In Vitro Leishmanicital Activity of *Momordica foetida* against *Leishmania Major*

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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 (IC_{50}) on promastigotes, percentage rates of macrophages infected by amastigotes and cytotoxicological concentration (CC_{50}) 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: Antileishmanicital, *Momordica foetida*, *Leishmania major,* leishmaniasis, promastigote, amastigotes 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 ahousehold 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 leishmaniasis 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*, (Cucurbitaceae) 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 diabeties. *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 cuteneous 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 part*s 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

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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 micro-filters 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, (Dorin*et al*., 2001). All the prepared compounds were stored at -4°C and retrieved only during use.

Evaluation of minimum inhibitory concentration (MIC): *Leishmania* promastigotes (10^6parasites/ml) were maintained in culture or in the presence of several concentrations (1mg/ml to 1µg/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-titter 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 thiazolyl 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 (IC_{50}) values generated. Percentage promastigotes viability was calculated using the formula:

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

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 4^0C 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 37^0C in 5% CO_2. Non-adherent cells were washed with phosphate buffered saline (PBS), and the macrophages incubated overnight in RPMI 1640 media. Adherent macrophages were infected with *L. major* promastigotes at a parasite/macrophage ratio of 6:1, incubated at 37^0C in 5% CO_2 for 4 hrs, free promastigotes removed by extensive washing with PBS, and the cultures incubated in RPMI 1640 medium for 24 hrs. Treatment of infected macrophages with the sample was done once. Pentostam® and amphotericin B were used as positive controls for parasite growth inhibition. The medium and drug was replenished daily for 3 days. After 5 days the mono-layers were washed with PBS at 37^0C, fixed in methanol and stained with 10% Giemsa solution. The number of amastigotes was determined by counting at least 100 macrophages in duplicate cultures, and results expressed as infection rate (IR) and multiplication index (MI) (Berman and Lee, 1984). IR = No. of infected macrophages in 100 macrophages.

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

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.

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

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by the Griess reaction for nitrites (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 HCI) 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 EssentialMedium supplemented with 10% FBS. The cells were cultured at37^{0}C in 5% CO2, harvested by trypsinization, pooled in a 50 ml vialand 100µl cell suspension (1 x 10^{5}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^{0}C in 5% CO_2 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^{0}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:

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

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, Amphotericin B and pentostam with 15.6µg/m, l7.8µg/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 $\pm$ 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 Amphotericin B were 125 ± 0.001µg/ml, 62.5 ± 0.002ug/ml 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.

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

Test samples	RPMI	H_2O extract	Met-extract	Pentostam	AmB
IC_{50} (µg/ml)	0 $\pm$ 0.01	15.6 $\pm$ 0.054	23.4 $\pm$ 0.053	11.7 $\pm$ 0.054	7.8 $\pm$ 0.053
MIC (µg/ml)	0 $\pm$ 0.09	125 $\pm$ 0.001	250 $\pm$ 0.03	62.5 $\pm$ 0.002	31.3 $\pm$ 0.001

PI (%)	10%	76 %	70 %	76%	88%
VP%	90%	24%	30%	24%	12%
SE	0.15	0.054	0.073	0.095	0.99
T statistic	106.82	-11.88	-16.21	-9.22	-2.24
P value	0.000	0.000	0.000	0.0.14	0.001

SD = Standard deviation, SE = Standard Error, IP =Parasites inhibition, VP = Viable parasites and AmB = Amphothericin B

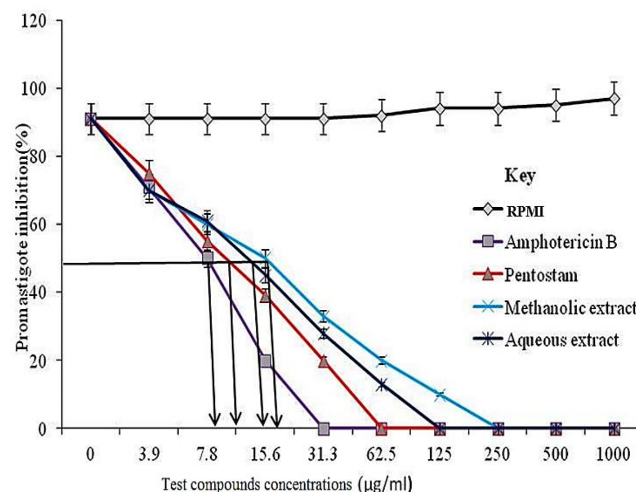

Figure 1: The trend showing percentage of promastigote inhibited by *M. foetida* extracts and control treatments

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 Amphotericin 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 inhibiting 30% parasites at 400µg/ml, 45% at 100µg/ml and 60% at 200µg/ml followed by aqueous extract which eliminated 35% at 50 µg/ml, 45% at 100 µg/ml and 58% at

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200 µg/ml. Pentostam killed 64% parasites infected macrophages at 50 µg/ml, 75% at 100 µg/ml and 82% at 200 µg/ml. Amphothericin 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 ($P \geq 0.05$) in antiamastigotes compared to *M. foetida* extracts.

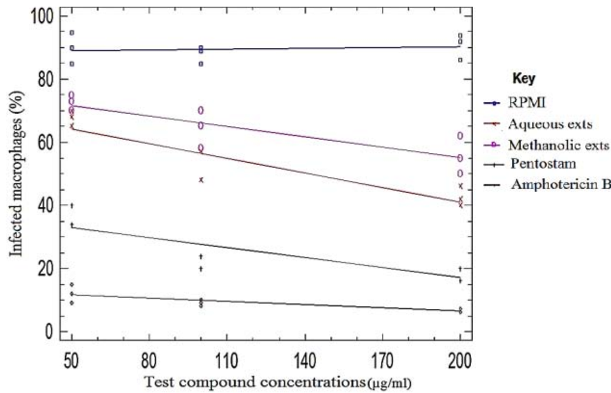

Figure 2: Macrophages infected by *L. major* amastigotes after treatments of *M. foetida* extracts compared with controls at concentration of 50µg/ml, 100µg/ml and 200µg/ml

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 ($P \leq 0.05$) 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 ($P \geq 0.05$) 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 ($P \geq 0.05$) between macrophage infections of *M. foetida* methanolic and aqueous extracts. The increase in *M. foetida* extracts and standard reference drugs concentrations significantly ($P \leq 0.05$) kill amastigotes reducing infections of macrophages compared to RPMI.

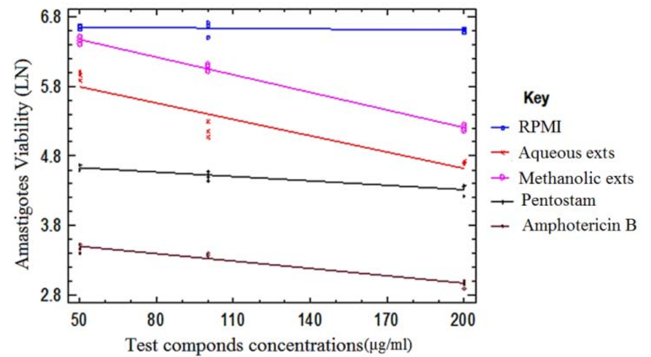

Figure 3: Effects of *M. foetida extracts,* Pentostam and Amphotericin B on *L .major* amastigotes viability

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 ($P \leq 0.05$) 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 ($P \leq 0.05$) was observed between *M. foetida* and standard reference drugs at concentration of 50µg/ml and 100µg/ml.

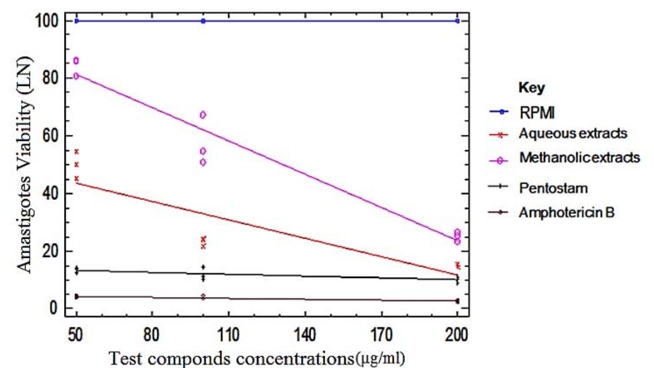

Figure 4: Multiplication index (MI) of infected macrophages by *L. major* amastigotes after treatment of *M. foetida* extracts and controls treatments

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

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(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 200 µg/ml. Methanolic and aqueous extracts showed small amount of NO production from 56% at 50µg/ml to 68% at 200µg/ml and 65% at 50µg/ml to 64% 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 enhace *L. major* amastigotes killing.

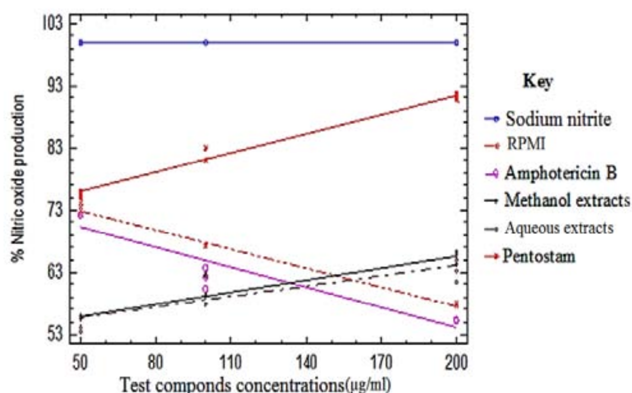

Figure 5: Nitric oxide productions by *L. major* infected macrophages after treatment with *M. foetida* extracts and controls

Cytotoxicity of *M. foetida* to Vero cells

The IC_{50} 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 $CC_{50} \leq$ 2µg/ml, moderate at CC_{50} between 2µg/ml-99µg/ml and not cytotoxic at $CC_{50} \geq$100µ/ml (Loomis & Hayes, 1996). Methanolic and aqueous extract had CC_{50} of 104µg/ml and 122.5µg/ml respectively indicating no toxic while Pentostam (CC_{50} of 76.2ug/ml) and Amphotericin B (IC_{50} of 88.6ug/ml) showed moderate cytotoxicity. The IC_{50} 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 IC_{50} values of aqueous extracts (80 ± 0.02%) and Pentostam (80 ±0.65%) both inhibited the same percentage of parasites. Methanolic extracts (72 ± 0.08%) showed the lowest inhibition of parasites while Amphotericin B (88 ± 0.95%) showed the highest inhibition of parasites.

Table 2: Statistical table for cytotoxicity test and% inhibition of parasites by test compound.

Treatments	CC_{50}µg/ml	%PI	IC_{50}	a/ b	P value
RPMI	0.000	0.00	-	4.51 ± 0.05	0.000
Methanol extracts	104 ± 0.1	72 0 ± 08	0.220.0 ± 5	-0.87 ± 0.4	0.010
Aqueous extracts	122.5 0 ± 07	80. 0 ± 02	0.26 0 ±.01	-0.64 ± 0.5	0.001
Pentostam	76.2 0 ±.12	80.6 ± 5	0.46 0 ±14	-0.49 ± 1	0.074
Amphotercin B	88.6 0 ± 19	88 0 ± 95	0.68 0 ±11	-0.28 ± 0.9	0.062

*CC_{50} = Cytotoxic concentration (CC < 2ug/ml = toxic, CC > 2ug/ml and < 99ug/ml = Moderate toxic and CC > 99ug/ml = non toxic), PI = Parasites inhibition, IC_{50} = 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 herbals some advantages over commercial drugs which includes affordability, accessibility and with no adverse effects. The lower IC_{50} and higher CC_{50} 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 cucurbitacins, kuguacins and momordicine (Chen, 2009), polyphenolic compounds and antioxidants (Molehin *et al.*, 2014) which are known for disrupting the cell membaranes 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-epoxycucurbita - 6, (23E)-diene, and momordicine (Chen, 2009) polyphenolic compounds, flavonoids and antioxidants (Molehin *et al.*,

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2014). These compounds have shown to have broad antiparasitic properties including antileishmanial activities by interrupting the cell membaranes 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 levels (Schepetkin & Quinn, 2006). 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. mojor* 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.

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