I**nsecticidal potential ofEucalp*tus citriodora* Hookand *Hyptissuavolens*(L.) Poit EthanolLeaves Extracts against Malaria mosquito (*Anopheles gambiae* Giles)**

The article is an original research article

Abstracts

Adult mosquitoes are known to transmit diseases, including malaria and filariasis. The larvicidal, pupacidal and mosquitocidalproperties of the leaves ethanol extracts of *E.citriodora* and *H. suaveolens*againstthe larva, pupa and adult *Anopheles gambiae*were examined.Phytochemical screening was also conducted on the selected plant species to determine the secondary metabolites. Ethanol was the solvent used for the extraction of the leaves using Soxhlet extractor at 60^{o}Cto determine the secondary metabolites.The larvae, pupa and adult mosquito were exposed to 0.2, 0.4, 0.6, 0.8 and 1.0 % of theextract at 28 ± 2^{o}C, 75 ± 5 % RH, 12L:12D photoperiod. Results showed that the leavesextracts of both plants caused high mortality of larvae, pupae and adults of *An. gambiae* at 1.0 % concentration level. The most effective extractwas *H. suaveolens* which caused 100 % mortality of larvae and pupae at 1.0 % concentration level within 48 h, while 100 % mortality of adult *An. gambiae* was observed at 1.0 % concentration within 4 h. Results also showed that*E. citriodora*leaves contains saponins, tannins, flavonoid, phenol, quinones and alkaloids, while that of *H. suaveolens*leaves contains saponins, tannins, flavonoid, phenol, alkaloids and sterols. As the extracts of both plants caused mortality at every stage of *An gambiae,* it can be suggested that the two plants could be integrated into vector control program of malaria and filariasis.

Keywords: *Eucalyptuscitriodora* and *Hyptissuaveolens, Anopheles gambiae,* larvicidal,pupacidal,mosquitocidal and phytochemicals.

Introduction

Mosquitoes are rural- urban insect which had been noted for their high commonness in many under-developing anddeveloping countries where insect pest management is still minimal. Mosquitoes of different species including *Aedes aegypti, Anopheleddirus, Culex quinquefasciatus, Aedes albopictus, Anopheles funestus, Anopheles arabiensis, Anopheles*

*annularis, Anopheles culcifaciesAnopheles stephensi*and *Anophels gambiae*, among others have been noted to be vector of different types of diseases among which malaria is the most prevalent in developing nations (Tawastin*et al.,* 2001; Joy *et al.,* 2003; Van Geertruyden*et al.,* 2004; Aina *et al.,* 2009; WHO 2010; Roll Back Malaria 2014; Akinkurolere*et al.,* 2011; Shankar *et al.,* 2013; Ileke*et al.,* 2014). The high incidence of malaria disease has been reported and it is the leading cause of death in most countriesof the world (Federal Ministry of Health 2012; Onwujekwe*et al.,* 2000).

Anopheles gambiae is a principal malaria vector in most countries of the world where the femaleswere responsible for the transmission of the malaria parasite called Plasmodium. There are other malaria vectors such as *Anopheles arabiensis,Anopheles pharoensis, Anopheles funetus, Anopheles nili, and Anopheles stephensi*which also transmit malaria in some countries of the world (Kloss and Zein 1993; Zein 2021; Balkew*et al.,* 2021). Various species of mosquitoes are abundant in more than 100 countries infecting over 700 million people every year globally (Akinkurolere*et al.,* 2011; Rahuman 2011). Presently there are over three hundred species in the world grouped in 39 genera and 135 subgenera (Remia and Logaswamy, 2010). *Anopheles gambiae* is one of the notorious species which have been incriminated for transmitting malaria parasites an estimated 3.3 billion people in 97 countries (Roll Back Malaria 2015; WHO 2015).

Indiscriminate and rampant use of the chemical insecticides in the control of mosquito vectors in order to prevent diseases vectored bythem has resulted in causing adverse effects on the environment (potential toxicity), depletion of the ozone layer, high operational cost, some of the mosquitoes develop resistance to the chemical insecticides, poor handling by peasant farmers andcommunity acceptance are affected. The factor mentioned above prompted the search for new methods of control. Use of plant extracts, essential oils and phytochemicals with larvicidal, mosquitocidal, and pupacidal potential are recognized as alternative to replace the chemical insecticides in mosquito control program. These botanicals and their derivatives also have excellent oviposition inhibition, insect repellence or insect growth regulatory effects, and they are found environmentally to be safe, bio-degradable and target specific. Several authors, from different part of India, timely reported the potential role of various plant extracts (Govidaranjan *et al.,* 2011; Prabhu *et al.,* 2011; Kumar *et al.,* 2011; Mandal 2011). Since these contain multiple

active ingredients with different mode of action, and thus lessening the chance of resistance development by the mosquito population.

Thelarvicidal, pupicidal and mosquitocidalactivities of the botanicals may be the effect of the toxic phytochemical present in the plants. This research was carried to analyze the phytochemicals present in the leaves of *E. citriodora* and *H.suaveolens*and to evaluate the toxicity of their extractson all the developmental stages of *An gambiae.*

Materials and methods

Collection of plant materials

The fresh leaves of*E. citriodora* and *H. suaveolens*were obtained from the campus of Ekiti State University, Ado Ekiti, Nigeria. Theauthentication of the leaveswere carried out by the herbarium curator in the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria.

Preparation of ethanol extracts from the plant materials

The leaves of*E. citriodora* and *H. suaveolens*were washed separately in clean water, cut into small pieces and air-dried for ten days on the laboratory tables. The dry leaves were pulverized into powders using a Binatone, electric grinder (ModelBL-400). The powders were kept in a black polythene bag and stored at the ambient temperature of 28±2^{o}C.Two hundred grams (200g) of each of the ground leaf material was weighed into a thimble and their oil was extracted with absolute ethanol at the temperature of 60^{o}C, using a Soxhlet extractor. The resulting extract was concentrated using rotary evaporator and then air-dried to remove any trace of ethanol. The extracts were poured into brown bottles, labeled and stored until needed for the experiment.

Rearing of mosquito larvae and pupae

Two black (opaque) plastic water-bath with large surface area were filled with rain water. The importance of the rain water was to simulate the natural breeding environment of mosquito and to attract adult mosquitoes for the purpose of breeding.Twenty grams (20 g) of yeast was sprinkled on the surface of the water and allowed to dissolve in order to feed the mosquitoes larvae. The containers filled with rain water and dissolved yeast also served as the bait for the

adult mosquitoes. The containers were arranged under shade trees outside the laboratory. Adult mosquitoes were able to visit the bait to lay their eggs which later undergo metamorphosis to develop into larvae and pupae. Afterward, the containers were moved to the laboratory.One of the containers was kept in a large netted cage for complete metamorphosis to take place and there was emergence of numerous adult mosquitoes which were exposed to the extracts of the test plants.

Toxic effect of the ethanolextracts of*E. citriodora* and *H. suaveolens*on the larvae the and pupae stages of *An. gambiae*

Serial concentrations of the extracts of *E. citriodora* and *H. suaveolens*leaves were prepared by adding 2.0µg of extracts to 100mL of distilled water to prepare, 0.2, 0.4, 0.6, 0.8 and 1.0 %.Five grams (5 g) of yeast powder was added to the extracts to serve as food for the larvae. Afterward, 20 larvae or pupae of *An. gambiae* were introduced into the beakers containing each of theextracts concentration (0.2, 0.4, 0.6, 0.8 and 1.0%). Untreated and water treatment control were also set-up. Larvae and pupae mortality were observed 48h after treatment, by introducing the larvae and pupa into distilled water to notice recovery. Larvae and pupae were considered dead when they failed to come to the surface for gaseous exchange and when they did not respond to probing on the abdomen with a sharp pin.

Fumigant effect of theethanol extracts of*E. citriodora* and *H. suaveolens*on adult *An. gambiae*

Ten adults mosquito were introduced into a test tube which was later plugged with cotton wool. Stripes of Whatman No. 1 filter paper (90 mm diameter) soaked in different concentrations (0.2, 0.4, 0.6. 0.8 and 1.0 %) of extracts were suspended into each test tube with the aid of a thread. Each treatment and the control was replicated 4 times and arranged in Complete Randomization Design (CRD)Adult mortality was observed, counted and recorded after 4h of application

Qualitative phytochemical screening of leavesethanol extracts of *Eucalyptus citriodora*

The phytochemical composition was determined following the procedure ofHarborne, J.B. (1973).

Saponins test (foam test): Two milliliters (2 mL) of extract was dissolved in 3 mL distilled water and shaken vigorously. The formation of a stable layer of foam indicated the presence of saponins in the sample.

Tannins test: Half milliliter (0.5 mL) of extract was dissolved in 1.0 mL of water and 1-2 drops of ferric chloride ($FeCl_3$) solution was added. The appearance of blue colour indicated the presence of tannins

Flavonoid test: Two milliliters (2 mL) of the filtrate was pipetted into a test tube and 5 drops of concentrated hydrochloric acid (HCl) and 2.0 g of magnesium filings were added to it. The appearance of red colour indicated the presence of flavonoids.

Phenol test: A pinch of ferric chloride ($FeCl_3$) was added to 2 mL of the extract and the appearance of green colour indicated the presence of phenol.

Quinone test: A few drops of concentrated H_2SO_4 was added to 2 mL of the extract. Appearance of red colour indicated the presence of quinones.

Test for alkaloids: The method of Harborne (1973) was used to determine the presence of alkaloid in the tested plants. A drop of Mayer's reagent was added by the side of the test tube to a few quantity of the filtrate. A creamy or white precipitate indicates the presence of alkaloid

Data Analysis

Data obtained were subjected to Analysis of Variance (Anova), while Turkey's test was used in separating the means.

Results

Effect of leaves ethanol extracts of*E. citriodora*and *H. suaveolens*on the mortality of larvae of *An. gambiae* within 48 h post-treatment.

The Mortality of the larvae of *An. gambiae*treated with the leaves extracts of*E. citriodora* and ***H. suaveolens***is presented in Table 1. The mortality of insect larvae varied with the species of plant used as well as the concentration of the extracts used. Extracts of*H. suaveolens*was able to achieve 100 % mortality within 48 h of treatment with 0.8 % concentrationand it is significantly ($P < 0.05$) higher than the mortality recorded in other concentration levels of the extracts. Moreover, all extracts concentrations of *H. suaveolens*achieved above 50 % larvae mortality. *E.citriodora* was only able to achieve 97.30 % larva mortality when treated with 1.0 % concentration level ofextracts within 48 h of exposure. All levels of concentrations recorded significantly higher larvae mortality than the control experiments.

Table 1: Percentage mortality of the larva of *A. gambiae* during 48 h of exposure to different concentration of leaves ethanolextracts of *E. citriodora* and *H. suaveolens*

Plant material	Percentage Concentration				
	0.2	0.4	0.6	0.8	1.0
E. citriodora	35.30 ± 0.75^{b}	46.10 ± 1.25^{b}	68.50 ± 2.40^{b}	86.20 ± 3.14^{b}	97.30 ± 3.25^{b}
H. suaveolens	52.50 ± 1.37^{a}	66.10 ± 2.13^{a}	87.30 ± 2.21^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Water treatment	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Untreated	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

Each value is mean± standard error of four replicates. Means in the same column followed by the same alphabet(s) are not significantly different at $p < 0.05$ using Tukey's test

Effect of leaves ethanolextracts of*E. citriodora* and *H. suaveolens*on the mortality of pupae of *An. gambiae* at 48 h post-treatment.

The mortality of the pupa of*An. gambiae* treated with the leavesextracts of *E. citriodora* and ***H. suaveolens***is presented in Table 2. The mortality of the pupae varied with the species of plant

used as well as the concentration levels of the extracts used. Extracts of *H. suaveolens*was able to cause 100 % mortality within 48 h of exposureto 1.0 % concentration and it is significantly ($P < 0.05$) higher than the mortality recorded in other concentrations of extracts. Moreover, not all extracts concentration levels of *H. suaveolens*achieved above 50 % larvae mortality because, only 44.50 %pupae mortality was achieved on exposure to 0.2 % concentration level. *E.citriodora* was only able to achieve 95.25 % larva mortality when treated with 1.0 % concentration level of exracts during the 48 h of exposure.All levels of concentrations recorded showed significantly higher pupae mortality than the control experiments.

Table 2: Percentage mortality of pupae of *An. gambiae* at 48 h of exposure to different concentration ofleaves ethanolextracts of *E. citriodora* and *H. suaveolens*

Plant material	Percentage Concentration				
	0.2	0.4	0.6	0.8	1.0
E. citriodora	38.25 ± 0.68^{b}	45.22 ± 2.18^{b}	59.25 ± 2.33^{b}	74.50 ± 3.21^{b}	95.25 ± 4.54^{b}
H. suaveolens	44.50 ± 1.14^{a}	56.30 ± 2.11^{a}	78.55 ± 2.60^{a}	85.15 ± 2.17^{a}	100.00 ± 0.00^{a}
Water treated	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Untreated	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

Each value is mean± standard error of four replicates. Means in the same column followed by the same alphabet(s) are not significantly different at $p < 0.05$ using Tukey's test

Effect of ethanol extracts of *E. citriodora* and *H. suaveolens*on mortality of adult *An. gambiae*

Thefumigant effect of extracts of*E.citriodora* and *H. suaveolens*on mortality of adult *An. gambiae* within 4 hafter treatment is presented in Table 3.Insect mortality increased with increased concentration level of the extractsand varied with the species of plants used. Generally, *An. gambiae* adults exposed to extracts of *E.citriodora* and *H. suaveolens* showed significantly higher ($P < 0.05$) mortality values than the control experiments.Extracts of *H. suaveolens* at concentration level of 0.8 % was able to cause 100 % adult mortality within 4 h of exposure.*E. citriodora* leaf extractwas also able to attain 93.25 % adult mortality on exposure to 1.0 % concentration level during the 4 h post-treatment.

Table 3: Fumigant effect of *E. citriodora* and *H. suaveolens* on the mortality of Adult *An. gambiae* at 4 hours post-treatment.

Plant material	Percentage Concentration				
	0.2	0.4	0.6	0.8	1.0
E. citriodora	42.20 ± 1.44a	52.25 ± 1.31^{b}	64.30 ± 2.41^{b}	79.25 ± 3.33^{b}	93.25 ± 3.32^{b}
H. suaveolens	46.25 ± 2.37^{a}	68.22 ± 2.10^{a}	85.50 ± 3.77^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
Water treated	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Untreated	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

Each value is mean± standard error of four replicates. Means in the same column followed by the same alphabet(s) are not significantly different at $p < 0.05$ using Tukey's test

Phytochemical screening of ethanol extracts of leaves ethanol extracts of *E. citriodora* and *H. suaveolens*

The results of the qualitative phytochemical screening of *E. citriodora* showed the presence of saponins, tannins, flavonoid, phenol, quinones and alkaloids in the leaves of *E. citriodora* while that of *H. suaveolens*showed the presences of saponins, tannins, flavonoid, phenol, alkaloids and sterols. (Table 4)

Table 4: **Qualitative phytochemical composition of *E. citriodora*and *H. suaveoles*leavesethanol extracts**

S/N	Phytochemical	*E. citriodora*	*H. suaveolens*
1	Saponins	+	+
2	Tannins	+	+
3	Flavonoids	+	+
4	Phenols	+	+
5	Sterols	+	+
6	Alkaloids	+	+
7	Quinone	+	-

Key: + = detected,

- = not detected

Discussion

The hazards caused by mosquitos and the conventional chemicals insecticides used in controlling them have necessitated the needs for development of new the management strategies for the management of mosquitos and other insects. Mosquito-borne diseases such as malaria fever, filariasis, dengue hemorrhagic fever, yellow fever, among others have created huge impact on human all over the world. The chemical insecticides used in controlling mosquitoes in order to prevent the diseases vectored by them, have created problems such as environmental pollution, poor application by illiterate farmers have led to the development of resistance by the vectors, also ozone depletion potentials of some chemical insecticides containing chloro-floro carbon is part of the problems (Shyamapada Mandal, 2011). These setbacks have necessitated the search for underutilized potential botanicals that could serve as alternatives to the active and hazardous chemical insecticides. (Ogungbite*et al*., 2014; Begum *etal.*,2003).

The results obtained from this work showed that the essential oils of the leaves of *E. citriodora* and *H. suaveolens*possess the potential to control the developmental and adult stage of *A. gambiae.*The ability of these essential oils to control the insects depends on the concentration level. Both the larva pupa and the adult stages weresusceptible to the essential oils of *E. citriodora* and *H. suaveolens*.Moreover, the oil of *H. suaveolens*was more potent on the developmental and the adult stage of *A. gambiae* because it caused 100% mortality of the larvae and pupa when treated with 1.0% concentration level of the essential oils of *H. suaveolens*during 48h of exposure to the essential oils, while the adult *A. gambiae* also recorded 100% mortality on exposure to 1.0% concentration level of the essential oils within 4h of exposure.

The high larvae and pupa mortality caused by the essential oils of *H. suaveolens* is similar to the result obtain by Okumu *et al.* (2007) who obtained 95% larvae mortality on exposure to extract of *Azadirachta indica.* The 100% mortality recorded when the adult *A. gambiae* was exposed to 1.0% essential oils of *H. suaveolens* is similar to the result obtain by Massebol*et al.* (2013) who obtained 90 % adult mortality of *A. arabiensis* on exposure to ethanol leaf extract of *Ocimumlamiifolium.*The ability of the essential oils of *E. citriodora* and *H. suaveolens* to cause high mortality of the pupa and larvae is due to the blockage of the respiratory apparatus by the

oils thereby, preventing gaseous exchange and the consequent suffocation and death of the developmental stages.(Adedire*et al.,* 2011)

The phytochemical analysis of the oils of *E. citriodora* and *H. suaveolens*revealed secondary metabolites such as alkaloids, flavonoids, tannins, saponnin, sterols, quinones and phenols which have insecticidal activities.All these phytochemical have been reported to cause reduction in growth and reduction inthe larvae and pupa survival as well as distrupting the life cycle of the insects (Yang *et al.,* 2006). These phytochemicals could have contributed to the high effectiveness of the essentialoils of *E. citriodora* and *H. suaveolens against A. gambiae*. These plants have also been used as insecticides against stored beetles (Ileke*et al.,* 2014; Obembe and Ogungbite 2017).

Conclusion

In the present research theleaves extracts of *E. citriodora* and *H. suaveolens* have shown a great insecticidal potential against the larva, pupa and adult mosquito. Hence, it could be integrated into malaria vector strategies to replace the hazardous chemical insecticides

References

Aina, S.A., Banjo, A.D., Lawal, O.A. and Jonathan, K. (2009). Efficacy of some plant extracts on *Anopheles gambiae* mosquito larvae. *Acad J Entomol.,* 2(1):31-35.

Akinkurolere, R.O., Adedire, C.O., Odeyemi, O.O., Raji, O. and Owoeye, J.A. (2011): Bioefficacy of extracts of some indigenous Nigerian plant on the developmental stages of mosquito (*Anopheles gambiae*). *Jordan Journal of Biological Science,* 4(4):237-242

Balkew, M., Mumba, P., Yohannes, G., Abiy, E., Getachew, D. and Yared, S. (2021). An update on the distribution, bionomics and insecticide susceptibility of *Anopheles stephensi*in Ethiopia 2018-2020. *Malar J.* 20:263

Begum, N., Shaarma, B. and Pandey, R.S. (2003): *Caloptropisprocera*and *Annona squamosal*: potential alternatives to chemical pesticides. *British J Appl Sci* Techno.,3(2): 254-267.

Federal Ministry of Health (2012). Focus on Nigeria. Nigeria: Federal Ministry of Health http://www.rbm.who.int/progressimpactsries/docs/report11-en.pdf

Govidarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K. and Anandan, A. (2011): Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi*(*Diptera: Culicidae*) *Asian Pac J Trop Biomed,* 1:43-48.

Harborne, J.B. (1973). Phytochemical Methods. Chapman and Hall, Limited, London. (1973) 49

Ileke, K.D., Afolabi, J.O., Ogungbite, O.C., Olayinka-Olagunju, J.O. and Akanbi, O.M. (2014): Mosquito activity of *Anarcardium occidentale, Afromomummelegueta, Garcina kola* and *Citrus sinesis*against the developmental stages of mosquito, *Anopheles gambiae* Giles. *J Mosquito Res* 4(3):21-26.

Ileke, K.D. andOgungbite, O.C. (2014): Entomocidalacivity of powders and extracts of four medicinal plants against *Sitophilus oryzae* (L), *Oryzaephilusmeractor*(Faur) and *Ryzoperthadominica*(Fabr.) *Journal J Biol Sci*.,7(1): 57-62.

Ileke, K.D., Ogungbite, O.C. (2015): Alstoniaboonei De wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease. *Journal of Coastal Life Medicine*, 3(7): 557-563.

Joy, A.D., Feng, X., Mu, J., Furuya, T., Chotivanich, K., Krettli A.U. (2003): Early origin and recent expansion of *Plasmodiumfalciparumi science* 300:318-321.

Kloos, H. and Zein, H.A. (1993): The ecology of health and disease in Ethiopia. Westview Press.

Kumar, S., Wahab, N., Warikoo, R. (2011): Bio-efficacy of *Mentha piperita* essential oil dengue fever mosquito, *Aedes aegypti* L., *Asian Pac J Trop Biome,* 1:90-93.

Mandal, S. (2011). Repellant activity of *Eucalyptus* and *Azadirachta*seed oil against the filarial mosquito *Culex quinquefasciatus*Say (*Diptera: Culicidae*) in India. *Asian Pac J Trop Biomed.* 1: 181-184.

Mandal, S. (2011): Activity of *Eucalyptus* and*Azadirachtaindica* seed oil against the filarial mosquito *Culex quinquefasciatus*Say (*Diptera: Culicidae*) in India, *Asian Pac J. Trop Biomed.* 1:181-184.

Obembe, O.M. and Ogungbite O.C (2017). Comparative Insecticidal Activities of some Botanical Powders and Pirimiphos-Methyl against *Callosobruchus maculatus* Fab.(Coleoptera: Bruchidae) infesting Cowpea Seeds. *MOJ Biology and Medicine,* 2 (4): 141- 145.

Ogungbite, O.C., Ileke, K.D. andAkinneye, J.O. (2014): Bio- pesticide treated jute bags: potential alternative method of application of botanical insecticides against *Rhyzoperthadominica*(Fabricius) infesting stored wheat. *Mol. Entomol.,* 5(4): 30-36.

Okumu, F.O., Knols, B.G.J. and Fillinger, U., (2017): Larvicidal eefects of aneem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. *Malar J.* 6:63.

Onwujekwe, O., Chima, R. and Okonkwo, P (2000): Economic burden of malaria illness on households versus that of all other illness episodes: a study in five malaria holo-endemic Nigeria communities. *Health policy,* 54:143-159.

Prabhu, K., Murugan, K., Nareshkumar, A., Ramasubramanian, N. andBragasdeeswaran, S. (2011). Larvicidal and repellent potential of *Moringa oleifera* against dengue vector, *Anophelesstephensi*Liston (*Insecta:Diptera:Culicidae*) *Asian Pac J Trop Biome.* 1:127-132.

Rahuman, A.A. (2011): Efficacies of plant medicinal extracts against blood-sucking parasites. *Parasitology research monograph*. 1:19-53.

Remia, K.M. andLogaswamy, S. (2010). Larvicidal efficacy of leaf extract of two botanical against the mosquito vector, *Aedes aegypti* (*Diptera: Culicida*), *Indian Journal of Natural products and Resources*, 1(2):208-212.

Roll Back Malaria (2011): Malaria key facts. http://www. Rollbackmalaria.org/keyfacts.html.

Roll Back Malaria (2012): Key malaria facts. Geneva: Roll Back Malaria http://www. Rollbackmalaria.org/keyfacts.html.

Shankar, B.S., Saravanan, T., Ragavi, M., Kaviya, G Anushree, A. and Samraj, A. (2013): Screening of local plant for their repellent activity against mosquitoes (*Diptera: Culicidae*). *J Mosquito Res.*, 3(14): 97-104.

Tawastin, A., Wratten, S.D., Scott, R.R., Thavara, U. andTechadamrongsin, Y. (2001). Repellency of volatile oils from plants against three mosquito vectors *J Vector Ecol.*, 26:76-82.

Van Geertruyden, J.P., Thomas, F., Erhart, A.D. and Alessandro, U. (2004): the contribution of malaria in pregnancy to prenatal mortality. *Am J Trop Med Hyg.*, 71:35-40/

World Health Organisation (2010). Malaria. Geneva: World Health Organization http://www.who.int/mediacentre/factsheets/fs094/en.

World Health Organization. (2015): World malaria report summary. WHO/HTM/GMP. http://www.who.int/malaria/publications/world_malaria_report.

Zein, A.Z. (2021): The ecology of health and disease in Ethiopia. Taylor and Francis group. P 540 pp.