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In vitro studies of larvicidal effects of some plant extracts against *Anopheles gambiae* larvae (Diptera: Culicidae)

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Larvicidal effects of plant extracts against larvae of *Anopheles gambiae* were studied in the malaria entomology laboratory at Mendefera. The aim of the study was to evaluate ethanol and hot water extracts from leaves of seven different plants, viz., *Azadirachta indica*, *Eucalyptus globulus*, *Tagetes minuta*, *Datura stramonium, Lantana camara*, *Ricinus communis* and *Jatropha curcas*, as natural larvicides against third instar larvae of *An. gambiae*. Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24 and 48 h (h) of exposure. The experiment was conducted in complete randomized design in three replications. Data were collected on mortality of mosquito larvae in all the treatments and then subjected to statistical analysis using one-way ANOVA. Most of the tested extracts showed more than 50% mortality. *J. curcas* (100±0.00%) and *R. communis* (99.44±0.56) gave significantly higher larval mortalities at 1000 ppm concentration after 48 h of exposure. The experiment also showed that ethanol extracts gave higher larval mortality than hot water extracts and the efficiency of the extracts increased with an increase in the exposure period of the larvae.

Key words: Mosquito vector, malaria, larvicidal activity, leaf extracts

INTRODUCTION

Vector borne diseases are among the major causes of illness and death in many developing countries. Mosquitoes (Diptera: Culicidae) are responsible for transmitting the most important vector borne diseases including malaria, lymphatic filariasis, Japanese encephalitis, and dengue as well as yellow fever and other forms of encephalitis (WHO, 2006). Malaria is transmitted through bites of parasite-infected Anopheles mosquitoes. *Anopheles arabiensis,* a sibling of *An. gambiae* Giles complex is the major malaria vector in

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Eritrea (Shililu et al., 2003; Sintasath et al., 2005). Malaria is an important cause of death and illness in children and adults in tropical countries (WHO, 2014). Half of the world's population is at risk from malaria infection especially in Africa and Asia. Most deaths due to malaria occur in children aged less than five years old in sub-Saharan Africa (WHO, 2014). Pregnant women are also at a higher risk of malaria infection.

In Eritrea malaria affects about 70% of the population and transmission is seasonal. Peak transmission occurs during the months of September to November in the central highland and western lowlands. A smaller malaria season is often observed during the months of March to April in the eastern lowlands (MoH, 2010). In 2012,2013 and 2014 the clinical malaria cases reported were 41,710; 41,091 and 26,368 respectively. Although the number of cases decreased drastically in 2014,malaria still remains one of the life threatening diseases of human in the country (NMCP,unpublished data)

Vector-borne diseases also result in school absenteeism, loss of productivity, aggravation of poverty, high costs for health care and a burden on public health services (WHO, 2012). The approach to combat this disease largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant waters that serve as breeding sites or by killing the adult mosquitoes using residual insecticides. At present the malaria control strategies in Eritrea include integrated programs involving habitat management, application of larval insecticides, use of insecticide-treated nets (ITNs), and indoor residual spraying (MoH, 2010).

Mosquito resistance to the currently-used insecticides and the emergence of multi drug-resistant strains of parasites has escalated the malaria problem in the affected countries. According to the studies conducted in Ethiopia (Abate and Hadis, 2011) *Anopheles arabiensis* was resistant to an array of insecticides, including DDT, permethrin, deltamethrin and Malathion. Similarly World Health Organization (WHO, 1992) has documented mosquito resistance to the aforementioned four classes of insecticides. Thus, synthetic insecticides have created several problems including the development of resistant insect strains, ecological imbalance and harm to mammals. These drawbacks of the hitherto developed insecticides, researchers in the area are working hard to find environmentally safe alternatives.

Botanical insecticides may serve as suitable alternatives to synthetics in future, as they are relatively safe, easily degradable and readily available in many parts of the world (Sivagnaname and Klyanasundaram, 2004). Although bio-pesticides of plant origin have been extensively used on agricultural pest control, a very limited extent has been used against insect vectors of public health importance (Das et al*.,* 2007). Because of these, many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active ingredients. Among the most important plant constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins (Shaalan et al., 2005). Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, oviposition deterrents and affect different activities of the organisms (Eich, 2008).

Although several compounds of plant origin have been reported as bio-pesticides and used for control of mosquito larvae, there is still a wide scope for the discovery of more effective plant products (Saxena and Yadav, 1986) particularly in the indigenous flora of lesser studied countries like Eritrea.

This investigation is aimed at evaluating and comparing the toxicity of different botanical extracts against the larvae of *An*. *gambiae*; comparing the effect of hot water and Ethanol solvent leaf extracts on the mortality of the larvae and establishing dose-response activities of the plant extracts that showed over 90% mortality after 24 h exposure against the malaria vector larvae. This work will have a great contribution to the efforts being made by the national malaria control program in controlling larvae of malaria vectors.

MATERIALS AND METHODS

Collection and preparation of plant materials

A total of seven plants belonging to diverse families and genera were collected from Mai-nefhi, Keren, Asmara and their vicinities. The collections were made in September and November 2014 and were identified at the Department of Plant Biology herbarium, EIT, Mai-nefhi (voucher No. is as shown in Table 1). The plants were selected based on available literature, medicinal and insecticidal properties. The plants species and their parts used for the study are given in Table 1.

The collected plant materials were washed in the laboratory using running tab water, and spread on papers to dry under shade for 20 days. The dried leaves were ground to a fine powder by means of domestic pestle and mortar. Each powdered plant material was sieved using kitchen strainer. The powdered leaves of each plant species was kept at room temperature in labeled glass bottles until used.

Extraction of plant materials

Preparation of hot water extracts

Hot water extract of selected plant biopesticides were prepared by suspending 30 g of dry leaf powder (weighed using digital balance) in 150 ml of boiled distilled water for one hour in a 500-ml flask. The same procedure was used for each plant species used. During this processing period flasks were kept on water bath at 60°C for maintaining constant temperature for one hour. Extracts were filtered through No. 1 Whatman filter papers and used as a stock solution. These stock solutions were evaporated to dryness at 60°C and then stored in a refrigerator until used.

Table 1. Plant species, local name, family name, place of collection and test organs used in the study.

Voucher No.	Plant species	Local name	Family name	Collection site	Tested organ
1733	*Azadirachta indica* A.Juss	Neem	Meliaceae	Keren	Leaf
1220	*Ricinus communis* L.	Gulie	Euphorbiaceae	Mai-nefhi	Leaf
1359	*Tagetes minuta* L.	Chena-amharai	Asteraceae	Mai-nefhi	Leaf
1271	*Eucalyptus globulus* Labill	Saedakelamitos	Myrtaceae	Betgergish	Leaf
1173	*Lantana camara* L.	Bun-tilian	Verbenaceae	Asmara	Leaf
1179	*Datura stramonium* L.	Mezerbae	Solanaceae	Mai-nefhi	Leaf
4553	*Jatropha curcas* L.	Jatropha	Euphorbiaceae	Keren	Leaf

Preparation of ethanol extracts

Thirty grams of dry leaf powder was suspended in 200 ml of 70% ethanol in a plastic sack covered 500 ml capacity beaker for 24 h in a flocculater SW1 (stirrer) at 150 rotation per minute(rpm). The suspension was filtered through No.1 Whatman filter papers. The procedure was repeated three times with the residue on the filter paper and similarly filtered. The two filtrates were combined and dried in a rotary evaporator at 45°C. The same procedure was repeated for each of the plant species used and the resulting extracts kept in petri-dishes were stored in a refrigerator until used.

Collection and rearing of mosquitoes

Anopheles larvae were collected from open drains at Mekremtere (Mendefera environ). The collected immature were put in a plastic container and then transported to the malaria entomology laboratory at Mendefera. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the mosquitoes were identified and their species confirmed using identification keys (manual) by an entomologist before rearing. Cyclic generations of Anopheles mosquitoes were maintained separately in cages measuring 30*30*30 cm in an insectary at a mean room temperature of 27±2°C and a relative humidity of 70-80%. For survival of the Adult mosquitoes, they were provided a 10% sugar solution soaked in woolen cotton.

Besides this for continuous maintenance of mosquito colony, the adult female mosquitoes were provided a live small pigeon for supply of blood meal. At the base/bottom of each cage Petri-dishes with moist cotton or filter papers were placed for egg oviposition. The eggs laid were then transferred to enamel larval trays in the larval rearing chamber where the larvae were fed on powdered yeast until the larvae become pupae. The pupae were then collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

Preparation of test and control solutions

Two hundred and fifty milligram of each of the crude leaf extracts (ethanol and hot water) was weighed using digital balance and placed in separate measuring flasks and dissolved in four milliliters of acetone. Each mixture was diluted to 250 ml using distilled water to prepare stock solution of 1000 ppm concentration. From each of the 250 ml of 1000 ppm stock solution, 125 ml was placed in separate beakers and used as 1000 ppm test concentration.

For dose-response activity, stock solutions of crude ethanol leaf extract of *R.communis* and *J.carcas* were diluted with distilled water up to 250 ml and were considered as 500 ppm concentrations. This sequential method was used to prepare serially diluted test concentrations of 250 and 125 ppm (Sakthivadivel and Daniel, 2008).

Four milliliters of acetone was diluted to 250 ml in standard measuring flasks by adding distilled water to serve as a negative control solution as per WHO guidelines (WHO, 2005).

Larvicidal bioassays

All bioassays were conducted at Mendefera malaria entomology insectarium. The Larvicidal effects of crude leaf extracts of the plants were assessed by following the WHO standard procedures (WHO, 2005). The assays were performed in two steps: (i) Detection of susceptibility of larvae to extracts; and (ii) Determination of larvicidal concentration of crude extracts in which above 90% mortality of larvae occurred.

The sensitivity of the larvae to the extracts was determined at single concentration (1000 ppm). Larvicidal bioassays were conducted after 24 and 48 h in glass beakers of 125 ml test solutions with three replicates of test concentrations in a complete randomized design. Batches of 20 third instar larvae were transferred into each test concentration of crude ethanol and hot water leaf extracts by means of droppers. The larvae were placed on a filter paper for removal of excess water and then placed in each test concentration. Larval mortalities were recorded after 24 and 48 h of exposure in each concentration of the test solutions. The failure of larvae to swim to the surface or their inabilities to go to the bottom in response to mechanical probing, were taken as indicators for larval mortality. The tests were repeated three times. The mortality percent was computed from the average of three replicates of three trials.

Based on the preliminary screening results, plant extracts which showed over 90% mortality of larvae after 24 h exposure, were subjected to dose response larvicidal bioassay. The desired mortality percentages were obtained from ethanol extracts of *J. curcas* and *R. communis* at 1000 ppm concentration. Different concentrations (125, 250, 500 and 1000 ppm) were prepared and tested for Larvicidal activity. Tests were conducted three times. The percentage mortality was recorded from the average of three replicates of three trials.

Data analysis

Data entry was done using Microsoft Excel. Mean percent mortalities and standard deviations were determined by one-way Analysis of Variance (ANOVA) using SPSS for windows, version 20. When significant difference was observed, the means were separated using Tukey's Studentized Range test at 5% level of significance.

Table 2. Larvicidal effect of crude leaf hot water and crude ethanol leaf extracts against larvae of *An.gambiae* after 24 and 48 h treatment application.

Treatments	Mean % Mortality ±SE			
	24 h		48 h	
	Crude leaf hot water extracts	Crude ethanol leaf extracts	Crude leaf hot water extracts	Crude ethanol leaf extracts
Negative control	3.33±1.18^{e}	2.78±0.88^{e}	5.56±0.56^{e}	3.89±0.73^{e}
Azadirachta indica	20.56±1.30^{d}	25.56±1.76^{d}	28.89±1.62^{d}	36.11±1.11^{d}
Datura stramonium	61.11±3.38^{a,b}	70.56±3.38^{b,c}	76.11±3.41^{a,b}	86.67±3.33^{b}
Eucalyptus globulus	41.67±1.67^{c}	66.67±2.50^{b,c}	64.44±1.55^{c}	86.11±1.11^{b}
Jatropha curcas	67.78±1.62^{a}	97.78±1.21^{a}	79.44±1.62^{a}	100.00±0.00^{a}
Lantana camara	55.00±2.64^{b}	72.78±1.69^{b}	72.22±3.13^{a,b,c}	91.11±2.47^{a,b}
Ricinus communis	65.56±2.06^{a}	92.22±1.21^{a}	78.89±2.56^{a}	99.44±0.56^{a}
Tagetes minuta	56.11±1.82^{b}	63.33±2.76^{c}	67.78±1.47^{b,c}	73.33±2.50^{c}
Mean%	46.38±1.96	61.73±1.92	59.11±1.99	72.07±1.70
CV%	5.7	3.8	3.5	2.7

*Values are mean (%) of the three replications of three trials ±SE. ANOVA followed by Tukey's test performed; Different superscripts in the column indicate significant difference at p< 0.05 levels.

RESULTS

Effect of crude hot water leaf extracts on mortality of mosquito larvae

Cumulative percent mortality of immature *An. gambiae* treated with different plant extracts at 1000 ppm concentration for 24 and 48 h exposure time is given in Table 2. The extracts of all the plants tested have caused significant (p<0.05) mortalities on the larvae of *An. gambiae*, compared to the negative control. In addition, it was observed that toxicity of the plant extracts increased with increase in exposure time.

At 24 h exposure time, *J. curcas* (67.78 ±1.62%) and *R. communis* (65.56 ±2.06%) gave significantly (p<0.05) highest mortalities followed by *D. stramonium* (61.11±3.38%) at 1000 ppm concentration. Extracts of *L. camara* and *T. minuta* were equally effective with 55±2.64 and 56.11±1.82% mortalities, respectively. The other treatments had lower effect on larval mortality.

At 48 h exposure time, extracts of *J. curcas*, *R. communis* and *D. stramonium* gave significant (p<0.05) mortalities of 79.4±1.62, 78.9±2.56 and 76.1±3.41%, respectively. The other treatments also caused significant (p<0.05) mortalities that ranged from 28.9±2.64 to 67.78±1.82% where the least effective extract was observed from treatment of *A. indica* (28.9±2.64%).

Effect of crude leaf ethanol extracts on mortality of mosquito larvae

Cumulative percent mortality of larvae of *Anopheles gambiae* treated with the different plant extracts with 1000 ppm concentration, for 24 and 48 h exposure time is given in Table 2.

With all the treatments bio-assayed, percent mortality of the mosquito larvae after 24 h exposure ranged from 2.78±0.88-97.78±1.21% with a mean of 61.73±1.92%. Extracts of *J. curcas* and *R. communis* gave significantly (P<0.05) highest mortalities of 97.78±1.21 and 92.22±1.21%, respectively. The range of larval mortality in other extracts was between 25.56±1.76 to 72.78±1.69% at the same exposure time, where *A. indica* gave the least effect on larval mortality.

Mortality was significantly high (p<0.05) after 48 h treatment application. The highest mortalities were obtained from extracts of *J. curcas* (100%) and *R. communis* (99.4±0.56%) followed by *L. camara* (91.1±2.47%). *D. stramonium* and *E. globulus* gave almost the same mortalities of 86.7±3.33 and 86.1 ±2.98%, respectively with no significant difference (p>0.05) between them while *T. minuta* gave 73.3±2.50% mortality. The least effective treatment was *A. indica* with 36.1±1.11% larval mortality.

Comparing the two solvent extracts ethanol gave significantly (p<0.05) higher mortality of larvae than hot water extracts at the two exposures times.

Dose-response activity

The toxicity of dose–response larvicidal bioassay is given in Table 3. According to preliminary screening results, two extracts (*J. curcas* and *R. communis*) that showed significantly (p<0.05) higher mortality after 24h exposure time were subjected to dose–response larvicidal bioassay test. The larvicidal effects of the two selected plant

Table 3. Mean % larval mortalities of ethanol leaf extract of *Jatropha curcas* and *Ricinus communis*.

Treatment	Concentration (ppm)	Mean % mortality±SE
Jatropha curcas	125	71.67±0.96[c]
	250	91.11±2.42[b]
	500	97.78±0.55[a]
	1000	97.78±0.55[a]
Ricinus communis	125	22.78±2.00[c]
	250	32.22±2.42[c]
	500	80.00±2.89[b]
	1000	92.22±2.00[a]
Negative control	0	2.22±0.55[d]

*Values are mean (%) of the three replications of three trials ±SE. ANOVA followed by Tukey's test performed; Different superscripts in the column indicate significant difference at p< 0.05 levels.

extracts were found to be dose dependent. With *R. communis* extracts, exposure to 1000ppm for 24 h produced significantly ($p<0.05$) greater mortality (92.22%) compared to exposures of lower concentrations (Table 3). In general, at 125 and 250 ppm concentrations crude ethanol leaf extracts of *R. communis* induced less than 50% mortality while this mortality increased to 80% at concentration of 500 ppm. Likewise crude methanol leaf extracts of *J. curcas* showed similar bio potency (97.78%) at 1000 ppm and 500 ppm concentrations while this mortality rate decreased to 91.11 and 71.67% at 250 and 125 ppm concentrations, respectively.

DISCUSSION

Exploring bioactive medicinal plants in vector management program is one of the eco-friendly approaches because they are easily biodegradable. Naturally plants are rich store houses for potential bioactive compounds which are gaining appreciation in recent times among the scientific communities. According to Berenbaum (1985) crude extracts of the plants may have mixtures of active compounds which act synergistically and their overall bioactivity was also greater than individual compounds (Chen et al., 1995).

Findings of this study highlight the potential of the different plant species bio assayed and their active ingredients on immobilization and toxic effects on the larvae of *An*. *gambiae*. Larvicidal effect of all the bio assayed extracts increased with an increase in exposure time. Dose-response effects of *J. curcas* and *R. communis* extracts also indicated that mortality means increased progressively with increasing doses. Bioactivity of the plants extracts were also significantly varied based on the solvent used for extraction which showed the superiority of ethanol extracts over hot water extracts. Similar results were obtained by Chaudhary et al. (2013) who reported potent mortality effects of ethanol extracts of some plants against *Meloidogyne incognita* (nematode), as compared to hot water extracts.

The larvicidal effects of leaf extracts of various plants have been reported by a number of researchers (Raj Kumar and Jebanesan, 2005). Innocent et al. (2008) suggested that the larvicidal effect of the root barks extracts of Lantana might be due to larvicidal chemicals present in the extracts. In this aspect, the findings of this study are in conformity with the finding of the previous researchers.

In this study, *J. curcas* and *R. communis* members of family Euphorbiaceae were found to be superior amongst all the tested plant extracts. Ethanolic extracts of *J. curcas* gave 100±0.00% mortality against the mosquito larvae after 48 h exposure time. Similar results were reported by Sakthivadivel and Daniel (2008), which showed crude petroleum ether leaf extract of *J. curcas* to have Larvicidal activity with the LC50 of < 100 ppm on the early 4th instar larvae of *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. Most parts of *J. curcas* plant is reported to be toxic (Kumar and Sharma, 2008), probably explaining the larvicidal activity shown in the present study.

R. communis gave 99.44±0.56 % mortality of the larvae treated 48 h after application of treatment. Similar results were reported by Aouinty et al. (2006) after analyzing aqueous extracts of *R. communis* leaves and *Tetraclinis articulate* wood on the 2nd and 4th instars larvae of *Culex pipiens*, *Aedes caspius*, *Culiseta longiareolata* and *Anopheles maculipennis*. The Larvicidal activity shown by *R. commuinis* could be due to the presence of the alkaloid, ricinine and the ricin protein which are toxic substances (Harborne and Baxter, 2001).

Ethanol extract of *L. camara* was found to be effective and recorded 91.11±2.47% mortality of the treated larvae

after 48 h exposure time. Similar results were reported by Nath et al. (2006) who found that leaf extract of *L. camara* showed larvicidal activity against *Cx. Quinquefasciatus* and *Ae. albopictus*. Another study by Innocent et al. (2008) showed the effect of the root barks extracts of Lantana against late 3rd or early 4th instar larvae of *An. Gambiae sensustricto.* This effect of lantana could be due to the presence of lantadenetriterpenoids and furanonaphthaquinones in Lantana species which may serve as an indicator for the plant's mosquito larvicidal properties.

It was observed in this study that *E. globulus* extract to have larvicidal activity. It showed 86.11±1.11% mortality against *An.gambiae* larvae 48h post treatment. This is comparable with the study of Sheeren (2006) who reported the larvicidal property of *E. globulus* using petroleum ether seed and leaf extracts against *C. pipiens* larvae with larval mortality of 100 and 80%, respectively at a dose of 1000 ppm.

Ethanol extract of *D. stramonium* was also found to have larvicidal activity at 1000 ppm dose with larval mortality of 86.67±3.33% at 48 h post treatment. This result is comparable to reports of Anitha and Geethapriya (2012) who observed the larvicidal activity of petroleum ether extract of *D. stramonium* with 100% mortality against 4th instar larvae of *Ae. aegypti*, at 1000 µg/ml dose. This effect may be due to the presence of neurotoxin compounds in this plant extracts as phytochemical analysis of *D. stramonium* indicated that it contains alkaloids and flavonoid ingredients (Anitha and Geethpriya, 2012).

T. minuta also gave significantly considerable larvicidal effect with both crude extracts but as noted above (Table 4) better results were obtained with ethanol extracts. These results are in conformity with the results of Macêdo et al. (1997) who reported that extracts of aerial parts of *T. minuta* showed larvicidal effects against *Ae. fluviatilis*.

Amongst all tested plant species Neem was reported as least effective extract, but some previous studies (Azim et al.,1998) reported high larvicidal potential of the plant against mosquito larvae. This low effect of Neem could be attributed to the method of extraction adopted and habitat (environment) of the plant species. Therefore, it requires further confirmation.

Considering the attained dose-mortality relationships, larval mortality increased with increase in concentration of the bio assayed extracts. *J. curcas* gave 97.78±0.55% mortality at both higher concentrations (500 and 1000 ppm). However this percentage mortality considerably decreased with lower concentrations of 250 and 125 ppm. With *R. communis,* mortality considerably decreased from 92.22±2.89 to 22.78±2.00% with decrease in dose of the extract. Comparable trends were depicted by El Tayeb et al. (2009) who studied the water extracts of *S. Argel* and *C. procera* as larvicides against certain mosquito species. They reported positive relationship between larval mortalities and the increase in the concentrations of extracts.

Moreover, the direct proportion detected between the mortality means and exposure time from 24 to 48 h confirmed what has been reported by several investigators (Abdul Rahuman et al., 2008; Abdu Zahir et al., 2009), which showed gradual buildup in mortalities in relation to time factor post treatments.

As shown above (Table 2), the variations among the extracts' activities could be ascribed to the difference in plant species and its environment, type of solvent used and method of extraction adopted in the test. The literature revealed that plants contain different quantity and quality of active compounds depending on the species and its habitat environment (Satti et al., 2010). Therefore, the superior mortalities manifested by the bio assayed plants could be attributed to the kinds of active ingredients occurred in these plants as compared to those of less effective.

Overall, the results of this study highlights the potential of the different plants for their use as larvicides which can serve as cheaper and environmentally benevolent alternatives to chemical larvicides for resource poor countries.

Conclusion

The present study showed that leaf extracts of the bio assayed plants possessing bioactive compounds to control immature stages of *An. gambiae*. Since the use of plants in insect control offers a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost, the use of indigenous plant based products by individuals and communities can provide a prophylactic measure for protection against mosquito borne diseases. There is also a need for promoting the use of herbal products through community based vector control programs.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Abate A, Hadis M (2011). Susceptibility of *Anopheles gambiaes.l*. to DDT, malathion, permethrin and deltamethrin in Ethiopia. J.Trop. Med. Int. Health 16:486-491.

Abdul Rahuman A, Bagavan A, Kamaraj C, Vadivelu M, Abdu Zahir A, Elango G, Pandiyan G (2008). Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* say (Diptera: Culicidae). J. Parasitol. Res. 104(3):637-643.

Abdu Zahir A, Abdul Rahuman A, Kamaraj C, Bagavan A, Elango G, Sangaran A, Senthil Kumar B (2009). Laboratory determination of efficacy of indigenous plant extracts for parasites control. J. Parasitol.

Res. 436:1-5.
Anitha R, Geethapriya D (2012). Larvicidal activity of plant extracts on *Aedes aegypti*, Asian Paci. J. Trop. Biomed. S1578-S1582.
Aouinty B, Oufara S, Mellouki F, Mahari S (2006). Preliminary evaluation of larvicidal activity of aqueous extracts from leaves of *Ricinus communis* L. and from wood of *Tetraclinisarticulata* on the larvae of four mosquito species: Culex pipiens, Aedes caspius, *Culiseta longiareolata* and *Anopheles maculipennis.* J. Biotech. Agron. Soc. Environ. 10: 67-71.
Azim M, Naqvi S, Ahmad I, Tabassum R, Anbreen B (1998). Toxicity of Neem leaves extract compared with Malathion against late 3rd instar larvae of *Culexfatigans*by WHO method. Turk. J. Zool. 22:213-8.
Berenbaum M (1985). Allochemical interactions among in plants. Rec. Adv. Phytochem. 19:139-169.
Chaudhary K, Haile A, Ayresea Z, Semereab G, Weldegergish T (2013). Nematicidal activity of Eritrean weed plants against the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. Nematropica 43:207-215.
Chen W, Isman M, Chiu S (1995). Antifeedant and growth inhibitory effects of limonoidtoosendanin and Meliatoosendanin extracts on the variegated cutworm *Prodromasaucia* (Lep.,Noctuidae). J. Appl. Entomol. 119:367-370.
Das N, Goswami D, Rabha B (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. J. Vector Borne Dis. 44:145-148.
Eich E (2008). Solanaceae and Convolvulaceae: Secondary Metabolites, Biosynthesis, and Chemotaxonomy. Springer-Verlag. Berlin Heidelberg.
El Tayeb F, Taha A, Mardi H, Sid Ahmed O (2009). Water extracts of Hargal, *Solenostemmaargel*, and Usher, *Calotropisprocera*, leaves as natural insecticides against mosquito larvae. J. Sci. Technol. 10(3):67-76.
Harborne J, Baxter H (2001).Chemical Dictionary of Economic Plants, Dictionary of Useful Plant Products. John Wiley and Sons. P 217.
Innocent E, Joseph C, Gikonyo N, Moshi M, Nkunya M. Hassanali A (2008). Mosquito larvicidal constituents from *Lantana viburnoides* (Verbenaceae). J. Vector Borne Dis. 45:240-244.
Kumar A, Sharma S (2008). An evaluation of multipurpose oil seed crop for industrial uses (Jatrophacurcas L.). J. Ind. Crops Prod. 28:1-10.
Macêdo M, Consoli R, Grandi T, Anjos A, Oliveira A, Mendes N (1997).Screening of Asteraceae plant extracts for larvicidal activity against *Ae. fluviatilis*. Mem. Inst. Oswaldo. Cruz. 92:565-570.
Ministry of health (MoH) (2010). Integrated vector management guidelines for the control of malaria and other vector-borne diseases in Eritrea.
Nath D, Bhuyan M, Goswami S (2006). Botanicals as Mosquito Larvicides. J. Def. Sci. 56:507-511.
Raj Kumar S, Jebanesan A (2005). Ovicidal activity of *Solanum trilobatum* L. (Solanaceae) leaf extract against *Culex quinquefasciatus* Say and *culex tritaeniorhynchus* Giles (Diptera: Culicidae). J. Int. Trop. Insect Sci. 24:340-342.
Sakthivadivel M, Daniel T (2008). Evaluation of certain insecticidal plants for the control of vector mosquitoes vis. *Culex quinquefaciatus*, *Anopheles stephensi* and *Aedes aegypti*. J. Appl. Entomol. Zool. 43:57-63.
Satti A, Hashim H, Nasr O (2010). Biologically active chemicals with pesticidal properties in some Sudanese plants. J. Int. Environ. Appl. Sci. 5(5):767-780.
Saxena S, Yadav R (1986). A preliminary laboratory evaluation of an extract of leaves of *Delonixregia*Raf as a disruptor of insect growth and development. J. Trop. Pest Manage. 32:58-59.
Shaalan E, Canyon D, Younes M, Abdel-Wahab H, Mansour A (2005). A review of botanical phytochemicals with mosquitocidal potential. J. Environ. Int. 31:1149-1166.
Sheeren M (2006). Larvicidal effects of Eucalyptus extracts on the larvae of *Culex pipiens* mosquito. J. Int. Agric. Biol. 8:896-897.
Shililu J, Ghebremeskel T, Mengistu S, Fekadu H, Zerom M, Mbogo C, Githure J, Gu W, Novak R, Beier JC (2003). Distribution of anopheline mosquitoes in Eritrea. Am. J. Trop. Med. Hyg. 69:259-302.
Sintasath D, Tewolde G, Lynch M, Kleinau E, Gustavo B, Shililu J, Brantly E, Graves P, Beier J (2005). Malaria prevalence and associated risk factors in Eritrea. Am. J. Trop. Med. Hyg. 72:682-687.
Sivagnaname N, Kalyanasundaram M (2004). Laboratory evaluation of methanolic extract of *Atalantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms. Mem. Inst. Oswaldo. Cruz. 99:115-118.
WHO (World Health Organization) (2005). Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/13. WHO. Geneva.
WHO (World Health Organization) (2006). Pesticides and their application for the control of vectors and pests of public health importance. WHO Pesticides Evaluation Scheme 113.
WHO (World Health Organization) (1992). Vector resistance to pesticides, Fifteenth Report of the WHO Expert Committee on Vector Biology and Control. Tech. Rep. Ser. Geneva.
WHO (World Health Organization) (2012). Guidance on policy-making for integrated vector management. WHO/HTM/NTD/VEM/2012.Geneva. Switzerland.
WHO (World Health Organization) (2014). World Malaria Report 2014.Geneva. Switzerland.