Antimalarial activity of *Cucumis metuliferus* and *Lippia kituiensis* against *Plasmodium berghei* infection in mice

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**Background:** The search for new antimalarial drugs has become progressively urgent due to plasmodial resistance to most of the commercially available antimalarial drugs. As part of this effort, the study evaluated the antimalarial activity of *Cucumis metuliferus* and *Lippia kituiensis*, which are traditionally used in Tanzania for the treatment of malaria.

**Materials and methods:** In vivo antimalarial activity was assessed using the 4-day suppressive antimalarial assay. Mice were infected by injecting via tail vein $1 \times 10^7$ erythrocytes infected by *Plasmodium berghei* ANKA. Extracts were administered orally; chloroquine (10 mg/kg/day) and dimethyl sulfoxide (5 mL/kg/day) were used as positive and negative controls, respectively. The level of parasitemia, survival time, packed cell volume (PCV) and variation in body weight of mice were used to determine the antimalarial activity of the extract.

**Results:** The ethyl acetate, methanolic and chloroform extracts of *C. metuliferus* and *L. kituiensis* significantly ($p<0.05$) inhibited parasitemia in a dose-dependent manner and prevented loss of body weight at the dose levels of 600 mg/kg and 1500 mg/kg, respectively. In addition, the extracts prolonged the mean survival time of *P. berghei*-infected mice compared to the non-treated control. The plant extracts did not show reduction of PCV except at the low dose of 300 mg/kg. The highest suppression was recorded at the dose level of 1,500 mg/kg. At this dose, *C. metuliferus* in chloroform, methanolic and ethyl acetate extracts had percentage suppression of 98.55%, 88.89% and 84.39%, respectively, whereas *L. kituiensis* in ethyl acetate, chloroform and methanolic extracts exhibited suppression of the pathogens of 95.19%, 93.88% and 74.83%, respectively.

**Conclusion:** It is worth reporting that the two plants induced suppression which is equivalent to that induced by chloroquine (*C. metuliferus* chloroform and *L. Kituiensis* ethyl acetate). The two plants have been demonstrated to be potential sources of antimalarial templates.

**Keywords:** malaria, *Cucumis metuliferus*, *Lippia kituiensis*, suppression, *Plasmodium berghei*, crude extracts

**Introduction**

Substantial progress has been made in fighting malaria since 2000. It has been estimated that the incidence of malaria has been reduced by 41% and the malaria mortality rates by 62% between 2000 and 2015. At the beginning of 2016, malaria was considered to be endemic in 91 countries and territories, which has reduced from 108 in 2000. This progress is associated with the wide-scale deployment of malaria control interventions. Despite this remarkable progress, malaria continues to have a devastating impact on people’s health and livelihoods. It is estimated that 212 million cases occurred globally in 2015, leading to 429,000 deaths; most of the cases were children aged <5 years. The burden of the disease is heaviest in Africa, where 82%
and 90% of all global cases and deaths, respectively, occurred. The report further indicates that 90% of all malaria deaths occur in African regions, of which 78% occur in children <5 years of age. Increased side effects of conventional drugs and development of resistance by malaria parasites have become a global concern, and thus call for continued efforts to search for antimalarial agents. Therefore, medicinal plants have made and continue to make, a great contribution to antimalarial chemotherapy as they contain molecules with a great variety of structures and biological activities. 

*Lippia kituiensis* and *Cucumis metuliferus* have been utilized by rural communities in Tanzania for the management of malaria. *L. kituiensis* belongs to the verbena families (Verbenaceae), which are aromatic due to their essential oils. Apart from its use for the management of malaria, its leaves are used as a culinary herb. Camphor isolated from *L. kituiensis* essential oil was found to have a strong repellent activity against maize weevil compared with N,N-diethyl toluamide (DEET). *L. kituiensis* is also used for the treatment of chronic joint pains, such as in osteoarthritis and/or rheumatoid arthritis, and also in different farms they use *L. kituiensis* for milk treatments in animals. *C. metuliferus* (Cucurbitaceae) is a monoecious annual herb with staminate flowers that grow wild. The family is commonly referred to as African horned cucumber, jelly melon in English. *C. metuliferus* is an annual climbing or rarely trailing herb; vegetative parts are rough with spreading hairs; its fruits occur in two forms – the bitter and non-bitter forms, which occur mostly in the wild state. It has been established that the fruit of *C. metuliferus* possesses antibacterial activity against *Salmonella gallinarum* in vitro. The pulp extract of *C. metuliferus* was shown to have an antiulcer property. Glycosides extracted from the fruit pulp of *C. metuliferus* possess antihyperglycemic activity against alloxan-induced diabetes mellitus in rats.

Despite the fact that *L. kituiensis* and *C. metuliferus* are used in Tanzania for the management of malaria, there is no other report on the antimalarial activity of the two plants. This paper reports antimalarial activity of methanolic, ethyl acetate and chloroform extracts of *C. metuliferus* and *L. kituiensis* against *Plasmodium berghei*-infected mice.

**Materials and methods**

**Collection of plant materials**

Fresh leaves of *L. kituiensis* and *C. metuliferus* were collected from Ugweno ward within Mwanga District, Kilimanjaro Region in Tanzania. Ugweno ward is situated at 3° 39’ 0” South and 37° 39’ 0” East in the Pare Mountains. Herbalists residing in Ugweno ward and a taxonomist (Mr Josephat Mboya) were consulted and involved in the identification and collection of plant materials. The voucher specimen coded (Voucher no 160 for *L. kituiensis* and 390 for *C. metuliferus*) was deposited at Nelson Mandela African Institution of Science and Technology.

**Extraction of the plant materials**

The leaves of *L. kituiensis* were cleaned and air-dried in the shade at room temperature. The powdered samples of *L. kituiensis* (1000 g) were weighed using a sensitive balance and extracted by chloroform, ethyl acetate and methanolic solvent. After 48 h, the mixture was filtered using a Whatman filter paper no 1 (W and R Balson Ltd, Whatman, England). The filtrates obtained were dried using a rotary evaporator, and the dried extract was kept until further use. The powdered samples of *C. metuliferus* (500 g) were weighed using a sensitive balance and extracted by chloroform, ethyl acetate and methanolic solvent, respectively, with occasional stirring. After 72 h, the mixture was filtered using a Whatman filter paper no 1. The filtrates obtained were dried using an oven (250 V, France Etuves, Chelles, France) set below 40°C, and the dried extract was transferred into a vial and then kept in a refrigerator until further use.

**Experimental animals**

Swiss albino male mice, weighing 25–32 g and 6–8 weeks old, were collected at Sokone University of Agriculture (SUA) and housed at Muhimbili University of Health and Allied Sciences (MUHAS) in plastic cages with softwood shavings and chips as bedding. They were exposed to a 12:12 dark-to-light cycle and provided with free access to pellet diet and clean drinking water. All mice were acclimatized to the room environment for 1 week before the beginning of the experiment.

**Malaria parasites**

Chloroquine-sensitive *P. berghei* (ANKA strain) was used for induction of malaria in experimental mice. Blood with *P. berghei* ANKA parasites used in the study were kindly donated by Dr Lindsay Stewart of the Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, United Kingdom.

**Materials and reagents**

Chloroquine (Addis Pharmaceuticals Factory Addis Ababa, Ethiopia), methanol (Okhla Industrial, New Delhi, India), Giemsa (Scientelab, Gaithersburg, MD, USA), trisodium citrate (Deluxe Scientific Surgico, India) and normal saline (Addis Pharmaceuticals Factory, Ethiopia), chloroform (Okhla Industrial, India), ethyl acetate (Okhla Industrial) and
dimethyl sulfoxide (DMSO) were used. All reagents were of analytical grade and procured from certified suppliers.

**Inoculum preparation**

The parasitemia of the previously *P. berghei*-infected donor mouse was determined. According to Waako et al., the appropriate inoculum for infecting mice had to be 1% parasitemia. After anesthetizing with chloroform, the blood from these mice was collected via cardiac puncture with a rising parasitemia of 44.40% into a test tube having 0.5% trisodium citrate, which was added as an anticoagulant. Then, the parasitemia was reduced to 1% by dilution using phosphate-buffered saline (PBS). A total of 9.77 mL of PBS was required to dilute 0.23 mL of blood from the donor mice with 44.40% parasitemia to obtain 10 mL of blood with 1% parasitemia, which was used to infect 50 experimental mice each receiving ~1.0×10⁷ parasitized cells.

**In vivo antimalarial determination**

In vivo antimalarial activity was determined by 4-day suppressive antimalarial assay. Chloroquine-sensitive *P. berghei* (ANKA strain) parasites obtained from the Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, United Kingdom were used for experimental purposes and were revived and stabilized in the host, according to Ravindran et al. The test was done by a method described by Fidock et al. After standard parasite inoculation (1×10⁷ infected red blood cells [iRBC], 0.2 mL), treatment was started 3 hours after infection and then continued for 3 consecutive days (ie, from day 0 [D0] to day 3 [D3]). On day 4 (D4), thin blood films were made from the tail of each mouse on a microscopic slide.

**Peripheral blood smears’ preparation**

For both models, thin smears of blood were made from the tail of each mouse on day 5 (D5). The smears were applied on microscopic slides, and blood was drawn evenly across a second slide to make thin blood films and they were allowed to dry at room temperature. Then, they were fixed with 100% methanol and stained with 10% Giemsa stain at pH 7.2 for 15 minutes.

**Parasitemia determination**

Each stained slide for each mouse was examined under a microscope (Olympus Corporation, Tokyo, Japan). The parasitemia was determined by counting the number of parasitized erythrocytes in random fields of the microscope image for both infected and donor mice. Percentage parasitemia and percentage suppression of both infected and donor mice were calculated as follows:

\[
\text{Percentage parasitemia} = \left( \frac{\text{Number of iRBCs}}{\text{Total number of RBCs}} \right) \times 100
\]

\[
\text{Percentage suppression} = \left( \frac{\text{Parasitemia in negative control} - \text{Parasitemia in study group}}{\text{Parasitemia in negative control}} \right) \times 100
\]

**Determination of packed cell volume (PCV)**

PCV of each mouse was measured before infection (D0) and after infection (D4). For this purpose, blood was collected from the tail of each mouse in heparinized microhematocrit capillary tubes. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge with the sealed ends downwards. The blood was centrifuged at 12,000 rpm for 5 minutes. The volume of the total blood and the volume of erythrocytes were measured, and PCV was calculated according to Mengistie, as given below.

\[
\text{PCV} = \left( \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \right) \times 100
\]

**Determination of mean survival time (MST)**

MST was determined according to Alli et al; mortality was monitored daily; the number of days from the time of infection up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period; and the MST was calculated for each group by using the following formula.

\[
\text{MST} = \left( \frac{\text{Sum of survival time (days) of all mice in a group}}{\text{Total number of mice in that group}} \right) \times 100
\]

**Determination of body weight**

According to Krettli et al, the change in body weight of each mouse in all groups was determined before infection (D0) and after treatment (D4) using a sensitive electrical balance (ADP 720 L; Adam Equipment, Cape Town, South Africa). Then, the body weight change for the extract-treated groups was compared with the control groups.
Ethical consideration
During experimental procedures, experimental animals were handled and cared for according to the internationally accepted laboratory animals’ use, care and welfare guideline from the Institute for Laboratory Animal Research (ILAR). Ethical clearance was requested and obtained from the National Institute of Malaria Research (NIMR) in Tanzania.

Data analysis
The data of the study are expressed as mean ± SD for each group of experiments. Data on the levels of parasitemia, variations in body weight and survival times were analyzed using a statistical analysis system (SAS). Values were considered statistically significant at p<0.05 and more significant at p<0.01. All results are represented as mean ± SD (n=5). Values with different superscripts are significantly different (p<0.05 and p<0.01).

Results
In vivo evaluation of the antiplasmodial activity of plant extracts
The chloroform, ethyl acetate and methanolic extract of C. metuliferus and L. kituiensis showed chemosuppressive activity against P. berghei infection in mice. Blood samples drawn from P. berghei-infected mice were subjected to a test to assess suppression of parasitemia. The chloroform, methanolic and ethyl acetate extracts of C. metuliferus significantly suppressed the parasitemia. The highest percentage suppression was obtained from chloroform (98%, 70% and 46%), followed by chloroform (93%, 69% and 46%) and least was methanolic (74%, 68% and 35%) in extract-treated mice at the dose levels of 300 mg/kg, 600 mg/kg and 300 mg/kg, respectively, as indicated in Table 1.

In the same way, the ethyl acetate, chloroform and methanolic extracts of L. kituiensis showed significant parasite suppression in all the doses administered. The highest suppression was obtained for ethyl acetate (95%, 70% and 42%), followed by chloroform (93%, 69% and 46%) and least was methanolic (74%, 68% and 35%) in extract-treated mice at the dose levels of 1500 mg/kg, 600 mg/kg and 300 mg/kg, respectively, as shown in Table 2.

Effect of extracts on PCV
In the 4-day suppressive test, PCV of P. berghei-infected mice was measured. The results showed a significant (p<0.05) reduction in PCV between D0 (inoculation day) and D4 in mice treated with chloroform, methanolic and ethyl acetate extracts of leaves of L. kituiensis at a dose level of 300 mg/kg, whereas mice treated with chloroform, methanolic and ethyl acetate extracts at dose levels of 600 mg/kg and 1500 mg/kg did not show significant PCV reduction (Table 3). In C. metuliferus, the mice treated with chloroform, methanolic and ethyl acetate extracts exhibited significant reduction (p<0.05) in PCV between D0 and D4 at a dose level of 300 mg/kg, whereas the mice treated with chloroform, methanolic and ethyl acetate extracts from the two plants at dose levels of 600 mg/kg and 1500 mg/kg did not show significant (p>0.05) PCV reduction (Table 4).

Effect of extract on survival times of mice
In the 4-day suppressive test, 300 mg/kg, 600 mg/kg and 1500 mg/kg extract-treated groups lived longer than the

Table 1 Antimalarial activities of ethyl acetate, methanolic and chloroform extracts of leaves of Cucumis metuliferus and mean survival time of Plasmodium berghei-infected mice

<table>
<thead>
<tr>
<th>Extraction solvents</th>
<th>C. metuliferus</th>
<th>Extract antimalarial activity on D4 postinfection</th>
<th>Mean survival time (days) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/kg)</td>
<td>% parasitemia ± SD</td>
<td>% suppression ± SD</td>
</tr>
<tr>
<td>Methanolic</td>
<td>300</td>
<td>30.09±0.78**</td>
<td>36.99±1.64</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>19.82±0.93**</td>
<td>58.91±1.68</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>5.49±0.30**</td>
<td>88.89±0.83</td>
</tr>
<tr>
<td>Chloroform</td>
<td>300</td>
<td>25.90±0.39**</td>
<td>45.81±1.22</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>14.37±0.95**</td>
<td>70.69±2.86</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>1.10±0.13**</td>
<td>98.55±0.7</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>300</td>
<td>34.03±0.98*</td>
<td>30.73±2.73</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>17.08±1.68**</td>
<td>64.23±3.53</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>8.95±0.47**</td>
<td>84.39±3.98</td>
</tr>
<tr>
<td>CQ</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>NC</td>
<td>47.15±1.74</td>
<td>-</td>
<td>6.0±1</td>
</tr>
</tbody>
</table>

Notes: Values are presented as mean ± SD; n=5; DMSO, negative control (1%). *Values are significantly different (p<0.05) from that of the negative control; **values are more significantly different (p<0.01) from that of the negative control.

Abbreviations: CQ, positive control (chloroquine); D4, day four; DMSO, dimethyl sulfoxide NC, negative control.
### Table 2 Antimalarial activities of ethyl acetate, methanolic and chloroform extracts of leaves of Lippia kituiensis and mean survival time of Plasmodium berghei-infected mice

<table>
<thead>
<tr>
<th>Extraction solvents</th>
<th>L. kituiensis Dose (mg/kg)</th>
<th>Extract antimalarial activity on D4 postinfection</th>
<th>Mean survival time (days) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>30.08±3.05*</td>
<td>35.94±5.49</td>
<td>9±1*</td>
</tr>
<tr>
<td>600</td>
<td>14.21±1.95**</td>
<td>68.76±3.34</td>
<td>12±2*</td>
</tr>
<tr>
<td>1500</td>
<td>12.46±0.52**</td>
<td>74.83±2.35</td>
<td>13±0**</td>
</tr>
<tr>
<td>Chloroform</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>25.61±2.17**</td>
<td>46.38±4.63</td>
<td>9±1*</td>
</tr>
<tr>
<td>600</td>
<td>14.44±0.20**</td>
<td>69.96±0.50</td>
<td>12±1.52*</td>
</tr>
<tr>
<td>1500</td>
<td>3.75±0.38**</td>
<td>93.88±2.47</td>
<td>14±0**</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>27.74±3.60*</td>
<td>42.42±8</td>
<td>8±0.57*</td>
</tr>
<tr>
<td>600</td>
<td>14.64±1.57**</td>
<td>70.14±3.85</td>
<td>10±1.52*</td>
</tr>
<tr>
<td>1500</td>
<td>2.53±0.13**</td>
<td>95.19±1.11</td>
<td>14±0**</td>
</tr>
<tr>
<td>CQ</td>
<td>10</td>
<td>0</td>
<td>14±0**</td>
</tr>
<tr>
<td>NC</td>
<td></td>
<td></td>
<td>6±1</td>
</tr>
</tbody>
</table>

Notes: Values are presented as mean ± SD; n=5; DMSO, negative control (1% DMSO); *Values are significantly different (p<0.05) from that of the negative control. **Values are more significantly different (p<0.01) from that of the negative control.

Abbreviations: CQ, positive control (chloroquine); D0, day zero; D4, day four; DMSO, dimethyl sulfoxide; NC, negative control.

### Table 3 Effect of chloroform, ethyl acetate and methanolic extracts of Lippia kituiensis on body weight and PCV of Plasmodium berghei-infected mice

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Dose (mg/kg)</th>
<th>PCV D0</th>
<th>D4</th>
<th>Body weight D0</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>300</td>
<td>51.23±1.21</td>
<td>43.32±5.6*</td>
<td>29.70±3.21</td>
<td>24.65±2.11*</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>50.12±2.32</td>
<td>48.13±1.3</td>
<td>30.11±1.63</td>
<td>28.24±2.12</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>59.31±1.2</td>
<td>57.91±1.2</td>
<td>32.22±1.01</td>
<td>31.90±2.11</td>
</tr>
<tr>
<td>Chloroform</td>
<td>300</td>
<td>51.23±1.50</td>
<td>44.21±2.3*</td>
<td>26.54±2.13</td>
<td>21.44±1.33*</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>51.32±2.3</td>
<td>49.23±1.2</td>
<td>29.33±1.55</td>
<td>27.12±1.5</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>50.18±2.1</td>
<td>49.91±1.2</td>
<td>30.45±1.03</td>
<td>29.93±1.02</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>300</td>
<td>49.23±1.5</td>
<td>43.32±5.3*</td>
<td>32.12±0.23</td>
<td>25.52±3.23*</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>51.34±3.1</td>
<td>48.23±2.1</td>
<td>28.14±2.11</td>
<td>25.23±2.11</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>50.32±1.2</td>
<td>49.32±0.1</td>
<td>28.15±1.23</td>
<td>27.25±2.55</td>
</tr>
<tr>
<td>CQ</td>
<td>10</td>
<td>55.41±1.03</td>
<td>54.66±0.52</td>
<td>30.23±0.44</td>
<td>29.92±0.12</td>
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<tr>
<td>NC</td>
<td></td>
<td>55.44±1.25</td>
<td>40.45±1.45*</td>
<td>32.17±1.4</td>
<td>25.13±4.35*</td>
</tr>
</tbody>
</table>

Note: Values are presented as mean ± SD; n=5. *The difference between day 0 and day 4 is significant (p<0.05).

Abbreviations: CQ, chloroquine (positive control); D0, day zero; D4, day four; DMSO, dimethyl sulfoxide; NC, negative control (1% DMSO); PCV, packed cell volume.

### Table 4 Effect of chloroform, ethyl acetate and methanolic extracts of Cucumis metuliferus on body weight and PCV of Plasmodium berghei-infected mice

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Dose (mg/kg)</th>
<th>PCV D0</th>
<th>D4</th>
<th>Body weight D0</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>300</td>
<td>48.32±1.61</td>
<td>40.32±5.6*</td>
<td>28.70±3.21</td>
<td>22.65±2.11*</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>49.32±2.32</td>
<td>47.13±1.3</td>
<td>30.11±1.63</td>
<td>28.24±2.12</td>
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<tr>
<td></td>
<td>1500</td>
<td>51.32±1.2</td>
<td>49.91±1.2</td>
<td>32.22±1.01</td>
<td>31.90±2.11</td>
</tr>
<tr>
<td>Chloroform</td>
<td>300</td>
<td>51.23±1.50</td>
<td>44.21±2.3*</td>
<td>26.54±2.13</td>
<td>20.44±1.33*</td>
</tr>
<tr>
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<td>27.12±1.5</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>53.23±2.1</td>
<td>52.32±1.2</td>
<td>30.45±1.03</td>
<td>29.93±1.02</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>300</td>
<td>50.23±1.5</td>
<td>40.32±5.3*</td>
<td>32.12±0.23</td>
<td>26.52±3.23*</td>
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<tr>
<td></td>
<td>600</td>
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<tr>
<td></td>
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<td>48.32±0.1</td>
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<td>27.25±2.55</td>
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<tr>
<td>CQ</td>
<td>10</td>
<td>55.41±1.03</td>
<td>54.66±0.52</td>
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<td>29.92±0.12</td>
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<tr>
<td>NC</td>
<td></td>
<td>55.44±1.25</td>
<td>40.45±1.45*</td>
<td>32.17±1.4</td>
<td>25.13±4.35*</td>
</tr>
</tbody>
</table>

Notes: Values are presented as mean ± SD; n=5. *The difference between day 0 and day 4 is significant (p<0.05).

Abbreviations: CQ, chloroquine (positive control); D0, day zero; D4, day four; DMSO, dimethyl sulfoxide; NC, negative control (1% DMSO); PCV, packed cell volume.
corresponding negative controls. Significant difference was observed in methanol, chloroform and ethyl acetate in dose level 300 mg/kg, 600 mg/kg and 1500 mg/kg in both C. metuliferus and L. kituiensis, as shown in Tables 1 and 2.

Effect of extract on body weights of mice

In the 4-day suppressive test, all extract-treated groups prevented loss of body weight except at a dose level of 300 mg/kg in C. metuliferus and L. kituiensis in chloroform, ethyl acetate and methanolic extract, between D0 and D4, as compared to negative control. However, there were no significant differences in weight at dose levels of 600 mg/kg and 1500 mg/kg in all solvents as shown in Tables 3 and 4.

Discussion

The antimalarial activities of the methanolic, chloroform and ethyl acetate extract of C. metuliferus and L. kituiensis, used in traditional medicine in Tanzania, against P. berghei infection in mice in 4-day suppressive test models are reported. Parasitemia refers to the level of infection by a particular parasite or the number of parasitized cells circulating in blood. A decline in parasitemia in a given organism is essential for the recovery from symptomatic malaria. In this study, the percentage parasitemia in the negative control group on D4 after infection with P. berghei parasites was 47.15% and chloroquine as a positive control had parasitemia of 0.00%, while none of the extracts reached this level. For plant extracts, the lowest parasitemia was observed in C. metuliferus with 1.1% chloroform extract at a dose level of 1500 mg/kg and in L. kituiensis with 2.53% ethyl acetate extract at a dose level of 1500 mg/kg. This result is in agreement with the antimalarial properties reported by Renata et al., which support the in vivo activities of C. metuliferus and L. kituiensis.

Chemosuppression is inversely related to parasitemia; the plant extracts which have been shown to reduce parasitemia to low levels have been shown to have a corresponding high chemosuppression. The study revealed that the highest suppression in C. metuliferus was obtained from chloroform (98%, 70% and 48%), followed by methanolic extracts (88%, 58% and 36%) and the least was from ethyl acetate (84%, 64% and 30%) in extract-treated mice at the dose levels of 1500 mg/kg, 600 mg/kg and 300 mg/kg, respectively. In the same way, the ethyl acetate, chloroform and methanolic extracts of L. kituiensis showed significant parasitemia suppression in all the doses administered. The highest suppression was obtained for ethyl acetate (95%, 70% and 42%), followed by chloroform (93%, 69% and 46%) and least was from methanolic (74%, 68% and 35%) extract-treated mice at the dose levels of 1500 mg/kg, 600 mg/kg and 300 mg/kg, respectively.

According to Ajaiyeoba et al., in vivo antimalarial activity of plant extracts can be categorized as moderate, good and very good, if the extract shows 50% or more chemosuppression. Hence, the current findings in both plants are very good and good 4-day suppressive antimalarial activity at the dose levels of 1500 mg/kg and 600 mg/kg, while at the lowest dose both plants have low 4-day suppression antimalarial activity. However, the highest peak chemosuppression was recorded in chloroform extracts of C. metuliferus with 98.53% (p<0.05), followed by ethyl acetate of L. kituiensis with 95.19%, which was equivalent to that induced by chloroquine. This observation was quite interesting since the extract was only a crude preparation. The chemosuppression showed by C. metuliferus and L. kituiensis was in agreement with other studies on medicinal plants used for malaria such as Artemisia annua, Annona senegalensis and Adhatoda schimperiana, which showed 90.48%, 96.20% and 97.80%, respectively. This observation also gives credit to the information provided by the traditional healers who provided information on plants traditionally used to treat malaria in Ugweno, northern Tanzania, and hence they can be trusted with ethnobotanical information in future studies. According to Ajaiyeoba, the antimalarial activity of the plant might be induced by the presence of bioactive compounds such as quassinoids and canthin alkaloids. However, the active compound(s) responsible for the observed activity need to be identified in future studies. However, the observed low antimalarial activity of plant extracts could be partly explained by the fact that many antimalarial traditional medicinal plants may lack direct antiplasmodial activity to cure the disease but their beneficial role could be in their antipyretic, analgesic and immune stimulatory effect as demonstrated in other studies. According to Guo et al., the effectiveness of a given extract can also be influenced by the rate of gastrointestinal uptake and the half-life in plasma metabolism of the active compound. The difference between the antimalarial activities of extracts of both plants in all the three solvents may indicate differences in the level and composition of active compounds. Additionally the significant change may be due to different solvents used in terms of polar and nonpolar solvent. More than that the administration of plant extracts on mice was done orally, due to this, some of the mice may have not taken the whole 100 mg/kg dosage and this may have affected the parasitemia in those groups.
Mean survival time is another parameter that evaluates the antimalarial activity of plant extracts as indicated in Tables 1 and 2. Plant materials that can prolong the survival time of infected experimental animals compared to the negative controls are considered as active agents against malaria.30,31 In this study, the mice treated with 300 mg/kg, 600 mg/kg and 1500 mg/kg of extract had significantly longer lives than negative controls in the 4-day suppressive test, which confirms that the tested extracts contain antimalarial compound which reduces the number of parasites and hence prolongs the survival time. The current finding was in agreement with other reports on medicinal plants used for malaria, such as Dodonaea angustifolia seed.32 However, the survival times of mice treated with the extract were shorter as compared to positive controls; this might be due to the fast elimination phase of the extract.

Body weight is another parameter that can be used to assess the effect of the extract in treated mice.33 In this experiment, the change in body weight of mice before and after treatment was determined; the results showed that there was significant (p<0.05) decrease in body weight between D0 and D4 of mice treated with chloroform, methanolic and ethyl acetate extract at a dose level of 300 mg/kg, for both C. metuliferus and L. kituiensis, as shown in Tables 3 and 4. This may be due to the depressant action on the appetite of the mice and the consequences of disturbed metabolic function and hypoglycemic effect of the parasite.34 Similar results were obtained for chloroform, methanol and ethyl acetate extracts at a dose level of 300 mg/kg for different species.35

The reduction in PCV values of P. berghei-infected mice signifies malaria infection. P. berghei-infected mice treated with extracts at 300 mg/kg exhibited a significant PCV reduction, while insignificant PCV reduction was recorded for infected mice treated with extracts at dose levels of 600 mg/kg and 1500 mg/kg. Similar results were obtained in studies reported from the same species of Ajuga remota used to treat malaria.36,37

Conclusion
In the present study, organic extracts of C. metuliferus had chemosuppression of 98.55% and those of L. kituiensis had a chemosuppression of 95% at a dose level of 1500 mg/kg. These chemosuppression levels of both plants were high especially for the organic extract, which was equivalent to that of chloroquine (100%). This shows that there is a possibility of the presence of antimalarial phytochemical in the leaves of these plants, which validates their ethnopharmacological use as antimalarial herbal remedies by the Ugweno community. Further study in vivo antimalarial activity should be done including measuring cerebral malaria symptoms as a parameter in the treated group. Screening for selected phytochemicals of L. kituiensis and C. metuliferus may provide insights into active antimalarial compounds in these plants. Therefore, phytochemical analysis of the plant is highly recommended to identify the active compounds associated with the interesting antimalarial activity. Further in vivo toxicity studies are also recommended to determine safe dose levels of these plants, and in vitro antiplasmodial studies, fractionation, purification and compound identification from the most active crude extracts are recommended.

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Disclosure
The authors report no conflicts of interest in this work.

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