages over commercial drugs which includes affordability, accessibility and with no adverse effects. The lower IC50 and higher CC50 of M. foetida extracts is an indication of its significance activity (P ≤ 0.05) against L. major promastigotes and amastigotes in comparison to standard reference drugs. In the study, aqueous extracts (125µg/ml) was observed to be as twice effective as methanolic extracts (250µg/ml) in inhibiting 50% of parasites proving to be potentially efficacious compounds.

The antileishmanial activities observed in this study could be due to the presents and 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 (Molehin, et al., 2014). The antileishmanial activities of M. foetida is in agreement with Chakraborty and Sundar (2010), who reported that catechins-flavonoid exhibited antibacterial activity by inhibiting the action of deoxyribonucleic acid (DNA) polymerase. The M. foetida plants was also reported to contain triterpenoids such as cucurbitins, kuguacins and momordicine (Chen, 2009), polyphenolic compounds and antioxidants (Molehin et al., 2014) which are known for disrupting the cell membranes of the L. major accounting for it inhibitory activity (Wong et al., 2014). The leishmanial inhibitory activity of M. foetida could also be attributed due to presence of three known analogues, 3beta, 7beta, 25-trihydroxycucurbita - 5, (23E) – diene - 19 - al, 3beta, 25 - dihydroxy-5beta, 19-epoxy cucurbita - 6, (23E)-diene, and momordicine (Chen, 2009) polyphenolic compounds, flavonoids and antioxidants (Molehin et al., 2014).

Cytotoxicity of M. foetida to vero cells

The IC50 values of the cytotoxicity of the samples to vero cells showed that M. foetida extracts were less toxic (P<0.05) compared to Pentostam and Amphotericin B as shown in Table 2. The MIC values revealed that concentrations of below 31.25µg/ml were not toxic for all the samples except RPMI which did not produce inhibition. Methanolic and aqueous extracts of M. foetida against vero cells showed no cytotoxicity based on scale values of classification of toxicity levels as; cytotoxic at CC50 ≤ 2µg/ml, moderate at CC50 between 2µg/ml-99µg/ml and not cytotoxic at CC50>100µg/ml (Loomis & Hayes, 1996). Methanolic and aqueous extract had CC50 of 104µg/ml and 122.5µg/ml respectively indicating no toxic while Pentostam (CC50 of 76.2ug/ml) and Amphotericin B (IC50 of 88.6ug/ml) showed moderate cytotoxicity. The IC50 values and percentage parasites inhibition of methanol extract, aqueous extract, Pentostam and Amphotericin B were 0.22ug/ml (72 ± 0.08%), 0.26ug/ml (80 ± 0.02%), 0.46ug/ml (80 ±0.65%) and 0.68ug/ml (88 ± 0.95%) respectively demonstrated significant (P ≤ 0.05) inhibition of > 70% of L. major parasites. Despite the difference in IC50 values of aqueous extracts (80 ± 0.02%) and Pentostam (80 ±0.65%) both inhibited the same percentage of parasites. Methanolic extract (72 ± 0.08%) showed the lowest inhibition of parasites while Amphotericin B (88 ± 0.95%) showed the highest inhibition of parasites.


Volume 4 Issue 9, September 2015

www.ijisr.net

Licensed Under Creative Commons Attribution CC BY

1824
2014). These compounds have shown to have broad antiparasitic properties including antileishmanial activities by interrupting the cell membranes of the *L. major* (Wong et al., 2014). *M. foetida* did not activate macrophages to kill parasites because it did not produce enough nitric oxide to activate macrophages to produce reactive oxygen and nitrogen metabolites. Among immune cells, macrophages play major roles in the immune system and are capable of destroying microorganisms, mainly by the production of intermediate metabolites, such as hydrogen peroxide and nitric oxide (Awasthi et al., 2004). It was suggested that immunostimulatory activity of Plant extracts associated with macrophage activation enhance macrophage Phagocytic immunostimulatory activity of Plant extracts associated with nitric oxide (Awasthi, 2000). The results obtained from the studies indicated that *M. foetida* extracts showed no toxicity in *vitro* assays compared to standard reference drugs. It has been reported that *M. foetida* has an important role on the host nonspecific immunity (Ziment, 1994).

5. Conclusion

This study scientifically demonstrates the antileishmanial potential of *M. foetida* revealing that *M. foetida* extracts contain active compounds against *L. major* promastigotes and amastigotes, which could serve as an alternative agent in the control of leishmaniasis. Further studies would therefore be needed to determine its antileishmanial activity, in *in vivo* clinical response and associated toxicities and its effectiveness against other forms of the parasite such as the visceralizing *L. donovani*, cutaneous *L. tropica* and *L. aethiopica*.

6. Acknowledgements

This investigation received supervision support and equipments from Kenya Medical Research Institute (KEMRI), Nairobi and University of Eldoret. All assistance from technical team and provision of Laboratory space are highly Appreciated.

References


