In Vitro Antileishmanial Activities of Olea europaea, Kigelia africana, Terminalia mollis, Croton Macroystachyus and Bridella micrantha, Kenyan Medicinal Plants

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MDW designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AC, MC and MMD managed the analyses of the study and reviewed the study design and all drafts of the manuscript. Author IJ managed the literature searches and laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Leishmaniasis is a major public health problem globally. Visceral leishmaniasis is known to be fatal if left untreated, while cutaneous leishmaniasis is the most neglected. The first-line treatment of leishmaniasis is based on pentavalent antimonial drugs which are expensive, requiring inpatient treatment and toxic. The Plants containing active compounds against other protozoan diseases may offer alternatives against leishmania parasites. This study determined the in vitro
antileishmanial activity of Olea europaea, Kigelia africana, Terminalia mollis, Croton macrostachyus and Bridella micrantha extracts. The plant samples were dried, pulverized into fine powders and extracted using ethanol at the Center for Traditional Medicine and Drugs Research, KEMRI. The in vitro assays were carried out at the Leishmania laboratory, Centre for Biotechnology Research and Development, KEMRI. In in-vitro assays the inhibitory concentrations (IC50) and Minimum Inhibition Concentration (MIC) on L. major promastigotes, percentage rates of macrophages infected by amastigotes and cytotoxicity on Vero cells were determined. For each parameter analyzed, differences among treatment groups exposed to different drugs were tested by logistic regression. Results showed that promastigote and amastigote growth inhibition was significantly affected by the crude extracts from the plants (P < 0.05) after 24 hours of exposure where the most effective drug was the standard drug (Amphotericin B) while among the crude extracts of the herbal drugs, T. mollis was the most effective against amastigote followed by C. macrostachyus while O. europaea was the least effective. Mammalian cell viability was significantly affected by the various test compounds (P < 0.05) after 24 hours exposure where % cell viability of herbal drugs, B. microstachyus, O. africana resulted to the most toxic effects by reducing the % cell viability to less than 50%. The study recommends the use of T. mollis in management of leishmaniasis in areas they occur. Further analysis of the active compounds that that affect efficacy of the plant extracts is advised.

Keywords: Cutaneous leishmaniasis; Leishmania major; plant extracts; promastigotes; amastigotes

1. INTRODUCTION

Considered as one of the neglected tropical diseases (NTD), Leishmaniasis is a major public health problem, with about 340 million people in 88 countries at risk and approximately 2 million infections and 70000 deaths annually [1]. The disease is caused by Leishmania species transmitted by female sand flies [2-4]. Promastigotes, transmitted by sand flies during a blood meal, invade and multiply as amastigotes within mammalian host macrophages [5] and finally accumulate in the liver and spleen [6,7]. Three general forms of clinical disease occur: cutaneous, muco-cutaneous and visceral leishmaniasis [8,9], but cutaneous is the most prevalent clinical form worldwide [10].

In Kenya, cutaneous leishmaniasis, caused by L. tropica is endemic in Nakuru and Laikipia counties while that caused by L. major is endemic in Baringo County. In recent years, Leishmania parasites have exhibited drug resistance leading to treatment failure [11,12]. Therefore, new effective and cheaper treatment approaches are urgently required [11,13]. Diverse bioactivity has been reported for Olea europaea, Kigelia africana, Terminalia mollis, Croton macrostachyus and Bridella micrantha [14-18]. However, a key pillar in the safe and efficacious use of medicinal drugs is to optimize the dose used. This requires determination of measures such as minimum inhibitory concentration (MIC) [19,20]. Efficacy of drugs available for the treatment of diseases is based on their ability to inhibit growth of pathogens.

Several studies have examined these plants for efficacy of their extracts for microbial growth [21,22] and inhibition of plasmodial growth [23-25] but there is limited data for anti-leishmanial activity. Despite medicinal properties of plants, the lack of proper dosing of herbal drugs put patients at risk of toxicity, especially as plant parts used have a mixture of metabolites [26]. Toxicity of medicinal plant extracts have been reported; nephrotoxicity and hepatotoxicity [27,28]. These findings support the need to have toxicity profiling of these plant extracts. Hence, regardless of how effective the drug is against the parasite, the toxicity of these plants must also be known before a decision on their adoption is made [29]. In light of the scanty data on efficacy and toxicity of the herbal medicine on leishmania parasites, the aim of this study was to evaluate the response of Leishmania major to therapy of Olea europaea, Kigelia africana, Terminalia mollis, Croton macrostachyus and Bridella micrantha extracts in vitro.

2. MATERIALS AND METHODS

2.1 Study Site

The tests and analyses were carried out at the leishmaniasis laboratory, Centre for Biotechnology Research and Development (CBRD) and Centre for Traditional Medicine and Drugs Research (CTMDR) Kenya Medical Research Institute (KEMRI). The experiments were carried out in Biosafety level 2 (BSL-2) facilities.
2.2 Sources of Plant Extracts

The barks of the five plants species: Olea europaea, Kigelia africana, Terminalia mollis, Croton macrostachyus and Bridella micrantha were collected from Baringo County in Kenya. They were authenticated the Department of Botany and the voucher specimens were deposited in the herbarium of the Museums of Kenya in Nairobi. The plant extracts were taken to the CTMDR, KEMRI Nairobi for hexane, Dichloromethane, ethyl acetate, methanolic and aqueous extraction. And further in vitro assays were carried out at the Leishmania laboratory, Centre for Biotechnology Research and Development (CBRD).

2.3 Sample Preparation and Extraction of Compounds of Plant Species

The stem barks 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. Cold sequential extraction were carried out on plant material with analar grade organic solvents of increasing polarity, which includes hexane, dichloromethane, ethyl acetate, methanol and aqueous. Six hundred milliliters of n-hexane were added to 300 g of the shredded specimen and flasks placed on a shaker and soaked for 48 h. The residue were filtered using a Buchner funnel under vacuum until the sample was dry. The samples were soaked further with 600 ml of hexane for 24 h until the filtrate remain clear. The filtrates were then concentrated under vacuum by rotary evaporation at 30 - 35°C [30]. The concentrates were transferred to a sample bottle and dried under vacuum; the weight of the dry extract were recorded and stored at -20°C until required for bioassay. The process were repeated sequentially for dichloromethane, ethyl acetate, methanol and aqueous. All the extracts (0.05 g/ml) were subjected to preliminary phytochemical screening following standard methods [31-33].

2.4 Experimental Animals (Mice)

A total of 48 eight week old female inbred BALB/c mice weighing 20 ± 2.1 g were used for all in vivo studies. The mice were obtained from the KEMRI animal facility. The animals were moved into the experimental room for acclimatization of one week before onset of experiments. The mice were housed in 15 cm × 21 cm × 29 cm transparent plastic cages bedded with wood shavings. They were fed on pellets (Mice pellets UNGA® feeds) and water as often as necessary. The wood shaving dressings in the cages were changed after every two days. The experimental room in the animal house were under lock and key and all cages clearly labeled with experimental details that included assigned treatment group numbers, dates of procedures and protocol SSC number. The experiments were done in compliance with Animal Care and Use Committee (AUC) guidelines of KEMRI. Standard Operating procedures (SOPs) available at Leishmania laboratory at CBRD included immunizing the animals using standard 21G needles, anaesthizing and killing them using painless method approved by ACUC (100 µl of Sagata). Animals were isolated and transported for incineration.

2.5 Culture of Leishmania Parasites

Metacyclic promastigotes of Leishmania major (strain IDUB/KE/83=NLB-144) were isolated from a female P. dubosci collected from Marigat, Baringo County, Kenya. The parasites were cultivated in Schneider’s Insect Medium supplemented with 20% heat inactivated foetal bovine serum, 100 µg/ml penicillin and 50 µg/ml streptomycin [34] and 250 µg/ml 5-fluorocytosine arabinoside.

2.6 Evaluation of Minimum Inhibitory Concentration (MIC)

The L. major promastigotes (10⁶ parasites/ml) were incubated at 26°C for 12 hr in fresh media (Schneiders Insect Media), supplemented with 20 % FBS in the absence or presence of several concentrations (1 mg/ml to mg/ml) of the extracts. Cell growth was determined microscopically using inverted microscope.

Inhibition percentage (I) was calculated using the following formula:

$$I(\%) = \frac{100 \times (\text{Absorbance of untreated cells} - \text{Absorbance of treated cells})}{\text{Absorbance of untreated cells}}$$

Where, the lowest concentration of the samples that prevented the growth of leishmania parasites in vitro was considered as their minimum inhibitory concentration.
2.6.1 Anti-promastigote assay

The viability of *Leishmania* parasites were evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay. Briefly, 10 mL of MTT (10 mg/mL) was added to each well of a micro-well plate and incubated for 3 h at 30°C. This was followed by addition of 100 L of 50% (v/v) isopropanol-10 % (w/v) sodium dodecyl sulfate (SDS) mixture to each well in order to dissolve insoluble formazan. Absorbance was measured at 560 nm using an ELISA plate reader after 30 min of incubation at room temperature. All assays were conducted in triplicate and compared to negative control and positive controls. Cell viability was evaluated by determining extract concentrations which inhibited 50 % of the cell population (IC50), and was obtained by plotting percentage of inhibition versus concentration of extract using Original Program.

Promastigote viability (%) = (average absorbance in duplicate drug wells-average blank wells/ Average absorbance control wells) x 100

2.6.2 Anti-amastigote assay

The anti-amastigote assay was carried out as described by [23]. Briefly, peritoneal macrophages were obtained from BALB/c mice. Mice were anaesthetized using 100μl pentobarbital sodium (sagaatal). The body surface was then disinfected with 70 % ethanol. The torso skin was torn dorsoventrally to expose the peritoneum. Using a sterile syringe and needle, 10 ml of sterile cold phosphate-buffered saline was injected into the peritoneum. After shaking the mouse, peritoneal macrophages were harvested by withdrawing the PBS. The contents were transferred into a sterile 50 ml centrifuge tube. The suspension was centrifugally washed at 2000 rpm for 10 minutes and the pellet resuspended 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 then infected with parasite/macrophage ratio of 6:1 and further incubated at 37°C in 5 % CO2 for 4 hours. Free macrophages were removed by extensive washing with PBS and the cultures incubated in RPMI for 24 hours. Treatment of infected macrophages with the samples was done once. Amphotericin B was used as a positive control drug for comparison of parasite inhibition. The medium and drug were replenished daily for 3 days. After 5 days, the monolayers were washed with PBS at 37°C, fixed in methanol and stained with Giemsa. Amastigotes were determined by counting at least 100 macrophages in duplicate cultures, and the results expressed as infection rate (IR) and multiplication index (MI) [24] as follows:

IR= No. of infected macrophages in 100 macrophages
MI= (No. of macrophages in experimental culture/100 macrophages/ No. of macrophages in 100 control cultures/1000 macrophages) x 100

The infection rate was used in calculations of the Association Index (AI). The association indices were determined by multiplying the % of infected macrophages by the number of parasites per infected cell. Association indices and interpreted as the number of parasites that actually infected the macrophages.

2.7 Cytotoxicity Assay Using Vero Cells

The assay was used to test the cytotoxicity of the individual plant extracts therapy against Vero cells. The assay was carried out as described elsewhere . Vero cells were grown in minimum essential medium (MEM) supplemented with 10 % FBS, penicillin (100 IU/ml) and streptomycin (100 μg/ml) in 25 ml cell culture flasks incubated at 37°C in a humidified 5% CO2 atmosphere for 24 hours. The Vero cells were harvested by trypsinization, and pooled in 50ml centrifuge tubes from where 100 μl of the cell suspension were put into 2 wells of rows A-H in a 96-well flat bottomed microtitre plate at a concentration of 1 x 10^5 cells per ml of the culture medium per well and incubated at 37°C in 5% CO2 in order to attach. The MEM was gently aspirated off and 150 μl of the highest concentration (1000 μg/ml) of the test extracts (AS, AF and AS/AF) was added and serially diluted by a factor of 3 up to a concentration of 1.37μg/ml at wells of row B. The microtitre plates containing the Vero cells and test extracts were incubated further at 37°C for 48 hours in a humidified 5% CO2 atmosphere. The control wells contained Vero cells and medium while the blank wells consisted of medium only. 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent were added into each well and incubated further for 2 to 4 hours until a purple precipitate (Formazan) is visible under the microscope (dead cells don’t metabolize MTT). The media
together with MTT reagent were gently aspirated off, then 100 µl of DMSO were added, and vigorously shaken for 5 minutes in order to dissolve formazan. The absorbance (optical density) for each well plate were measured using a micro-titer plate reader at wavelength of 562 nm. Cell viability was calculated at each concentration. The IC\textsubscript{50} values of the extracts were determined automatically using the Chemosen software program.

2.8 Data Analysis

All data collected was entered, organized and managed using EXCEL spreadsheet for Windows. All statistical analyses were performed with a version of STATISTICA 10.0 statistical packages. Normality of data distributions was checked by means of the skewness and kurtosis to determine any need for applying appropriate data transformation procedures. Percentage data was arcsine transformed before subjecting it to statistical analysis of Variance. All analyzed results were declared significant at P < 0.05.

3. RESULTS

3.1 Efficacy of Crude Extracts of Medicinal Plants on Parasite Growth Inhibition, Parasite Loads and Infection Rates in-Vitro

Efficacy of varying concentrations of extracts from O. europaea, K. africana, T. mollis, C. macrostachyus, B. micrantha and standard drug (Amphotericin B) on promastigotes of L. major is shown in Fig. 2. The promastigote growth inhibition was significantly affected by the crude extracts from the plants (P < 0.05) after 24 h of exposure.

The % growth inhibition estimated for the promastigote form of parasite fully fitted the logistic regression model. Based on the model parameter coefficients of C, the most effective drug against promastigotes was the standard drug (Amphotericin B). Based on the gradient analysis of logistic regression of the crude extracts of the herbal drugs, T. mollis was the most effective against amastigote followed by C. macrostachyus while O. europaea was the least effective in terms of optimal efficacy.

Table 1 describes the optimal efficacy, concentration at optimal efficacy, IC\textsubscript{50} and IC\textsubscript{90} of the test drugs against promastigote forms of the parasites. There were significant differences in the optimal efficacy of the test drugs (P < 0.05).

Table 2 describes the optimal efficacy, concentration at optimal efficacy, LC\textsubscript{50} and LC\textsubscript{90}, of the test drugs against Amatigote forms of the parasites. There were significant differences in the optimal efficacy of the test drugs (P < 0.05). The optimal efficacy of the standard drugs was 100% even though the test drugs did not achieve this efficacy levels against promatigotes.

The optimal efficacy of the standard drugs was 100% even though the test drugs did not achieve this efficacy levels against promatigotes. Among the test drugs T. mollis was the most effective against amastigote followed by C. macrostachyus while O. europaea was the least effective in terms of optimal efficacy. None of the non standard test compound achieved IC\textsubscript{50} except the combined therapy of Croton (472.6 mg/l).

The efficacy of crude extracts from O. europaea, K. africana, T. mollis, C. macrostachyus, B. micrantha and standard drug (Amphotericin B) on amastigotes of L. major are shown in Fig. 3. The amastigote growth inhibition was significantly affected by the various test compounds (P < 0.05) after 24 h of exposure.

The % growth inhibition estimated for the amastigote form of parasite fully fitted the logistic regression model. Based on the model parameter coefficients of C, the most effective drug was Amphotericin B, which was a standard drug. Among the herbal test drugs that were used in the current study, the T. mollis was found to be the most effective drugs followed by C. macrostachyus while O. europaea was the least effective.

Table 2 describes the optimal efficacy, concentration at optimal efficacy, LC\textsubscript{50} and LC\textsubscript{90}, of the test drugs against Amatigote forms of the parasites. There were significant differences in the optimal efficacy of the test drugs (P < 0.05). The optimal efficacy of the standard drugs was 99.8% even though the test drugs did not achieve this efficacy levels against promastigotes. Among the test drugs T. mollis was the most effective against amastigote followed by C. macrostachyus while O. europaea was the least effective in terms of optimal efficacy. There was significant (P < 0.05) difference in the LC\textsubscript{50} with the lowest LC\textsubscript{50} occurring in standard drug followed by crude extracts from T. mollis and least in O. europaea. None of the non standard test compound achieved LC\textsubscript{90} except the standard drug, crude extracts of T. mollis and C. macrostachyus.

3.2 Toxicity Extracts of O. europaea, K. africana, T. mollis, C. macrostachyus and B. micrantha on Vero Cells

Results of the efficacy of plant extracts on the viability of mammalian cell are shown in Fig. 4. Mammalian cell viability was significantly affected by the various test compounds (P < 0.05) after
24 hours exposure. The % cell viability estimates of mammalian cells treated with fully fitted the logistic regression model describing a dose response treatment ($R^2 = 0.9756$). Subject to 24 hours treatment with extracts, the model equation was $\log \left( \frac{\rho}{1-\rho} \right) = 100 - 14.2924C - 1.0440C^2 + 0.1445C^3$ and the parameter significance (P-value) obtained for each coefficient were: $\beta_0 (P = 0.1351)$, $\beta_1 (P = 0.0001)$, $\beta_2 (P = 0.1857)$ and $\beta_3 (P = 0.0091)$. The % cell viability estimates of mammalian cells treated with the standard drug and other herbal drugs, fully fitted the logistic regression model describing a dose response treatment ($R^2 = 0.9815$). The % cell viability estimates of mammalian cells treated with $T. mollis$, fully fitted the logistic regression model describing a dose response treatment ($R^2 = 0.9961$). Based on the gradient analysis of logistic regression of the crude extracts of the herbal drugs, $B. micrantha$, $O. africana$ resulted to the most toxic effects by reducing the %cell viability to less than 50%.

![Fig.1. The 96 well plate design for the cytotoxicity assay of the extracts using MTT and Vero cells](image1)

**Key:**
- Blank wells had maintenance media and plant extracts only (Negative control)
- Wells will have Vero cells, maintenance media and MMT
- Wells will have Vero cells, maintenance media and plant extracts where concentration will decrease from H to B

The direction of extracts’ dilution will decrease upwards from H to B in that order. Concentration of extracts (drugs) were in the order of 1000, 333.33, 111.11, 37.04, 12.35, 4.12 and 1.37 μg/ml, A will not have any drug (Positive control)

![Fig. 2. Promastigote growth inhibition following treatments with various test drugs](image2)

**Fig. 2. Promastigote growth inhibition following treatments with various test drugs**
Table 1. Optimal efficacy, LC$_{50}$ and LC$_{90}$ of test drugs against promastigote form of the parasites for 24 h period

<table>
<thead>
<tr>
<th>Test drugs</th>
<th>Parameter and statistics</th>
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<tbody>
<tr>
<td></td>
<td>Concentration (µg/ml)</td>
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<tr>
<td>Optimal efficacy (%)</td>
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<tr>
<td>Concentration at optimal efficacy</td>
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<tr>
<td>IC$_{50}$</td>
<td>228</td>
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<td>IC$_{50}$</td>
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</table>

Table 2. Optimal efficacy, LC$_{50}$ and LC$_{90}$ of test drugs against amastigote form of the parasites for 24 h period

<table>
<thead>
<tr>
<th>Test drugs</th>
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<tbody>
<tr>
<td></td>
<td>Concentration (µg/ml)</td>
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<td>Optimal efficacy (%)</td>
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<tr>
<td>Minimum concentration at optimal efficacy</td>
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<td>IC$_{50}$</td>
<td>52.8</td>
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<tr>
<td>IC$_{90}$</td>
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Fig. 3. Amastigote growth inhibition following treatments with various test drug

Fig. 4. Laboratory values of mammalian cell mortality, (CC50, and CC90 of the test compounds
4. DISCUSSION

In BALB/c infected with *L. major*, the standard drug (Amphotericin B) acting as a positive control was the most effective in inhibiting growth of promastigotes after 24 h of exposure which concurs with several published studies [35-37]. Amphotericin B exhibits a higher affinity for ergosterol which is present in fungi and *Leishmania* spp. membranes, explaining the relative efficacy of Amphotericin B [38]. The promatigote inhibition activity of Amphotericin B is attributed to its ability to bind ergosterol in the parasite membrane or sequester cholesterol in the host membrane, thereby inhibiting the macrophage-parasite interaction which is necessary for macrophage infection [39]. In addition, Amphotericin B is responsible for: i) lipid peroxidation favoring membrane cell destruction [40]; ii) inhibition of endosome-lysosome fusion [41]; and iii) immunoadjuvant action by promoting the production of IFN-γ that contributes to macrophage activation [42]. Nevertheless, there are issues especially with drug resistance which has necessitated the need for search for new, effective and safer drugs in the management of Leishmaniasis.

The study also established that among the plant extracts, *T. mollis* and *C. macrostachyus* exhibited the most potency in inhibiting the growth of promastigotes and were comparable to the standard drug. The activity of *T. mollis* against several parasites has been previously attributed to punicalin, ellagic acid and their derivatives [43-45]. These compounds also have high solubility in methanol and could therefore be in large quantitative in the present sample. *T. mollis* extract also contain other active compounds including urolithins and benzopyranones, which are cystein protease inhibitors [46-48]. The plant has been tested and showed good antioxidant activity attributed to ellagittannins, ellagic acid, as well as condensed tannins such as catechin, epicatechin, galloカテchin and apigalocatechin as well as garlic acid and derivatives [49,50].

The extracts of *C. macrostachyus* was the second most potent plant against *Leishmania* promatigotes. A number of pharmacological studies confirmed the antileishmanial activities *C. macrostachyus* [51] due to its phytochemical constituents. Phytochemical studies indicated that the *Croton* plants are endowed with many classes of secondary metabolites mainly alkaloids, flavonoids, terpenoids and essential oils such as mono and sesquiterpenoids [52,53].

Of these, terpenoids are the predominant secondary metabolite constituents in the genus chiefly diterpenoids, which belongs to the neoclerodane, clerodane, kaurane, phorbol, labdane and trachylobane skeletal types [54]. Further fractionation of the biologically active crude extracts of the plant parts of many croton species has afforded the isolation and characterization of specific compounds which belong mainly to the groups of alkaloids, flavonoids and terpenoids. Phytochemical screening showed the existence of secondary metabolites such as phenolic compounds, tannins, terpenoids, alkaloids, saponins, free anthraquinones, phytosterols, polyphenols and wthanoïdes. Fractionation and characterization approaches on the most biologically active crude extracts led to the isolation of many secondary metabolites from the given medicinal plant [55]. Cyclohexane diepoxides such as crotepoxide, lupeol and betulin, cis-clerodane, crotomacrine, 3β-Acetoxy tetraxer-14-en-28-oic acid, trachylin-19-oic acid, trachylin-18-oic acid are among the isolated compounds from various parts of *Croton macrostachyus* [56].

*Olea europaea* was the least effective in inhibiting the growth of promastigotes. *Olea europaea* contains many different compounds, specifically biophenols [57]. The most abundant biophenol is oleuropein, a secoiridoid composed from elenolic acid and hydroxytyrosol, which is considered the major bitter constituent [58]. Other bio-phenols such as verbascoside, apigenin-7-glucoside, luteolin-7-glucoside and hydroxytyrosol are present in lower quantities [59]. Most of these compounds have known potency against microbes such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* [60] but not *Leishmania* species.

In BALB/c infected with *L. major*, the efficacy of varying concentrations of extracts from the test plants showed similar patterns to those of amastigotes. Herbal drug *Terminalia mollis* was the most effective followed by *C. macrostachyus* and *K. africana* also showed good promastigote growth inhibition while *O. europaea* was the least effective. The short-term efficacy of the test compounds against a *Leishmania* infection which evolved for 4 weeks prior to drug administration was demonstrated. The activity of these test compounds appear to act in similar manner as in the promastigotes. Although data in this study do not provide a direct measure of the parasite killing (1- [amastigote load at the end of the treatment/amastigote load before the start of the treatment]), they show induction of effective...
leishmanicidal activity. The prolonged effect of the test compounds on parasite growth suggests a low catabolism and/or slow clearance, resulting in a long biological half-life for the test drugs.

The liver plays a pivotal role in the excretion of toxins and drugs. The fact that the liver has high capacity for drugs uptake makes them vulnerable to toxicity. Overall, liver cell pathology in infected mice treated with different test drugs showed considerable differences indicating sign of toxicity. In the L. major infected BALB/c mice treated with Amphotericin B and T. mollis, large section of the liver showed normal physiology thus asserting the low toxicity of Amphotericin B and T. mollis to macrophage and cells. Low toxic effects of Amphotericin at the right doses has been previously reported mostly based on different mode of formulation [61-63]. However, the treatment with antimonials has several side effects, such as: pancretitis, malaise, diarrhoea and cardiac arrhythmia [64] which means that even if they are of low toxicity to the cells, there is a need to search for safer alternatives. Mild granulomas were encountered more frequently in the L. major treated with T. mollis showing signs of mild toxicity. Cell toxicity is caused by exogenous toxicant which can damage cells, especially when the toxicant can cause cell death and serious organ dysfunction. The mechanisms of cell toxicity are widely involved. It has long been proved that toxicant may induce overproduction of Nitric oxide (NO), reactive oxygen species (ROS) and the subsequent oxidative stress [65]. The high level of NO, ROS and the subsequent oxidative burst have been identified as one main mechanism of severe cell toxicity or even organ dysfunction [66]. Toxicity agents may also induce and release compounds that directly damage DNA, causing cell apoptosis and toxicity [67]. The infected mice treated with B. micrantha and O. europaea suffered several toxic effects suggesting autophagy. This suggests that extracts’ bioactive ingredients were more likely to affect the host cells by inducing apoptosis in cells with provoked autophagy [68]. The current study differ with other finding is compatible especially Song et al. who showed that O. europaea extract reduced Prostaglandin E2 (PGE2) production without exhibiting any cytotoxic activity on balb/c mice cells [69].

5. CONCLUSION

There were differences in the efficacy of varying concentrations of extracts from O. europaea, K. africana, T. mollis, C. macrostachyus, B. micrantha and standard drug (Amphotericin B) on promastigotes of L. major. The promastogote and amastigote growth inhibition was significantly affected by the crude extracts from the plants after 24 h of exposure. The % growth inhibition estimated for the promastigote and amastigote showed that the most effective drug was the standard drug (Amphotericin B) while among the crude extracts of the herbal drugs, T. mollis was the most effective against amastigote followed by C. macrostachyus while O. europaea was the least effective in terms of optimal efficacy.

6. RECOMMENDATIONS

1. The study recommends the use of T. mollis in management of leishmaniasis in areas they occur. However, further analysis of the active compounds that that affect efficacy of the plant extracts is advised.
2. Further investigations for potential applications of new natural antioxidants require anyway, elucidation of the chemical composition of phenolic and flavonoid in vivo studies in order to better establish the functionality of the examined plant species.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was done in accordance with ethical guidelines of Maseno University and the Kenya Medical Research Institute.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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