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INSECTICIDAL ACTIVITIES OF RICINUS COMMUNIS EXTRACTS ON PHLEBOTOMUS DUBOSCQI ADULTS

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ABSTRACT

Sand flies are small haematophagous insects that transmit *Leishmania* parasites through the bite of infected female phlebotomine sand flies. Roots, leaves and seeds of *Ricinus communis* are extensively used in different ways especially in the treatment of rheumatic arthritis, paralysis, epilepsy and distension of the uterus. This study sought to determine the adulticidal effects of *Ricinus communis* (Euphorbiaceae) leaf extracts on *Phlebotomus duboscqi* in the laboratory. A comparative experimental design using extracts obtained from the leaf and bark of *Ricinus communis* plant was used. The study was carried out at the Kenya Medical Research Institute, Centre for Biotechnology Research and Development, Kenya. Aqueous, methanol and ethyl acetate extracts were prepared from *Ricinus communis* plant. Thirty-five day old adult *Phlebotomus duboscqi* flies were aspirated into plastic rearing jars partially filled with plaster of Paris and fitted with screen tops. They were fed on *R. communis* extract laced with 10% sucrose. Sand flies that fed on 10% sucrose solution soaked in cotton wool pads and placed onto the screen tops were used as controls. There was no significant difference when bark and leaf extracts were compared (P=0.061). *R. communis* extracts from Narok showed insecticidal effects against adults. At 48 hours post treatment, the LC_{50} was 121.15 µg/ml and 126.21 µg/ml for bark and leaf extracts respectively. *P. duboscqi* adults were found to be highly susceptible to methanol extracts. Therefore, *R. communis* extracts have insecticidal effects on adult *P. duboscqi*; hence *R. communis* should be used against sand flies and *Leishmania in situ*.

KEYWORDS: *P. duboscqi, Ricinus communis,* adulticidal, mortality.

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 adulticidal effects of *R. communis* (Euphorbiaceae) extract on *Phlebotomus duboscqi* flies.

2. MATERIALS AND METHODS

2.1 Study site

The study was 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 mortality of adult sand flies (*P. duboscqi*).

2.3 Sand Fly Colony

A colony of *P. 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°C, 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, 600ml of methanol were added to 300g 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°C. 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°C 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 (1mg/ml to 20mg/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 Bioassay

Insecticidal effects of the plant extracts were determined as previously described[8] with slight modifications. Thirty-five day old adult *P. duboscqi* were carefully aspirated into plastic rearing jars partially filled with plaster of Paris and fitted with screen tops. 10% sucrose solution was used to prepare 125 µg/ml, 250 µg/ml and 500 µg/ml of 0.6 % *R. communis* extract by serial dilution and used in the feeding of the flies. Cotton wool pads were soaked in the preparations and placed on the screen tops. Two triplicate series with 10 flies each of *P. duboscqi* was used for each dilution. The first triplicate contained 30 females and the second triplicate had 30 males in each jar. 60 specimens were assayed for each dilution and gender. Sand flies that fed on 10% sucrose solution soaked in cotton wool pads and placed onto the screen tops were used as controls. Males and females were not nested together. Mean lethal concentration designated LC_{50}, was determined at 12, 24, 36, 48 and 72 h of exposure. The set up was maintained at 27±2°C, relative humidity of 78-83 % and a 12: 12 (light: dark) photoperiod. The experiment was stopped when all the flies in the controls had died.

3. RESULTS

3.1 Mean mortality of *Phlebotomus duboscqi* after feeding on *R. communis* extracts

Once, adult sand flies were subjected to *R. communis* extract, both male and female sand flies were killed after feeding on the extract that was mixed with 10% sucrose. Mortality values between male and female sand flies were not significantly different ($F = 1.51$, $P = 0.218$). However, individual sexes showed significant mortality when different extract concentrations were used. Low or no mortality was recorded at 1, 4, 8, 12, 24 hours post treatment and hence cumulative mortality was reported at 96 hours post feeding (table 1). Cumulative mortality was 24.05 ± 2.35 (n=30) and 23.75 ± 2.95 (n=30), for male and female respectively when *P. duboscqi* flies were fed on *R. communis* leaf extracts. At the same time, *R. communis* bark extract caused a cumulative mortality of 19.33 ± 1.67 and 22.90 ± 2.15 for males and females respectively. LC_{50} and LC_{90} at 48 hours was 121.15 µg/ml and 173.78 µg/ml respectively for *R. communis* bark extract.

Table 1: Cumulative mortality of female *P. duboscqi* after feeding on *R. communis* extracts.

Extract	8hrs	12hrs	24hrs	48hrs	72hrs	96hrs	Total dead flies
MeOHL	2	1	4	4	8	5	24
MeOHB	3	3	6	2	5	8	27
AqL	1	0	2	5	8	3	19
AqB	4	4	3	6	4	2	23
EACL	0	2	8	4	3	4	21
EACB	5	2	3	1	6	5	23
Total dead flies	15	12	26	22	34	27	137

KEY: MeOHL-methanol leaf, MeOHB-methanol bark, AqL-aqueous leaf, AqB-aqueous bark, EACL-ethyl acetate leaf, EACB-ethyl acetate bark

Significant female mortality was recorded in methanol extract of both leaf and bark *R. communis* extracts at 48 hours. When female sand flies were fed on leaf extracts, recorded mortality was 15.57 ± 0.63 (n=30), 19.67 ± 1.45 (n=30) and 21.00 ± 0.58 (n=30) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. A significant difference was noted (F=13.38, $DF_{6, 14}$, *P*=0.001). The LC_{50} and LC_{90} for females was 126.21 µg/ml (χ^2 = 23.4) and 292.86 µg/ml (χ^2 = 31.3) respectively. Equally, significant male mortality was recorded (*P*<0.001) with LC_{50} and LC_{90} being 146.78 µg/ml (χ^2 =29.01) and 323.59 µg/ml (χ^2 =39.20) respectively.

Likewise, mortality increased with increase in concentration when sand flies fed on bark extracts. At 48 hours post feeding, recorded mortality was 17.23 ± 1.15 (n=30), 20.67 ± 0.33 (n=30) and 25.01 ± 0.58 (n=30) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively and the observed difference was significant (F=79.75, $DF_{6, 14}$, *P*=0.001). LC_{50} and LC_{90} for females was 125.05 µg/ml (χ^2 = 18.9) and 288.40 µg/ml (χ^2 = 26.2) respectively. Mortality for males was 13.63 ± 2.55 (n=30), 15.64 ± 1.37 (n=30) and 22.25 ± 0.55 (n=30) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. A significant difference (*P*=0.001) occurred when comparing female and male mortality at a concentration of 250 µg/ml. Mortality increased steadily in both sexes at a concentration of 500 µg/ml and as time of exposure increased, mortality rate approached 100%. Sand fly mortality was significantly higher in *R. communis* extract treatments than in the control treatments (*P* < 0.001) (Fig. 1).

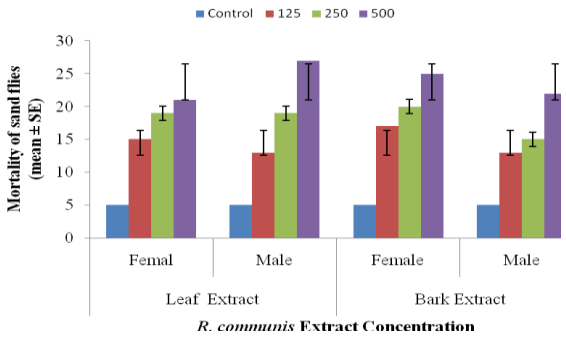


Figure 1: Comparing male and female mortality rates of *P. duboscqi* after feeding on methanol *Ricinus communis* extracts.

For aqueous extract treatment, similar mortality rates were recorded which were significantly higher than those of the controls. Significant mortality rates were noted when comparing males and females (F=63.58, *P*<0.001). At 96 hours post feeding on *R. communis* leaf extract, male mortality was 14.00 ± 1.47, 16.67 ± 0.33 and 20.48 ± 2.32 (n=30) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. Female mortality rates were 6.40 ± 2.31, 11.00 ± 0.67 and 15.85 ± 0.55 (n=30) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. LC_{50} and LC_{90} values were 121.15 µg/ml (χ^2 = 16.31) and 301.39 µg/ml (χ^2 = 23.42) respectively. Cumulatively, more males were dying as compared to the females, although the difference was not significant (*P*=0.131) (fig. 2).

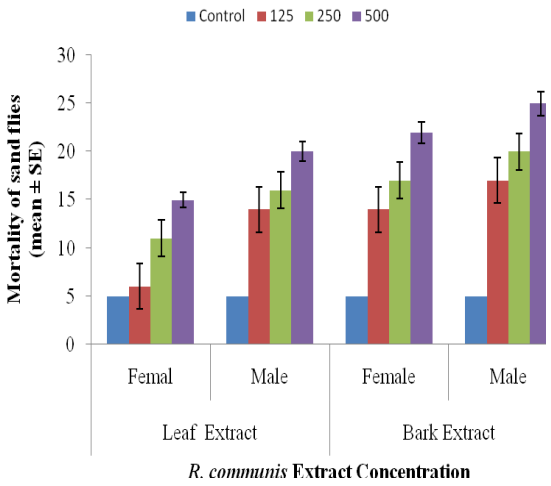


Figure 2: Mortality rates of *P. duboscqi* sand flies after feeding on aqueous *R. communis* extract.

In the ethyl acetate extract treatment, males in both *R. communis* leaf and bark bioassays were affected more than the females with mortality of over 60% in the lowest concentration of 125 µg/ml at 48 hours of exposure and beyond. There was also significant mortality difference between the concentrations used (F=37.12, *P*=0.01). The LC_{50} for females at 48 hours was 169.25 µg/ml (χ^2= 31.7) while the LC_{50} for males at 48 hours was 134.02 µg/ml (χ^2= 26.5). Female mortality was 11.33 ± 2.33, 13.63 ± 1.45 and 17.23 ± 1.85 (n=30) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively. For males, mortality was 12.67 ± 1.63, 16.00 ± 2.83 and

21.42 ± 1.30 (n=30) at 125 µg/ml, 250 µg/ml and 500 µg/ml respectively (fig. 3).

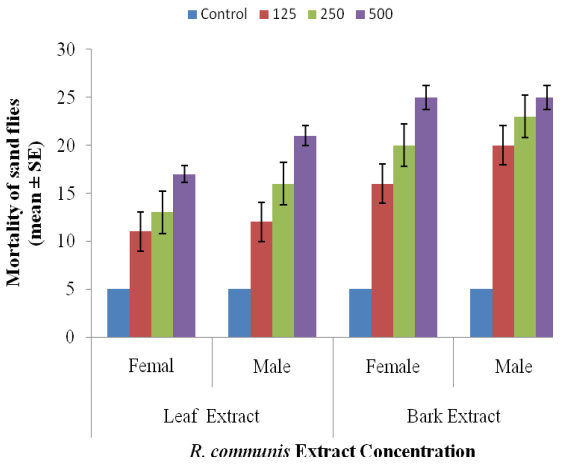
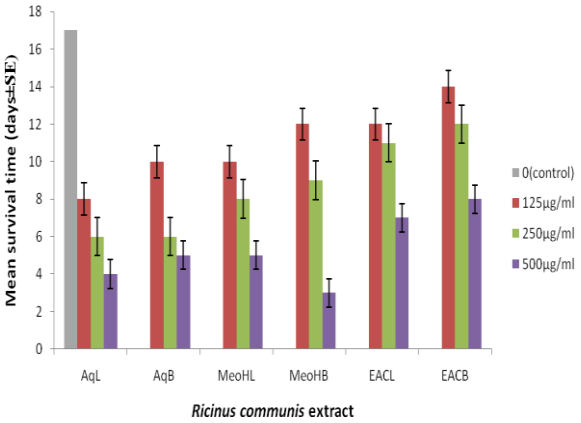


Figure 3: Comparison of male and female *P. duboscqi* mortalities after feeding on *R. communis* ethyl acetate extract.

3.2 Mean survival time after feeding on *R. communis* extracts

Mean survival time decreased with increase in concentration of the extract used. At a concentration of 125 µg/ml there was no significant difference in survival time (P=0.31) for sand flies that had fed on methanol, aqueous and ethyl acetate extracts. However, there was a significant difference when compared to the control group (P<0.001). Longevity in days was highest in the sand flies that had fed on ethyl acetate *R. communis* leaf extract, 12.67 ± 1.33 days (n=30, P=0.011) followed by the sand flies that had fed on methanol extract, 10.44 ± 1.46 days (n=30 P=0.011) while the sand flies that had fed on aqueous extract had the lowest longevity of 8.67 ± 0.33 days (P=0.021). Sand flies in the control group had lived for 17.37 ±1.63 days.

Significant decline in longevity was noted when high extract concentrations were used. At a concentration of 250 µg/ml, mean survival time was 6.00±0.58 days and 8.37 ± 2.01 days for sand flies that had fed on aqueous and methanol extracts respectively. However, sand flies in the control group lived for 15.00 ± 1.30 under similar conditions. The sand flies that had fed on ethyl acetate extract had the highest longevity of 11.60 ± 2.54 days (P=0.021). At 500 µg/ml, longevity was further suppressed across all the three extracts used. In the sand flies that had fed on aqueous extract, mean survival time was 4.41 ± 1.58 days, significantly different compared to the sand flies in the control group (P<0.001). Feeding the sand flies on methanol and ethyl acetate extracts resulted in 5.95 ± 0.55 and 7.81 ± 1.18 days of survival respectively (fig. 4).



Key: AqL-aqueous leaf, AqB-aqueous bark, MeOHL-methanol leaf, MeOHB-methanol bark, EACL-ethyl acetate leaf, EACB-ethyl acetate bark

Figure 4: Mean survival time of *P. duboscqi* sand flies after feeding on different *R. communis* extracts.

4 DISCUSSION

Extensive use of synthetic compounds in the control of vectors of medical, agricultural and veterinary importance has led to the build-up of pesticide resistance, negative impacts on the environment and they are not target specific. Due to this, botanical insecticides have been found to be suitable alternatives for new and selective agents for the treatment of important tropical diseases and the control of vectors.[9] In the present study, results obtained from *R. communis* extracts concur with early research showing that botanical insecticides are potential in controlling vectors of medical importance.

This study has shown that *R. communis* compounds extracted from either leaves or the bark have the potential of killing sand flies through ingestion or contact. The killing effect of an insecticide depends not only on the active compounds contained in an insecticide but also the part of the insect in contact with the insecticide. Insecticidal and antimicrobial agents including quercetin, gallic acid, flavone or kaempferol responsible for pest repellence, deterrence or toxicity have specifically been isolated from the *R. communis* plant.[10] This may explain why ingestion of *R. communis* extract caused high mortality in *P. duboscqi* sand flies. Similar to this study is past research which had shown that ingestion or contact with *R. communis* extracts caused high mortality of up to 100% in various insect vectors.[11;12]

The observation that low concentrations produced low mortality rates while high concentrations gave mortalities of up to 100% shows that *R. communis* has insecticidal properties. This is in agreement with past research which had shown that high concentrations of *Acalypha fruticosa* and *Tagetes minuta* extracts had insecticidal effects against *Phlebotomus duboscqi* sand flies.[13] This means that most plants contain compounds including repellents, feeding deterrents, toxins, and

growth regulators which are insecticidal to haematophagous arthropods and because of this, plants have been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance biting insects.[14]

The efficacy of most plant extracts varies with insect species or the developmental stage, and also the formulation type and concentration tested.[10] Larval stages are highly susceptible compared to adult insects. In this study, the recorded mortality was concentration dependent and despite the low concentrations used mortality was recorded with very low LC_{50} values indicating the high insecticidal properties present in *R. communis*. Biologically active flavonoids like quercetin and rutin have been identified in extracts of *R. communis* and may be responsible for these high insecticidal properties.[15] Furthermore, quercetin is a flavonoid which has been found to interfere with the iron metabolism.[16]

The current results are in agreement with past research which has shown that most medicinal plants have excellent results in controlling vectors including sand flies. Plants like *Azadirachta indica*, *Ricinus communis*, *Solanum jasminoides*, *Bougainvillea glabra* and *Capparis spinosa* have been shown to act as future alternatives for the control of sand flies.[17] Extracts from these plants contain a wide range of active compounds which can act concertedly on physiological processes of the vectors to either kill or deform them. This might be the reason why the mean survival time of sand flies fed with *R. communis* extracts was significantly low compared to the untreated sand flies.

In this study, sugar solution was mixed with the crude extracts to act as bait for the flies. Female sand flies need blood for egg production, but sugar is their main source of energy and the only food taken by males.[18] It has been shown that sand flies can feed on aqueous sucrose solutions mixed with noxious plant juices and have their lifespan greatly reduced.[19] Therefore, the sugar that was mixed with the crude extracts might have attracted more sand flies which in turn fed more on the crude extracts leading to their death. This property of sugar feeding behaviour in sand flies has been shown to influence longevity, fecundity, dispersal, host seeking behavior and ultimately blood feeding and disease transmission.[18]

Among the extracts used, methanolic extract was highly efficacious followed by ethyl acetate extract and the least was aqueous extracts. Phytochemical analysis of methanolic extractions shows the presence of tannins, saponins, flavonoids, alkaloids and terpenoids[16] which acts in a variety of ways to cause death. Besides, killing arthropods, *R. communis* extracts have been found to be active against a wide range of protozoa, bacteria and fungi.[20;21]

5. CONCLUSION

R. communis extracts have insecticidal properties against adult sand flies hence extracts of *R. communis* should be processed into insecticides that can be used against all sand fly stages in line with WHO guidelines.

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Competing interests: Authors have declared that no competing interests exist.

Author's contributions

1. Dr. Samuel Mong'are was the Principal investigator. He co-ordinated all the aspects of the research, did literature search and prepared the manuscript.
2. Prof. Peter K. Ngure assisted the principal investigator with the logistics of the study in the field and elsewhere. He also assisted in statistical data analysis and proof reading of the manuscript.
3. Prof Zipporah Ng'ang'a and Dr. Philip M. Ngumbi provided expert ideas on leishmaniasis in the country. They also provided expertise in training and co-ordination of research activities in the laboratory. All authors read and approved the final manuscript before submission to the journal.

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.

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