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Ovicidal, Larvicidal and Pupicidal Efficacy of Crude Methanol and Hexane Extract of *Urtica massaica* Mildbri 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 cm^3/100ml (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 thurigiensis*

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 (Ko¨rpe *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

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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 on mosquitocidal 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 thurigiensis 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 (35^0 16' 46'' E, 0^0 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/100ml of distilled water. A second quantity of 80ml of the stock's solution was prepared but toped up with 120 ml of distilled water. This solution was then apportioned in two beakers of equal capacity (100ml) each with a concentration of 40 ml/100ml (s/w). One of this was picked and 100ml distilled water added and later apportioned in two beakers of equal capacity with each having 20ml/100ml (s/w). This procedure was repeated until serial dilution of 80, 40, 20, 10, 5 and 2.5 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. Baccillus thurigiensis israeliensis (Bti)

Baccillus thurigiensis israeliensis (Bti) used in this study was obtained from CGHR/KEMRI. 80 mg of it was dissolved in 1l 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;

$$\% \text{ egg mortality} = \frac{\text{Number of hatched laevae}}{\text{Total number of eggs exposed}} \times 100$$

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.

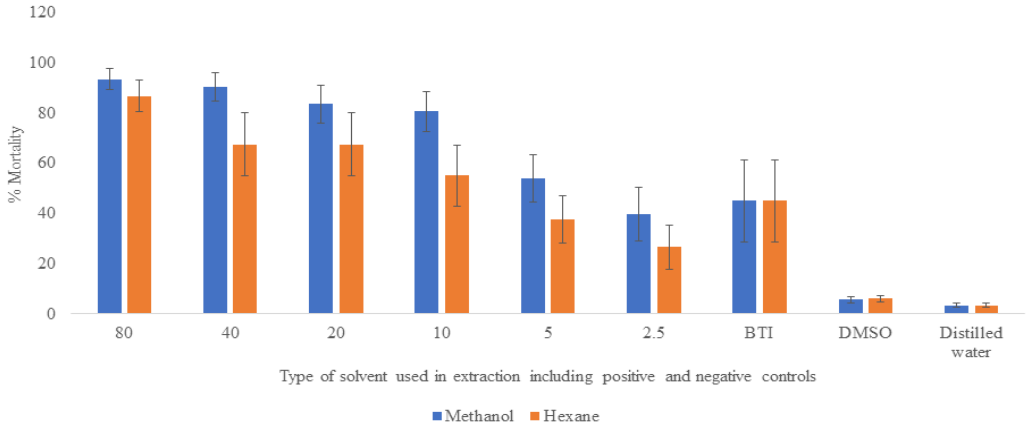

Figure 1: Effects of solvent of extraction on mortality of *An. gambiae* aquatic stages: Bti**=** *Bacillus thurigiensis israelensis* and **DMSO=** Dimethyl sulfoxide

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)]

Dose (ml/100ml)	Parts of Plant extracted for botanicals			df	F	p
	Leaves	Stem	Roots			
80	86.92 ± 7.97	99.96 ± 0.04	83.75 ± 7.29	2	1.899	0.184
40	70.96 ± 17.16	98.38 ± 1.58	76.38 ± 8.38	2	1.723	0.212
20	64.21 ± 18.02	93.21 ± 4.87	69.25 ± 10.68	2	1.558	0.243
10	53.17 ± 17.20	86.04 ± 7.93	64.29 ± 11.54	2	1.705	0.215
5	31.33 ± 9.53	49.00 ± 12.38	56.88 ± 12.35	2	1.294	0.303
2.5	22.50 ± 12.81	34.33 ± 13.83	42.58 ± 9.27	2	0.693	0.515
BTI	50.67 ± 20.45	34.67 ± 19.89	49.83 ± 20.97	2	0.194	0.826
DMSO	6.83 ± 1.68	4.67 ± 0.80	6.00 ± 1.77	2	0.542	0.592
Distilled water	4.17 ± 0.87	2.00 ± 0.86	4.00 ± 0.86	2	1.958	0.176

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)]

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

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)]

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

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)]

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

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)]

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

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 thurigiensis 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 Ogungbite, 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 eclode 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 thurigiensis 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 busting 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 Mgbemena, (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,Garcina 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 synagestically against *An. gabiae* 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 thurigiensis israelensis* (Bti) were used purely for experimental purposes only.

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