Ovicidal, Larvicidal and Pupicidal Efficacy of Crude Methanol and Hexane Extract of *Urtica massaica* Mildbr on *Anopheles gambiae* Giles

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**Abstract**

This study was designed to evaluate ovicidal, larvicidal and pupicidal potency of methanol and hexane extracts of leaves, stems and roots of *Urtica massaica* Mildbr. against aquatic stages of *Anopheles gambiae* Giles. The effectiveness of the extracts was evaluated using the WHO protocol. One-way analysis of variance was performed for statistical justifications of the insecticidal property of the extracts with p considered significant at p < 0.05. It was found that potency of extracts was dose dependent. Extracts from the stem were more potent than the roots or leaves. Mortalities of the aquatic stages were however, significantly different (p < 0.05) irrespective of stage. Third larval instars (L3s) were more susceptible than eggs or pupae. Doses of 80 cm³/100ml (s/w) and above matched the WHO > 80% mortality for an effective insecticide. It was concluded that higher doses of the crude extracts of *U. massaica* were potent against *An. gambiae*.

**Keywords:** *Urtica massaica*, Extracts, Methanol, Hexane, *Anopheles gambiae*, Bacillus thuringiensis

1. **Introduction**

Mosquitoes serve as vectors of several diseases, causing serious health problems and death in humans (Alayo et al., 2015). Among diseases transmitted by mosquitoes is malaria whose causative agent is *Plasmodium* parasites. Malaria is responsible for morbidity, mortality, low birth weight, stillbirths, and early infant death mainly in tropical and subtropical areas (Karunamoorthi et al., 2014). About 3.2 billion people are at risk of malaria (WHO, 2017) infection.

Presently, protection against mosquito bites is through vector control from the use of insecticide treated nets (ITNs) and larval source reduction. This approach has greatly reduced the frequency of contact between mosquitoes and humans and is considered a big win towards the fight against vector borne diseases since the current lack of effective prophylactic vaccine or well-established preventive measure (Soonwera, 2015) at the moment would mean escalation in the current sorry state of such disease burden. Moreover, the continual application of synthetic insecticides in the management of insect vectors is disadvantageous as it is non target specific (Sanghong et al., 2015; Soonwera, 2015; Govindarajan et al., 2016), bringing about disturbance of natural ecosystems, leading to development of resistance in vector population and in some cases resulting in resurgence of vector borne diseases.

To mitigate these challenges, scientists have turned their attention to the use of natural products as an alternative strategy to the control of insect vector population. This is because the natural products are not only a rich source of bioactive phytochemicals but are also safe, biodegradable and non-toxic (Asadollahi et al., 2019). These products are, therefore, an excellent source of green insecticides that are eco-friendly and also seen as the solution to the inevitable environment and human health challenges.

*Urtica massaica* Mildbr, commonly known as stinging nettle, is a perennial herb (Ayan et al., 2006) from the family of *Urticaceae*. It grows naturally in the borders of fields, roads and forests and is mostly found in the wet parts of the highlands in Kenya. It is a vegetable (Grubben, 2004) among other uses. Leaf and root extracts of this plant are rich in proteins, vitamins, minerals, amino acids (Westfall, 2001) and polyphenols that have found use as food and in the pharmaceutical industries (Kregiel et al., 2018). Though the extracts are toxic (Oloro et al., 2015) and with potential for teratogenicity (Wabai et al., 2018), they have been known to cure stomach aches, malaria, bruises, injuries, fractures, venereal diseases, rheumatism, urethral leak, hepatic diseases (Grubben, 2004) as well as manage diabetes (Ketera and Mutiso, 2012; Kamau et al., 2017). Methanolic extracts of *U. massaica* have also been demonstrated to have antimicrobial (Koître et al., 2013) as well as fungal potential (Kamalakannan et al., 2012; Kipruto et al., 2019).

It is believed that there are a lot of other potential benefits of this wonder herb that have not been exposed, and the present study was designed to enlighten us on this. In this...
study, potency of methanolic and hexane extracts of leaves, stems and roots of U. massaica is evaluated against An. gambiae Giles aquatic stages under laboratory conditions. This is to generate and inform on effect of mosquitoicidal effect of these extracts and avail this information that is presently unknown.

2. Materials and Methods

2.1. Study area, experimental mosquitoes and study design

Eggs, third larval instars (L3s) and pupae of An. gambiae mosquitoes kept at the insectary of the Entomological Laboratory at the Centre for Global Health Research and reared following standard techniques as describe by Das et al. (2007) and Yugi et al. (2014) were used in the experiments described in this study. A completely randomized informal ‘after-only with control’ experimental design (Kothari, 2004) was used to investigate the ovicidal, larvicidal and pupicidal effect of crude methanol and hexane extracts of U. massaica on the aquatic stages. Solvent, dose and U. massaica extracts were taken as independent while observed mortalities as dependent variables respectively. Bacillus thuringiensis israelensis (Bti) was taken as positive while dimethyl sulfoxide (DMSO) and distilled water were taken as negative control.

2.2. Plant materials

Fresh leaves, stem and roots of U. massaica were collected from Kambi Somali in Eldoret on May 2017. The site is at an altitude of (35° 16' 46'' E, 0° 31' 41'' N) and an elevation of 2118 meters above sea level. The plant was identified and a voucher specimen number JOY2017/001 issued. The voucher was later deposited at the School of Biological Sciences, University of Nairobi herbarium.

2.3. Methanol and hexane extracts of Urtica massaica

Two hundred grams of ground powdered leaves of U. massaica were soaked in 400ml of absolute methanol for 1hr after which the suspension was filtered using Whatman No. 1 filter paper and the filtrates freeze-dried using the Edwards Modulyo Freeze-drying machine to remove the solvent. The derived paste was stored as freeze-dried stock for later use. The procedure was repeated for stem and root grounded powders respectively. Hexane extracts of the respective plant parts was also derived following a similar procedure.

2.4. Preparation of stocks solution

One (1) g of crude methanol stock’s extracts of U. massaica leaves was weighed and dissolved in 100ml of dimethyl sulfoxide (DMSO). Eighty milliliters (ml) of this solution was obtained and topped up with 20 ml of distilled water to make 100 ml. This solution made a 80 ml/100 ml distilled water was obtained. A similar procedure was used to prepare stock’s and serially dilute solution for stems and roots as well as for crude hexane extracts of similar parts of U. massaica.

2.5. Bacillus thuringiensis israelensis (Bti)

Bacillus thuringiensis israelensis (Bti) used in this study was obtained from CGHR/KEMRI. 80 mg of it was dissolved in 11 of distilled water to make a stock’s solution. Bti has demonstrated efficacy as larvicide against mosquitoes (Uragayala et al., 2018; Derua et al., 2019), and it is on this basis that it was used as a positive control to compare to that of the test botanical extracts from U. massaica. 

2.6. Empirical activities

Three bioassay experiments were conducted to evaluate ovicidal, larvicidal and pupicidal potency of crude methanol and hexane extracts of leaf, stem and root of U. massaica against An. gambiae aquatic stages. In each experimental arrangement, three sets of plastic containers measuring 6 cm × 5.7 cm × 3.5 cm were used. To each container, approximately 33ml of a particular solution of either crude methanol or hexane extracts of leaf, stem or root of U. massaica was added. Four replicates for each concentration including appropriate controls were used. Standard WHO procedures and thresholds were used to assess effectiveness of the extracts as insecticides at a mortality rate of > 80% (WHO, 2005).

2.6.1. Ovicidal, Larvicidal and Pupicidal Bioassays

Freshly laid eggs of An. gambiae were collected from adult culturing cages, counted in batches of 10 under a dissecting microscope (Leica Zoom 2000) at ×10 magnification using fine tipped painting brushes and placed in smaller Whatman No. 1 filter papers. Each of such filter paper was then gently placed in containers. Mortality of the eggs was assessed 48 hrs post treatment by observing the eggs under dissecting microscope (Leica Zoom 2000) at ×10 magnification and noting if the egg was dead or alive. A dead egg was one that was non-hatched and with unopened opercula and a live egg was one that had hatched or with open operculum. Abbot’s formula (1925) was employed to correct percentage viability of eggs if control inhibition of egg hatching was between 5 % and 20 %. Egg mortality was calculated using the formula;

\[
\% \text{ egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs exposed}} \times 100
\]

Batches of ten freshly transformed third larval instars (L3) were collected and transferred by means of a dropper to plastic containers. The larvae were left exposed overnight. Moribund and dead larvae were put in a pail of hot water and dispensed in a septic tank. Larval mortality was registered 24 hours post exposure and mortality calculated using the formula;

\[
\% \text{ Larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of exposed larvae}} \times 100
\]

Ten early stage pupae (pupae metamorphosing from L4 larvae within a two-hour window) were randomly picked
from a tray containing such pupae using a dropper and placed individually in plastic containers. The mouth of each container was covered with mosquito netting to prevent emerged adult from escape. The pupae were exposed overnight and mortality rate determined 24 h later. This experiment was replicated four times. Mortality was calculated as well as corrected using Abbott’s formula (Abbott, 1925) as shown below;

\[
\text{% Mortality} = \frac{\text{Number of dead pupae}}{\text{Total number of pupae introduced}} \times 100
\]

\[
\text{% Corrected mortality} = \frac{\text{Percent mortality in test} - \text{Percent mortality in control}}{100 - \text{Percent in control}} \times 100
\]

2.7. Statistical Analysis

Data was entered in excel spreadsheets and the relationship between the effect of the crude methanol and hexane extracts of *U. massaica* on exposed eggs, larvae and pupae of *An. gambiae* were determined. Descriptive statistics was used to express the effect of the solvent of extraction, dose and part of plant used on exposed mosquito stages. One-way analysis of variance (ANOVA) was used to determine the level of significance of the impact of the extracts on the exposed mosquitoes. Student’s t test was used to compare effect of solvent on potency of extracts. All statistical analysis was performed using statistical package for social scientists (SPSS) version 22.

Figure 1: Effects of solvent of extraction on mortality of *An. gambiae* aquatic stages: Bti= *Bacillus thurigiensis israelensis* and DMSO= Dimethyl sulfoxide

Table 1: Effects of crude extracts from different parts of *U. massaica* on mortality of exposed *An. gambiae* eggs, larvae and pupae [% mortality is expressed as mean ± standard error of mean (SEM)]

<table>
<thead>
<tr>
<th>Dose (ml/100ml)</th>
<th>Parts of Plant extracted for botanicals</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stem</td>
<td>Roots</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>86.92 ± 7.97</td>
<td>99.96 ± 0.04</td>
<td>83.75 ± 7.29</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>70.96 ± 17.16</td>
<td>98.38 ± 1.58</td>
<td>76.38 ± 8.38</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>64.21 ± 18.02</td>
<td>93.21 ± 4.87</td>
<td>69.25 ± 10.68</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>53.17 ± 17.20</td>
<td>86.04 ± 7.93</td>
<td>64.29 ± 11.54</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>31.33 ± 9.53</td>
<td>49.00 ± 12.38</td>
<td>56.88 ± 12.35</td>
<td>2</td>
</tr>
<tr>
<td>2.5</td>
<td>22.50 ± 12.81</td>
<td>34.33 ± 13.83</td>
<td>42.58 ± 9.27</td>
<td>2</td>
</tr>
<tr>
<td>Bti</td>
<td>50.67 ± 20.45</td>
<td>34.67 ± 19.89</td>
<td>49.83 ± 20.97</td>
<td>2</td>
</tr>
<tr>
<td>DMSO</td>
<td>6.83 ± 1.68</td>
<td>4.67 ± 0.80</td>
<td>6.00 ± 1.77</td>
<td>2</td>
</tr>
<tr>
<td>Distilled water</td>
<td>4.17 ± 0.87</td>
<td>2.00 ± 0.86</td>
<td>4.00 ± 0.86</td>
<td>2</td>
</tr>
</tbody>
</table>

Notes: df = degree of freedom; F = the F statistical factor; P = probability for the level of significance. P was taken as significant at \( p < 0.05 \). BTI = *Bacillus thurigiensis israelensis*; DMSO = dimethyl sulfoxide.
Table 2: Effects of dose of methanolic or hexane crude extracts of *U. massaica* on mortality of *An. gambiae* eggs, larvae and pupae [% mortality is expressed as mean ± standard error of mean (SEM)]

<table>
<thead>
<tr>
<th>Dose (ml/100ml)</th>
<th>Exposed aquatic stages of <em>Anopheles gambiae</em></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs Larval Instar 3 (L3) Pupae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>88.13 ± 7.16b 100.00 ± 0.00b 82.50 ± 7.93b</td>
<td>2</td>
<td>2.097</td>
<td>0.157</td>
</tr>
<tr>
<td>40</td>
<td>76.54 ± 10.10b 100.00 ± 0.00b 69.17 ± 15.67b</td>
<td>2</td>
<td>2.236</td>
<td>0.141</td>
</tr>
<tr>
<td>20</td>
<td>61.67 ± 9.75a 100.00 ± 0.00a 65.00 ± 16.82a</td>
<td>2</td>
<td>3.578</td>
<td>0.054</td>
</tr>
<tr>
<td>10</td>
<td>51.00 ± 7.54b 100.00 ± 0.00b 62.92 ± 8.08b</td>
<td>2</td>
<td>2.616</td>
<td>0.106</td>
</tr>
<tr>
<td>5</td>
<td>40.13 ± 7.85a 70.00 ± 12.37a 27.08 ± 7.54a</td>
<td>2</td>
<td>5.350</td>
<td>0.018</td>
</tr>
<tr>
<td>2.5</td>
<td>28.58 ± 2.96b 54.58 ± 16.58b 16.25 ± 6.73b</td>
<td>2</td>
<td>3.493</td>
<td>0.057</td>
</tr>
<tr>
<td>BTI</td>
<td>81.33 ± 15.67a 35.67 ± 19.10a 18.17 ± 15.80a</td>
<td>2</td>
<td>3.710</td>
<td>0.049</td>
</tr>
<tr>
<td>DMSO</td>
<td>6.00 ± 1.63b 7.50 ± 1.59b 4.00 ± 0.82b</td>
<td>2</td>
<td>1.581</td>
<td>0.238</td>
</tr>
<tr>
<td>Distilled water</td>
<td>3.00 ± 0.82b 3.33 ± 1.05b 3.83 ± 0.98b</td>
<td>2</td>
<td>0.193</td>
<td>0.827</td>
</tr>
</tbody>
</table>

Notes: df = degree of freedom; F = the F statistical factor; P = probability for the level of significance. P was taken as significant at p < 0.05. Bti = *Bacillus thurigiensis israelensis*; DMSO = dimethyl sulfoxide. Rows having mean percentage mortality superscripted with letter “a” indicate a significant influence of dose on exposed *An. gambiae* aquatic stages

Table 3: Comparative performance of hexane and methanol crude extracts of *U. massaica* leaves on *An. gambiae* eggs, larvae and pupae [% mortality is expressed as mean ± standard error of mean (SEM)]

<table>
<thead>
<tr>
<th>Part of plant</th>
<th>Mosquito stage</th>
<th>Solvent</th>
<th>N</th>
<th>Mean ± SEM</th>
<th>t</th>
<th>df</th>
<th>P (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>eggs</td>
<td>Methanol</td>
<td>6</td>
<td>68.71 ± 9.84a</td>
<td>6.983</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>25.79 ± 7.05a</td>
<td>3.656</td>
<td>5</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>Methanol</td>
<td>6</td>
<td>87.50 ± 9.44a</td>
<td>9.266</td>
<td>5</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>61.67 ± 17.93a</td>
<td>3.439</td>
<td>5</td>
<td>0.018</td>
</tr>
<tr>
<td>Pupae</td>
<td></td>
<td>Methanol</td>
<td>6</td>
<td>73.33 ± 17.17a</td>
<td>4.271</td>
<td>5</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>12.08 ± 10.62b</td>
<td>1.138</td>
<td>5</td>
<td>0.307</td>
</tr>
</tbody>
</table>

Notes: df = degree of freedom; t = the t statistical factor for student t test; P = probability for the level of significance. P was taken as significant at p < 0.05 for a two tailed test. N = total number of considered samples for the t test; Rows having mean percentage mortality superscripted with letter “a” indicate a significant influence of *U. massaica* leaf extracts on exposed *An. gambiae* aquatic stages

Table 4: Comparative performance of hexane and methanol crude extracts of *U. Massaica* stems on *An. gambiae* eggs, larvae and pupae [% mortality is expressed as mean ± standard error of mean (SEM)]

<table>
<thead>
<tr>
<th>Part of plant</th>
<th>Mosquito stage</th>
<th>Solvent</th>
<th>N</th>
<th>Mean ± SEM</th>
<th>t</th>
<th>df</th>
<th>P (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>eggs</td>
<td>Methanol</td>
<td>6</td>
<td>74.13 ± 11.00a</td>
<td>6.740</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>62.21 ± 12.37a</td>
<td>5.029</td>
<td>5</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>Methanol</td>
<td>6</td>
<td>98.33 ± 1.31a</td>
<td>75.173</td>
<td>5</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>80.42 ± 12.89a</td>
<td>6.241</td>
<td>5</td>
<td>0.002</td>
</tr>
<tr>
<td>Pupae</td>
<td></td>
<td>Methanol</td>
<td>6</td>
<td>69.17 ± 19.51a</td>
<td>3.545</td>
<td>5</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>75.00 ± 15.26a</td>
<td>4.914</td>
<td>5</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Notes: df = degree of freedom; t = the t statistical factor for student t test; P = probability for the level of significance. P was taken as significant at p < 0.05 for a two tailed test. N = total number of considered samples for the t test; Rows having mean percentage mortality superscripted with the same letter indicate a significant influence of *U. massaica* stem extracts on exposed *An. gambiae* aquatic stages

Table 5: Comparative performance of hexane and methanol crude extracts of *U. Massaica* roots on *An. gambiae* eggs, larvae and pupae [% mortality is expressed as mean ± standard error of mean (SEM)]

<table>
<thead>
<tr>
<th>Part of plant</th>
<th>Mosquito stage</th>
<th>Solvent</th>
<th>N</th>
<th>Mean ± SEM</th>
<th>t</th>
<th>df</th>
<th>P (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>eggs</td>
<td>Methanol</td>
<td>6</td>
<td>52.17 ± 7.00a</td>
<td>7.455</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>63.04 ± 10.40a</td>
<td>6.062</td>
<td>5</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>Methanol</td>
<td>6</td>
<td>86.67 ± 11.45a</td>
<td>7.569</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>97.92 ± 2.08a</td>
<td>47.000</td>
<td>5</td>
<td>0.000</td>
</tr>
<tr>
<td>Pupae</td>
<td></td>
<td>Methanol</td>
<td>6</td>
<td>50.83 ± 4.22a</td>
<td>12.056</td>
<td>5</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexane</td>
<td>6</td>
<td>42.50 ± 5.16a</td>
<td>8.230</td>
<td>5</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Notes: df = degree of freedom; t = the t statistical factor for student t test; P = probability for the level of significance. P was taken as significant at p < 0.05 for a two tailed test. N = total number of considered samples for the t test; Rows having mean percentage mortality superscripted with the same letter indicate a significant influence of *U. massaica* root extracts on exposed *An. gambiae* aquatic stages
3. Results

Solvent of extraction had an impact on potency of extracts as ovicides, larvicides as well as pupicides. Methanol extracts were more potent than hexane extracts regardless of the dose. Potency of extracts reduced with reduced dose irrespective of solvent of extraction. Doses of 80 ml/100ml killed >80% of exposed An. gambiae immature stages (Figure 1). Both methanol and hexane extracts were more potent than Bacillus thuringiensis israelensis (Bti); however, none of the observed mortalities were significantly different (p > 0.05) regardless of dose or solvent of extraction. Similarly, stem extracts were more potent than root or leaf regardless of dose though the observed mortalities did not differ significantly (p > 0.05), irrespective of dose (Table 1).

Larvae (L3) were more susceptible to U. massaica crude extracts than either eggs or pupae. Mortalities were, however, dose dependent with doses of 10 ml and above killing all exposed L3. Interestingly, preparations of Bti were more effective on eggs than L3 or pupae. Observed mortalities for 20 ml were significantly different (p < 0.05), while the rest were not (p > 0.05) (Table 2).

Methanol extracts of leaves were more potent than hexane extracts of the same part for exposed eggs and L3 in all observed cases. Mortalities from exposure to methanol extracts of leaves were significantly different for all stages at p < 0.05, while that of exposure to hexane extracts were significantly different for eggs and L3 but not for pupae (Table 3). A similar trend was observed for extracts of stem albeit with slight difference (Table 4).

Hexane extracts of roots were more potent than methanol extracts for the same part to exposed eggs and L3. The trend, however, was different for pupae where methanol extracts were more potent than those of hexane. Observed mortalities were, however, significantly different at p < 0.05 (Table 5) irrespective of solvent of extraction or stage used.

4. Discussion

Aquatic mosquito stages (eggs, larvae and pupae) are “sitting ducks” as they are unlikely to escape from the habitat and, therefore, easy to control than the highly mobile winged adults. A control program focused on eliminating mosquito eggs; larvae or pupae is likely to be more effective in reducing mosquito population (Chung et al., 2009; Conti et al., 2010). If such a strategy employs the use of botanicals, then it may not only be used to mitigate the problem of vector resistance, but also help to reduce the undesirable effect on human health and environment resulting from the use of synthetic insecticides (Govindarajan et al., 2016). By virtue of the fact that growing of plants universally encourages as a strategy to increase vegetation on planet earth, the plant-based insecticides will not only be readily available in many areas, but the product can be easily and cheaply acquired.

In this study the most vulnerable aquatic stage to the toxic effect of U. massaica was the larvae (L3). This is because they were totally annihilated, especially when exposed to high doses. This finding was consistent with that of Plectranthus glandulosus and Callistemon rigidus leaves extracts against fourth larval instars (L4s) of Ae. aegypti, An. gambiae and Cx. Quinquefasciatus (Pierre et al., 2014) and ethanol and water extracts of Phytolacca dodecandra against all larval stages of An. gambiae (Yugi et al., 2015).

It was observed that high doses of the extracts were even more lethal to the L3. Indeed doses higher than 10 ml killed all exposed L3. Of the exposed aquatic stages, only L3 feed. Eggs and pupae do not feed. It is safe to say that the mode of action of the toxic extracts was due to gut poisoning and that the toxic effect of the extracts was delivered most effectively through the gut. It could be said that the L3 might have accumulated (through ingestion) large doses of the poison in their gut while feeding, and that the higher doses were responsible for the observed fatalities (Nathan et al., 2005; Akinkurolere et al., 2011; Ileke and Ogungbile, 2015). This finding was similar to that observed for Terminalia chebula against larvae and eggs of An. stephensi, Ae. aegypti and Cx quinquefasciatus (Thangapandi et al., 2017) where it was noted that higher doses of the botanicals yielded better mortality rates on mosquito immatures. In this study, doses of 80ml and above met WHO threshold for an effective insecticide (>80% mortality) irrespective of solvent used in the extraction.

It was also observed that exposed An. gambiae eggs and pupae failed to hatch or eclove to adults respectively. An. gambiae eggs as well as pupae are non-feeding and could not ingest toxic U. massaica extracts. The fact that the eggs failed to hatch or pupae to moult to adults demonstrate that the mode of action of the toxicants was not only enteric but topical as well. This finding was similar to that of Phytolacca dodecandra plant extracts against An. gambiae eggs (Yugi and Kiplimo, 2017) and pupae (Yugi et al., 2017).

Interestingly, solutions of Bacillus thuringiensis israelensis (Bti), inhibited more eggs from hatching than it killed exposed L3. Bti has proven bioefficacy against mosquito larvae (Uragayala et al., 2018; Derua et al., 2019). It affects the midgut epithelium of affected larvae (de Barjac 1978) by enhancing swelling and bursting of cells herein causing severe damage to the gut wall (de Barjac 1978; Kalfon et al., 1984) and death to the larvae. Earlier, it had been shown that though Bti had effect on oviposition behaviour of mosquitoes, it had no effect on either the adults or their eggs (Futami et al., 2011). The observation made herein of Bti on An. gambiae eggs is, therefore, unique and is neither finding support nor given meaning by the demonstrations mentioned above.

Indeed, extracts of plants, prepared using specific solvents had been shown to influence bioactivity, probably because of the concentration of active components present therein (Oliveira et al., 2010). This was also reported for crude benzene, hexane, ethyl acetate, chloroform and methanol extracts of leaf of T. chebula against A. stephensi, A. aegypti, and C. quinquefasciatus (Thangapandi et al., 2017). In the current study, methanol extracts were more potent than hexane extracts, an observation that was similar to that made by Munusamy et al. (2016) on ovicidal and larvicidal activities of some plant extracts on Aedes aegypti L. and Culex quinquefasciatus Say (Diptera: Culicidae). It
would seem here that methanol facilitated optimal extraction of the botanicals due to its high polarity.

In the current study, it was found that extracts from the stem were more potent followed by roots and then leaves though mortalities arising from the exposures were not significantly different irrespective of dose. For some time, it had been known that different parts of plants (leaves, fruits, seeds, roots and bark) contained polyphenols or secondary metabolites (flavanols, anthocyanins and phenolic acids). The polyphenols are the components responsible for free radical scavenging activity (Mathew and Abraham 2006) as well as unique biological activity (Govindarajan et al., 2008) including mosquitocidal properties (Niraimathi et al., 2010; Ramkumar et al., 2015).

Concentrations of botanicals have been known to be differentially distributed within plant parts, with parts of plants with higher concentrations demonstrating high bioassay potency (Yugi and Kiplimo, 2017). In the present study, extracts from the stem killed a higher percentage of exposed aquatic stages than extract from leaves or roots irrespective of solvent or dose. It would be correct to assume that stems of U. massaica contain a higher concentration of botanicals than either leaves or roots. If this be true, then the findings of this study are consistent with that of Mgbechema, (2010), Anupam et al., (2012) and Yugi and Kiplimo, (2017) that reported on differential vertical distribution of polyphenols commensurate with the reported levels of bio-potency of extracts from different plant parts. This however was inconsistent with the findings by Rafajlovska et al. (2013) that showed that the concentration of botanicals in stinging nettles did indeed differ in distribution vertically along the length of stinging nettles but that the leaves and not the stem had higher quantities of the polyphenols followed by stems and then roots. This was confirmed by Pinelli et al. (2008) who demonstrated in their study that roots of stinging nettles indeed contained the least concentration of botanicals.

The present study clearly proves, therefore, that crude extracts of U. massaica has impressive ovicidal, larvicidal and pupicidal properties against An. gambiae, and that methanol and hexane extracts of leaves, stem and roots of this herb have insecticidal ability. This puts U. massaica in the same category with plants with insecticidal properties such as Anacardium occidentale, Afromomum melegueta, Garcinia kola and Citrus sinensis (Ileke et al., 2014) and a few others with ovicidal, larvicidal and pupicidal potential against Anopheles gambiae and Aedes aegypti mosquitoes (Raveen et al., 2017). Although the effects of pure samples of U. massaica were never experimented on either singly or synergistically against An. gambiae aquatic stages, it may be postulated that the complex mixtures of active components of crude methanol and hexane extracts (Oliveira et al., 2010) of different parts of U. massaica acted synergistically to show greater overall bioactivity compared to the individual constituents (Sumroiphon et al., 2006).

It is, therefore, our submission that although the findings of this study proves the mosquitocidal potential of U. massaica extracts on aquatic stages of An. gambiae mosquitoes, we recommend that extracts be isolated to pure compounds to determine their impacts before the development of natural mosquitocidal products to complement synthetic insecticides is done.

Authors' contributions

YJO conceived the concept, conducted the statistical analysis and wrote the manuscript. YJO and SV designed, supervised and guided the experiments. KRT cultured the experimental mosquitoes and conducted the experiments.

Competing interests

The authors declare that they have no competing interests and that U. massaica and Bacillus thuringiensis israelensis (Bti) were used purely for experimental purposes only.

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