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Phytochemical composition and larvicidal properties of plants used for mosquito control in Kwale County, Kenya

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Abstract
The present study determined phytochemical constituents and larvicidal activities of *Tagetus minuta* L., *Adansonia digitata* Linn., *Ocimum suave*, *Plectranthus barbatus* A., *Azadirachta indica* A. Juss., *Lantana camara* L. Phytochemical analysis established that saponins were present in all the plants. Alkaloids and flavonoids were present in 83% of all the extracts. Tannins and sterols were found in 67% of the plants. Terpenoids were present in 33% of the plants while glycosides were present in 16% of the plants. Larvicidal activity was tested on 4th instar larva of *Aedes aegypti*. At the concentration of 1mg/ml, all the extracts exhibited 100% larval mortality except aqueous extract of *L. camara* that killed 90% of the larvae. In aqueous extracts, *T. minuta* extract was most active with LD_{50} of 0.61 and LD_{95} 0f 2.256. Acetone extracts of *T. minuta* and hexane extract of was *O. suave* were most active as they caused 100% larval mortality at all tested concentrations thus LD_{50} and LD_{95} could not be determined for these extracts. These findings indicate that the selected plants have larvicidal activity. More studies are needed to evaluate the activities of the plants against other types of mosquito larvae and for possible development of larvicides that are safe to use and environment friendly.

Keywords: *Aedes aegypti*, *Tagetus minuta* L., *Adansonia digitata* Linn., *Ocimum suave*, *Plectranthus barbatus* A., *Azadirachta indica* A. Juss., *Lantana camara* L., Larvicidal activity, mortality

Introduction
Mosquitoes have almost a worldwide distribution occurring in all continents except in Antarctica [1, 2]. Their bite leads to allergic reactions, dermatitis and secondary infections [3]. They are vectors in transmission of malaria, filariasis, yellow fever, dengue, Japanese encephalitis, chikungunya, O'Nyong nyong, rift valley fever and west Nile virus [4]. Mosquito control can be achieved by biological, physical and chemical methods [5]. Biological control is difficult as mosquito predators such as fish, parasites and pathogens do not lead to rapid control of the larvae [6-8]. The predators also feed on beneficial organisms and cannot be used in polluted or temporary water areas such as puddles and vehicle ruts that form only in rainy seasons [9]. Physical control and habitat change are unattainable since it is impossible to eliminate all aquatic habitats of the mosquitoes like vehicle ruts, puddles, irrigation ditches, burrow pits, foot/hoof prints, edges of boreholes, swamps and rice fields. Other mosquito habitats are sources of water and/or food such as rice paddies [1].
Mosquito breeding sites are increased by activities like production of debris and pools that hold water and water storage containers around living premises [10]. Frequent use of insecticides for mosquito control has resulted in vector resistance for all classes of insecticides and undesirable effects on non-target organisms [9]. Insecticide treated bed nets reduce airflow making it hot to sleep under the nets besides been implicated as a factor for respiratory problems for those who sleep under them [4, 11-13]. Repellents are applied to exposed areas of skin to provide temporary protection from mosquitoes. Synthetic repellents such as *N*, *N*-diethyl-m-toluamide (DEET), are expensive for everyday use and there are concerns about their toxicity and safety [5].
Organchlorine larvicides and insecticides lead to vector resistance. They persist in soil, plant and animal tissues and cause death to fish and other aquatic life [14]. Plants have been used since ancient times. *Artemisia absinthium*, *Ferula asafetida*, *Cassia* spp, *Ficus carica*, *Allium sativum*, *Urgenia maritima* and *Citrus medica* were used as insect deterrents and for personal protection [15]. Few plants have been studied for their larvicidal activity and only those

Belonging to the families *Asteraceae, Verbenaceae*, *Meliaceae*, and *Rutaceae* have been reported as a potential source of secondary metabolites for larval control [16, 17]. In the family of *Meliaceae* 4% methanol concentration of leaf extracts of *Dysoxylum malabaricum* killed more than 97% of first instars, 92% of fifth instars, 93% of pupae and 91% of adults *An. Stephensi*, *Melia azedarach* methanol Leaf and seeds showed strong larvicidal activity against *An. Stephensi* while methanol leaf extract of *Azadirachta indica* Showed an acute and chronic LC_{50} and 95% CL at 824 and 265 ppm against *Cx. Pipens* [18].

Among the *Asteraceae*, methanolic leaf extracts *Chromolaena odorata* L resulted in 100 per cent larval mortality after 24 h exposure at the concentrations of 220, 900 and 10,000 ppm respectively [19]. In another study on larvicidal activities of plants of *Asteraceae* family on *Anopheles arabiensis* larvae by Maharaj *et al*., 2012 [20], dichloromethane root extracts of *Litogyne gariepina* (DC.) Anderb. Exhibited 100% mortality in 96 hrs, aqueous leaf extracts of *Pentzia globosa* less. Had 100% mortality in 48 hrs while dichloromethane leaf extracts of *Psiadia punctulata* (DC.) Oliv. & Hiern ex Vatke had 100% larval mortality in 144 hrs. Aqueous extracts of whole plant of *Vernonia natalensis* Oliv. & Hiern had 100% larval mortality in 48 hrs.

Ghosh *et al*., 2012 [18] document several plants of the *Asteraceae* family that had larvicidal activity against various types of mosquito larvae. Among these were petroleum ether leaf extracts of *Artemisia Annua* against *Anopheles stephensi* larvae whose LC_{50} value was 16.85 ppm after 24 h and 11.45 ppm after 48 hour of exposure. Others were acetone leaf extracts of *Ageratum conyzoides* against laravae of *Cx. Quinquefasciatus* in which potent larvicidal activity was noticed while acetone twig extracts of *Ageratina adenophora* had appreciable larvicidal activity against *Ae. aegypti* and *Cx. Quinquefasciatus* whereby at 24 h, LC_{50} value of the extract was found to be 356.70 ppm for *Ae. aegypti* and 227.20 ppm for *Cx. Quinquefasciatus*. The LC_{50} value of methanol leaf extract of *Chrysanthemum indicum* against *Cx. Tritaeniorhynchus* was 42.29 mg/ml after 24 hours.

Studies on mosquito larvicidal activity of *Verbenaceae* species include the findings that acetone and methanol extracts of *V. payos* root bark and the acetone column chromatography fractions thereof exhibited larvicidal activity against larvae of *An. gambiae* within 72 hours [21]. In another study, it was proved that the extracts of the leaves of *Duranta erecta* Linn both have larvicidal activity on the larvae of *Cx. Quinquefasciatus* as exemplified by complete inhibition of adult emergence from the larvae at low concentrations of methanol and water [22] while methanol leaf extract of *Vitex negundo* against larvae of *Cx. Quinquefasciatus* had LC_{50} value of 212.57 ppm [18].

Examples of plants in the *Rutaceae* family that have larvicidal activity are dichloromethane stem extract of *Macrostylis squarrosa* Bartl. & H.L.Wendl. And leaves of *Toddalia asiatica* (L.) Lam.that had 100% mortality in 24hrs and 72hrs respectively [20]. Others are ethanol extracts of seeds of *Citrus reticulata* activity against larvae *Cx. Quinquefasciatus* and *Ae. Aegypti* whose LC_{50} values against *Ae. aegypti* and *Cx. quinquefasciatus* larvae was 2,267.71, and 2,639.27 ppm respectively [18]. The aim of this study was to evaluate larvicidal activity of plants used for mosquito control in Msambweni district, Kwale County, Kenya's south coast.

2. Materials and methods

2.1 Study area

The plants used during the present study were collected in Msambweni district, coordinates 4.47^0S 39.48^0E. It is in Kwale county of Kenya's south coast, is hot and humid throughout the year with annual mean temperatures ranging between 23 ^{0}C and 34 ^{0}C and average relative humidity ranging between 60% and 80%. The area has monsoon climate, hot and dry from January to April while June to August is the coolest period. Rainfall is in two seasons with short rains from October to December and long rains from March/April to July [23].

2.2 Selection and collection of plant material

This study was initiated to establish whether the six plants that have been reportedly used traditionally as anti mosquitoes have larvicidal activity. Selection of plants for this study was based on the ethnobotanical and ethnopharmacological surveys carried on the area [24] coupled with review of relevant literature on ethnomedicinal plants used in East Africa and the Kenya's south coast that have been reported to have activity against mosquito larvae. Field collection and initial identification of the plants was done with the assistance of traditional herbal practitioners from Msambweni District. The plants were further identified by a plant taxonomist at the Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi where voucher specimens were deposited. The plant parts were harvested during the optimal season of the months of September and November 2012. These are the months when plants in Msambweni district have adequate foliage due to rains and material of best quality is ensured [23]. After harvesting, the plant parts were cleaned with water and stored in dry bags. The collected plant material was then transported to the Department of Public Health, Pharmacology and Toxicology, University of Nairobi.

2.3 Preparation of plant material

The plant parts were scrutinized for any foreign matter or moulds and cleaned with distilled water. They were then chopped into small pieces and air dried under shade at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. When the plant material dried, it was ground into powder using a laboratory mill. The powdered plant material obtained was packed in 500gram portions and stored in clean air tight polythene papers [25].

2.4 Extraction

One thousand grams (1000 grams) of each plant powder was extracted separately using water, acetone and hexane. Water extraction was done by placing each powder in conical flasks and distilled water was added until the powder was fully submerged. Stirring and shaking of the mixture was done to ensure proper mixing. The conical flasks were corked tightly with stoppers. Shaking was done regularly to allow for percolation for four days. On the fifth day filtration was done using Whatman No.1 filter paper and the resultant liquid was collected in sterilized beakers, covered tightly in aluminium foil and stored in a refrigerator at $^+4^0$ C pending freeze-drying. Freeze drying was done using Virtis Bench Top 3® Model freeze drier (The Virtis Company, Newyork), at the Department of Veterinary Anatomy and Physiology, University of Nairobi. The freeze dried material was used for subsequent larvicidal laboratory tests. For acetone extraction, the plant powder was extracted separately by placing the powder in conical flasks and analytical grade acetone was added until the powder was fully submerged. Stirring was done to ensure proper mixing and shaking was done regularly to allow percolation. On the fifth day, the extracts were filtered using Whatman No.1 filter paper into another conical flask. Acetone was removed in a rotary evaporator at 60^0c and collected for recycling. The resultant viscous substance was dried and stored in amber coloured bottles and in a refrigerator at $+4^0$C and was eventually used for larvicidal laboratory tests. Hexane extraction was done by placing the powder in a conical flask and hexane was added until the powder was fully submerged. The conical flask was corked with appropriate stopper. For four days, thorough stirring was done to ensure proper mixing and percolation. On the fifth day, the extracts were filtered using Whatman No.1 filter paper into another conical flask. Hexane was removed in a rotary evaporator at 60^0c and collected for recycling. The resultant viscous substance obtained was dried and stored in amber coloured bottles in a refrigerator and maintained at $+4^0$C until larvicidal laboratory testing.

2.5 Phytochemical Screening

Phytochemical screening was done to determine presence or absence of secondary metabolites such as tannins, alkaloids, flavonoids, saponins, sterols, anthraquinones, terpenoids, sterols and glycosides. This was done according to established procedures [26- 28].

2.6 Determination of Larvicidal activity

Larvae of *Aedes aegypti* were used for larvicidal determination. The eggs of *Aedes aegypti* were collected on a filter paper and reared in trays containing tap water maintained at 28±2°C. On hatching, the larvae were fed on yeast powder and glucose until they moulted to fourth instar by the seventh day. Larvicidal tests were carried out on newly emerged 4^{th} instar larvae reared under standard conditions. The stock solution was prepared according to WHO, 2005 [29]. Dilutions of the extracts of 1, 0.5, 0.25 and 0.125% were made from stock solution [30-33]. The aqueous extracts were made in distilled water, acetone extracts in analytical grade acetone while hexane extracts in 3% DMSO [18]. One (1) ml of the dilution was made up to 250 ml with distilled water. Twenty five (25) larvae were exposed to different concentrations of the extracts. Larvae were considered dead if they were immobile, unable to reach the water surface and lacked head to tail flexion in response to tapping the beaker with a probe [9]. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed [29]. Mortality was recorded after 24 hours and determined using Abbott's formula [34].

2.7 Statistical analysis

The average larval mortality data was subjected to probit analysis for calculating LD_{50}, LD_{95} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit using the SPSS V22 (Statistical Package of Social Sciences) software [35]. Results with $P< 0.05$ were considered to be statistically significant.

3. Results

The study area is shown in figure 1 below. Phytochemical results are presented in table 1 below. Secondary metabolites were present in extracts ranging from sixteen (16) percent to hundred (100) percent. Saponins were present in all the plant extracts while alkaloids and flavonoids were each present in eighty three (83) percent of all the extracts tested. Tannins and sterols were each found in sixty seven (67) percent of all the plants tested. Terpenoids were present in thirty three (33) percent of the plants while glycosides were present in sixteen (16) percent of the plants. *Adansonia digitata* had alkaloids, tannins, saponins, sterols and flavonoids while terpenoids and glycosides were absent. *O. suave* had alkaloids, tannins, saponins, sterols, terpenoids and flavonoids while glycosides were absent. *Azadirachta indica* extracts had saponins, sterols and flavonoids while alkaloids, tannins, terpenoids and glycosides were absent. *Tagetes minuta* had alkaloids, tannins, saponins and flavonoids while sterols, terpenoids and glycosides were absent. Alkaloids, tannins, saponins, terpenoids and flavonoids were present in *Plectranthus barbatus* while sterols were absent. *Lantana camara* had alkaloids, saponins, flavonoids, glycosides while tannins, sterols, and terpenoids were absent.

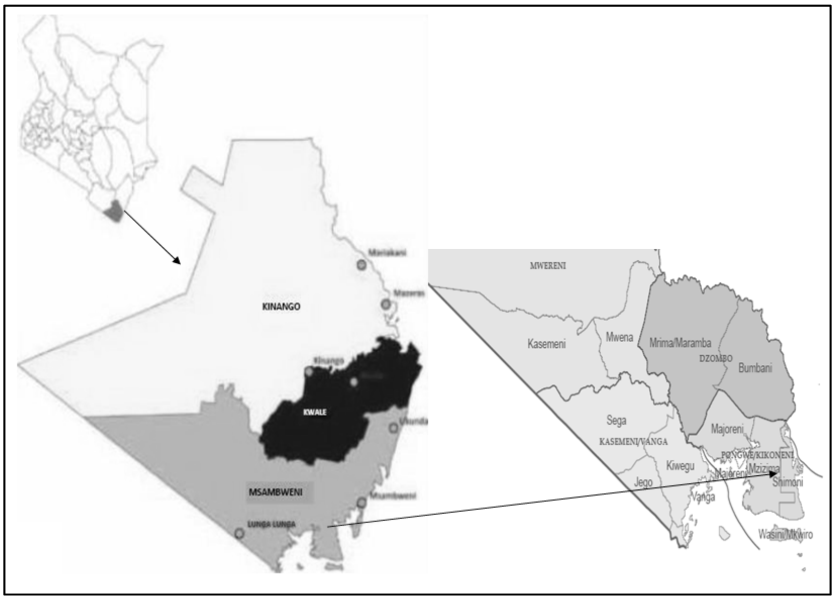

Fig 1: Map of Kenya showing Kwale County, Msambweni district and Shimoni location

Table 1: phytochemical composition of extracts of six plants used for mosquito control in kwale county, Kenya

Family	Plant species	Life form	Part used	Alk	Tan	Sap	Ster	Terp	Flav	Gly
Asteraceae	*Tagetus minuta*	Tree	Leaves	+	+	+	+	-	+	-
Bombacacea	*Adansonia digitata* Linn.	Tree	Leaves	+	+	+	+	-	+	-
Labiatae	*Ocimum suave*	Herb	Leaves	+	+	+	+	+	+	-
Labiatae	*Plectranthus barbatus*	Shrub	Leaves	+	+	+	-	+	+	+
Meliacea	*Azadirachta indica*	Tree	Leaves	-	-	+	+	-	+	-
Verbenaceae	*Lantana camara L*	Shrub	Leaves	+	-	+	-	-	+	+

Key: Alk: Alkaloids**; Tan:** Tannins**; Sap:** Saponins**; Ster:** Sterols**; Terp:** Terpenoids**; Flav:** Flavonoids**; Gly:** Glycosides

3.1 Larvicidal activity

3.1.1 Determination of LD_{50} and LD_{95} at 95% confidence interval

Acetone extracts of *A.digitata* had more activity followed by its aqueous and the hexane extract. Aqueous extracts of *O. suave* had better activity than the acetone extract. However, at the tested hexane concentrations, both LD_{50} and LD_{95} could not be determined since larvae were killed in all the concentrations. Acetone and aqueous extracts of *A. indica* had greater activity than the hexane extracts. Aqueous extract of *T. minuta* has better than the hexane extract. The acetone extract of *T. minuta* was most active than both the aqueous and hexane extracts. Acetone extracts of *P. barbatus* were more active followed by the hexane extracts then aqueous extracts. Acetone extracts of *L. camara* were more active followed by hexane and then aqueous extracts. Acetone extracts were more active followed by the aqueous and the hexane extracts.

Table 2: Larvicidal efficacy of six plants used for mosquito control in Kwale County, Kenya

Family	Plant species	Life form	Part used	Aqueous		Acetone		Hexane	
				LD 50	LD 95	LD 50	LD 95	LD 50	LD 95
Asteraceae	*Tagetus minuta*	Tree	Leaves	0.743	2.123	0.64	1.135	1.101	3.223
Bombacacea	*Adansonia digitata* Linn.	Tree	Leaves	2.598	3.987	2.453	4.492	-	-
Labiatae	*Ocimum suave*	Herb	Leaves	1.366	3.784	1.363	3.524	1.858	3.31
Labiatae	*Plectranthus barbatus*	Shrub	Leaves	0.61	2.256	-	-	1.053	2.601
Meliacea	*Azadirachta indica*	Tree	Leaves	1.914	4.065	1.501	2.47	2.694	3.33
Verbenaceae	*Lantana camara L*	Shrub	Leaves	2.227	5.436	1.638	3.32	2.823	4.216

3.1.2 Larval mortality

The extracts showed larvicidal activity which was dependent on the type of the extract and the concentration. At the highest concentration of 1mg/ml, all the extracts exhibited a hundred (100) percent mortality of the tested larvae except aqueous extract of *Lantana camara* that killed ninety (90) percent of the larvae. Acetone had most larvicidal activity of hundred percent at 0.5mg/ml of the extracts except for *Ocimum suave* where its mortality effect was only fifty (50) percent of the larvae.

4. Discussion

Mosquito borne diseases are a major cause of morbidity and mortality in the Sub-Saharan Africa as well as being obstacles to socio-economic development [36]. In Kenya 170 million working days are lost each year because of malaria illness [37]. Rift Valley Fever is endemic to Africa and the Middle East with outbreaks of resulting in human illness and livestock losses in Kenya [38].

Larval control is easier and effective compared to other methods of mosquito control which have serious limitations [1, 5, 9, 39]. The larvicidal activity was dose dependent and at the highest concentration of 1mg/ml, all extracts exhibited 100% mortality. This could be due to presence of secondary metabolites especially alkaloids and flavonoids. Alkaloids have long history of use as insecticides. They include sabadilla obtained from the seeds of *Schoenocaulon officinale* whose mode of action is similar to that of the pyrethrins. Nicotine, nornicotine and anabasine, are synaptic poisons that mimic the neurotransmitter acetylcholine. They cause symptoms of poisoning to the insects similar to organophosphate and carbamate insecticides [40]. The flavonoid rotenone has insecticidal properties acting as a mitochondrial poison, which blocks the electron transport chain and prevents energy production [41]. Thus the presence of alkaloids and flavonoids in all the plants studied could have contributed to their larvicidal activity. Other secondary metabolites which have been previously studied and found to have larvicidal activity include saponins [30] and tannins [42] whose presence in the study plants could have contributed to larvicidal activity.

Lantana species have been shown to have larvicidal activity in previous studies. Dose dependent larvicidal effect of methanol and ethanol extract of *lantana camara* on larvae of *Aedes aegypti* has been demonstrated [43]. This study showed dose and extract type dependent larvicidal effect of *lantana camara*. A study done earlier established that aqueous leaf extracts of *A. indica* exhibited 100% mortality on 3rd instar larvae after twenty four hours [44]. Results from this study showed that aqueous leaf extracts of *A. indica* had 100% mortality on the tested mosquito larvae after twenty four (24) hours. This supports findings of that previous study. This study showed that *Tagetes minuta* has larvicidal activity with LC_{50} values of 0.61 to 2.6mg/ml. Larvicidal activity of *Tagetes minuta* is probably due to presence of terpenoids, saponins, alkaloids and flavonoids. This study recorded larvicidal activity of *Ocimum suave*, *Plectranthus barbatus* and *Adansonia digitata* as causing 100% mortality at 1mg/ml which was the highest concentration tested.

5. Conclusion

Mosquito borne diseases infect over one billion people worldwide annually with over one million resultant deaths [45]. These diseases have deleterious effects on the economies of such countries due to loss of man hours and their overall financial implication in terms of hospital bed occupancy. It is therefore imperative to find ways of controlling mosquitoes that are relatively harmless to non-target organisms and present little risks to users and consumers. Plant-based products are culturally acceptable, economical and locally available. Of the methods of controlling mosquitoes, larviciding approach is the more proactive, pro-environment, target specific and safer approach than controlling adult mosquitoes. Larvae have low mobility in the breeding habitats and are easy to control in these habitats. All the plants in this study had hundred percent (100%) mortality on *Aedes aegypti* larvae after twenty four hours. These plants should further be evaluated for possible development of efficacious, environment friendly larvicides that are not harmful to the users.

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6. References

1. Service MW. Lecture notes on medical entomology. Blackwell scientific publications, London, 1986; 37-48
2. Foster WA, Walker ED. Mosquitoes. In: Medical and Veterinary Entomology. Edn 1 Academic press/Elsevier Science, 2002, 203.
3. Kitchen LW, Kendra LL, Coleman RE. The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets. Journal of Vector Ecology. 2009; 34(1):50-61.
4. Ogoma SB, Lweitoijera DW, Ngonyani H, Furer B, Russell TL. Screening mosquito house entry points as a potential method for integrated control of endophagic filariasis, arbovirus and malaria vectors. PLoS Neglected Tropical Diseases 2010; 4(8):e773. *doi:10.1371/journal.pntd.0000773*
5. Jahn A, Kim SY, Choi J, Kim D, Ahnc YJ, Yong SY, *et al*. A bioassay for mosquito repellency against *Aedes aegypti*: Method validation and bioactivities of DEET analogues. Journal of Pharmacy and Pharmacology. 2010; 62:91-97.
6. Chandra G, Bhattacharjee I, Chatterjee SN, Ghosh A. Mosquito control by larvivorous fishIndian Journal of Medical Research 2008; 127:13-27.
7. Service MW. Medical entomology for students. Cambridge university press, Cambridge, UK, 2004, 22-24.
8. WHO. Larval source management: A supplementary measure for malaria vector control. World health organization, Geneva, Switzerland, 2013, 24.
9. Maniafu BM, Wilber L, Ndiege IO, Wanjala CC, Akenga TA. Larvicidal activity of extracts from three *Plumbago* spp against *Anopheles gambiae*. Mem Inst Oswaldo Cruz 2009; 104(6):813-817.
10. Tolle MA. Mosquito-borne diseases. Current Problems in Paediatric and Adolescence Healthcare 2009; 39:97-140.
11. Alaii J, Hawley W, Kolczak M, Ter Kuile F, Gimnig J, Vulule J, Phillips-Howard PA. Factors affecting use of permethrin treated bednets during a randomised-controlled trial in Western Kenya. American Journal of Tropical Medical Hygiene. 2003; 68(4):137-141.
12. Galvin KT, Petford N, Ajose F, Davies D. An exploratory qualitative study on perceptions about mosquito bed nets in the Niger Delta: what are the barriers to sustained use? Journal of Multidisciplinary Healthcare 2011; 4:73-83.
13. Nnodim AU, Urang ES, Uzoije TA. Effectiveness of Insecticide-Treated Mosquito Nets (Itns) In the Control of Malaria Disease among Slum Dwellers in Port Harcourt Metropolis, Rivers State. Research on Humanities and Social Sciences 2014; 4(3):79-85.
14. Matasyoh JC, Wathuta EM, Kariuki ST, Chepkorir R, Kavulani J. *Aloe* plant extracts as alternative larvicides for mosquito control. African Journal of Biotechnology 2008; 7(7):912-915.
15. Moore SJ, Lenglet AD. An overview of plants used as insect repellents. In: Traditional medicinal plants and malaria, CRC press, Florida 2004, 393-415.
16. Innocent E, Joseph CC, Gikonyo NK, Nkunya MH, Hassanali A. Constituents of the essential oil of *Suregada zanzibariensis* leaves are repellent to the mosquito, *Anopheles gambiae* s.s. Journal of Insect Science. 2010; 10:57.
17. Mwangi RW, Rembold H. Growth inhibiting and larvicidal effects of *Melia volkensii* extracts on *Aedes aegypti* larvae. Entomologia Experimentalis Et Applicata 1988; 46:103-108.
18. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian Journal of Medical Research. 2012; 135:581-598.
19. Sukhthankar JH, Kumar H, Godinho MHS, Kumar A. Larvicidal activity of methanolic leaf extracts of plant, *Chromolaena odorata* L. (Asteraceae) against vector mosquitoes. International Journal of Mosquito Research 2014; 1(3):33-38.
20. Maharaj R, Maharaj V, Crouch NR, Bhagwandin N, Folb PI, Pillay P, *et al*. Screening of selected ethnomedicinal plants from South Africa for larvicidal activity against the mosquito *Anopheles arabiensis*. Malaria Journal 2012; 11:320.
21. Mokua GN, Mbwambo ZH, Ochola BJ, Innocent E, Lwande W, Hassanali A. Chemical composition and evaluation of mosquito larvicidal activity of *Vitex payos* extracts against *Anopheles gambiae* Giles S.S larvae. Spatula DD. 2013; 3(3):113-120.
22. McConnell MS, Balasubramani1 M, Rajan K, Gerald IAJ. Evaluation of the larvicidal activity of the leaf extracts of Duranta erecta Linn. (Verbenaceae) on the larvae of Culex quinquefascitatus (Say) (Culicidae) Journal of Biopesticides, 2010; 3(3):582-585.
23. Muthaura CN, Rukunga GM, Chhabra SC, Mungai GM, Njagi ENM. Traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast Journal of Ethnopharmacology 2007; 114:377-386.
24. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kiama SG. Antimalarial herbal remedies of Msambweni, Kenya. *Journal of Ethnopharmacology*. 2010; 128:424-432.
25. Wagate CG, Mbaria JM, Gakuya DW, Nanyingi MO, Kareru PG, Njuguna A *et al*. screening of some Kenyan medicinal plants for antibacterial activity. Phytotherapy research 2010; 24:150-153.
26. Fawole OA, Ndhlala AR, Amoo SO, Finnie JF, Van-Staden J. Anti-inflammatory and phytochemical properties of twelve medicinal plants used for treating gastrointestinal ailments in South Africa. Journal of Ethnopharmacology. 2009; 123:237-243.
27. Ndam LM, Mih AM, Fongod AGN, Tening AS, Tonjock RK, Enang JE, *et al*. Phytochemical screening of the bioactive compounds in twenty (20) Cameroonian

medicinal plants. International Journal of Current Microbiology and Applied Sciences. 2014; 3(12):768-778.
28. Ngbede J, Yakubu RA, Nyam DA. Phytochemical screening for active compounds in *Canarium schweinfurthii* (atile) leaves from Jos North, Plateau State, Nigeria. Research Journal of Biological Sciences. 2008; 3(9):1078-1078.
29. WHO Guidelines for laboratory and field testing of mosquito larvicides, World Health Organization, Geneva, 2005, 7-12.
30. Wiseman Z, Chapagain BP. Larvicidal effects of aqueous extracts of *Balanties aegyptiaca* (desert date) against the larvae of *Culex pipens* mosquitoes. African Journal of Biotechnology 2005; 4(11):1351-1354.
31. Singh RK, Dhiman RC, Mittal PK. Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). Journal of Vector Borne Diseases 2006; 43:88-9.
32. Shanmugasundaram R, Jeyalakshmi T, Dutt SM, Murthy PB. Larvicidal activity of neem and karanja oil cakes against mosquito vectors, *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.). Journal of Environmental Biology 2008; 29(1):43-45.
33. Tinneke LSV, Puput NT. Larvicidal activity of *Syzygium polyanthum* W. leaf extract against *Aedes aegypti* L larvae. Progress in Health Sciences 2015; 5(1):102-106.
34. Ansari MA, Mittal PK, Razdan RK, Sreehari U. Larvicidal and mosquito repellent activities of Pine (*Pinus longifolia*, Family: Pinaceae) oil. Journal of Vector Borne Diseases 2005; 42:95-99.
35. IBM, IBM® SPSS® statistics version 22. www.ibm.com 2013
36. Karunamoorthi K, Sabesan S. Laboratory evaluation of dimethyl phthalate treated wristbands against three predominant mosquito (Diptera: Culicidae) vectors of disease. European Review of Medical and Pharmacological Sciences 2010; 14 (5):443-448.
37. USAID. Malaria, USAID- Kenya, 2011.
38. LaBeaud AD, Sutherland J, Muiruri S, Muchiri EM, Gray LR, Zimmerman PA *et al*. Arbovirus prevalence in mosquitoes, Kenya. Emerging Infectious Diseases 2011; 17(2). *www.cdc.gov/eid*
39. Howard AFB, Zhou G, Omlin FX. Malaria mosquito control using edible fish in Western Kenya: preliminary findings of a controlled study. BMC Public Health 2007; 7:199-204.
40. Hayes WJ Jr. Pesticides Studied in Man. Williams & Wilkins, Baltimore, 1982, 92-93.
41. Hollingworth R, Ahmmadsahib K, Gedelhak G, McLaughlin J. New inhibitors of complex I of the mitochondrial electron transport chain with activity as pesticides. Biochemical Society Transactions 1994; 22:230-33.
42. Khana VG, Kannabiran K. Larvicidal effect of *Hemidesmus indicus, Gymnema sylvestre* and *Eclipta prostrate* against *Culex quinquefasciatus* mosquito larvae. African Journal of Biotechnology 2007; 3:307-311.
43. Kumar MS, Maneemegalai S. Evaluation of Larvicidal Effect of Lantana Camara Linn against Mosquito Species *Aedes aegypti* and *Culex quinquefasciatus*. Advances in Biological Research 2008; 2(3-4):39-43.
44. Rathy MC, Sajith U, Harilal CC. Plant diversity for mosquito control: A preliminary study. International Journal of Mosquito Research 2015; 2(1):29-33.
45. WHO. A global brief on vector-borne diseases. World health organization, Geneva, Switzerland, 2014, 9.