Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Assessing the potential effects of selected Kenyan medicinal plants on longevity of *Phlebotomus duboscqi* (Diptera: Psychodidae)

Mong'are samuel[1]*, Ng'ang'a Zipporah[2], Maranga Rosebella[1], Osiemo Zipporah[1], Ngure Peter[3], Ngumbi Philip[4], Mutai Charles[4], Karanja Robert[4], Ireri Laban[5], Tonui Willy[6]

1 Jomo Kenyatta University of Agriculture and Technology, P.O Box 62000, Nairobi, Kenya
2 Institute of Tropical Medicine and Infectious Diseases, P.O Box 62000, Nairobi, Kenya
3 Daystar University, P.O Box 44400-00100, Nairobi, Kenya
4 Kenya Medical Research Institute, KEMRI, P.O. Box 43640 - 00100 Nairobi, Kenya
5 Division of Vector Borne and Neglected Tropical Diseases, Ministry of Public Health and Sanitation, Embu, Kenya
6 National Biosafety Authority, Kenya.

ARTICLE HISTORY

Received: 13.11.2012

Accepted: 12.12.2012

Available online: 10.02.2013

Keywords:

Longevity, *Phlebotomus duboscqi, Tarchonanthus camphoratus, Acalypha fruticosa, Tagetes minuta, bioassays, medicinal plants*

***Corresponding author:**

Email : mongaresam25@yahoo.co.uk
Tel : +254726927699 / +254734706063

ABSTRACT

Phlebotomus duboscqi (Diptera: Psychodidae) has been incriminated as the vector of *Leishmania major*, the causative agent of zoonotic cutaneous leishmaniasis (ZCL) in various parts of the world. This study sought to describe the influence of *Tarchonanthus camphoratus* (Asteraceae), *Acalypha fruticosa* (Euphorbiaceae) and *Tagetes minuta* (Asteraceae) crude extracts on longevity of *Phlebotomus duboscqi*. These medicinal plants were prepared from the dried aerial parts followed by grinding into a fine powder and then soaking the plant materials in methanol and ethyl acetate solvents for 48 hours. After 48 hours, the materials were filtered and dried out using a rotary evaporation at 30-35°C. The extracts obtained were later prepared into appropriate concentrations for bioassay. Groups of ten female sand flies were aspirated into vials where they were fed on a mixture of the plant extracts and sucrose solution. The crude extracts reduced the survival time of *P. duboscqi* significantly ($P<0.05$). It was found out that *P. duboscqi* flies that had fed on *A. fruticosa* extract had a life span of 7 days, *T. minuta* 7 days and *T. camphoratus* 9 days as compared to a life span of 12 days in *P. duboscqi* flies that formed the control group. The observation that *A. fruticosa*, *T. minuta* and *T. camphoratus* have effect on longevity of *P. duboscqi* implies that these plants can be used as a natural means of reducing transmission of leishmaniasis by reducing the life span of *Phlebotomus duboscqi* eventually killing them.

INTRODUCTION

Phlebotomine sand flies have been known as the vectors of *Leishmania*. These pathogens cause a group of diseases known as leishmaniases in more than 88 countries in the World. Leishmaniasis infects an estimated 14 million people, and each year about two million new cases occur [1]. Other than leishmaniasis, sand flies are also vectors of other human pathogens such as Bartonella and viruses belonging to three different genera: (i) the *Phlebovirus* (family Bunyaviridae) including sandfly fever Sicilian virus, sandfly fever Naples virus, Toscana virus and Punta Toro virus; (ii) the *Vesiculovirus* (family Rhabdoviridae) including Chandipura virus and (iii) the *Orbivirus* (family Reoviridae) including Changuinola virus [2]. Sand flies (*P. duboscqi, P. papatasi, P. sergenti*, and *Sergentomyia schwetzi*) have also been infected and found to develop Rift valley fever in the laboratory [3]. This shows that sand flies are vectors of potentially deadly pathogens. However, no viral disease transmitted by sand flies to humans has been reported so far.

The global prevalence of leishmaniasis has risen in recent times because of an increase in international travel, human alteration of both vector and host habitats, and concomitant factors that increase susceptibility, such as human immunodeficiency virus infection and malnutrition. Recent international conflicts have also contributed to an increase in and

spread of leishmaniasis in previously unaffected countries [4]. Leishmaniasis is a major tropical disease with a wide clinical spectrum of cutaneous, mucocutaneous and visceral involvement whose presentation is often varied and diagnosis can be challenging. The type of disease expressed depends both on the type of *Leishmania* species and on the zymodeme expressed on that species. Clinical manifestation depends on the parasite species and the host's specific immune responses to *Leishmania* antigens [5].

These findings show that there is need to control either the vector or eradicate the disease. Experts are advocating for control of the vector hence interrupting disease transmission [6]. Therefore, one of the approaches for control of these Phlebotomine-borne diseases is the interruption of disease transmission by killing or preventing sand flies from biting human beings. Chemical insecticides or potential herbal products can be used in interrupting the transmission of sand fly-borne diseases at the individual as well as at the community level. Several medicinal plants have been used traditionally for controlling many insect vectors. Some of these include *Nicotiana tabacum* leaves, pyrethrum from *Chrysanthemum cinererifolium* (sp) flowers, *Tagetes minuta*, *Warbugia ugandensis*, Neem among others [7]. These traditional medicinal plants work in different ways; they may be insecticidal, repellent or antifeedant or by reducing the survival time of the vectors that have fed on the plant extracts.

Normally, female sand flies need a blood meal for egg production, but sugar is their main source of energy and the only food taken by males [8]. The sugar feeding behavior of sand flies, therefore, influences survival time and fertility, dispersal, host seeking behavior and ultimately blood feeding and disease transmission [9]. In this study we assessed the potential of *Tarchonanthus camphoratus, Acalypha fruticosa* and *Tagetes minuta* crude extracts on the life span of *Phlebotomus duboscqi*. These plants are used in treating various diseases in Kenya and repelling biting flies including sand flies [10]. We hypothesized that these plants can be used as a natural means of reducing transmission of leishmaniasis by reducing the life span of *Phlebotomus duboscqi* eventually killing them.

MATERIALS AND METHODS

Phlebotomus duboscqi Neveu Lemaire sand fly colony was used. This colony is being reared at the Kenya Medical Research Institute for research purposes. This colony was established using periodically field captured sand flies and inbreeding. The sand flies were blood fed on Syrian golden hamsters for egg laying. Adult sand flies were kept in cages where they were fed on slices of apples as a source of energy. The set up was maintained at 25±1°C and a relative humidity of 78-83 % in the insectary.

Floral and foliar parts of *Tarchonanthus camphoratus, Acalypha fruticosa,* and *Tagetes minuta* were used in the investigation. These plant parts were dried then ground using an electrical mill in readiness for extraction. The sample extraction procedure was carried out as described elsewhere [11]. Briefly, sequential extraction was carried out on plant material with analar grade organic solvents of increasing polarity. The solvents used include methanol and ethyl acetate to prepare two different extracts. Three hundred milliliters 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 300 ml of methanol for 24 hours until the filtrate remained clear. The filtrate was then concentrated under vacuum by rotary evaporation at 30 - 35°C. The concentrated extracts were transferred to a sample bottle and dried under vacuum and stored at 4°C until required for bioassay.

Bioassay on Longevity of P. Duboscqi

Longevity of *P. duboscqi* was assessed according to the methods of Moura [12]. Briefly, two day old female *P. duboscqi* adults were placed in plastic vials partially filled with plaster of Paris and fitted with screen tops. The flies were then fed on dark corn Karo syrup laced with the crude extracts in the ratio of 1:1. The flies were fed *ad libitum* and the number of days they lived was noted. Their longevity was compared with flies that were fed on dark corn syrup diluted with distilled water. Three replicates were used in this experiment with a total of 10 sand flies per replicate.

Data Analysis

All experiments were carried out in triplicate. The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for the experimental groups was done by analysis of variance (ANOVA) to analyze the significance of the results and student's *t* test. *P* values of < 0.05 were considered significant.

RESULTS

This study demonstrated that crude extracts from *T. camphoratus, A. fruticosa* and *T. minuta* reduced the survival time of *P. duboscqi*. Survival time decreased with increase in concentration of the plant extract used. At a concentration of 2.5 mg/ml methanol extracts, longevity in days was highest in the sand flies that had fed on *T. camphoratus* extract, 10.67±.33 days (mean±SE, n=30, *P*=0.011). This was followed by the sand flies that had fed on *T. minuta* extract that had lived for 9.44±1.46 days (mean ± SE, n=30 *P*=0.011). However, the sand flies that had fed on *A. fruticosa* extract had the lowest longevity of 8.67±0.33 days.

Further decline in survival time was observed when higher extract concentrations were used. At 5 mg/ml, lowest survival time was observed in sand flies that had fed on *A. fruticosa* extract, 6.00±0.58 days (mean±SE, n=30, *P*=0.001). Sand flies in the control group lived for 12.00±1.00 (mean±SE) under similar conditions. The sand flies that had fed on *T. camphoratus* had the highest longevity of 9.60±2.54 days (mean±SE, n=30, *P*=0.021).

At 10 mg/ml, longevity was further suppressed across all the three extracts used. In the sand flies that had fed on *A. fruticosa* extract lived for 4.40±0.58 days (mean±SE, n=30, *P*=0.001). This difference was significant as compared to the sand flies that had formed the control group. Feeding the sand flies on *T. minuta* and *T. camphoratus* extracts resulted in 5.24±0.56 and 7.62±0.28 days of survival (mean±SE) respectively (Fig. 1).

In the ethyl acetate crude extract, there was a steady decline in longevity in the sand flies that had fed on the three crude extracts as compared to control group. At 2.5 mg/ml, sand flies that had fed on *A. fruticosa, T. camphoratus* and *T. minuta* crude extracts lived for 11.34±0.26, 10.67±1.33 and 10.44±0.66 days (mean±SE) respectively. At 5 mg/ml concentration, survival time was 7.54±1.46, 9.67±1.33 and 9.00±1.00 (mean±SE) days in the sand flies that had fed on *A. fruticosa, T. camphoratus* and *T. minuta* crude extracts respectively. Longevity in the control group was 12.48±1.12 (mean±SE) days at the same extract concentration.

At 10 mg/ml, survival time was greatly depressed in all the three extracts. The sand flies that had fed on *A. fruticosa* extract survived for 4.42±1.38 days (mean±SE, n+30, *P*=0.001) while those that had fed on *T. camphoratus* had the highest survival time, 7.67±0.33 (mean±SE, n=30, *P*=0.017). The sand flies that had fed on *T. minuta* crude extract lived for 5.45±1.55 days (mean±SE, n=30, *P*=0.012) (fig. 2). The difference in longevity of the sand flies that had fed on methanol extracts and those that had fed on ethyl acetate extracts was not significant.

Combined Effects of the Crude Extracts on Longevity of P. Duboscqi

The extracts evoked a significant decline in survival time; however, the difference was not significant as compared to the individual plant extracts (*P*>0.05). Feeding the sand flies on 2.5 mg/ml methanol extracts of *A. fruticosa* + *T. minuta, A. fruticosa* + *T. camphoratus* and *T. minuta* + *T. camphoratus* extract combinations resulted in 11.00±1.00, 11.84±1.46 and 12.34±0.44 days respectively. This difference was not significant as compared to the sand flies that formed the control group.

There was further decline in survival time when 5 mg/ml extract concentration was used. The sand flies that had fed on *A. fruticosa* + *T. minuta* extract combination had the least survival time of 6.74±1.06 days (mean±SE, n=30, *P*=0.002). This was followed by the sand flies that had fed on *A. fruticosa*+*T. camphoratus* and *T. minuta* + *T. camphoratus* extract

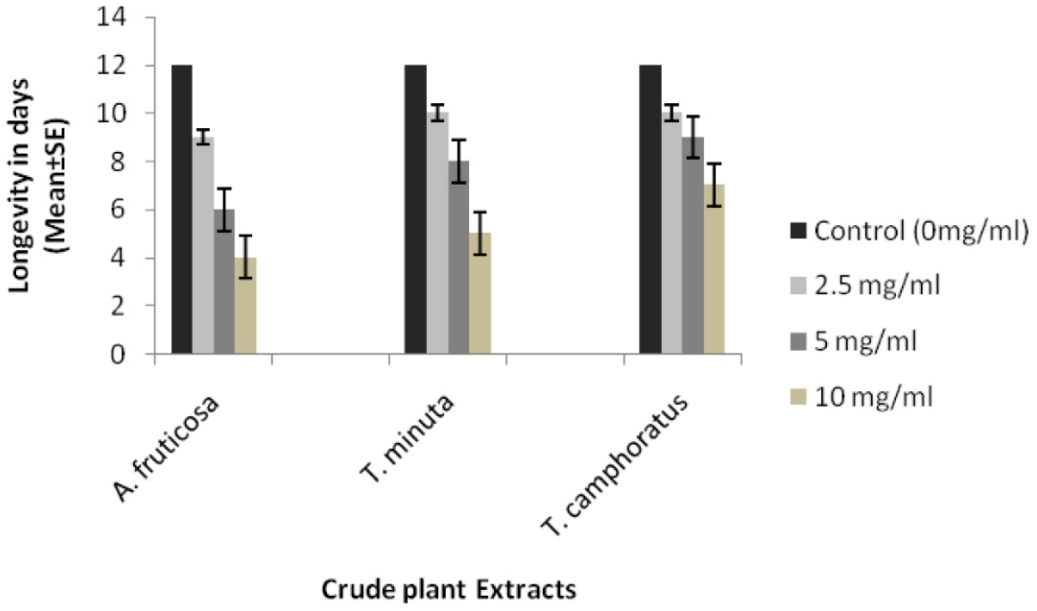

Figure 1: Longevity of *P. duboscqi* after feeding on the methanol crude extracts of *A. fruticosa, T. minuta* and *T. camphoratus*

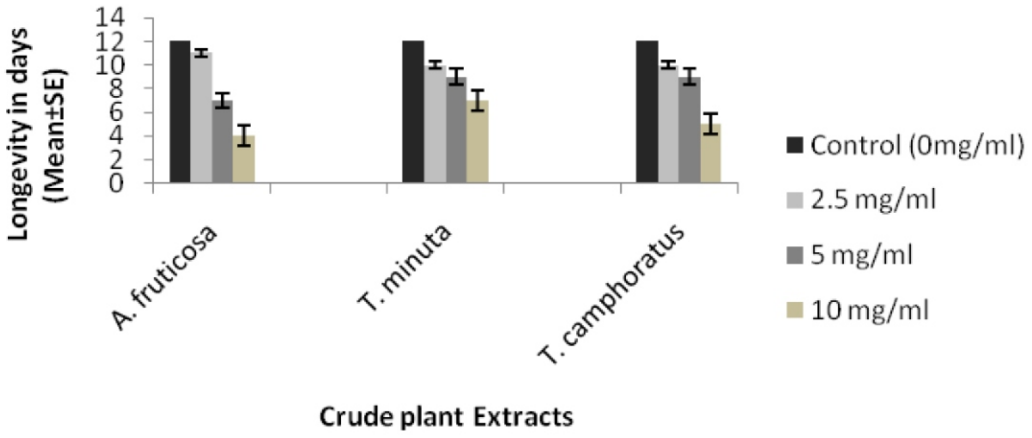

Figure 2: Longevity of *P. duboscqi* after feeding on *A. fruticosa, T. minuta* and *T. camphoratus* ethyl acetate crude extracts

combinations that lived for 10.67±0.33 (mean±SE, n=30, *P*=0.47) and 9.00±0.58 (n=30, *P*=0.31) days respectively. This difference was not significant as compared to the sand flies that formed the control group (fig. 3).

In the ethyl acetate extract combinations, at 2.5 mg/ml, the sand flies that had fed on the crude extracts of *A. fruticosa* + *T. minuta* combination had survival time of 10.10±0.55 (mean±SE, n=30, *P*=0.012), *A. fruticosa* + *T. camphoratus* 11.33±0.67 (mean±SE, n=30, *P*=0.012), *T. minuta* + *T. camphoratus* 12.67±0.88 (mean±SE, n=30, *P*=0.08). This difference was not significant when compared to the control group.

At 5 mg/ml, longevity of the sand flies that had fed on the crude extracts of *A. fruticosa* + *T. minuta* was 8.18±1.52 (mean±SE, n=30, *P*=0.011), *A. fruticosa* + *T. camphoratus* 11.33±0.67 (mean±SE, n=30, *P*=0.012), *T. minuta* + *T. camphoratus* 10.22±0.88 (mean±SE, n=30, *P*=0.012). When 10 mg/ml concentration was used, the sand flies that had fed on the extracts of *A. fruticosa* + *T. minuta* combination was 4.33±0.67 (mean±SE, n=30, *P*=0.001), *A. fruticosa* + *T. camphoratus* 7.33±0.67 (mean±SE, n=30, *P*=0.002), *T. minuta* + *T. camphoratus* 7.67±0.33 (mean±SE, n=30, *P*=0.002) (fig. 4). These results showed that both methanol and ethyl acetate crude extracts combinations did not result in any significant difference

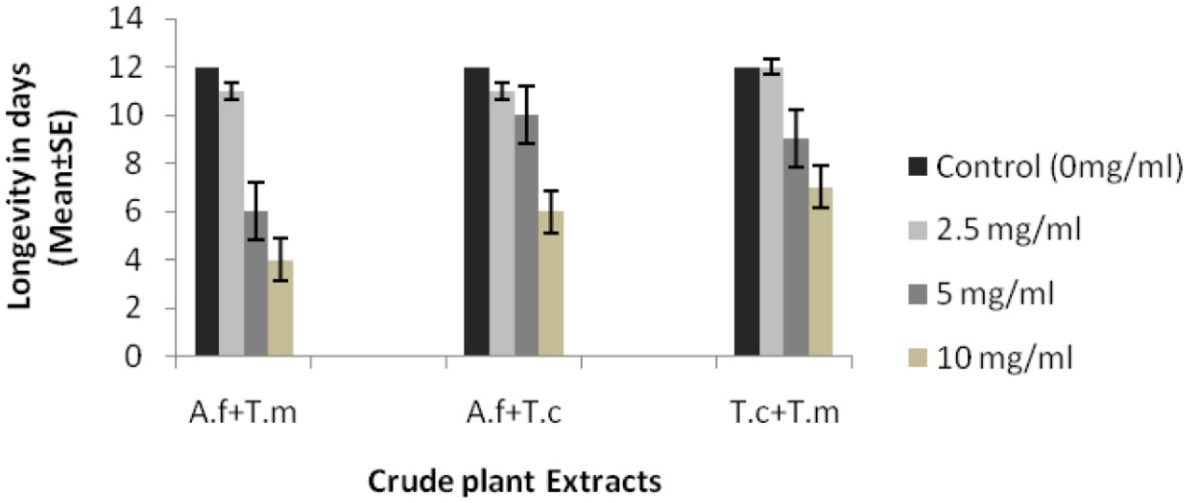

Figure 3: Longevity of P. duboscqi in days after feeding on the combined methanol crude extracts.

Key A. f + T. m= *Acalypha fruticosa* + *Tagetes minuta*

A. f + T. c= *Acalypha fruticosa* + *Tarchonanthus camphoratus*

T. c + T. m= *Tarchonanthus camphoratus* + *Tagetes minut*

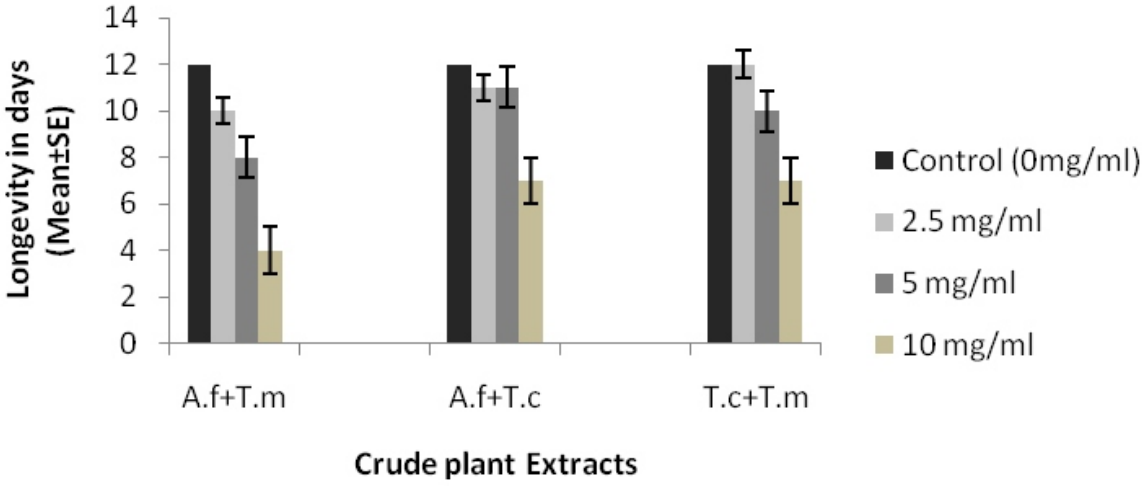

Figure 4: Longevity of P. duboscqi in days after feeding on the combined ethyl acetate crude extracts

Key A. f + T. m= *Acalypha fruticosa* + *Tagetes minuta*

A. f + T. c= *Acalypha fruticosa* + *Tarchonanthus camphoratus*

T. c + T. m= *Tarchonanthus camphoratus* + *Tagetes minuta*

between the combinations and the individual extracts.

DISCUSSION

Longevity and post oviposition survival time differed significantly after feeding on the crude plant extracts. Sand flies that fed on *A. fruticosa* and *T. minuta* crude extracts lived for a shorter period at a concentration of 10 mg/ml as compared to those that fed on *T. camphoratus*. Higher effects were observed in methanolic extracts than in ethyl acetate extracts. This implies that methanol and ethyl acetate solvents might have extracted different active compounds from the plants leading to the difference in their effects.

Female sand flies require a blood meal for the synthesis of eggs. However, they require sugar meals to supply energy and support synthesis of eggs. In the laboratory, the sugar solution which was mixed with the crude extracts acted as bait for the flies to feed on the insecticidal extract. Studies have shown that sand flies can feed on aqueous sucrose solutions mixed with noxious plant juices and have their lifespan greatly reduced [13; 14]. Normally, vectors feed on plant secretions, juice and nectar hence they end up feeding on insecticidal substances. This greatly reduces their survival time. The sugar that was mixed with the crude extracts might have attracted more females than males to the crude extracts. This might have led to females feeding more on the extracts than males. This probably explains why females lived for a short time as compared to the male sand flies. It has been shown that plant sugars attract more female sand flies than male sand flies [15], may be that is why females lived for a shorter period than males because they fed more on the crude extracts.

It has been shown that female sand flies need a blood meal for egg production, but sugar is their main source of energy and the only food taken by males [8]. The sugar feeding behavior of sand flies, therefore, influences survival time and fertility, dispersal, host seeking behavior and ultimately blood feeding and disease transmission [9]. Female sand flies obtain sugar meals mainly from honeydew excreted by aphids and coccids [15] and by feeding directly on tissues of plants in the field [16; 17]. In arid areas, there is evidence that availability of suitable sugar sources is a limiting factor for sand fly fitness and survival [18]. A previous study showed that flowering *Tamarix nilotica* is a highly attractive sugar source for sand flies [9].

In this study, we found out that *P. duboscqi* flies that had fed on *A. fruticosa* and *T. minuta* extracts had their life span greatly reduced to 7 days, those that had fed on *T. camphoratus* had a life span of 9 days as compared to 12 days in the control experiment. This is in agreement with the findings of Schlein. [13]. Their study found out that feeding *P. papatasi* on *Solanum jasminoides, Bougainvillea glabra and Ricinus communis* reduced their lifespan from 33 days to 8 days, 9 days and 11 days respectively [13]. These findings are in sharp contrast to sand flies kept on blood meal only. Their life span stretches 1 or 2 months [19] in the laboratory. This study has shown an insight on the potential of medicinal plants as sources of insecticides against sand flies. However, we need to consider conditions outside the laboratory in further field trials.

CONCLUSION

This study revealed that the plants *Acalypha fruticosa*, *Tagetes minuta* and *Tarchonanthus camphoratus* have a potent effect on the longevity of *P. duboscqi*. *Acalypha fruticosa* crude extract was more effective in reducing the survival time of *P. duboscqi* followed by *T. minuta* crude extract and the least in efficacy was *T. camphoratus* crude extract. We therefore propose that these herbs be considered for further and intensive studies on insecticidal trials on dipterans for possible harnessing in the manufacture of future botanical insecticides.

ACKNOWLEDGEMENT

We wish to appreciate the support of the center for biotechnology research and development director for allowing us to use his laboratory. We are indebted to the Director, Kenya Medical Research Institute (KEMRI) for providing the infrastructure and logistical support for the study.

Source of Funding

This study was funded by the International Foundation for Science (IFS) through a grant offered to Dr. Willy Tonui. There are no competing interests.

REFERENCES

1. World Health Organization, (2007). Report on global surveillance of epidemic-prone infectious diseases.
2. Depaquit J, Grandadam M, Fouque F, Andry P, Peyrefitte C. (2010). Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review . Euro Surveill. 15(10): pii=19507.
3. Dohm D J, Rowton E D, Lawyer P G, O'Guinn M, Turell MJ. (2000). Laboratory transmission of Rift Valley fever virus by Phlebotomus duboscqi, Phlebotomus papatasi, Phlebotomus sergenti, and Sergentomyia schwetzi (Diptera: Psychodidae). Journal of Medical Entomology. 37(3):435-8.
4. Rosypal A C, Troy G C, Zajac A M, Duncan R B, Waki K, Chang K P and Lindsay D S. (2003). Emergence of zoonotic canine leishmaniasis in the United States: Isolation and immunohistochemical detection of *Leishmania infantum* from foxhounds from Virginia. Journal of Eukaryotic Microbiology 50: 691693.
5. Roberts M T M (2006). Current understandings on the immunology of Leishmaniasis and recent developments in prevention and treatment. *British Medical Bulletin* 75 and 76: 115130.
6. Hailu A, Mudawi M A, Royce C, Wasunna M. (2005). Visceral Leishmaniasis: New Health tools are needed. PLoS Medicine 2: 7.
7. Hartzell A. and Wilcoxon F. (1941). A survey of plant products for insecticidal properties. Contrib Boyce Thompson Institute.
8. Killick-Kendrick R. (1999). The biology and control of Phlebotomine sand flies. Clinical Dermatology. 17(3):279-89.
9. Muller G C and Schlein Y. (2004). Nectar and honeydew feeding of *Phlebotomus papatasi* in a focus of *Leishmania major* in Neot-Hakikar oasis. Journal of Vector Ecology 29: 154-158.
10. Ireri L N, Kongoro J, Ngure P, Mutai C, Langat B, Tonui W, Kimutai A and Mucheru O. (2010). The potential of the extracts of *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae) and *Tarchonanthus camphoratus* L. (Compositae) against *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae), the

vector for *Leishmania major* Yakimoff and Schokhor . *J Vector Borne Dis* 2010; 47 (9): 168-174

11. Harborne J B. (1994). Phytochemical methods: A guide to modern Techniques of Plant Analysis. Chapman and Hall Ltd, London, pp 279.
12. Moura T R, Oliveira F, Novais F O, Miranda J C, Clarencio J. (2002) Enhanced *Leishmania braziliensis* Infection Following Pre-Exposure to Sand fly Saliva. PLoS Neglected Tropical Diseases 1 (2): 84.
13. Schlein, Y., Jacobson R. L. and Muller G. R. (2001). Sand fly feeding on noxious plants: a potential method for the control of leishmaniasis. American journal of Tropical medicine and Hygiene 65 (4): 300-303.
14. Ireri L N, Kongoro J, Ngure P K, Tonui W (2011). Laboratory Evaluation Of Selected Medicinal Plant Extracts In Sugar Baits And Larval Food Against Phlebotomus Duboscqi Neveu Lemaire (Diptera: Psychodidae), A Vector For Cutaneous Leishmaniasis In Kenya. WebmedCentral PARASITOLOGY 2(3):1-7 Pp (WMC001651)
15. Muller G C, Revay E E. and Schlein Y. (2011). Relative attraction of the sand fly Phlebotomus papatasi to local flowering plants in the Dead Sea region. Journal of Vector Ecology 36: S187S194.
16. Schlein Y. and Jacobson R L. (1994). Mortality of Leishmania major in *Phlebotomus papatasi* caused by plant feeding of the sand flies. American Journal of Tropical Medicine and Hygiene 50: 20-27.
17. Schlein, Y. and Muller,G.C.. (1995). Assessment of plant tissue feeding by sand flies (Diptera: Psychodidae) and mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 32:882-887.
18. Schlein.Y. and Jacobson R. L. (2002). Linkage between susceptibility of Phlebotomus papatasi to Leishmania major and hunger tolerance. Parasitology 125: 343-348.
19. Mukhopadhyay S. and Mandal C. (2006). Glycobiology of *Leishmania donovani*. *Indian Journal of Medical Research* 123: 203-220.