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Ovicidal and Larvicidal Effects of *Ricinus communis* L. (*Euphorbiaceae*) Extracts on *Phlebotomus duboscqi*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MS and N. Peter designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NZ and N. Philip provided expert ideas on leishmaniasis in the country. Author JI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the Ovicidal and larvicidal effects of *Ricinus communis* (Euphorbiaceae) extracts on *Phlebotomus duboscqi*.
Study Design: A comparative experimental design using extracts obtained from the leaf and bark of *Ricinus communis* plant.
Study Site: Kenya Medical Research Institute, Centre for Biotechnology Research and Development (CBRD), Nairobi Kenya from January to July, 2015.
Methodology: Aqueous, methanol and ethyl acetate extracts were prepared from *Ricinus*

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communis plant. Freshly laid eggs were moistened with 1 ml of each extract separately during the incubation period. Larvae were also fed on larval food mixed with the powdered crude extract and a second group was fed on larval food sprinkled with 5 ml of each extract daily.
Results: No significant difference when bark and leaf extracts were compared (P=0.061). 250 µg/ml and 500 µg/ml of extract eroded all the chorionic membranes of the egg shell while egg hatchability was significantly inhibited with only 7%, 9% and 26% of eggs hatching at 500 µg/ml of aqueous, methanol and ethyl acetate extracts respectively (P<0.001). 100% larval mortality noted when at 500 µg/ml methanol extract. The larval period was prolonged to 87 days with the life cycle lasting for 101 days.
Conclusion: *R. communis* extracts have deleterious effects on hatching of eggs, larval and pupal development and adult emergence of *P. duboscqi*; hence *R. communis* should be used against sand flies and *Leishmania in situ*.

Keywords: P. duboscqi; Ricinus communis; ovicidal; larvicidal; mortality; sculpturing.

1. INTRODUCTION

Sand flies in the genus *Phlebotomus* spread a viral agent pappataci virus (an arbovirus) that causes sand fly fever (pappataci fever) as well as protozoan pathogens (*Leishmania* spp) that causes leishmaniasis [1]. In Kenya, the two prevalent forms of leishmaniases are cutaneous leishmaniasis and visceral leishmaniasis caused by *Leishmania major* and *Leishmania donovani* respectively. The vectors are *P. duboscqi* for *L. major* and *P. martini* for *L. donovani* [2]. Species in three genera, *Phlebotomus, Lutzomyia* and *Sergentomyia*, suck blood from vertebrates but only the former two transmit leishmaniases to man [3] infecting more than 350 million people in more than 80 countries world wide [4].

Vector control programmes for sand flies include spraying houses and other habitats with insecticides. The effectiveness of these spraying programmes is not the only issue for concern but their side effects are also important on health and environment, and their potential for sustainability, which depends on the cost of the insecticides and their application. Sand flies have also developed resistance to the chemicals [1]. Moreover, the sand fly characteristically feeds at dusk, and, being a weak flier, tends to remain close to its breeding area, not too high from the ground. This makes it difficult to spray the immature stages which are inaccessible and dispersed in animal burrows.

Ricinus communis (Castor bean plant) is a small wooden tree which grows to about 6 meters in height widespread throughout tropical regions as ornamental plants. Stems of *R. communis* have Anticancer, antidiabetic and antiprotozoal activity [5]. The aerial parts of *R. communis* extract have been shown to possess insecticidal activity against a wide range of haematophagus insects [6]. Further research shows that *R. communis* extract has larvicidal effects with 100% killing activities for *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes albopictus* larvae [5]. Both cutaneous and visceral leishmaniases are andemic in Kenya and are transmitted by bites of infected female *Phlebotomus* sand flies. There are also plenty of medicinal plants with toxic phytoconstituents but their efficacy has not been tested against such vectors. Therefore, this study sought to assess the ovicidal and larvicidal effects of *R. communis* (Euphorbiaceae) extract on *Phlebotomus duboscqi* flies as an alternative for controlling sand flies. This is because the available chemical insecticides pose adverse effects to the user and the environment. Sand flies have also developed resistance to the chemicals, mainly to DDT and in some cases to Malathion and pyrethroids [1]. There is need to explore substances of natural origin because they are likely to lower the high cost of treatment, reduce resistance of drugs & insecticides and also reduce environmental pollution.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out from January to July, 2015. Ovicidal and larvicidal experiments were carried out at the Kenya Medical Research Institute, Centre for Biotechnology Research and Development (CBRD), Nairobi.

2.2 Study Design

A comparative experimental design using extracts obtained from the leaf and bark of *R. communis* plant. Efficacy of the different

extracts was assessed based on their effects on egg sculpturing and on their ability to inhibit larval metamorphosis and adult emergence.

2.3 Sand Fly Colony

A colony of *Phlebotomus duboscqi* Neveu Lemaire which is being reared at KEMRI for research purposes was used. Female sand flies were blood fed using Syrian golden hamsters for egg development. Blood feeding of *P. duboscqi* involved anaesthetizing a hamster with sodium pentobarbitone, shaving its lower belly and introducing it into the cage containing sand flies. Almost an equivalent number of males were included for purposes of copulation. Temperature was maintained at 25±1℃ relative humidity of 78-83% and a 12:12 (light:dark) photoperiod.

2.4 Collection and Preparation of *Ricinus communis*

Stems, floral and foliar parts of *R. communis* were collected from Suswa, Narok County, Kenya. Botanical identification was carried out with the help of taxonomists from the National Museums of Kenya. All the collected parts of the plants were left to dry completely under a shade for one month and then transported to the laboratory where they were left to dry further under room temperature.

Extraction of castor bean leaf and bark was carried out as described earlier [7]. Briefly, 600 ml of methanol were added to 300 g of the shred specimen and flasks placed on a shaker and soaked for 48 hours. The residue was filtered using a Buchner funnel under vacuum until the sample dried. The sample was soaked further with 600 ml methanol for 24 h until the filtrate remained clear. The filtrate was then concentrated under vacuum by rotary evaporation at 30 - 35℃. The concentrate was later transferred to a sample bottle and dried under vacuum using a rotary evaporator; the weight of the dry extract was recorded and stored at 4℃ until required for bioassay. The process was repeated for ethyl acetate and water.

2.5 Evaluation of Toxicity Test

Fifteen *Phlebotomus duboscqi* flies were fed on sugar solution mixed with the crude extracts in the ratio of 1:1 of several concentrations (1 mg/ml to 20 mg/ml) of test compounds. Mortality was assessed daily by counting dead flies in order to evaluate the minimum inhibition concentration (MIC). The lowest concentration of the samples that killed the sand flies was considered the MIC.

2.6 Experiment 1: Ovicidal Effects of *Ricinus communis* Extracts

Five blood-fed *P. duboscqi* females were aspirated into each oviposition vial partially filled with plaster of Paris and fitted with screen tops. They were fed on drops of Karo dark corn syrup (Best Foods, CPC International, Inc., Englewood Cliffs, NJ) placed on the screen tops of the oviposition vials. The set up was then observed for 7-10 days for egg laying. Various concentrations of *R. communis* extract were prepared and adjusted to parts per million (µg/ml). A total of 130 freshly laid eggs were then moistened with 1 ml of the aqueous extract of *R. communis* on 0 day post-oviposition. Another group (the control) was moistened with 1ml distilled water. These eggs were then incubated at 25±2℃ and 70±5% RH for hatching while being moistened with 1ml of extract daily. Two eggs from each vial were picked daily using a water-moistened applicator stick. They were then transferred to a microscope slide where a drop of gum chloral was applied. The eggs were then covered with a cover slip and left to dry for one day. They were then observed using a light microscope for any morphological changes on the chorionic membrane. The numbers of eggs that were hatching were recorded. The same experiment was repeated for methanolic and ethyl acetate leaf and bark extracts of *R. communis.* Percentage hatchability was calculated as follows:

$$\%\ \text{hatchability} = \frac{\text{Number of larvae hatched}}{\text{Total number of eggs}} X\ 100$$

2.7 Experiment 2: Larvicidal Effects of *R. communis* Extracts

Larvicidal effects of *R. communis* were assessed previously described [8]. Briefly, after hatching, the larvae were put into four groups and treated as follows: first group of the larvae were fed on larval food mixed with the powdered crude extract (1:1), second group was fed on larval food sprinkled with 5ml of the aqueous extract, third group was fed on powdered extract only while the fourth group (control) consisted of larvae fed on larval food only. In each group, 100-150 larvae were used for bioassays.

Preliminary screening of extracts was carried out at a range of concentrations ranging from 0.016 to 20,000 µg/ml; each extract was mixed with the larval diet ingredients during preparation. Based on preliminary screening results, all of the extracts were subjected to concentration-response bioassays for larvicidal activities against *P. duboscqi.* Larvae were observed daily for any deaths. Any dead larvae were removed and mounted using gum chloral, left to dry for a day then examined for any morphological changes. These larvae were also examined for any transformation to second, third, fourth instars, pupae and adult emergence. The following parameters were evaluated: the length of the larval and pupal periods; the larval and pupal viability, Mean lethal dosage (LD_{50}) after every 24 hours was determined.

$$\% \text{ Observed Mortality} = \frac{\text{Test Mortality} - \text{Control Mortality}}{100 - \textit{Control Mortality}} X\ 100$$

3. RESULTS

3.1 Effect of *R. communis* Extract on the Egg

When 125 µg/ml of *R. communis* leaf extract was used to moisten the incubated eggs, all the eggs were seen to have thin and smooth chorionic layers with very little sculpturing. This observation was made microscopically at 7 days post oviposition. The eggs were also swollen within the first 48 hours post treatment. At a concentration of 250 µg/ml of *R. communis* leaf extract, the exochorionic membrane of the egg was eroded. There was faint sculpturing of the egg hence loss of most of the exochorion layer. At 500 µg/ml, the egg lost its colour becoming more translucent and swollen compared to the control. Exochorion and mesochorion layers of the egg were eroded by the extract and a transverse line was seen on the egg representing the plastron (plate 1). The egg in the control vial was amber in colour, outer chorion was sculptured with a series of ridges cross-linked irregularly and both poles were rounded.

Treating eggs with *R. communis* bark extract showed similar effects as those seen under *R. communis* leaf extract. However, there was little loss of exochorion under 125 µg/ml extract concentration and severe loss of exochorion and mesochorion at 500 µg/ml (plate 2)

At 14 days post treatment, the egg lost all the exochorion, mesochorion and endochorion as shown below (plate 3). These eggs never hatched.

3.2 Effect of Extract on Egg Hatchability

Increase in concentration of the extract lead to increased inhibition of egg hatchability. However, there was no significant difference in hatchability when bark and leaf extracts were used (*P*=0.061). Egg hatching in the treatment group was first observed at 11 days post-oviposition. On average, the incubation period of eggs treated with *R. communis* extract was 15±37 days while in the control group, hatching occurred 10±0.15 days post-oviposition. 500 µg/ml concentration had 100% inhibition on egg hatchability in both leaf and bark extracts.

In the leaf aqueous extract of *R. communis*, % hatchability was 38% (43.33±1.66) at 125 µg/ml, 29% (35.33±1.45) at 250 µg/ml, 7% (15.00±2.88) at 500 µg/ml while in the control experiment hatchability was 73% (92.67±3.92). Significant effects were observed when comparing hatchability in the control group with hatchability in all the three concentrations (*P*<0.001). In the methanolic leaf extracts of *R. communis*, % hatchability was 34% (39.03±2.33) at 125 µg/ml, 21% (29.67±2.40) at 250 µg/ml, 9% (13.33±1.67) at 500 µg/ml while in the untreated group; hatchability was 84% (96.67±7.26). Severe effects were observed at 500 µg/ml with a significant effect (*P*<0.001).

In the ethyl acetate extract 37% (47.00±1.53) of the eggs hatched at a concentration of 125 µg/ml, 36% (41.00±2.08) at 250 µg/ml, 26% (29.67±7.68) at 500 µg/ml while 73% (108.67±1.85) hatched in the control group (*P*=0.016). No significant difference when comparing hatchability of eggs at a concentration of 125 µg/ml and 250 µg/ml (*P*=0.073). However, a significant difference was noted when comparing hatchability in the treatment group with those in the control group (*P*=0.002) (Fig. 1).

Bark extracts of *R. communis* showed more severe effects on egg hatchability when compared to the extracts from the leaves. Severe effects were exhibited by the methanolic extracts at a concentration of 500 µg/ml where 5% (10.00±1.33) of the eggs hatched. At a concentration of 250 µg/ml, 15% (22.33±0.67) of eggs hatched while 29% (38.13±1.67) of eggs hatched at 125 µg/ml. In the control group, 75% (98.67±2.33) of eggs hatched. The observed difference was significant (P=0.001).

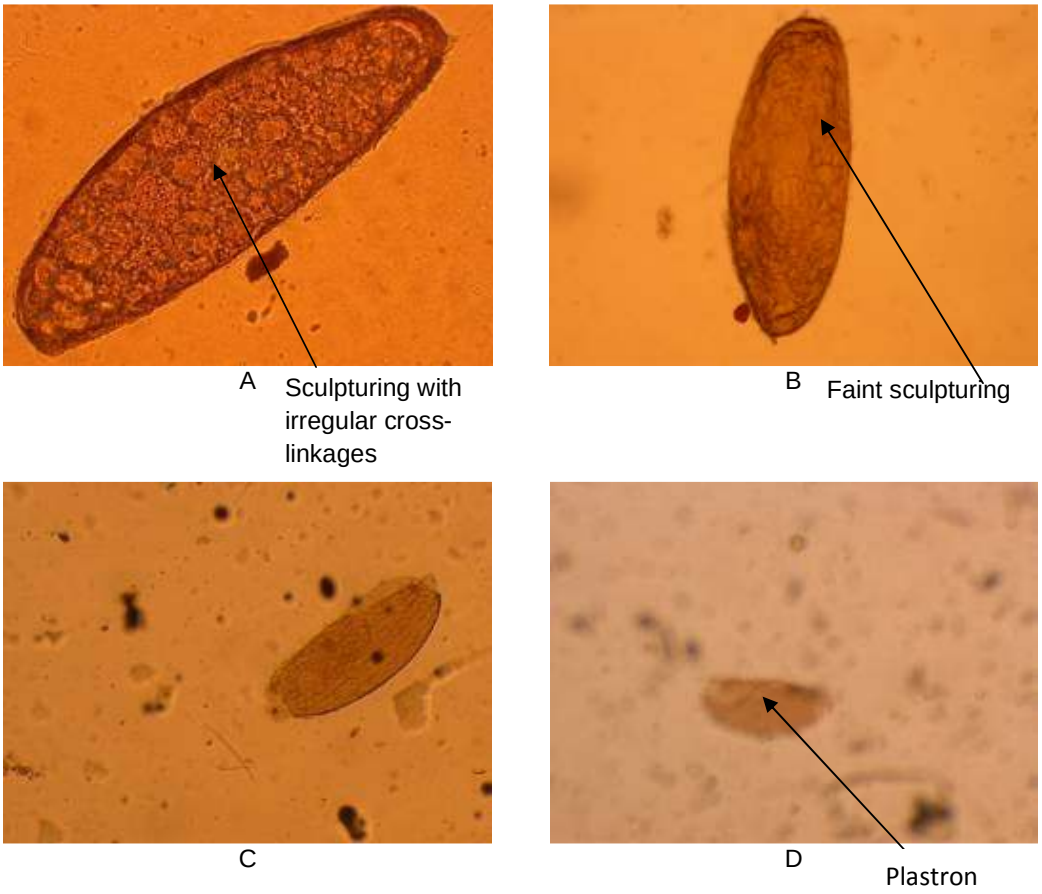

Plate 1. (a) A normal (control) *P. duboscqi* egg at X200 magnification, (b) egg treated with 125 µg/ml *R. communis* leaf extract, (c) *P. duboscqi* egg at 250 µg/ml *R. communis* leaf extract (d) *P. duboscqi* egg at 500 µg/ml *R. communis* leaf extract

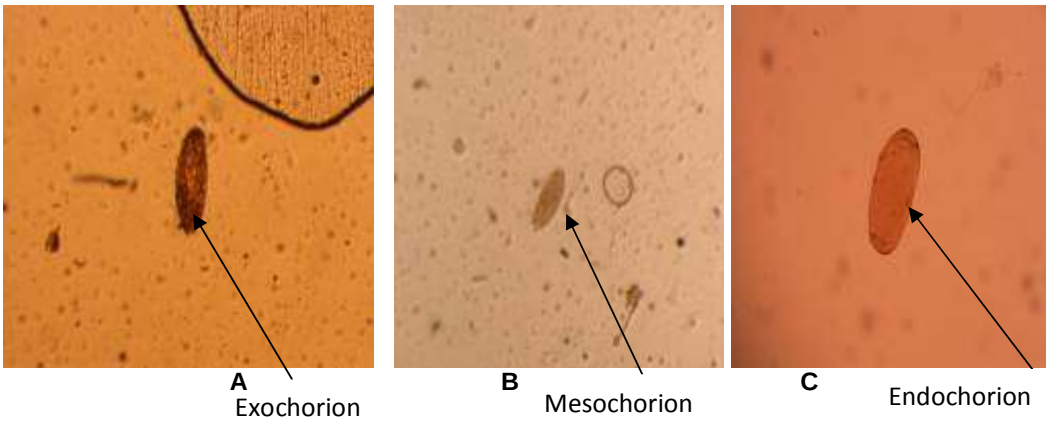

Plate 2. (a) *P. duboscqi* egg treated with 125µg/ml extract (b) *P. duboscqi* egg treated with 250µg/ml extract (c) *P. duboscqi* egg treated with 500 µg/ml extract

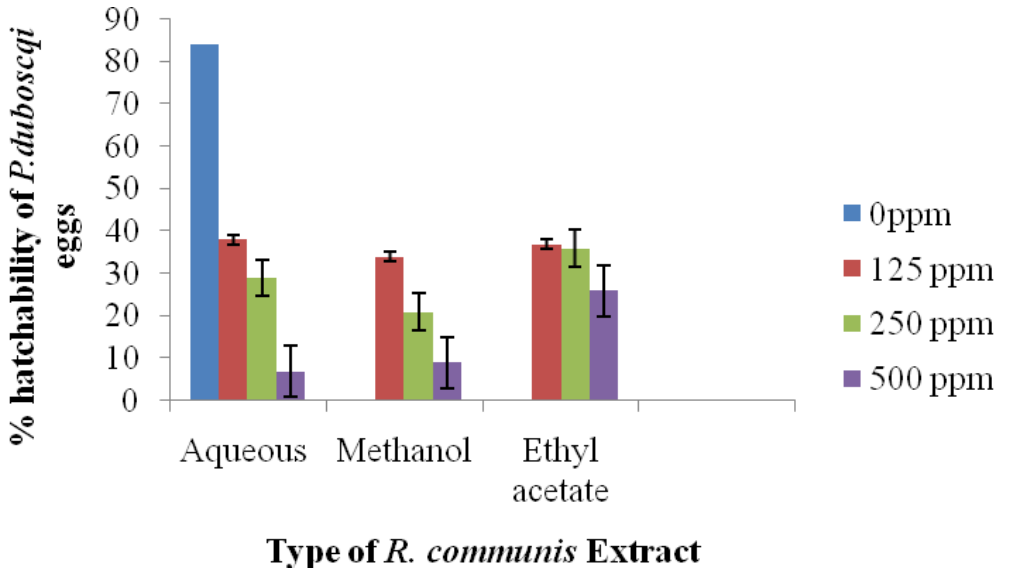

Fig. 1. % Hatchability of *P. duboscqi* eggs treated with *R. communis* Leaf extracts

Plate 3. Egg at 500 µg/ml bark extract (14 days post treatment) lost all the chorionic membranes

In the ethyl acetate extracts of *R. communis*, percentage hatchability was 35% (60.00±1.66) at 125 µg/ml, 30% (35.13±3.60) at 250 µg/ml, while at 500 µg/ml, 19% (20.66±1.86) of eggs hatched compared to 88% (105.00±3.33) in the control group (*P*=0.000). Moderate effects of the extracts on egg hatchability were observed in the aqueous extracts where 40% (45.15±0.65) of the eggs hatched at 125 µg/ml, 25% (30.33±0.87) at 250 µg/ml and 8% (17.00±1.67) of eggs hatched at 500 µg/ml (Fig. 2). 86.3% (98.58±2.42) of eggs from the control group had hatched (*P*=0.001). The extracts of *R. communis* at 500 µg/ml showed significant reduction in the hatchability rate compared to the other concentrations and the control group; in the methanolic extract (*F*=19. 87, df= 3, 11, *P*=0.001), in aqueous extract (*F*=44. 61, df= 3, 11, *P*=0.001) and in ethyl acetate extract (*F*=39. 83, df= 3, 11, *P*=0.001).

3.3 Larvicidal Effects of *R. communis* Extracts

Methanolic extract was the most active against *P. duboscqi* larvae followed by aqueous extract then ethyl acetate extract. The LC_{50} values for *R. communis* bark extracts at 24 hours post treatment were 241.45 µg/ml, 219.14 µg/ml and 310.10 µg/ml for aqueous, methanol and ethyl acetate extracts respectively. At 48 hour post treatment, LC_{50} values observed were 192.20 µg/ml, 187.32 µg/ml and 262.40 µg/ml for aqueous, methanol and ethyl acetate extracts respectively.

When *R. communis* leaf extracts were used against *P. duboscqi* larvae, the values for LC_{50} at 24 hours post treatment were 249.55 µg/ml, 228.51 µg/ml and 322.99 µg/ml for aqueous, methanol and ethyl acetate extracts respectively. Also at 48 hours post treatment, LC_{50} values changed to 205.30 µg/ml, 199.38 µg/ml and 287.44 µg/ml for aqueous, methanol and ethyl acetate extracts respectively. LC50 values decreased further with increase in the post treatment period.

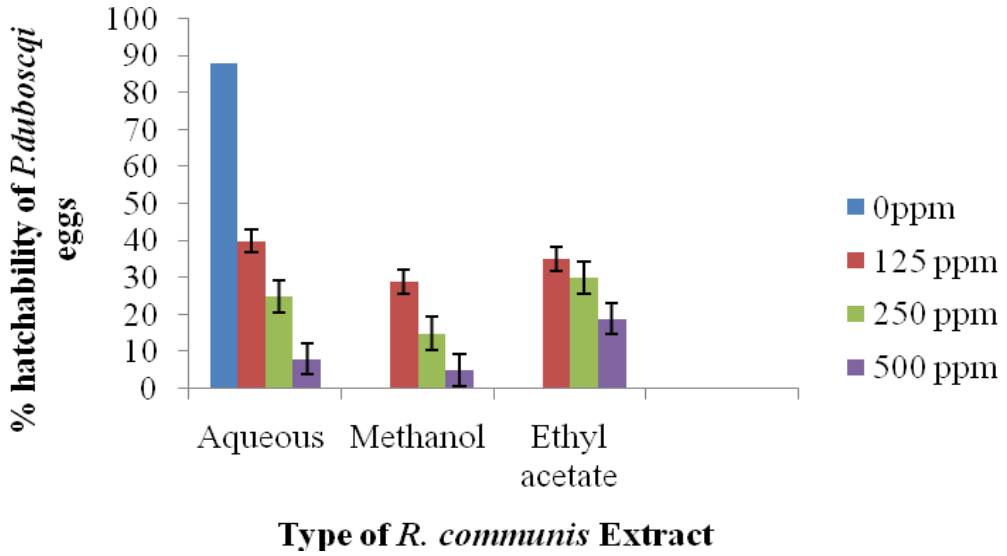

Fig. 2. % Hatchability of *P. duboscqi* eggs treated with *R. communis* bark extracts

3.4 Larvicidal Effects of *R. communis* Bark Extract

After emergence, all the first instar larvae treated with 125 µg/ml aqueous extract showed normal developmental features without any deformity. They all survived and moulted to the second instar larvae despite extract contact. No larval death was recorded due to the extract treatment. However, the extract was suspected to deter larvae from feeding as evidenced by the small size of the larvae and almost empty gut (plate 5). The larvae had 2 caudal bristle and settae from each segment. When the concentration was increased to 250 µg/ml and 500 µg/ml, larval mortality was observed.

At the second larval instar, inhibition to feeding was observed. Percent mortality increased with the dead larvae having a head detached from the gut. Later, the gut was seen to be split (disintegrated) then rotting followed and finally the larvae darkened (Plate 4). Most dead larvae were found attached to the larval cage surface. Conversely, when larvae were fed on powdered leaves, no mortality was observed and all the larvae were very healthy.

Further larval mortality occurred at the 3rd and 4th instar stages. Observed percentage mortality was 15.61% (21±1.45), 57.78% (62±3.61) and 74.89% (90±3.75) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively in larvae treated with aqueous extract. In the negative control group, larval mortality was significantly low; 11.10% (9±0.35). The observed difference was significant (*P*=0.002).

Larval period was 63.01±1.37 days, 60.33±1.45 days and 80.55±0.35 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. On the contrary, the larval duration was 27.37±0.17 days in the control group. Further, only 9.33±2.58, 11.45±1.35 and 4.11±0.99 pupae were viable at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. At the same time, the pupal duration lasted for 13.23±0.27 days, 16.44±2.54 days and 21.00±1.55 days for 125 µg/ml, 250 µg/ml and 500 µg/ml respectively against 9.47±0.33 days for the control group. All the larvae were viable without any deformity although, only 4.00±1.37, 6.33±1.37 and 1.00±1.55 emerged as adults. The emerged adults had normal developmental features although a high percentage died within the first 24 hours of emergence. In the control group, 41.00±1.55 adults emerged. The life cycle of *P. duboscqi* was significantly long lasting for 91.00±2.35 days, 91.25±1.45 days and 116.05±1.37 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. In the control group, the life cycle was completed in 46.77±1.33 days (*P*=0.019).

Severe effects on larvae were observed in the methanolic extract treated group. Mortality was high and most larvae had their guts detached from the head then disintegration and rotting of the larvae started almost immediately (plate 6). Mortality reached 100% at the 3rd instar larvae hence the life cycle was never completed.

Plate 4. Second larval instar treated with 250 µg/ml of *R. communis* leaf extract. arrows indicate; A-gut detaching from head, B-gut fully detached from the head, C-split gut, D- rotting larvae

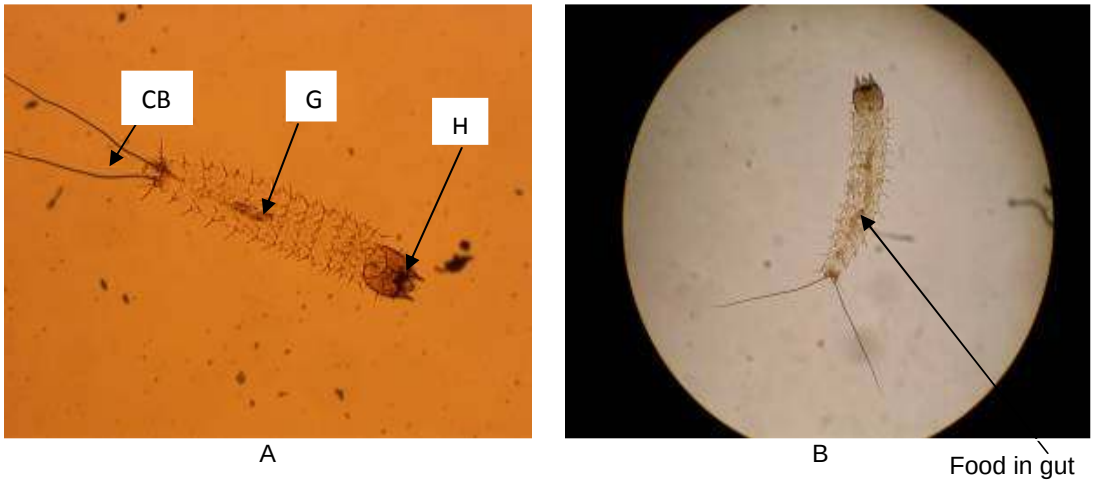

Plate 5. (A) first larval instar under 125 µg/ml extract concentration with almost empty gut: CB-caudal bristle, G-gut, H-head (B) First larval instar (control) which is fed

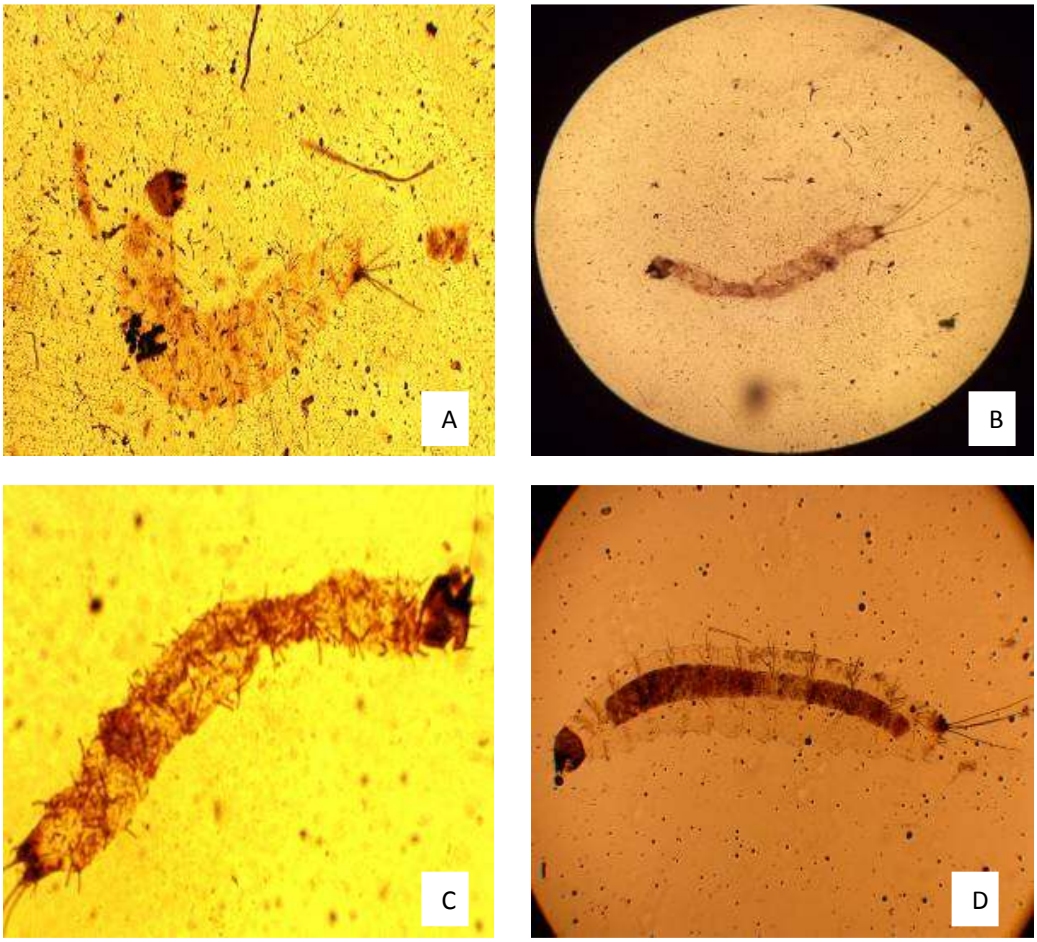

Plate 6. Second instar larvae treated with methanol extract of *R. communis*; (A) Almost dead larvae (B) Rotting larvae (C) rotting larvae (D) Viable larvae from the control group

In the methanol extract larval mortality was 40.00% (48.33±2.33), 100 % (125.65±2.40) and 100 % (135.03±2.67) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. Larval duration was 68.11±0.21 days, 66.33±0.67 days and 87.10±1.67 days in 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. The pupal duration was 19.25±0.15 days at 125 µg/ml. However, at 250 µg/ml and 500 µg/ml larval mortality was 100%, hence there was no pupation. Adults that emerged were 3.67±2.33 (mean ± SE) and they had no deformity. The entire life cycle lasted for 101.01±2.67 days, 103.12±0.68 days and 140.35±1.75 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively.

Ethyl acetate extract had moderate effect on larvae hence larval viability was high. Despite this, mortality was observed and it started with detachment of gut followed by splitting and finally rotting of the larvae (plate 7).

In the larval group that had contact with ethyl acetate extract, percentage larval mortality was 37.5% (50.45±0.58), 43.75% (45.33±0.88) and 48.75% (41.33±2.67) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively.

In the group treated with ethyl acetate extract, the larval period was significantly low. The larval duration was 37.10±1.37 days, 43.45±0.65 days and 48.33±1.67 days at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. This was in contrast to 28.00±1.65 days in the larvae that formed the control. The pupal duration was extremely long compared to larvae treated with aqueous and methanol extracts. The duration was 37.10±0.27 days, 38.54±1.46 days and 40.00±1.67 days at 125 µg/ml, 250 µg/ml and

Plate 7. Second larval instar treated with ethyl acetate extract of *R. communis*; A-larvae with deterred feeding, B-Gut starting to detach from head, C- gut splitting D-gut splitting, E-dead larvae

500 µg/ml respectively. The entire life cycle lasted for 89.23±1.67 days, 96.21±2.33 days and 103.24±1.54 days in 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. In the control group, the life cycle was completed in 46.77±1.33 days.

4. DISCUSSION

4.1 Effect of *R. communis* on Egg Shell Membranes

Synthetic chemical pesticides have been used widely as the main method for controlling insect pests. However, there are many problems associated with the extensive use of these compounds, such as build-up of pesticide resistance, negative impact on natural enemies, in addition to negative environmental and health impacts [6]. It has been proven that plant extracts and plant compounds are eco-friendly, target-specific, less expensive, and highly efficacious pesticides for the control of vectors [9]. Therefore, natural products are proving to be potential sources of new and selective agents for the treatment of important tropical diseases and the control of vectors.

The observation that *P. duboscqi* eggs treated with *R. communis* extract lost their chorionic layers shows that active compounds in *R. communis* are capable of eroding the exochorion, mesochorion and endochorion layers of the egg leaving a thin chorionic layer on the egg. This concurs with past research which showed that moistening incubated *P. duboscqi* eggs with *Mundulea sericea* [10] led to massive loss of chorionic layers. At a scanning electron microscopy level, the egg shell of all phlebotomine sand flies is characterised by the outer chorion forming a series of ridges cross-linked in various places to form a specific pattern [11]. All these cross-linkages are lost to an extent of revealing the hidden plastron once incubated eggs are moistened with plant extracts [12]. These corrosive effects of *R. communis* extracts are due to the presence of certain phytoconstituents in the plant especially ricinine, a water soluble glycoprotein concentrated in various parts of the plant [5].

The insect egg shell has several roles that it performs so as to ensure viability of the embryo. The egg shell serves as a boundary between the external environment and the developing embryo. Other vital functions of the egg shell include protection against desiccation, enemies and enhancing gaseous exchange [11]. Therefore, any change in the structure of the egg shell will affect the developing embryo. The loss of chorionic structures may account for the loss of embryo viability that was observed in this study and the subsequent low number of emerging larvae. The embryo might have died due to desiccation or poor regulation of gaseous exchange due to the loss of chorionic membranes.

4.2 Effect of *R. communis* Extract on Hatchability of Eggs

In this study, hatchability of eggs was found to be inversely proportional to the extract concentration. Aqueous and methanolic extracts showed significant reduction in egg hatchability revealing the harmful effects of these extracts. However, at 125 µg/ml concentration, a significant number of eggs hatched showing the inability of the extracts to reach the embryo. This observation is in agreement with past research on mosquitoes which revealed that hatchability rate is inversely proportional to the concentration of *Artemisia annua* extract [13].

In the lower concentrations, the extracts eroded the chorionic egg layers but did not inhibit the eggs from hatching. This might be due to the inability of the extract to penetrate the egg membranes to reach the embryo. However, at 500 µg/ml concentration, 95% inhibition of egg hatching was observed meaning that the high concentration of the toxic compounds reached the embryo and inhibited or killed it. Our findings are in agreement with past research which showed that sand flies feeding on *R. communis* and Bougainvillea leaves produced fewer larvae compared to the control and post hoc analysis revealed that fewer eggs had hatched [14]. Hexane extracts of *Andrographis lineat, A. paniculata and Tagetes erecta* showed 100% ovicidal activity against *An. Subpictus* [15]. Elsewhere, hatchability of eggs was reduced by petroleum ether extracts of *Piper nigrum* and *Jatropha curcas* by 59% and 58% respectively at the lowest concentration of 2 µL/ml [16]. This reduction in egg hatchability shows that plant extracts possess compounds which are toxic to the developing embryo.

Besides inhibiting hatching, *R. communis* extracts also had pronounced effect on the incubation period of eggs and developmental period of larvae. Under normal insectary conditions, phlebotomine eggs take averagely 10 days to hatch into larvae but when these eggs were moistened with the extract, the incubation period took 15 days. This may suggest that bioactive compounds from plant extracts can have pronounced effects on the developmental period of various insect stages. This may reveal that, exposure of insect vectors to active botanical derivatives can result in an extension of the duration of development [15] hence, exposing susceptible stages to the effects of these botanicals.

Comparing the effects of leaf and bark extracts shows that *R. communis* bark extracts have severe effects on hatchability of *P. duboscqi* eggs. Acute application of extracts in all of the concentrations did not significantly inhibit hatchability of eggs. At 125 µg/ml concentration of bark methanol extract, 29% of eggs hatched while at 500 µg/ml, only 5% of eggs hatched. In the methanol leaf extract, 34% and 9% of eggs hatched at 125 µg/ml and 500 µg/ml respectively. This implies that the active compounds in *R. communis* especially ricinine is differentially concentrated in leaves and bark. Ethyl acetate and aqueous extracts showed moderate effects.

4.3 Effect of *R. communis* Extract on Larvae

At 125 µg/ml extract concentration, no mortality or deformity was observed. This observation is in agreement with past research which showed that feeding *P. duboscqi* larvae with *Mundulea sericea* powdered leaves resulted in the survival of all first instar larvae [10]. Also no mortality was recorded when larvae were fed on powdered extracts. An explanation for this may be due to the low content of active compounds in the low concentrated or powdered extract. Therefore, the extract was unable to penetrate the larval cuticle hence no toxic effect was experienced by the larvae.

Increasing the concentration to 250 µg/ml and 500 µg/ml saw significant larval mortality with deformity especially when using *R. communis* bark extracts. High larvicidal activity was seen against 2^{nd}, 3^{rd} and 4^{th} larval instars. Methanol extract was more effective in mortality as shown by the LC_{50} values. Similar results 0% larval viability rate with methanol leaf extracts of

R. communis at 24000 µg/ml [6]. Elsewhere, it was revealed that in topical and ingestion toxicity tests, aqueous extracts and oil emulsion of castor bean plant caused significantly higher *Plutella xylostella* larval mortalities than the control [17]. Extracts from *R. communis* have been shown to possess larvicidal and insecticidal activities against a wide range of insects of medical, veterinary and agricultural importance [6]. Phytochemical studies of *R. communis* extract reveal the presence of steroids, saponins, alkaloids, flavonoids, and glycosides which might be responsible for the observed mortalities. Besides, the killing effects of these extracts may have been due to the presence of ricinine compound in the bark extract [18] which probably killed the larvae through ingestion and contact.

A relatively high mortality was noted when *R. communis* bark extracts were used as opposed to leaf extracts. This might be due to the difference in the levels of ricinine in these extracts since insecticidal effects of plant extracts vary according to plant species, insect species, geographical varieties and plant parts used [19]. It has also been shown that roots and bark have a high partitioning for the photosynthates or exudates which act as toxins [20]. Past investigation suggests that ricinine is the compound responsible for this activity but it occurs in minor concentrations in the leaf [6]. Several compounds in the extract might be responsible for the killing action of *R. communis* extracts. Ricin is known to cause larval mortality by causing acute cell death by inactivating ribosomal RNA hence inhibiting protein synthesis. Also the burning effect of the extract might have caused larval mortality by contact [17].

The effectiveness of an insecticide depends on several factors including chemical nature of the molecule, amount of active ingredients used, frequency of applications, time interval between applications, size and age of insect, area and time of contact with the insect [21]. In the current study, larvae of *P. duboscqi* were sprayed with 5 ml of the extracts on a two day interval throughout the experimental period. Hence, larvae were completely in contact with the extract and this may explain why mortality was very high. It has also been shown that plants produce numerous chemicals, many of which produce chemical factors and metabolites that have larval, adulticidal or repellent activities against different species of insects [19]. Where larvae survived, the explanation could be because the extract did not break the cuticle to penetrate into the vital internal organs hence toxicity was very low. This was observed in all the low concentration throughout the experimental period.

These extracts have a wide range of biological activity against vectors and the toxic effects of these extracts may be comparable to extracts from other medicinal plants. From this study, it was observed that the killing of larvae by these extracts was stepwise through lysis of the gut. First there is disintegration of the gut which detaches first from the head. Later, splitting of the gut was observed at specific points. After this, rotting occurred which caused darkening of the larvae and the final step in the mechanism of larvae killing was the rotting of the whole larvae. This concurs with past research on *Leishmania infantum* which showed that saponins in *R. communis* extract increased membrane permeability of the parasite leading to their lysis [22] and eventual death.

R. communis extracts were also found to prolong the larval, pupal and the entire life cycle duration for *P. duboscqi* flies. These developmental stages were either delayed or inhibited completely (like the pupal stage) because most plants have been found to contain compounds like repellents, feeding deterrents, toxins, and growth regulators [23] which have varying effects to the insects. The long larval duration of up to 87 days in methanolic extract shows that this plant extract can act as a growth regulator. This way, this extract could be effective against all the developmental stages of sand flies. Some plant phytochemicals have a toxic effect on insect adults and larvae by interfering in their growth, development or reproduction [14]. Also Neem oil has been shown to act as larvicidal, oviposition inhibitor and growth regulator against *Culex quinquefasciatus, Anopheles culicifacus, Anopheles stephensi* and *Aedes aegypti* [15].

5. CONCLUSION

Effective control of sand flies is very challenging because the immature stages reside in inaccessible places. *R. communis* and other plants have sugars which sand flies feed on hence; feeding sand flies on *R. communis* can have deleterious effects on hatching of eggs, larval and pupal development and adult emergence as revealed by this study. The findings showed that *R. communis* extracts inhibited the life cycle of *P. duboscqi* at the larval

stage showing that the spraying of *R. communis* extracts on sand flies could greatly reduce their abundance. Therefore, to reduce *Leishmania* infections and other effects of sand flies to humans, *Ricinus communis* plants should be planted as barriers for sand flies in leishmaniasis endemic areas.

CONSENT

This section is not applicable because we didn't use human subjects. It was a laboratory based study; hence only institutional ethical approval was sought and granted.

ETHICAL APPROVAL

Approval to conduct this investigation was granted by Kenya Medical Research Institute's ethical review committee, Scientific Steering Committee and Animal Care and Use Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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